

GENERAL STRUCTURE OF MONOGRAPHS

① ACETAZOLAMIDE

② *Acetazolamidum*

③ $C_4H_6N_4O_3S_2$; 222.25

④ acetazolamide; 00063

⑤ N-[5 - (aminosulfonyl) -1,3,4-thiadiazol-2-yl] acetamide

⑥ [59-66-5]

It contains at least 98.0% and at most 102.0% of $C_4H_6N_4O_3S_2$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white or almost white powder.

Solubility. Very slightly soluble in water, slightly soluble in ethanol, practically insoluble in chloroform, ethyl ether, and carbon tetrachloride. Soluble in dilute alkalis.

IDENTIFICATION

⑦ **A.** The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the acetazolamide SQR spectrum similarly prepared. Should the sample spectrum be not identical to the standard one, dissolve separately the sample and standard in ethanol, evaporate to dryness, and repeat the test with the residues.

⑧ **B.** The ultraviolet absorption spectrum (5.2.14), in the ranging from 230 nm to 260 nm, 0.003% solution to (w/v), in 0.01 M sodium hydroxide, exhibits maxima at 240 nm and absorbance from 0.49 to 0.52. The ultraviolet absorption spectrum ranging from 260 nm to 350 nm, 0.00075% solution (w/v), in 0.01 M sodium hydroxide, exhibits maximum at 292 nm and absorbance from 0.43 to 0.46.

⑨ **C.** In a test tube, add 20 mg of the sample, 4 mL of 2 M hydrochloric acid, and 0.2 g of zinc powder. To place the lead acetate paper strip on opening of the tube. There is detachment of hydrogen sulfide and paper becomes darker.

D. To dissolve 25 mg of the sample in mixture of 0.1 mL of sodium hydroxide SR and 5 mL of water. To add 1 mL of copper sulfate SR. It produces greenish-blue precipitate.

PURITY TESTS

Appearance of solution. To dissolve 1 g of the sample in 10 mL of M sodium hydroxide. The solution obtained is not more opalescent than the *Reference suspension II* (5.2.25) and is not more intensely colored than the *Reference color solution* (5.2.12) prepared as described below.

Reference color solution: to mix 4.8 mL of the *Ferric chloride base solution*, 1.2 mL of *Cobaltous chloride base solution*, and 14 mL of 1% hydrochloric acid (w/v). To dilute 12.5 mL of the solution with 87.5 mL of 1% hydrochloric acid (v/v).

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF_{254} silica gel as support and a mixture of ammonium, ethyl acetate, and isopropyl alcohol (v/v) (20:30:50) as mobile phase. To separately apply to the plate 20 μ L of each of the solutions freshly prepared, as described below.

Solution (1): 5 mg/mL solution of the sample in mixture of ethanol and ethyl acetate (1:1).

Solution (2): to dilute 1 mL of *Solution (1)* to 100 mL with mixture of ethanol and ethyl acetate (1:1).

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (2)* (1.0%).

Heavy metals (5.3.2.3). To use *Method III*. At most 0.002% (20 ppm).

Sulfates (5.3.2.2). To dissolve 0.96 g of the sample in 20 mL water, heat to boiling to obtain total dissolution. To cool down by stirring and filter it. To proceed as described in *Limit test for sulfates*, by using 1 mL of standard sulfuric acid. At most 0.05% (500 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample, in an oven, at 100 °C - 105 °C. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To dissolve 0.2 g of the sample in 25 mL of dimethylformamide. To titrate with 0.1 M SV ethanolic sodium hydroxide, to potentiometrically determine the endpoint. Each mL of 0.1 M SV ethanolic sodium hydroxide is equal to 22.225 mg of $C_4H_6N_4O_3S_2$.

PACKAGING AND STORAGE

In airtight closed containers, protected from light.

LABELLING

To comply with the legislation in force.

① Name of Monograph

② International Nonproprietary Names-INN

③ Molecular formula and molecular mass (g/mol)

④ Brazilian Common Denomination - DCB And DCB number

⑤ Chemical name (according to Iupac rules)

⑤ Chemical name (according to Iupac rules)

⑥ CAS Registry

⑦ Reagents (description in chapter 14)

⑧ Reference chemical substance - SQR
(Full list: www.anvisa.gov.br/farmacopeia)

⑨ General method no.

AVOCADO

Persea folium

Small fusiform crystals of calcium oxalate occur in parenchyma cells close to the ribs. At the base of the leaf blade, there are two other small collateral bundles along the edge, facing the adaxial face.

Persea americana Mill. - LAURACEAE

The vegetable drug consists of dried leaves containing at least 0.4% of total flavonoid expressed in apigenin and 0.14% of volatile oil.

SCIENTIFIC SYNONYM

Persea gratissima Gaertn. f.

CHARACTERISTICS

Organoleptic characteristics. The leaf is odorless and tastes slightly astringent.

MACROSCOPIC DESCRIPTION

Simple leaves, elliptic, oblong or oval-coriaceous, semi-coriaceous, entire margins, roughly wavy; blade from 8.0 cm to 20.0 cm long and from 4.0 cm to 9.0 cm wide; petiole up to 5 cm long and from 3 mm to 4 mm wide at the base; when fresh, they are dark green on the adaxial face, slightly bright and nearly flat, and the lower face has lighter green color, and somewhat dull rough, light brown dry leaves. Midrib prominent on the lower face, with secondary oblique ribs, also prominent, giving rise to tertiary ribs that anastomose in thin weft.

MICROSCOPIC DESCRIPTION

The leaf blade is amphistomatic and dorsiventral symmetry. The epidermis, in front view, on the adaxial face, is formed by polygonal cells with slightly sinuous-walled cells and rare unicellular trichomes, short to long, thick-walled; usually the lower surface is formed by smaller cells, rectangular or rounded, with periclinal walls slightly convex. The cuticle is grainy and the stomata are anomocytic with 3-4 subsidiary cells. The tector trichomes are common in young leaves and rare in adult leaves. In cross section, the epidermis is uniseriate on both sides with thick cuticle. On the adaxial face, the cells are elongated in the transverse direction. The mesophyll consists of one or two layers of palisade cells, elongated, presenting many mucilage secretory idioblast and volatile oil, voluminous, and rounded. The spongy parenchyma has few layers of irregular cells with large intercellular spaces. There may be a differentiated conformation of the mesophyll along the secretory idioblasts, composed of parenchyma cells, tangentially elongated and flattened, thick-walled. The midrib shows a developed collateral vascular bundle, surrounded by a sclerenchymatic sheath practically continuous.

MICROSCOPIC DESCRIPTION OF POWDER

The powder meets all the requirements for the species, unless the macroscopic characters. Characteristics: dark green color; fragments of the epidermis facing the adaxial face with isodiametric polygonal cells, covered with a thick cuticle; fragments of the epidermis facing the lower face, with smaller cells; fragments of the epidermis facing the lower face with anomocytic stomata; fragments of the epidermis facing the lower face with tector trichomes; whole tector trichomes accompanied by epidermal cells or isolated; fragments of tector trichomes; fragments of mesophyll with rounded secretory idioblasts; fragments of rib, as described, accompanied by cells containing fusiform crystals.

IDENTIFICATION

To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel, with 250 µm thickness as support and a mixture of ethyl acetate, formic acid, and water (80:10:10) as mobile phase. To separately apply to the plate 10 µl of *Solution (1)* freshly prepared, as described below.

Solution (1): to prepare 20% tincture (w/v) of pulverized leaves with 65% ethanol (v/v) by maceration or percolation.

To develop the chromatogram. To remove the plate, and let it drying. To nebulize with anisaldehyde SR. To examine under visible light. To observe five principal yellowish spots: on top of the chromatogram, an isolated spot and a two spots very close slightly below; in the middle part of the chromatogram, there are two spots. At the bottom of the chromatogram, observe a pinkish spot and another one, lower, blue color.

PURITY TESTS

Foreign matter (5.4.2.2). At most 2.0%.

Water (5.4.2.3). At most 12.0%.

Total ashes (5.4.2.4). At most 5.0%.

Sulfated ashes (5.4.2.6). At most 10.0 %.

DOSAGE

Volatile oils

To proceed as described in *Determination of volatile oils in vegetable drugs* (5.4.2.7). To use 1000 mL flask containing 500 mL of water as distillation liquid. To use dry stretched plant, not bruised.

Sample solution: to add 10 mL of *Stock solution* to a 25 mL volumetric flask with 2 mL 5% aluminum chloride solution (w/v) in 50% ethanol solution (v/v) and top up with 50 % ethanol solution (v/v). To take a reading after 30 minutes.

Blank solution: To add 10 mL of *Stock solution* to 25 mL volumetric flask and top up with 50% ethanol solution (v/v).

To measure the absorbance of the *Sample solution* at 425 nm, by using Blank solution to adjust zero. The content of total flavonoid, expressed in apigenin per 100 g of dry drug, is then calculated as:

$$\text{TFT} = \text{Abs} \times 250 / (m - \text{PD}) \times 336,5$$

To immediately proceed to determine the volatile oil from 100 g of the scratched drug. To distill for 4 hours.

Total flavonoids

To proceed as described in *Visible absorption spectrophotometry* (5.2.14). To prepare the solutions as described below.

Stock solution: to accurately weigh about 0.5 g of pulverized drug (800 µm) and transfer to a 100 mL round bottom flask. To add 1 mL of 0.5% aqueous methenamine (w/v), 30 mL of ethanol solution, and 2 mL of hydrochloric acid to the drug. To heat it in a heating mantle for 30 minutes under reflux. To use cotton to filter the mixture to a 100

mL volumetric flask. To put the residue of the drug back and the cotton to round bottom flask, add extra 30 mL of 50% ethanol solution (v/v), and heat again under reflux for 15 minutes. To use cotton again to filter to the same 100 mL volumetric flask. To repeat the operation, to put back again the residue of the drug and cotton to a round bottom flask, add 30 mL of 50% ethanol solution (v/v), heat it under reflux for 15 minutes, and filter to the same 100 mL volumetric flask. After cooling down, top up the 100 mL flask with a 50% ethanol solution (v/v).

$$\text{TFT} = \frac{\text{Abs} \times 250}{(m - \text{PD}) \times 336,5}$$

where

TFT = content of total flavonoid;

Abs = absorbance of the *Sample solution*;

250 = dilution factor;

m = mass of the drug (g);

PD = loss on drying;

336.5 = specific apigenin absorptivity.

PACKAGING AND STORAGE

In tightly closed containers, protected from light and heat.

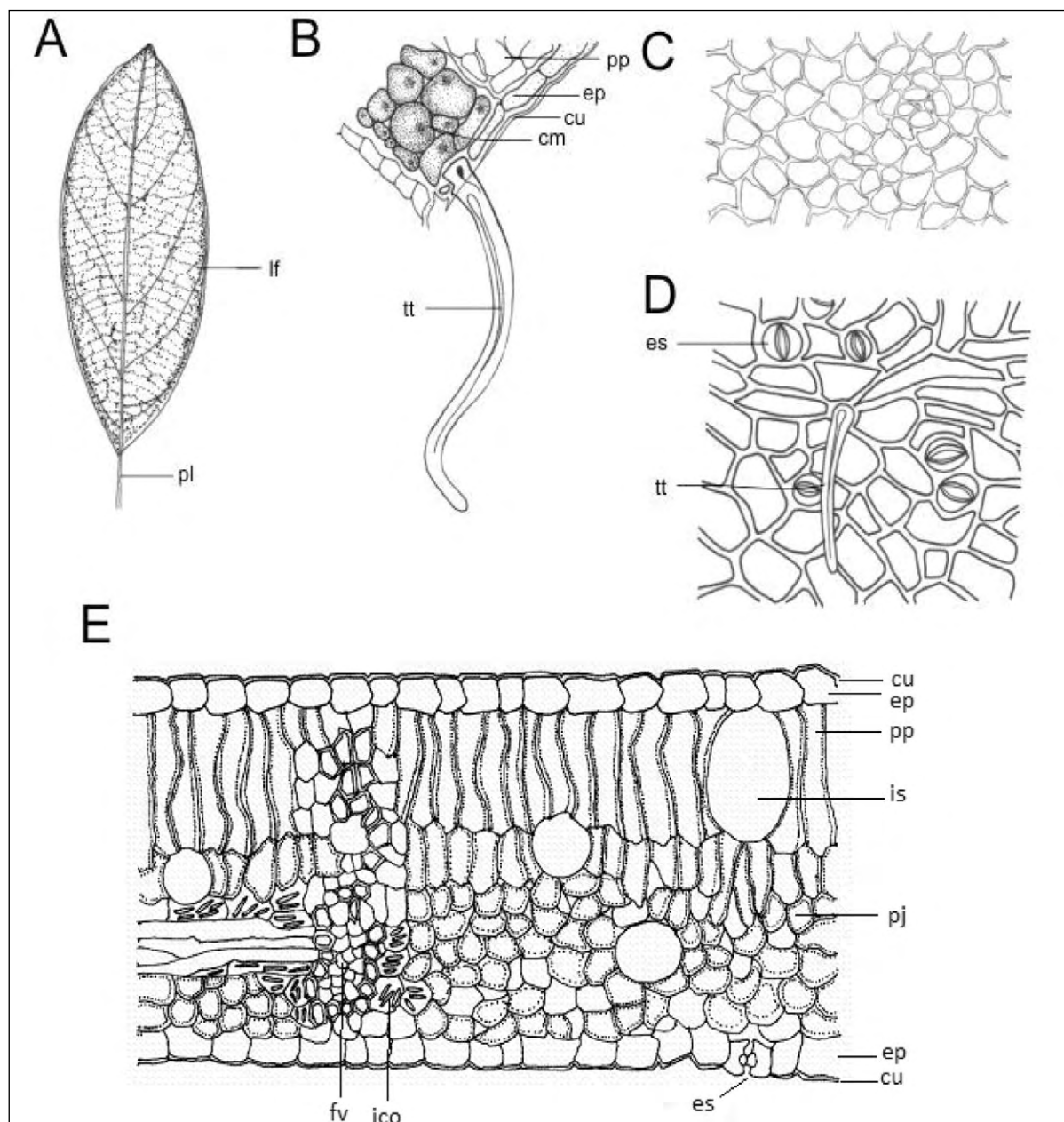


Figure 1 - Macroscopic and microscopic aspects in *Persea americana* Mill.

Additional information of Figure 1.

A — leaf in front view: leaf blade (lf); petiole (pl). **B** — partial detail of the epidermis facing the lower face in cross-section: palisade parenchyma (pp); epidermis (ep), cuticle (cu); cell containing mucilage (cm); tector trichomes (tt). **C** — partial detail of the epidermis facing the adaxial face, in front view: stomata (es); tector trichomes (tt). **D** — partial detail of the epidermis facing the lower face, in front view: stomata (es); tector trichomes (tt). **E** - details of leaf blade in cross-section: cuticle (cu); epidermis (ep); palisade parenchyma (pp); secretory idioblast (is); spongy parenchyma (pj); stomata (s); idioblast with crystals of calcium oxalate (ico), vascular bundle (fv).

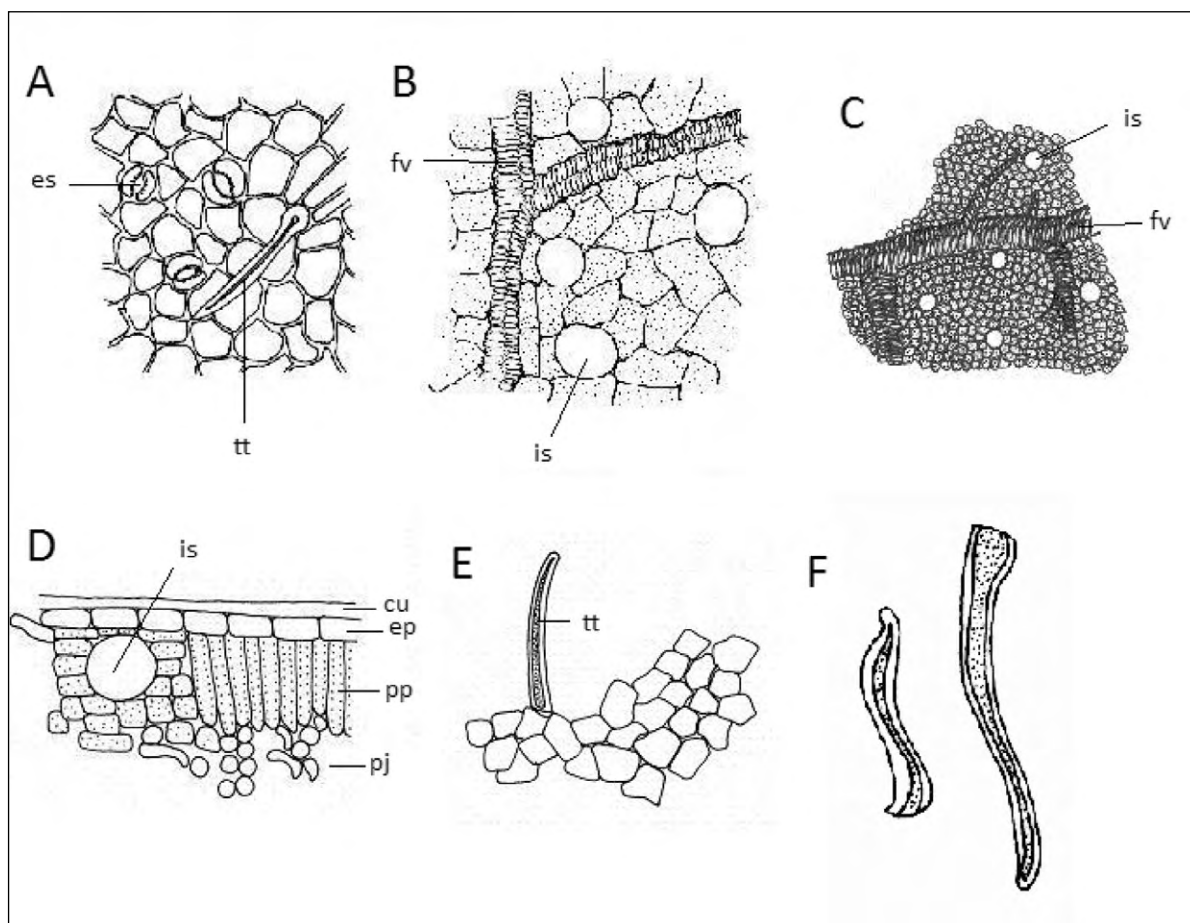


Figure 2 - Aspects of microscopy of powder in *Persea americana* Mill.

Additional information of **Figure 2**.

A — fragment of the epidermis facing the lower face: stomata (es); tector trichomes (tt). **B** and **C** — fragments of the leaf blade in front view, with emphasis on vascular bundles and secretory idioblasts: vascular bundle (fv); secretory idioblast (is). **D** - fragment of the leaf blade in cross section showing secretory idioblast with cells with different conformation: secretory idioblast (is); cuticle (cu); epidermis (ep); palisade parenchyma (pp); spongy parenchyma (pj). **E** — fragment of the epidermis: tector trichomes (tt). **F** — fragments of trichomes

ACETATE, DEXAMETHASONE, CREAM

It contains at least 90.0 % and at most 110 % of the amount stated of $C_{24}H_{31}FO_6$.

IDENTIFICATION

The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

BIOLOGICAL SAFETY TEST

Counting of the total viable micro-organisms (5.5.3.1.2). It complies with the test.

Survey and identification of pathogens (5.5.3.1.3).

It complies with the test.

DOSAGE

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 240 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μ m), kept at 40 °C; flow of *Mobile phase* of 1.2 mL/min.

Mobile phase: mixture of methanol and water (65:35).

Standard solution: to accurately weigh about 20 mg of dexamethasone acetate SQR and transfer to a 100 mL volumetric flask. To add 50 mL of methanol and put it in ultrasound to dissolve. To top up with methanol and mix it. To transfer 5 mL of this solution to 50 mL volumetric flask, top up with *Mobile phase*, and make it homogeneous.

Sample solution: to transfer amount of the sample carefully weighed equivalent to 2 mg of dexamethasone acetate. To add 40 mL of methanol and put it in ultrasound, by stirring with the glass rod to dissolve. To quantitatively transfer to 100 mL volumetric flask, top up with the same solvent, and make it homogeneous.

To inject replicates of 20 μ L of the *Standard solution*. The relative standard deviation for the replicate areas for the peaks recorded is not greater than 2.0%.

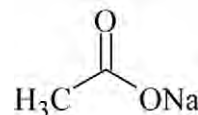
Procedure: to separately inject 20 μ L of *Sample and standard solutions*, record the chromatograms, and measure area under the peaks. To calculate the content of $C_{24}H_{31}FO_6$ from the sample, from the responses obtained for the *Sample and Standard solutions*.

PACKAGING AND STORAGE

In tightly closed container, protected from excessive heat.

LABELLING

To comply with the legislation in force.

ACETATE, SODIUM
Natrii acetat

$C_2H_3NaO_2$; 82.03

$C_2H_3NaO_2 \cdot 3H_2O$; 136.08

sodium acetate; 00087

trihydrate sodium acetate; 00088

Sodium salt of acetic acid (1:1)

[127-09-3]

Sodium salt of hydrate acetic acid (1:1:3) [6131-90-4]

It contains at least 99.0% and at most 101.0% of $C_2H_3NaO_2$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Colorless, transparent crystals, or white crystalline powder, granular, or white flake. Odorless and mild acetous odor, with saline taste slightly bitter. It effloresces if exposed to warm, dry air.

Solubility. Very soluble in water, and soluble in ethanol.

IDENTIFICATION

A. It responds to the reactions of acetate ion (5.3.1.1).

B. It responds to the reactions of sodium ion (5.3.1.1).

PURITY TESTS

Appearance of solution. The 10% aqueous solution (w/v) is clear (5.2.25) and colorless (5.2.12).

pH (5.2.19). To prepare a solution containing 5% (w/v) of $C_2H_3NaO_2$ and proceed as described for *Determination of pH*. From 7.5 to 9.2.

Insoluble matter. To dissolve the equivalent to 20 g of anhydrous sodium acetate with water to 150 mL. To prepare this solution in a beaker and heat to boiling. To cover the beaker with a watch glass and leave it in a water bath for an hour. To filter by using a pre-weighed filter, wash, and dry at 105 °C to obtain constant weight. At most 0.05% (500 ppm).

Calcium and magnesium. To dissolve the equivalent to 0.2 g of anhydrous sodium acetate with water to 20 mL. To add 2 mL of the following reagents: 6 M ammonium hydroxide, ammonium oxalate SR, and 12% dibasic sodium phosphate (w/v). No turbidity is developed for 5 minutes.

Potassium. To dissolve the equivalent to 3 g of anhydrous sodium acetate with water to 5 mL. To acidify the solution with a few drops of *M* acetic acid, and add 5 drops of sodium cobaltinitrite SR. No precipitate is formed.

Arsenic (5.3.2.5). To dissolve the equivalent to 1 g of anhydrous sodium acetate in 35 mL of water and proceed as described in *Limit test for arsenic*. At most 0.0003% (3 ppm).

Chlorides (5.3.2.1). The equivalent to 1 g of anhydrous sodium acetate does not present more chlorides than the equivalent to 0.5 mL of 0.02 *M* SV hydrochloric acid. At most 0.035% (350 ppm).

Iron (5.3.2.4). To proceed as described in *Method I* by using 10 mL of the solution obtained *Appearance of solution*. To use 1 mL of the *Iron standard solution* (10 ppm). At most 0.001% (10 ppm).

Heavy metals (5.3.2.3). To use the *Method I*. To dissolve equivalent to 4.2 g of anhydrous sodium acetate in 50 mL volumetric flask. To top up to volume with water and proceed as described in *Limit test for heavy metals*, by using *Lead standard solution* (2 ppm Pb). At most 0.001% (10 ppm).

Sulfates (5.3.2.2). The equivalent to 10 g of anhydrous sodium acetate does not present more sulfates than the equivalent to 0.50 mL of 0.01 *M* SV sulfuric acid. At most 0.005% (50 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 105 °C until obtaining constant weight. The hydrate form loses from 38% to 41% by weight; the anhydrous form loses no more than 1%.

DOSAGE

To proceed as described in *Titrations in non-aqueous medium* (5.3.3.5). To weigh amount equivalent to 0.2 g of sodium acetate previously desiccated, and dissolve it in 25 mL of glacial acetic acid. To warm it, if necessary, to obtain complete solubilization. To add 2 drops of 1-naphtholbenzein. To titrate with 0.1 *M* SV perchloric acid. To perform sample blank correction and adjust it as required. Each mL of 0.1 *M* SV perchloric acid is equal to 8.203 mg of $C_2H_3NaO_2$.

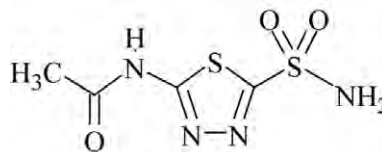
PACKAGING AND STORAGE

In tightly closed containers.

CATEGORY

Pharmaceutical adjuvant used in dialysis solutions.

ACETAZOLAMIDE Acetazolamidum



$C_4H_6N_4O_3S_2$; 222.25

acetazolamide; 00063

N-[5 - (aminosulfonyl) -1,3,4-thiadiazol-2-yl] acetamide
[59-66-5]

It contains at least 98.0% and at most 102.0% of $C_4H_6N_4O_3S_2$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white or almost white powder.

Solubility. Very slightly soluble in water, slightly soluble in ethanol, practically insoluble in chloroform, ethyl ether, and carbon tetrachloride. Soluble in dilute alkalis.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the acetazolamide SQR spectrum similarly prepared. Should the sample spectrum be not identical to the standard one, dissolve separately the sample and standard in ethanol, evaporate to dryness, and repeat the test with the residues.

B. The ultraviolet absorption spectrum (5.2.14), ranging from 230 nm to 260 nm, 0.003% solution to (w/v), in 0.01 *M* sodium hydroxide, exhibits maxima at 240 nm and absorbance from 0.49 to 0.52. The ultraviolet absorption spectrum ranging from 260 nm to 350 nm, 0.00075% solution (w/v), in 0.01 *M* sodium hydroxide, exhibits maximum at 292 nm and absorbance from 0.43 to 0.46.

C. In a test tube, add 20 mg of the sample, 4 mL of 2 *M* hydrochloric acid, and 0.2 g of zinc powder. To place the lead acetate paper strip on opening of the tube. There is detachment of hydrogen sulfide and paper becomes darker.

D. To dissolve 25 mg of the sample in mixture of 0.1 mL of sodium hydroxide SR and 5 mL of water. To add 1 mL of copper sulfate SR. It produces greenish-blue precipitate.

PURITY TESTS

Appearance of solution. To dissolve 1 g of the sample in 10 mL of *M* sodium hydroxide. The solution obtained is not more opalescent than the *Reference suspension II* (5.2.25) and is not more intensely colored than the *Reference color solution* (5.2.12) prepared as described below.

Reference color solution: to mix 4.8 mL of the *Ferric chloride base solution*, 1.2 mL of *Cobaltous chloride base solution*, and 14 mL of 1% hydrochloric acid (w/v). To dilute 12.5 mL of the solution with 87.5 mL of 1% hydrochloric acid (v/v).

Related substances. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF₂₅₄ silica gel as support and a mixture of ammonium, ethyl acetate, and isopropyl alcohol (v/v) (20:30:50) as mobile phase. To apply separately to the plate 20 µl of each of the solutions freshly prepared, as described below.

Solution (1): 5 mg/mL solution of the sample in mixture of ethanol and ethyl acetate (1:1).

Solution (2): to dilute 1 mL of *Solution (1)* to 100 mL with mixture of ethanol and ethyl acetate (1:1).

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (2)* (1.0%).

Heavy metals (5.3.2.3).

To use the *Method III*. At most 0.002% (20 ppm).

Sulfates (5.3.2.2). To dissolve 0.96 g of the sample in 20 mL water, heat to boiling to obtain total dissolution. To cool down by stirring and filter it. To proceed as described in *Limit test for sulfates*, by using 1 mL of standard sulfuric acid. At most 0.05% (500 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample, in an oven, at 100 °C - 105 °C. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To dissolve 0.2 g of the sample in 25 mL of dimethylformamide. To titrate with 0.1 M SV ethanolic sodium hydroxide, to potentiometrically determine the endpoint. Each mL of 0.1 M SV ethanolic sodium hydroxide is equal to 22.225 mg of C₄H₉N₄O₃S₂.

PACKAGING AND STORAGE

In airtight closed containers, protected from light.

LABELLING

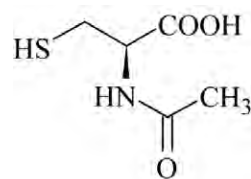
To comply with the legislation in force.

THERAPEUTIC CLASS

Diuretic.

ACETYLCYSTEINE

Acetylcysteinum



C₅H₉NO₃S; 163.19
acetylcysteine; 00067
N-Acetyl-L-cysteine
[616-91-1]

It contains at least 98.0% and at most 102.0% of C₅H₉NO₃S, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white or almost colorless powder.

Solubility. Freely soluble in water and ethanol and practically insoluble in methylene chloride.

Physical and chemical constants.

Melting range (5.2.2): 104 °C to 110 °C.

Specific rotation (5.2.8): +21° to +27°, in relation to the desiccated substance. To add 1.25 g of the sample, 1 mL of 1% disodium edetate (w/v), 7.5 mL of sodium hydroxide SR in 25 mL volumetric flask and mix it. To top up to volume with phosphate buffer pH 7.0.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the acetylcysteine SQR spectrum similarly prepared.

B. To dissolve about 1 g of the sample in 20 mL of water and add 0.05 mL of 5% sodium nitroprusside (w/v) and 0.05 mL of ammonium hydroxide. The dark violet color develops.

PURITY TESTS

Appearance of solution. The solution of the 5% sample (w/v) is clear (5.2.25) and colorless (5.2.12).

pH (5.2.19). 2.0 to 2.8. To determine in 1% solution (w/v) in carbon dioxide-free water.

Heavy metals (5.3.2.3). To carefully moisten 2 g of the sample, dropwise, with 2 mL of nitric acid and proceed as described in *Method III*. At most 0.001% (10 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample in oven at 70 °C under reduced pressure to obtain constant weight. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 2 g of the sample. At most 0.5%.

DOSAGE

To employ one of the methods described below.

A. To accurately weigh about 0.14 g of the sample, dilute in 60 mL of water, and add 10 mL of 2 M hydrochloric acid. To cool down in an ice bath, add 10 mL of potassium iodide SR, and titrate with 0.05 M SV iodine, thus potentiometrically determining the endpoint or by using 1 mL of starch SI as indicator. Each mL of 0.05 M SV iodine is equal to 16.319 mg of $C_3H_9NO_3S$.

B. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 214 nm; 300 mm column and 3.9 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), flow of *Mobile phase* of 1.5 mL/min.

Mobile phase: to dissolve 6.8 g of monobasic potassium phosphate in 1000 mL of water. To filter and adjust the pH to 3.0 with phosphoric acid.

Internal standard solution: to transfer approximately 1 g of DL-phenylalanine to 200 mL volumetric flask and top up with 0.05% sodium metabisulphite (w/v) freshly prepared. To make it homogeneous.

Sample solution: to accurately weigh 1 g of the sample and transfer to a 100 mL volumetric flask. To top up with 0.05% sodium metabisulphite (w/v) and make it homogeneous. To transfer 5 mL of this solution and 5 mL of *Internal standard solution* to 100 mL volumetric flask and top up with 0.05% sodium metabisulphite (w/v).

Standard solution: to accurately transfer about 0.1 g of acetylcysteine SQR to 10 mL volumetric flask and top up with 0.05% sodium metabisulphite (w/v). To transfer 5 mL of this solution and 5 mL of *Internal standard solution* to 100 mL volumetric flask and top up with 0.05% sodium metabisulphite (w/v), thus obtaining 0.5 mg/mL solution.

To inject 5 µL of *Standard solution*. The resolution between the acetylcysteine and DL-phenylalanine peaks should be greater than 6. The relative standard deviation for replicate areas of the peaks recorded is less than 2.0%.

Procedure: to separately inject 5 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks

related to acetylcysteine and DL-phenylalanine. To calculate the content of $C_3H_9NO_3S$ from the sample, from the responses of relationship acetylcysteine/DL-phenylalanine with the *Sample and standard solutions*.

PACKAGING AND STORAGE

In tightly closed containers.

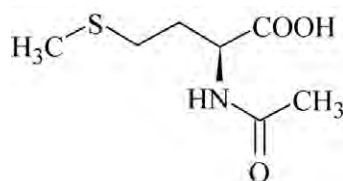
LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Mucolytic.

ACETYL METHIONINE Acetylmethioninum



$C_7H_{13}NO_3S$; 191.25
acetyl methionine; 00074
N-Acetyl-L-methionine [65-82-7]

It contains at least 98.0% of $C_7H_{13}NO_3S$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White crystalline powder, typical mild unpleasant odor and slightly bitter flavor.

Solubility. Soluble in water, acetone, and boiling ethanol.

Physical and chemical constants.

Melting range (5.2.2): 114 °C to 116 °C.

IDENTIFICATION

To dissolve 10 mg of the sample in 1 mL of distilled water and successively add by stirring 1 mL of 5 M sodium hydroxide, 1 mL of glycerol, and 0.3 mL of 5% sodium nitroprusside (w/v). To heat from 35 °C to 40 °C, for 10 minutes, and cool down in an ice bath for 2 minutes. To add 1.5 mL of hydrochloric acid SR and stir it. The red-purple color develops.

PURITY TESTS

Appearance of solution. To dissolve 0.2 g of the sample in 2 mL of distilled water. The resulting solution is clear (5.2.25).

To add 38 mL of distilled water and set apart this solution for the other tests.

Iron (5.3.2.4). To use *Method I*. To determine in 10 mL of the solution obtained in *Appearance of solution*. At most 0.005% (50 ppm).

Chlorides (5.3.2.1). To determine in 10 mL of the solution obtained in *Appearance of solution*. At most 0.015% (150 ppm).

Heavy metals (5.3.2.3). To determine in 10 mL of the solution obtained in *Appearance of solution*. At most 0.002% (20 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 105 °C for 4 hours to obtain constant weight. At most 2.0%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To accurately weigh about 0.3 g of the sample and transfer to a erlenmeyer flask with lid. To add 100 mL of water, 5 g of dibasic potassium phosphate, 2 g of monobasic potassium phosphate, and 2 g of potassium iodide. To stir to obtain total dissolution. To add 50 mL of 0.05 *M* SV iodine, stir it, and let it rest for 30 minutes. To titrate the iodine in excess with 0.1 *M* SV sodium thiosulfate, add 3 mL of starch SI near the endpoint, and continue titration until the blue color disappears. To perform sample blank correction and adjust it as required. Each mL of 0.05 *M* SV iodine is equal to 9.562 mg of $C_8H_{11}N_5O_3$.

PACKAGING AND STORAGE

In tightly closed containers.

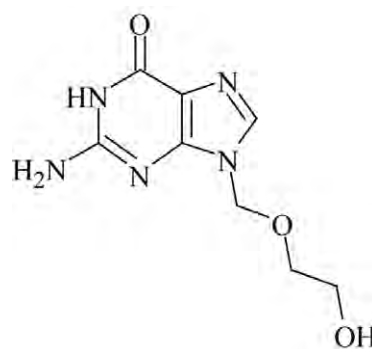
LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Lipotropic.

ACYCLOVIR Aciclovirum



$C_8H_{11}N_5O_3$; 225.20

aciclovir; 00082

2-Amino-1,9-dihydro-9-[(2-hydroxyethoxy) methyl]-6H-purin-6-one
[59277-89-3]

It contains at least 98.0% and at most 101.0% of $C_8H_{11}N_5O_3$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. Crystalline, white or almost white powder.

Solubility. Sparingly soluble in water, freely soluble in dimethyl sulfoxide, and very slightly soluble in ethanol. Soluble in dilute mineral acid solutions and alkalis.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the aciclovir SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14), from 200 nm to 350 nm, 0.015% solution (w/v) prepared in 0.1 *M* hydrochloric acid, has absorption maximum at 255 nm and shoulder around 274 nm, similar to those observed in the spectrum of a similar solution of aciclovir SQR.

C. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method **B**, corresponds to that of main peak of *Standard solution*.

PURITY TESTS

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF_{254} silica gel as support and a mixture of chloroform, methanol, and ammonium hydroxide (80:20:2) as mobile phase. To apply separately to the plate 5 µl of each of the solutions freshly prepared, as described below.

Solution (1): to transfer 0.1 g of the sample to 10 mL volumetric flask, dissolve in dimethyl sulfoxide, and top up with the same solvent, to obtain 10 mg/mL solution.

Solution (2): 0.2 mg/mL aciclovir SQR solution in dimethyl sulfoxide.

Solution (3): 0.1 mg/mL aciclovir SQR solution in dimethyl sulfoxide.

Solution (4): 0.05 mg/mL aciclovir SQR solution in dimethyl sulfoxide.

Solution (5): 0.01 mg/mL aciclovir SQR solution in dimethyl sulfoxide.

Procedure: To develop the chromatogram. To remove the plate, dry the spots in a current of air. To examine under ultraviolet light (254 nm). Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (2)*, *Solution (3)*, *Solution (3)*, and *Solution (5)*. The sum of impurities observed does not exceed 2.0%.

Limit for guanine. To proceed as described in *Dosage method B*. To calculate the content of guanine from the sample, from the responses obtained for the peak related to guanine in the *Sample and Standard solutions*. At most 0.7%.

Water (5.2.20.1). At most 6.0%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. To dissolve 0.15 mg of the sample in 60 mL of glacial acetic acid. To titrate with 0.1 M SV perchloric acid, to potentiometrically determine the endpoint. To perform sample blank correction and adjust it as required. Each mL of 0.1 M SV perchloric acid is equal to 22.52 mg of $C_8H_{11}N_5O_3$.

B. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 254 nm; 250 mm column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μ m to 10 μ m), flow of *Mobile phase* of 3 mL/min.

Mobile phase: mixture of water and glacial acetic acid (100:0.1).

Guanine solution: to accurately transfer about 8.75 g of guanine to a 500 mL volumetric flask, and dissolve it in 50 mL of 0.1 M sodium hydroxide. To top up it with water and make it homogeneous.

Sample solution: to accurately transfer about 0.1 g of the sample to a 200 mL volumetric flask by using 20 mL of 0.1 M sodium hydroxide. To add 80 mL of water, leave it in ultrasound for 15 minutes, mechanically stir for 15 minutes. To top up with water, and make it homogeneous. To transfer 10 mL to 50 mL volumetric flask, top up with 0.01 M sodium hydroxide, and make it homogeneous.

Standard solution: to accurately transfer about 25 g of aciclovir SQR to a 50 mL volumetric flask, dissolve it with 0.5 mL of 0.1 M sodium hydroxide. To top up it with water and make it homogeneous. To transfer 10 mL of this solution to a 50 mL volumetric flask, add 2 mL of Guanine solution, top up to volume with 0.01 M sodium hydroxide, and make it homogeneous, to obtain a 0.1 mg/mL concentration of aciclovir SQR and 0.7 μ g/mL of guanine.

The relative retention times are about 0.2 to guanine and 1 to aciclovir. The tailing factor to peak analyzed should not be greater than 2.0. The resolution between aciclovir and guanine should not be less than 2.0. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2 %.

Procedure: to separately inject 20 μ L of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_8H_{11}N_5O_3$ from the sample, from the responses obtained for the *Sample and standard solutions*.

PACKAGING AND STORAGE

In airtight containers, at a temperature below 25 °C.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antiviral.

ACICLOVIR, TABLETS

It contains at least 95.0 % and at most 105 % of the amount stated of $C_8H_{11}N_5O_3$.

IDENTIFICATION

A. The ultraviolet absorption spectrum (5.2.14) ranging from 230 nm to 350 nm, of sample solution obtained in *Dosage*, exhibits maxima at 255 nm and shoulder about 264 nm.

B. To proceed as described in *Limit for guanine*. The principal spot obtained with *Solution (2)* corresponds in position, color, and intensity to that obtained with *Solution (3)*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 900 mL

Equipment: blade, 50 rpm

Time: 45 minutes

Procedure: after testing, remove aliquot from the dissolution medium, filter, and dilute in 0.1 M hydrochloric acid, to obtain suitable concentration. To measure the absorbance of the solutions at 255 nm (5.2.14), by using the same solvent to adjust zero. To calculate the amount of $C_8H_{11}N_5O$ dissolved in the medium, by comparing the readings obtained with aciclovir SQR solution (w/v) at 0.001 % concentration (w/v). Alternatively, make the calculations taking into account that $A(1\%, 1\text{ cm}) = 560$ at 255 nm, in 0.1 M hydrochloric acid.

Tolerance: not less than 80% (Q) of the stated amount of $C_8H_{11}N_5O_3$ dissolves within 45 minutes.

PURITY TESTS

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF_{254} silica gel as support and a mixture of 13.5 M ammonium hydroxide, methanol, and methylene chloride (2:20:80) as mobile phase. To apply separately to the plate 2 μ L of each of the solutions freshly prepared, as described below.

Solution (1): to weigh and pulverize the tablets. To stir for 15 minutes the amount of powder equivalent to 0.25 g of aciclovir with 10 mL of dimethyl sulfoxide. To filter it.

Solution (2): to dilute 0.7 volumes of *Solution (1)* to 100 volumes with dimethyl sulfoxide.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (2)* (0.7%).

Limit for guanine. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using F_{254} cellulose as support and a mixture of n-propyl alcohol, 13.5 M ammonium hydroxide, and 5% ammonium sulfate (w/v) (10:30:60) as mobile phase. To apply separately to the plate 10 μ L of each of the solutions freshly prepared, as described below.

Solution (1): to weigh and pulverize the tablets. To transfer amount of powder equivalent to 0.25 g of aciclovir to

50 mL volumetric flask. To add 25 mL of 0.1 M sodium hydroxide, stir for 10 minutes, and top up with 0.1 M sodium hydroxide. To decanter the undissolved material before applying the plate.

Solution (2): to transfer 1 mL of *Solution (1)* to 10 mL volumetric flask and top up with 0.1 M sodium hydroxide.

Solution (3): to dissolve 5 mg of aciclovir SQR in 10 mL of 0.1 M sodium hydroxide.

Solution (4): to dissolve 5 mg of guanine in 100 mL of 0.1 M sodium hydroxide.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). Any secondary spot related to guanine in the chromatogram obtained with *Solution (1)* is not more intense than that obtained with *Solution (4)* (1.0%). To discard the spots present in the application point of the solvent.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3).

It complies with the test.

DOSAGE

To weigh and pulverize 20 tablets. To transfer amount of powder equivalent to 0.1 g of aciclovir to 100 mL volumetric flask, add 60 mL of 0.1 M sodium hydroxide, leave it in ultrasound for 15 minutes, and top up with 0.1 M sodium hydroxide. To make it homogeneous and filter it. To transfer 15 mL of the filtrate to a 100 mL volumetric flask, add 50 mL of water, 5.8 mL of 2 M hydrochloric acid, and top up with water. To transfer 5 mL of this solution to 50 mL volumetric flask, top up with 0.1 M acetic acid, make it homogeneous, thus obtaining 0.0015% solution (w/v). To prepare a standard solution at the same concentration by using the same solvent. To measure the absorbance of the resulting solutions at 255 nm (5.2.14), by using 0.1 M hydrochloric acid to adjust zero. To calculate the amount of $C_8H_{11}N_5O_3$ in the tablets, from the readings obtained. Alternatively, make the calculations taking into account that $A(1\%, 1\text{ cm}) = 560$ at 255 nm, in 0.1 M hydrochloric acid.

PACKAGING AND STORAGE

In airtight containers, at a temperature below 25 °C.

LABELLING

To comply with the legislation in force.

ACICLOVIR, CREAM

It contains at least 90.0 % and at most 110 % of the amount stated of $C_8H_{11}N_5O_3$.

IDENTIFICATION

A. The ultraviolet absorption spectrum (5.2.14), from 230 nm to 350 nm, of sample solution obtained in *Dosage*, has maximum at 255 nm and shoulder around 274 nm, similar to those observed in the spectrum of a similar solution of aciclovir SQR.

B. The principal spot in the chromatogram of *Solution (2)*, in the *Limit for guanine*, corresponds in position and intensity to that obtained with the *Solution (3)*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

PURITY TESTS

Limit for guanine. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using F_{254} cellulose as support. To apply separately to the plate 10 μ l of each of the solutions freshly prepared, as described below.

Solution (1): to weigh amount of cream equivalent to 30 mg of aciclovir, transfer to graduated centrifuge tube, add 3 mL of 0.1 M sodium hydroxide, and stir to obtain the dispersion of the cream. To add 5 mL of mixture of chloroform and *n*-propyl alcohol (1:2), stir, centrifuge, and use the top layer.

Solution (2): to transfer 1 mL of *Solution (1)* to 10 mL volumetric flask and top up with 0.1 M sodium hydroxide.

Solution (3): to dissolve 6 mg of aciclovir SQR in 10 mL of 0.1 M sodium hydroxide.

Solution (4): to dissolve 6 mg of guanine in 100 mL of 0.1 M sodium hydroxide.

To initially develop the chromatogram by using ethyl acetate as mobile phase and let it touch the entire length of the plate. To remove the plate and let it air drying. To develop the chromatogram again by using a mixture of *n*-propyl alcohol, 13.5 M ammonium hydroxide, and 5% ammonium sulfate (w/v) (10:30:60) as mobile phase. To remove the plate and let it air drying. To examine under ultraviolet light (254 nm). Any secondary spot related to guanine in the chromatogram obtained with *Solution (1)* is not more intense than that obtained with *Solution (4)* (1%). To discard the spots present in the application point of the solvent.

BIOLOGICAL SAFETY TEST

Counting of the total viable micro-organisms (5.5.3.1.2). It complies with the test.

Survey and identification of pathogens (5.5.3.1.3). It complies with the test.

DOSAGE

To transfer amount of sample equivalent to 7.5 mg of aciclovir to a separatory funnel, by using 50 mL of 0.5 M sulfuric acid, and stir. To add 50 mL of ethyl acetate, stir, wait for separation of the phases, and collect the lower aqueous phase. To wash the organic phase with 20 mL of 0.5 M sulfuric acid, collect the aqueous phase, and combine with previous mixture. To transfer the aqueous combined to 100 mL volumetric flask and top up with 0.5 M sulfuric acid. To make it homogeneous and filter, by discarding the first few milliliters of the filtrate. To transfer 10 mL of this solution to 50 mL volumetric flask and top up with water. To prepare a aciclovir SQR solution at the same concentration by using the same solvent. To measure the absorbance of the resulting solutions at 255 nm (5.2.14), by using 0.1 M sulfuric acid to adjust zero. To calculate the amount of $C_8H_{11}N_5O_3$ in the cream, from the readings obtained.

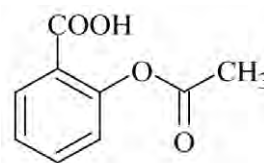
PACKAGING AND STORAGE

In tightly closed containers, in a dry place, at 15 °C - 25 °C.

LABELLING

To comply with the legislation in force.

ACETYLSALICYLIC ACID Acidum acetylsalicylicum



$C_9H_8O_4$; 180.16
acetylsalicylic acid; 00089
2 - (acetyloxy) benzoic acid
[50-78-2]

It contains at least 99.5% and at most 101.0% of $C_9H_8O_4$, in relation to the desiccated substance.

DESCRIPTION

Physical and chemical characteristics. White, crystalline powder, or colorless crystals; in general, it is odorless. Melting point (5.2.2): it melts around 143 °C.

Solubility. Sparingly soluble in water, very soluble in ethanol, soluble in ethyl ether.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the acetylsalicylic acid SQR spectrum similarly prepared.

B. To mix small amount of the sample with water and heat for a few minutes. To cool down. To add 1-2 drops of ferric chloride SR. The red-violet color develops.

C. To weigh 0.2 g of the sample. To add 4 mL of 2 M sodium hydroxide and boil it for 3 minutes. To cool down. To add 5 mL of M sulfuric acid. Crystalline precipitate is produced. To filter, wash the precipitate with water, and dry in an oven at 105 °C. The precipitate shows melting range (5.2.2) between 156 °C and 161 °C.

D. To heat the filtrate obtained in *Identification*, test C., with 2 mL of ethanol and 2 mL of sulfuric acid. Ethyl acetate, with typical odor, is formed.

PURITY TESTS

Appearance of solution. To dissolve 1 g of the sample in 9 mL of ethanol. The solution is clear (5.2.25) and colorless (5.2.12).

Related substances. To proceed as described in *Visible absorption spectrophotometry* (5.2.14). To transfer 0.3 g of the sample to a 100 mL volumetric flask and dissolve with 10 mL of 0.1 M tetrabutylammonium hydroxide in ethanol. After 10 minutes, add 8 mL of 0.1 M hydrochloric acid and 20 mL 1.9% of sodium tetraborate (w/v). Then, make it homogenous. To add 2 mL of 1% 4-aminoantipyrine (w/v), by constantly stirring, and 2 mL of 1% potassium ferricyanide (w/v). After 2 minutes, dilute to 100 mL with water. Let it rest for 20 minutes. To measure the absorbance of the resulting solutions at 505 nm, in cuvettes of 1 cm, by using water to adjust zero. The absorbance should be less than 0.25.

Salicylic acid. To accurately weigh 0.1 g of the sample, dissolve in 5 mL of ethanol, add 15 mL of ice water, and 1-2 drops of 0.5% ferric chloride (w/v). Let it rest for 1 minute. To transfer to Nessler tube. For the preparation of the standard solution, dissolve 5 mg of salicylic acid in 100 mL of ethanol. To transfer 1 mL of this solution to Nessler tube and add 1-2 drops of 0.5% ferric chloride (w/v), 0.1 mL of acetic acid, 4 mL of ethanol, and 15 mL of water. The color of the sample solution is not more intense than that of the standard solution. At most 0.05% (500 ppm).

Heavy metals (5.3.2.3). To dissolve 2 g of the sample in 25 mL of acetone and add 1 mL of water. To add 1.2 mL of thioacetamide SR ml and 2 mL of acetate buffer pH 3.5. Let it rest for 5 minutes. Any color developed is not darker than that of a standard prepared with 25 mL of acetone, 2 mL of *Lead standard solution* (10ppmPb), 1.2 mL of thioacetamide SR, and 2 mL of acetate buffer pH 3.5. At most 0.001% (10 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample, in a desiccator, at room temperature, under reduced pressure, to obtain constant weight. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To accurately weigh about 1 g of the sample, transfer to a 250 mL erlenmeyer flask with lid, and dissolve in 10 mL of ethanol. To add 50 mL of 0.5 M SV sodium hydroxide. Let it rest for 1 hour. To add 0.2 mL of phenolphthalein SI, as an indicator, and titrate with 0.5 M SV hydrochloric acid. To perform sample blank correction and adjust it as required. Each mL of 0.5 M SV sodium hydroxide is equal to 45.040 mg of $C_9H_8O_4$.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Analgesic, antipyretic, anti-inflammatory non-steroid; antiplatelets; it is also used to relieve migraine and ischemic heart disease.

**ACETYLSALICYLIC ACID
TABLETS**

It contains at least 95.0 % and at most 105.0 % of the amount stated of $C_9H_8O_4$.

IDENTIFICATION

A. To weigh and pulverize the tablets. To transfer amount of powder equivalent to 0.5 g of acetylsalicylic acid to a centrifuge tube and stir with 10 mL of ethanol for a few minutes. To centrifuge it. To remove the clear supernatant and evaporate to dryness in a water bath at 60 °C for 1 hour. To desiccate the residue in a vacuum oven at 60 °C for 1 hour. The infrared absorption spectrum (5.2.14) of the residue, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the acetylsalicylic acid SQR spectrum similarly prepared.

B. To weigh and pulverize the tablets. To transfer amount of powder equivalent to 0.5 g of acetylsalicylic acid and dissolve in 10 mL of 5 M sodium hydroxide. To boil it for 2-3 minutes. To cool it down. To add an excess of M sulfuric acid. Crystalline precipitate and typical odor of acetic acid are produced. To add ferric chloride SR to the solution. The intense violet color develops.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness (5.1.3.1). It complies with the test.

Friability (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). At most 5 minutes.

Uniformity of dosage units (5.1.6). It complies with the test. For 100 mg tablets, proceed as described in *Dosage*, by using 0.1 M volumetric solutions.

DISSOLUTION TEST (5.1.5)

Dissolution medium: acetate buffer 0.05 M pH 4.5, 500 mL

Equipment: blades, 50 rpm

Time: 30 minutes

Procedure: After testing, remove aliquot from the dissolution medium, filter, and dilute, if necessary, in acetate buffer 0.05 M pH 4.5, to obtain suitable concentration. To measure the absorbance of the solutions at 265 nm (5.2.14), by using the same solvent to adjust zero. To calculate the amount of $C_9H_8O_4$ dissolved in the medium, by comparing the readings obtained with 0.008 % acetylsalicylic acid SQR solution (w/v) prepared before using it. Ethanol can be used to dissolve the standard before dilution in 0.05 M acetate buffer pH 4.5. The volume of ethanol must not exceed 1% of the total volume of the standard solution.

Tolerance: not less than 80% (Q) of the stated amount of $C_9H_8O_4$ dissolves within 30 minutes.

PURITY TESTS

Salicylic acid. To weigh and pulverize the tablets. To transfer amount of powder equivalent to 0.2 g of acetylsalicylic acid to 100 mL volumetric flask. To add 4 mL of ethanol and stir. To dilute to 100 mL with cold water, by keeping the temperature below 10 °C. To immediately filter and transfer 50 mL of the filtrate to Nessler tube. To prepare the 0.01% salicylic acid solution SQR (w/v). To transfer 3 mL of this solution to a Nessler tube, add 2 mL of ethanol and enough water to 50 mL. To add 1 mL of ammonium ferric sulfate SR2 to sample and standard solutions. The violet color produced with the sample solution should not be more intense than that obtained with the standard solution.

DOSAGE

To weigh and pulverize 20 tablets. To transfer amount of powder equivalent to 0.5 g of acetylsalicylic acid to 250 mL erlenmeyer flask, and add 30 mL of 5 M SV sodium hydroxide. To carefully boil it for 10 minutes and titrate the alkali in excess with 0.5 M SV hydrochloric acid, by using phenol red SI as indicator. To perform sample blank correction and adjust it as required. Each mL of 0.5 M SV sodium hydroxide is equal to 45.040 mg of $C_9H_8O_4$.

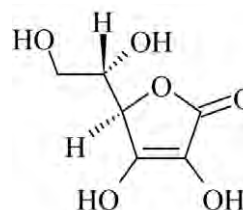
PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

ACID, ASCORBIC
Acidum ascorbicum



$C_6H_8O_6$; 176,12
 ascorbic acid; 00104
 L-ascorbic acid
 [50-81-7]

It contains at least 99.0% and at most 100.5% of $C_6H_8O_6$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, fine, white or slightly yellowish powder. In the solid state, it is stable in air, but in the solution it oxidizes rapidly. Its aqueous solution is clear.

Solubility. Freely soluble in water, slightly soluble in ethanol and acetone, insoluble in ethyl ether, chloroform, petroleum ether, and benzene.

Physical and chemical constants.

Melting range (5.2.2): 189 °C to 192 °C with decomposition.

Specific rotation (5.2.8): +20.5° to +21.5°. To determine in 10% solution (w/v) in carbon dioxide-free water.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide,

has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the ascorbic acid SQR spectrum similarly prepared.

B. To add alkaline cupric tartrate SR to an aliquot of the 2% solution (w/v) and let it rest at room temperature. Color change is observed due to slow reduction of cupric tartrate. Upon heating, the reduction is faster.

PURITY TESTS

pH (5.2.19). 2.2 to 2.5. To determine in 5% aqueous solution (w/v).

Heavy metals (5.3.2.3). To dissolve 1 g in 25 mL of water. At most 0.002% (20 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample, in a vacuum desiccator, on sulfuric acid, for 24 hours. At most 0.4%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To accurately dissolve about 0.2 g of the sample in a mixture of 100 mL of water and 25 mL of *M* sulfuric acid. Then, add 3 mL of starch SI and immediately titrate with 0.05 *M* SV iodine. Each mL of 0.05 *M* SV iodine is equal to 8.806 mg of $C_6H_8O_6$.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Vitamin.

ACID, ASCORBIC TABLETS

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_6H_8O_6$.

IDENTIFICATION

To weigh and pulverize the tablets. To use the powder to prepare 2% ascorbic acid solution (w/v) in ethanol, make it homogeneous, and filter it.

To proceed as described in *Identification*, tests **A.** and **B.**, in the monograph *Ascorbic acid*, by using 2 mL of the solution obtained.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). At most 30 minutes.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL

Equipment: blades, 50 rpm

Time: 45 minutes

Procedure: After testing, remove aliquot from the dissolution medium and dilute, if necessary, with water, to obtain suitable concentration. To make it homogenous and filter it. To transfer amount equivalent to approximately 2 mg of ascorbic acid to 50 mL erlenmeyer flask, add 5 mL of metaphosphoric acetic acid SR, and titrate with dichlorophenol indophenol standard solution to obtain pink color for 5 seconds. To perform sample blank correction with mixture of 5.5 mL of metaphosphoric acetic acid SR and 15 mL of water. To calculate the amount of ascorbic acid dissolved from the titer of dichlorophenol indophenol standard solution.

Tolerance: not less than 75% (Q) of the stated amount of $C_6H_8O_6$ dissolves within 45 minutes.

DOSAGE

To employ one of the methods described below.

A. To weigh and pulverize 20 tablets. To use the amount of powder equivalent to 0.2 g of ascorbic acid. To proceed as described in *Dosage*, in the monograph *Ascorbic acid*.

B. To weigh and pulverize 20 tablets. To use the amount of powder equivalent to 0.15 g of ascorbic acid. To dissolve in a mixture of 30 mL of water and 20 mL of *M* sulfuric acid. To titrate with 0.1 *M* SV ammonium ceric sulfate, by using ferroin SI as indicator. Each mL of 0.1 *M* SV ammonium ceric sulfate is equal to 8.806 mg of $C_6H_8O_6$.

PACKAGING AND STORAGE

In airtight, opaque containers.

LABELLING

To comply with the legislation in force.

ACID, ASCORBIC INJECTABLE SOLUTION

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_6H_8O_6$.

IDENTIFICATION

A. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF₂₅₄ silica gel as support and a mixture of ethanol and water (120:20) as mobile phase. To

separately apply to the plate 2 µl of each of the solutions freshly prepared, as described below.

Solution (1): to dilute the injectable solution in water, to obtain a 5 mg/mL ascorbic acid solution.

Solution (2): 5 mg/mL aqueous solution of ascorbic acid SQR.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

B. To dilute the injectable solution in ethanol to obtain a 2% concentration (w/v) and filter it. To proceed as described in *Identification*, test **B.**, in the monograph *Ascorbic acid*.

C. To transfer volume of injectable solution equivalent to 50 mg of ascorbic acid to the test tube. To add 0.2 mL of 2 M nitric acid and 0.2 mL of 0.1 M silver nitrate. Gray precipitate is produced.

D. It responds to the reactions of sodium ion.

(5.3.1.1) CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

pH (5.2.19). 6.1 to 7.1.

PURITY TESTS

Limit for oxalate. To transfer volume of injectable solution equivalent to 50 mg of ascorbic acid with water to 5 mL. To add 0.2 mL of glacial acetic acid and 0.5 mL of calcium chloride SR. No turbidity occurs within 1 minute.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

Bacterial endotoxins (5.5.2.2). At most 1.2 UE/ mg of ascorbic acid.

DOSAGE

To transfer volume of injectable solution equivalent to about 0.2 mg of ascorbic acid to erlenmeyer tube. To add 100 mL of carbon dioxide-free water and proceed as described in *Dosage*, in the monograph *Ascorbic acid*, from "and 25 mL of M sulfuric acid..." Each mL of 0.05 M SV iodine is equal to 8.806 mg of $C_6H_8O_6$.

PACKAGING AND STORAGE

In type I glass containers, protected from light.

LABELLING

To comply with the legislation in force.

ACID, BENZOIC

Acidum benzoicum



$C_7H_6O_2$; 122.12
benzoic acid; 00115
Benzoic acid
[65-85-0]

It contains at least 99.5% and at most 100.5% of $C_7H_6O_2$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White, crystalline powder or colorless crystals, odorless or slight typical odor.

Solubility. Slightly soluble in water, soluble in boiling water, freely soluble in ethanol, ethyl ether, and fatty acids.

Physical and chemical constants

Melting range (5.2.2): 121 °C to 124 °C.

IDENTIFICATION

A. To prepare a saturated benzoic acid solution in water and filter it twice. To add ferric chloride SR solution to a portion of the filtrate. Orange precipitate is formed. To add 1 mL of 3 M sulfuric acid to another portion of 10 mL of the filtrate and cool down the mixture. White precipitate is formed; it is soluble in ethyl ether in about 10 minutes.

B. To dissolve 5 g of the sample in 100 mL of ethanol. It responds to the reactions of benzoate ion (5.3.1.1).

PURITY TESTS

Appearance of solution. To dissolve 5 g of the sample in 100 mL of ethanol. The resulting solution is clear (5.2.25).

Oxidizable substances. To dissolve 2 g of the sample in 10 mL of boiling water, cool down, and filter it. To add 1 mL of 5% sulfuric acid (v/v) and 0.2 mL of 0.02 M potassium permanganate to the filtrate. Pink color is developed for at least 5 minutes.

Carbonizable substances. To dissolve 0.5 g of the sample in 5 mL of sulfuric acid SR. After 5 minutes, this solution is not more intensely colored than the solution prepared by diluting 12.5 mL of the *Color H solution* (5.2.12) to 100 mL with hydrochloric acid SR.

Halogenated compounds and halides.

Note: all glassware used must be free from chlorides. To do this, fill the glassware with 50% nitric acid solution (w/v) and leave it in ultrasonic bath overnight. In the next day, wash the glassware with water and keep it full of water. It is recommended to have a specific glassware for running the test.

Solution (1): to dissolve 6.7 g of the sample in a mixture of 40 mL of 0.1 M sodium hydroxide and 50 mL of ethanol and top up to 100 mL with water. In 10 mL of this solution, add 7.5 mL of sodium hydroxide SR solution, 0.125 g of nickel-aluminum alloy, and heat in a water bath for 10 minutes. To cool down at room temperature, filter, and wash with 3 portions of ethanol (3 mL each). To wash with 25 mL of water.

Solution (2): to prepare this solution similarly to *Solution (1)*, but without using the sample.

Solution (3): chloride standard solution (8 ppm Cl)

To separately add 10 mL of *Solution (1)*, 10 mL of *Solution (2)*, 10 mL of *Solution (3)*, and 10 mL of water to four 25 mL volumetric flasks. To add 5 mL of ferric ammonium sulfate SR1, 2 mL of nitric acid SR, and 5 mL of mercury thiocyanate SR to each vial. To top up the volume of each flask to 25 mL with water. To put in a water bath at 20 °C for 15 minutes. To proceed as described in *Visible absorption spectrophotometry (5.2.14)*. To measure the absorbance of the *Solution (1)* at 460 nm, by using the *Solution (2)* to adjust zero. To measure the absorbance of the *Solution (3)* at 460 nm, by using the resulting solution with 10 mL of water to adjust zero. The absorbance of *Solution (1)* is not greater than the absorbance of the *Solution (3)* (300 ppm).

Heavy metals (5.3.2.3). To use *Method III*. To weigh 5 g of the sample and dissolve in 100 mL of ethanol. To prepare a standard solution by using ethanol as solvent. At most 0.001% (10 ppm).

Water (5.2.20.1). To dissolve the sample in a mixture of methanol and pyridine (1:2). At most 0.7%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.05%.

DOSAGE

To accurately weigh about 200 g of the sample and dissolve in 20 mL of ethanol. To titrate with 0.1 M SV sodium hydroxide, by using phenol red SI to obtain violet color corresponding to the endpoint of the titration. Each mL of 0.1 M SV sodium hydroxide is equal to 12.212 mg of $C_7H_6O_2$.

PACKAGING AND STORAGE

In tightly closed, opaque containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antimicrobial.

ACID, BORIC
Acidum boricum

H_3BO_3 ; 61.83
boric acid; 00116
Boric acid [10043-35-3]

It contains at least 99.5% and at most 100.5% of H_3BO_3 , in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White crystalline powder, greasy to touch, or colorless shiny crystals.

Solubility. Soluble in water, freely soluble in boiling water, and 85% glycerol (v/v); soluble in ethanol.

IDENTIFICATION

To dissolve under mild heating 0.1 g of the sample in 5 mL of methanol. To add 0.1 mL of sulfuric acid and ignite the solution. Flame with green edges is observed.

PURITY TESTS

Appearance of solution. To dissolve 3.3 g of the sample in 80 mL of boiling water. To cool down and dilute to 100 mL with carbon dioxide-free water. The solution is clear (5.2.25) and colorless (5.2.12).

pH (5.2.19). 3.8 to 4.8. To determine in the solution obtained, in *Appearance of solution*.

Solubility in ethanol. To dissolve 1 g of the sample in 10 mL of boiling ethanol. The preparation is colorless (5.2.12) and not more opalescent than the *Reference suspension II (5.2.25)*.

Organic impurities. To gradually warm the sample to red. No darkening occurs.

Heavy metals (5.3.2.3). To use the *Method I*. To dissolve 1 g of the sample in 23 mL of water, add 2 mL of *M* acetic acid, and continue as described in *Limit test for heavy metals*. At most 0.002% (20 ppm).

Sulfates (5.3.2.2). To determine in 2.7 g of the sample. At most 0.045% (450 ppm).

Loss on drying (5.2.9). To desiccate on silica gel for 5 hours. At most 0.5%.

DOSAGE

To accurately weigh about 1 g of the sample and dissolve in 100 mL of water with 15 g of mannitol under heating. To titrate with *M* SV sodium hydroxide by using 0.5 mL of phenolphthalein SI as an indicator to change pink color. Each mL of *M* SV sodium hydroxide is equal to 61.832 mg of H_3BO_3 .

PACKAGING AND STORAGE

In tightly closed containers.

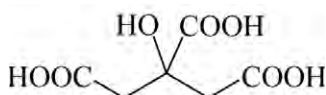
LABELLING

To comply with the legislation in force.

CATEGORY

Antiseptic and pharmaceutical adjuvant.

ACID, CITRIC
Acidum citricum



$\text{C}_6\text{H}_8\text{O}_7$; 192.12

$\text{C}_6\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O}$; 210.14

citric acid; 00134

2-Hydroxy-1,2,3-propanetricarboxylic acid

[77-92-9]

2-Hydroxy-1,2,3-propanetricarboxylic acid hydrate (1:1)

[5949-29-1]

It contains at least 99.5% and at most 100.5% of $\text{C}_6\text{H}_8\text{O}_7$, in relation to the anhydrous substance.

DESCRIPTION

Physical and chemical characteristics. White, crystalline powder, or colorless and clear crystals. It effloresces if exposed to warm, dry air. The hydrate form is slightly deliquescent in moist air. Melting range (5.2.2): 153 °C with decomposition.

Solubility. Very soluble in water, and freely soluble in ethanol.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated at 105 °C for 2 hours, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the citric acid SQR spectrum similarly prepared.

B. To dissolve 1 g of the substance in 10 mL of water. The solution is highly acidic.

C. To dissolve 0.5 g of the substance in 5 mL of water and neutralize with *M* sodium hydroxide. To add 10 mL of calcium chloride SR and heat to boiling. A white precipitate is formed.

D. It responds to the reactions of citrate ion (5.3.1.1).

PURITY TESTS

Appearance of solution. To dissolve 2 g of the sample in water and top up the volume to 10 mL with the same solvent. The solution is clear (5.2.24) and colorless (5.2.12).

Readily carbonisable substances. To exactly transfer about 0.5 g of the sample pulverized to a test tube previously washed with sulfuric acid containing 5 mL of sulfuric acid. To heat for 1 hour at 90 °C. The solution should be only yellow, not brown.

Oxalic acid. To weigh equivalent to 0.8 g of citric acid and dissolve in 4 mL of water. To add 3 mL of hydrochloric acid and 1 g of zinc granules. To boil for 1 minute and cool down for 2 minutes. To transfer the supernatant liquid to a test tube containing 0.25 mL of 1% phenylhydrazine chloride solution (w/v) and heat to boiling. To rapidly cool down, transfer to a graduated tube, and add an equal volume of hydrochloric acid and 0.25 mL of potassium ferricyanide SR. To stir and let it rest for 30 minutes. The pink color developed in the solution should not be more intense than that developed by the oxalic acid standard prepared similarly by using 4 mL of 0.01% oxalic acid solution (w/v).

Aluminum (5.3.2.10). To accurately weigh about 20 g of the sample and proceed as described in *Limit test for aluminum* by using 40 mL of 2 ppm standard. At most 0.2 ppm (0.00002%) when citric acid is used for dialysis solutions.

Sulfates (5.3.2.1). To dissolve 3.2 g of the sample in 40 mL of water and proceed as described in *Limit test for sulfates*, by using 1 mL of standard solution of 0.005 *M* sulfuric acid. At most 0.015% (150 ppm).

Heavy Metals (5.3.2.3). To use *Method I*. To accurately weigh about 2 g of the sample and dissolve in sodium hydroxide SR. To dilute to 25 mL with water and proceed as described in *Limit test for heavy metals*. After adding thioacetamide reagent and diluting with water, make it homogeneous and heat at 80 °C, and let it rest for 2 minutes. At most 0.001% (10 ppm).

Water (5.2.20). Anhydrous form: to determine 2 g of the sample. At most 1%. Hydrate form: to determine 0.5 g of the sample. From 7.5 to 9.0 %.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

BIOLOGICAL SAFETY TEST

Citric acid for preparing parenteral preparation complies with the following additional tests.

Sterility (5.5.3.2.1). It complies with the test.

Bacterial endotoxins (5.5.2.2). At most 0.5 EU/mg of anhydrous citric acid, if the finished product is not subjected to a subsequent procedure to remove the bacterial endotoxins.

DOSAGE

To accurately weigh about 0.5 g of the sample and dissolve in 50 mL of water, by gently heating, if necessary, to obtain total dissolution. To titrate with *M* SV sodium hydroxide by using phenolphthalein SI as an indicator. Each mL of *M* SV sodium hydroxide is equal to 64.040 mg of $C_6H_8O_7$.

PACKAGING AND STORAGE

In tightly closed containers.

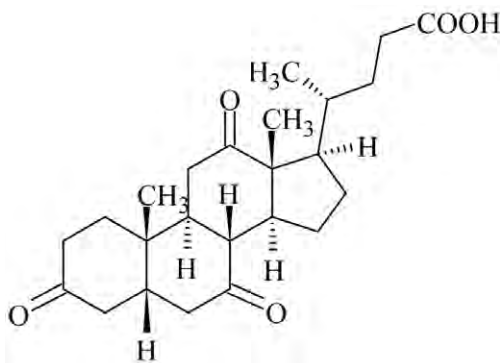
LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Acidulant.

ACID, DEHYDROCHOLIC
Acidum dehydrocholicum



$C_{24}H_{34}O_5$; 402.52

dehydrocholic acid; 00157

(5 β)-3,7,12-trioxocolan-24-oic acid
[81-25-2]

It contains at least 98.5% and at most 101.0% of $C_{24}H_{34}O_5$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White, odorless powder with bitter taste.

Solubility. Very slightly soluble in water and ethyl ether, slightly soluble in glacial acetic acid, ethanol, and chloroform.

Physical and chemical constants

Melting range (5.2.2): 231 °C to 240 °C. The range between the beginning and end of melting does not exceed 3 °C.

Specific rotation (5.2.8): 29.0° to +32.5°. To determine at 2% solution (w/v) in dioxane.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the dehydrocholic acid SQR spectrum similarly prepared.

B. To dissolve 5 mg of the sample in 1 mL of sulfuric acid and a drop of formaldehyde solution. To add 5 mL of water after 5 minutes. The solution becomes yellow and fluorescence greenish-blue.

PURITY TESTS

Barium. To dissolve 2 g of the sample in 100 mL of water and boil for 2 minutes. Add 2 mL of hydrochloric acid and SR boil for 2 minutes, cool and filter. To wash the filter with water and top up the volume to 100 mL with the same solvent.

To add 1 mL of *M* sulfuric acid to 10 mL of the filtrate. The resulting solution should not become turbid or precipitate.

Heavy metals (5.3.2.3). To use *Method III*. To use 1 g of the sample, by heating the solution at 80 °C before adding thioacetamide SR. At most 0.002% (20 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample, in an oven at 105 °C for 2 hours. At most 1.0%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.3%.

BIOLOGICAL SAFETY TEST

Counting of the total viable micro-organisms

(5.5.3.1.2). Total aerobic bacteria: At most 1000 UFC/g. Fungi and yeasts: At most 100 UFC/g.

DOSAGE

To accurately weigh about 0.25 g of the sample previously desiccated and dissolve in 30 mL of ethanol. To stir to total solubilization, by warming, if necessary, and add 2 drops of phenolphthalein SI and 30 mL of water. To titrate with 0.1 *M* SV sodium hydroxide. Each mL of 0.1 *M* SV sodium hydroxide is equal to 40.252 of $C_{24}H_{34}O_5$.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Cholagogue.

ACID, STEARIC
Acidum stearicum

stearic acid; 00182

Octadecanoic acid

[57-11-4]

Mixture of stearic acid ($C_{18}H_{36}O_2$, 284.48) and palmitic acid ($C_{16}H_{32}O_2$, 256.43). It may contain antioxidant.

DESCRIPTION

Physical characteristics. White to yellowish-white powder, or white crystals, flaky, and waxy, or white to slightly yellowish solid mass. Mild odor, similar to not rancid sebum.

Solubility. Practically insoluble in water, freely soluble in chloroform and ethyl ether, soluble in ethanol and petroleum ether.

Physical and chemical constants

Freezing temperature: above 54 °C.

IDENTIFICATION

It complies with the requirements of the test *Index of acidity*, in *Purity Tests*.

PURITY TESTS

Acidity. To stir 5 g of the melted sample with an equal volume of hot water, for 2 minutes, cool down, and filter it. To add a drop of methyl orange SI to the filtrate. No reddish color is developed.

Index of acidity (5.2.29.7). 194 to 212.

Index of Iodine (5.2.29.10). At most 4.0.

Paraffin and other unsaponifiable substances. To boil in a volumetric flask about 1 g of sample with 30 mL of water, and 0.5 g of anhydrous sodium carbonate. The resulting solution, while hot, is clear or at most slightly opalescent.

Heavy metals (5.3.2.3). To use *Method III*. At most 0.001% (10 ppm).

Sulfated ashes (5.2.10). To determine in 4 g of the sample. At most 0.1%.

BIOLOGICAL SAFETY TEST

Counting of the total viable micro-organisms

(5.5.3.1.2). Total aerobic bacteria: At most 1000 UFC/g. Fungi and yeasts: At most 50 UFC/g.

Survey and identification of pathogens (5.5.3.1.3). It complies with the test.

PACKAGING AND STORAGE

In tightly closed containers.

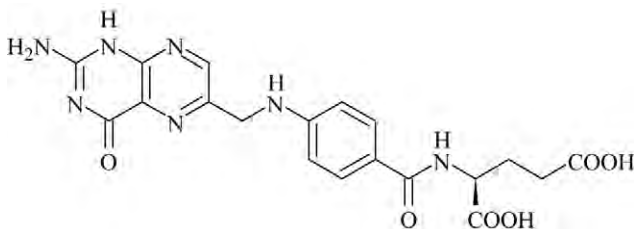
LABELLING

To comply with the legislation in force.

CATEGORY

Raw material for preparing sodium stearate, magnesium, zinc, and other pharmaceutical adjuvants.

ACID, FOLIC
Acidum folicum



$C_{19}H_{19}N_7O_6$; 441.40

folic acid; 00194

N-[4 - [[[2-amino-3,4-dihydro-4-oxo-6-pteridinyl)methyl] amino] benzoyl]-L-glutamic acid
[59-30-3]

It contains at least 97.0% and at most 102.0% of $C_{19}H_{19}N_7O_6$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. Crystalline, orange-yellow, odorless powder.

Solubility. Practically insoluble in water, insoluble in ethanol, acetone, chloroform, and ethyl ether. Soluble in alkalis. Soluble in hydrochloric acid and sulfuric acid; it produces pale yellow solution.

Physical and chemical constants

Specific rotation (5.2.8): +18° to +22°, in relation to the anhydrous substance. To determine in 1.0% solution (w/v) in 0.1 M sodium hydroxide.

IDENTIFICATION

A. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using G silica gel as support and a mixture of ethanol, n-propyl alcohol, and concentrated ammonia solution (60:20:20) as mobile phase. To apply separately to the plate 2 µL of each of the solutions freshly prepared, as described below.

Solution (1): 0.5 mg/mL solution of the sample in mixture of concentrated ammonia solution and methanol (2:9).

Solution (2): 0.5 mg/mL solution of folic acid SQR in mixture of concentrated ammonia solution and methanol (2:9).

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (365 nm). The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

PURITY TESTS

Chromatographic purity. To proceed as described in *Dosage*. To use the *Concentrated sample solution* as *Test solution*.

Procedure: To inject 10 µL of *Test solution*. To record the chromatogram at least twice the retention time of folic acid and measure the areas under the peaks. The sum of the areas of all peaks, other than that corresponding to folic acid, is not greater than 2.0% of the sum of the areas of all the peaks recorded, including that one corresponding to folic acid. To disregard the peaks related to the solvent.

Water (5.2.20.1). At most 8.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.2%.

DOSAGE

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 280 nm; 250 mm column long and 4.0 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (3 µm to 10 µm), at room temperature; flow of *Mobile phase* of 1.2 mL/min.

Mobile phase: to transfer 2 g of monobasic potassium phosphate to 1000 mL volumetric flask. To add 650 mL of water, 15 mL of 0.5 M tetrabutylammonium hydroxide in methanol, 7 mL of M phosphoric acid, 270 mL of methanol, and make it homogeneous. To adjust pH to 5.0 with M phosphoric acid or 6 M ammonium hydroxide. To top up to volume with water, make it homogeneous, and filter.

Note: to protect the following solutions from direct light.

Internal standard solution: to dissolve 50 mg of methylparaben in 1 mL of methanol, dilute to 25 mL with *Mobile phase*, and make it homogeneous.

Concentrated sample solution: to accurately transfer about 0.1 g of the sample to a 100 mL volumetric flask. To add 40 mL of *Mobile phase* and 1 mL of 10% ammonium hydroxide (v/v). To top up to volume with *Mobile phase* and make it homogeneous.

Sample solution: To transfer 4 mL of the *Concentrated sample solution* and 4 mL of the *Internal standard solution* to a 50 mL volumetric flask. To top up to volume with *Mobile phase* and make it homogeneous.

Stock standard solution: to prepare 1 mg/mL folic acid SQR solution in *Mobile phase*, by using 1 mL of 10% ammonium hydroxide (v/v) per 100 mL of solution.

Standard solution: to transfer 4 mL of the *Stock standard solution* and 4 mL of the *Internal standard solution* to a 50 mL volumetric flask. To top up to volume with *Mobile phase* and make it homogeneous.

To inject replicates of 10 µL of the *Standard solution*. The resolution between methylparaben and folic acid should not be less than 2.0. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2.0%.

Procedure: to separately inject 10 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of C₁₉H₁₉N₇O₆ from the sample, from the responses obtained for folic acid/methylparaben with the *Standard solution* and *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Hematopoietic.

ACID, FOLIC TABLETS

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_{19}H_{19}N_7O_6$.

IDENTIFICATION

To weigh and pulverize the tablets. To stir amount of powder equivalent to 50 mg of folic acid with 100 mL of 0.1 M sodium hydroxide heated at 40 °C - 50 °C. To cool down and filter it. To adjust the filtrate to pH 3.0 with hydrochloric acid. To cool down the solution up to 5° C, filter, and wash the precipitate with cold water until the washings do not respond to the reaction of chlorides (5.3.1.1). To wash the precipitate with acetone and dry at 80 °C for 1 hour. To transfer 10 mg of the residue to 100 mL volumetric flask, dissolve in 0.1 M SV sodium hydroxide, and top up the volume with the same solvent. To transfer 5 mL of this solution to a 50 mL volumetric flask and top up with 0.1 M sodium hydroxide, thus obtaining 0.001% solution (w/v). The ultraviolet absorption spectrum (5.2.14), ranging from 230 nm to 386 nm, of the resulting solution, exhibits maxima at 256 nm, 283 nm, and 365 nm. The ratio between the absorbance values measured at 256 nm and 365 nm is between 2.80 and 3.00.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 500 mL

Equipment: blades, 50 rpm

Time: 45 minutes

Procedure: After testing, remove aliquot from the dissolution medium and proceed as described in *Dosage*.

Tolerance: not less than 75% (Q) of the stated amount of $C_{19}H_{19}N_7O_6$ dissolves within 45 minutes.

DOSAGE

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 283 nm; 250 mm column long and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), at room temperature; flow of *Mobile phase* of 1 mL/min.

Mobile phase: mixture of 0.05 M monobasic potassium phosphate and acetonitrile (93:7). To adjust the pH of the mixture to 6.0 with 5 M sodium hydroxide.

Sample solution: To weigh and pulverize 20 tablets. To transfer amount of powder equivalent to 20 mg of folic acid to a 100 mL volumetric flask, add 50 mL of 0.1 M sodium hydroxide, and top up to volume with the same solvent. To make it homogeneous. To centrifuge, transfer 5 mL of the supernatant to a 100 mL volumetric flask, and top up with *Mobile phase*.

Standard solution: to accurately transfer about 20 mg of folic acid SQR to a 100 mL volumetric flask, and top up with 0.1 M sodium hydroxide. To make it homogeneous. To transfer 5 mL of this solution to 100 mL volumetric flask and top up with *Mobile phase*.

Procedure: to separately inject 10 µL of *Sample and standard solutions*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of $C_{19}H_{19}N_7O_6$ in the tablets, from the responses obtained for the *Sample and standard solutions*.

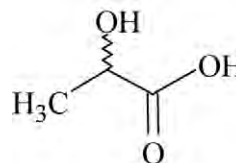
PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

ACID, LACTIC Acidum lacticum



$C_3H_6O_3$; 90.08
lactic acid; 00274
2-hydroxypropanoic acid
[50-21-5]

Mixture of 2-hydroxypropanoic acid and its condensation products, such as lactic-lactol acid, polylactic, and water. The balance between lactic acid and polylactic acids depends upon the concentration and temperature. Lactic acid is usually a racemate ((*RS*)-lactic acid), but the isomer S (+) may predominate. It contains at least 88% and at most 92.0% of $C_3H_6O_3$.

DESCRIPTION

Physical characteristics. Viscous colorless or slightly yellow liquid.

Solubility. Miscible with water, ethanol, and ethyl ether.

Physical and chemical constants

Specific rotation (5.2.8): $-0,05^{\circ}$ to $+0,05^{\circ}$, for the racemic lactic acid.

IDENTIFICATION

A. To dissolve 1 g of the sample in water. The solution is highly acidic (pH less than 4).

B. It responds to the reactions of the lactate ion (5.3.1.1).

PURITY TESTS

Appearance of solution. To dissolve 5 g of the sample in 42 mL of *M* sodium hydroxide and dilute to 50 mL with water. The solution is not darker than the *Standard solution color SC F (5.2.12)* with 7 mL of water.

Sugars and other reducing substances.

To add 5 drops of the sample to 10 mL of hot alkaline cupric tartrate SR. No red precipitate is produced.

Readily carbonisable substances. To wash a test tube with sulfuric acid and let drain for 10 minutes. To add to the test tube 5 mL of sulfuric acid and carefully add 5 mL of the sample so as to not mix the liquids.

To keep the tube at 15°C . After 15 minutes, no dark color develops at the interface of both acids.

Substances insoluble in ether. To dissolve 1 g of the sample in 25 mL of ethyl ether. The solution is not more opalescent than the solvent used for the test.

Oxalic acid, citric acid, and phosphoric acid. To add ammonia SR with weak pH (8 to 10) to 5 mL of the solution obtained in *Appearance of solution*. To add 1 mL of calcium chloride SR solution. To heat in water bath for 5 minutes. Any opalescence in the solution before or after heat treatment is not more intense than that of a mixture of 1 mL of water and 5 mL of the solution obtained in *Appearance of solution*.

Calcium (5.3.2.7). To dilute 5 mL of the solution obtained in *Appearance of solution* to 15 mL with water and proceed as described in *Limit test for calcium*. At most 0.02% (200 ppm).

Chlorides (5.3.2.1). To add a few drops of 0.1 *M* silver nitrate to 10 mL of solution of the 1% sample (w/v) acidified with nitric acid. No opalescence is immediately produced.

Sulfates (5.3.2.2). To add 2 drops of hydrochloric acid and 1 mL barium chloride SR to 10 mL of solution of the 1% sample (w/v). No turbidity is produced.

Heavy metals (5.3.2.3). To use *Method III*. At most 0.001% (10 ppm).

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To accurately transfer about 1 g of the sample to vial with a lid, add 10 mL of water, and 20 mL of *M* sodium hydroxide. To close the vial and let it rest for 30 minutes. To add 0.5 mL of phenolphthalein SI and titrate with *M* SV hydrochloric acid to pink color disappears. Each mL of *M* sodium hydroxide is equal to 90.080 mg of $\text{C}_3\text{H}_6\text{O}_3$.

PACKAGING AND STORAGE

In tightly closed containers.

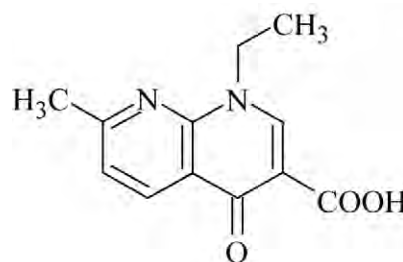
LABELLING

To comply with the legislation in force.

CATEGORY

Buffering agent.

ACID, NALIDIXIC
Acidum nalidixicum



$\text{C}_{12}\text{H}_{12}\text{N}_2\text{O}_3$; 232.24
nalidixic acid; 00294

1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carboxylic acid
[389-08-2]

It contains at least 99.0% and at most 101.0% of $\text{C}_{12}\text{H}_{12}\text{N}_2\text{O}_3$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white to almost white, or pale yellow powder.

Solubility. Practically insoluble in water, soluble in methylene chloride, sparingly soluble in ethanol and acetone. Soluble in dilute alkalis.

Physical and chemical constants

Melting range (5.2.2): 225°C to 231°C .

IDENTIFICATION

The identification test **A.** may be omitted if the tests **B.**, **C.**, and **D.** are conducted.

A. The infrared absorption spectrum (**5.2.14**) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the nalidixic acid SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (**5.2.14**), ranging from 230 nm to 350 nm, 0.0005% solution (w/v), in 0.01 M sodium hydroxide, exhibits maxima at 258 nm and 334 nm. The ratio between the absorbance values measured at 258 nm and 334 nm is between 2.2 and 2.4.

C. The principal spot in the chromatogram of *Solution (2)* in the *Related substances* corresponds in position, color, and intensity to that principal spot obtained in the chromatogram of *Solution (3)*.

D. To dissolve 0.1 g of the sample in 2 mL of hydrochloric acid. To add 0.5 mL of 10% 2-naphthol (w/v) in ethanol. The red-orange color develops.

PURITY TESTS

Absorption of light. To dissolve 1.5 g of the sample to 50 mL volumetric flask with methylene chloride and top up with the same solvent. To make it homogeneous. The absorbance of solution (**5.2.14**) measured at 420 nm is not greater than 0.10.

Related substances. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using F_{254} silica gel as support and a mixture of ammonia SR, methylene chloride, and ethanol (10:20:70) as mobile phase. To apply separately to the plate 10 µL of each of the solutions freshly prepared, as described below.

Solution (1): to dissolve 0.2 g of the sample in methylene chloride and top up to 10 mL with the same solvent. To make it homogeneous.

Solution (2): to transfer 1 mL of *Solution (1)* to 20 mL volumetric flask and top up with methylene chloride.

Solution (3): to dissolve 10 mg of nalidixic acid SQR in methylene chloride and top up to 10 mL with the same solvent. To make it homogeneous.

Solution (4): to transfer 2 mL of *Solution (2)* to 10 mL volumetric flask and top up with methylene chloride.

Solution (5): to transfer 1 mL of *Solution (4)* to 10 mL volumetric flask and top up with methylene chloride.

Solution (6): to transfer 1 mL of *Solution (4)* to 25 mL volumetric flask and top up with methylene chloride.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (5)* (0.1%) and not more than a spot is more intense than that obtained with *Solution (6)* (0.04 %).

Heavy metals (5.3.2.3). To use *Method III*. At most 0.002% (20 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample, in an oven at 100 °C - 105 °C for 2 hours. At most 0.5 %.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To accurately dissolve about 0.25 g of the sample in 30 mL of dimethylformamide, previously neutralized, by using thymolphthalein SI as indicator. To titrate with 0.1 M SV lithium methoxide, by using thymolphthalein SI as indicator. To use a magnetic stirrer and avoid absorption of atmospheric carbon dioxide.

Each mL of 0.1 M SV lithium methoxide is equal to 23.224 mg of $C_{12}H_{12}N_2O_3$.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antibacterial.

ACID, NALIDIXIC TABLETS

It contains at least 95.0 % and at most 105.0 % of the amount stated of $C_{12}H_{12}N_2O_3$.

IDENTIFICATION

A. To weigh and pulverize the tablets. To use the amount of powder equivalent to 1 g of nalidixic acid, add 50 mL of chloroform, stir for 15 minutes, filter, and evaporate the filtrate to dryness. The residue obtained responds to *Identification test A.*, in the monograph Nalidixic acid.

B. To dry the residue from test **A.**, *Identification*, at 105 °C, for 2 hours. The ultraviolet absorption spectrum (**5.2.14**), ranging from 230 nm to 350 nm, 0.0008% solution (w/v), in 0.01 M sodium hydroxide, exhibits maxima at 258 nm and 334 nm.

C. To dry the residue from test A., *Identification*, at 105 °C, for 2 hours. Melting temperature (5.2.2): around 228 °C.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

PURITY TESTS

Related substances. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF₂₅₄ silica gel as support and a mixture of 5 M ammonia, methylene chloride, and ethanol (20:30:50) as mobile phase. To apply separately to the plate 10 µl of each of the solutions freshly prepared.

Solution (1): to dissolve number of tablet equivalent to 0.1 g of nalidixic acid in 50 mL of methylene chloride, stir for 15 minutes, filter, and evaporate to dryness.

To dissolve the residue in 5 mL of methylene chloride.

Solution (2): to dilute the *Solution (1)* to 200 mL volumetric flask and top up with methylene chloride. To dilute the resulting solution (1:2) with methylene chloride.

Solution (3): to dilute *Solution (2)* (1:2.5) in methylene chloride.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). Any secondary spot obtained in the chromatogram with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (2)* (0.25%) and no more than one spot is more intense than that obtained with *Solution (3)* (0.1 %).

DISSOLUTION TEST

Dissolution medium: phosphate buffer pH 8.6, 900 mL

Equipment: blade, 60 rpm

Time: 30 minutes

Procedure: after testing, remove aliquot from the dissolution medium, filter, and dilute in 0.01 M sodium hydroxide, to obtain suitable concentration. To measure the absorbances of the solutions at 334 nm (5.2.14) by using a mixture of 0.01 M sodium hydroxide and *Dissolution medium* at the same rate as the test solution to adjust zero. To calculate the amount of nalidixic acid dissolved in the medium, by comparing the readings obtained with nalidixic acid SQR solution (w/v) at 0.00055% concentration (w/v), 0.01 M sodium hydroxide.

Tolerance: not less than 80% (Q) of the stated amount of nalidixic acid dissolves within 30 minutes.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To weigh and pulverize 20 tablets. To transfer amount of powder equivalent to 0.1 g of nalidixic acid to a 200 mL volumetric flask, add 150 mL of M sodium hydroxide, stir for 3 minutes, and top up to volume with the same solvent. Let it rest for 15 minutes. To transfer 2 mL to 200 mL volumetric flask and top up with water. To prepare standard solution under the same conditions. To measure the absorbance of the resulting solution at 334 nm, by using 0.01 M sodium hydroxide to adjust zero. To calculate the content of nalidixic acid in the sample from the readings obtained. Alternatively, make the calculations taking into account that A (1%, 1 cm) = 494 at 334 nm, in 0.01 M sodium hydroxide.

PACKAGING AND STORAGE

In airtight closed containers, protected from light.

LABELLING

To comply with the legislation in force.

ACID, NALIDIXIC ORAL SUSPENSION

It contains at least 92.5 % and at most 107.5 % of the amount stated of C₁₂H₁₂N₂O₃.

IDENTIFICATION

To transfer 5 mL of the sample to a separatory funnel, add 30 mL of water, and 20 mL of 10% decahydrated sodium carbonate (w/v). To make it homogeneous. To extract with 2 portions of chloroform (30 mL each). To acidify the aqueous solution with 5 M hydrochloric acid, add 40 mL of chloroform, and stir. To collect a chloroform layer and transfer to a separatory funnel, wash with 10 mL of water, and add 0.5 mL of 5 M hydrochloric acid. Then, filter the chloroform layer through cotton and evaporate the filtrate to dryness. The ultraviolet absorption spectrum (5.2.14), ranging from 230 nm to 350 nm, 0.0008% solution (w/v), in 0.1 M sodium hydroxide, exhibits maxima at 258 nm and 334 nm.

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

BIOLOGICAL SAFETY TEST

Counting of the viable micro-organisms (5.5.3.1.2). It complies with the test.

Survey and identification of pathogens (5.5.3.1.3). It complies with the test.

DOSAGE

To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. To transfer the volume or mass of oral suspension equivalent to 0.12 g of nalidixic acid to a 100 mL volumetric flask. To add 0.01 M sodium hydroxide, stir, and top up to volume with the same solvent. To transfer 2 mL of this solution to a 250 mL volumetric flask and top up with 0.01 M sodium hydroxide. To filter it, if necessary. To measure the absorbance of the resulting solution at 334 nm, by using 0.01 M sodium hydroxide to adjust zero. To calculate the amount of $C_{12}H_{12}N_2O_3$ in the oral suspension, by taking into account that $A(1\%, 1\text{ cm}) = 494$ at 334 nm in 0.01 M sodium hydroxide

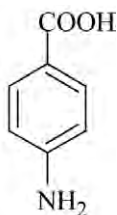
PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

ACID, PARA-AMINOBENZOIC
Acidum 4-aminobenzoicum



$C_7H_7NO_2$; 137.14
para-aminobenzoic acid; 00098
4-aminobenzoic acid
[150-13-0]

It contains at least 98.5% and at most 101.5% of $C_7H_7NO_2$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline powder or white or yellowish-white needles with bitter taste. It gets darker if exposed to air and light.

Solubility. Sparingly soluble in water, freely soluble in ethanol, soluble in warm glycerol, slightly soluble in ethyl ether, sparingly soluble in chloroform. Freely soluble in alkali hydroxides and carbonate solutions and sparingly soluble in hydrochloric acid solutions SR.

Physical and chemical constants

Melting range (5.2.2): 186.0 °C to 189.5 °C.

IDENTIFICATION

A. The ultraviolet absorption spectrum (5.2.14), ranging from 200 nm to 400 nm, of 1% solution (w/v) in isopropyl alcohol, exhibits maxima at 288 nm. The absorbance at 288 nm is approximately 1.370.

B. To dissolve 0.05 g of the sample in mixture of 1 mL of sodium hydroxide SR and 1 mL of distilled water. By following this sequence, gather 0.5 mL of potassium iodide SR, 0.5 mL of hydrochloric acid SR, and 0.5 mL of sodium hypochlorite SR. Brown precipitate is formed.

C. To dissolve 0.01 g of the sample in 2 mL of hydrochloric acid, by heating it, if necessary. To cool down at about 10 °C and add 1 mL of sodium nitrite SR, and 3 mL of 2-naphthol SR. Red color is formed.

PURITY TESTS

Heavy metals (5.3.2.3). To suspend 1 g of the sample in 15 mL of distilled water and add amount of 6 M ammonium hydroxide to obtain dissolution. To add acetic acid SR to obtain a mixture slightly acidic to litmus paper and add extra 2 mL of the same acid. To proceed as described in *Method I*. At most 0.002% (20 ppm).

Loss on drying (5.2.9). At most 0.2%.

Sulfated ashes (5.2.10). At most 0.1 %.

DOSAGE

To accurately weigh about 250 mg of the sample previously desiccated and transfer it to an erlenmeyer flask. To add 5 mL of hydrochloric acid and 50 mL of distilled water. To stir to obtain total dissolution, by heating, if necessary. To cool down at about 15° C, add 25 g of crushed ice, and slowly titrate with 0.1 M SV sodium nitrite until a drop produces a blue color when touched, in a porcelain plate, by glass rod moistened by iodine starch solution SI. The titration is ended up when the mixture is at rest longer than 1 minute and a drop reproduces blue color by observing with the iodine starch solution SI. Each mL of 0.1 M SV sodium nitrite is equal to 13.714 mg of $C_7H_7NO_2$.

PACKAGING AND STORAGE

In tightly closed, opaque containers.

LABELLING

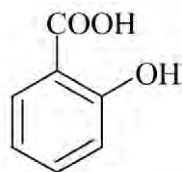
To comply with the legislation in force.

THERAPEUTIC CLASS

Protective, topic.

ACETYLSALICYLIC ACID

Acidum salicylicum



$C_7H_6O_3$; 138.12
salicylic acid; 00340
2-hydroxybenzoic acid
[69-72-7]

It contains at least 99.5% and at most 101.0% of $C_7H_6O_3$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Spongy, white, and crystalline powder or white crystals, usually in the form of fine needles, odorless, sweet-tasting at first, becoming sour. The synthetic product is white and odorless. The resulting from natural substances is slightly pink or yellow, with faint odor of methyl salicylate.

Solubility. Sparingly soluble in water, very soluble in acetone, freely soluble in ethanol and ethyl ether, slightly soluble in chloroform and fatty oils.

Physical and chemical constants.

Melting range (5.2.2): 158 °C to 161 °C.

IDENTIFICATION

The identification tests **B.** and **C.** may be omitted if the test **A.** is conducted.

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the salicylic acid SQR spectrum similarly prepared.

B. To perform cold solubilization of 0.1 g of the sample in sulfuric acid. To add a few crystals of sodium nitrate. The red color develops.

C. To add a drop of ferric chloride SR to a saturated aqueous solution of the sample. Purple color develops. By adding ammonium hydroxide, it changes to brown-green color. Strong mineral acids, some bases, and other salts impede this reaction.

PURITY TESTS

Readily carbonisable substances. To dissolve 0.5 g of the sample in 5 mL of sulfuric acid. No distinctly brown color is developed before 20 minutes.

Phenol. To dissolve 0.5 g of the sample in 10 mL of sodium carbonate SR, stir with 10 mL of ethyl ether, and let it rest to ether phase decants. To desiccate the ether phase with sodium sulfate and filter it. A volume of 5 mL of the filtrate, left to spontaneous evaporation, leaves no more than 0.001 g of residue. To dissolve the residue in hot water, add ammonium hydroxide and a few drops of sodium hypochlorite SR. The blue color develops.

Chlorides (5.3.2.1). To dissolve by heating 1.5 g of the sample in 75 mL of distilled water. To cool down, add distilled water to top up the initial volume, and filter it. A volume of 25 mL of the filtrate does not contain more chloride than the corresponding in 0.10 mL of 0.02 M hydrochloric acid. At most 0.014% (140 ppm).

Sulfates (5.3.2.2). To add 2 drops of hydrochloric acid and 5 drops of barium chloride SR to 25 mL of the filtrate obtained from *Chlorides*.

The resulting preparation is not more opalescent than 0.1 mL of 0.01 M sulfuric acid. At most 0.02% (200 ppm).

Heavy metals (5.3.2.3). To dissolve 1 g of the sample in 25 mL of acetone. To add 2 mL of water, 2 mL of acetate buffer pH 3.5, and 1.2 mL of thioacetamide SR. To make it homogeneous and let it rest for 5 minutes. The color produced is not more intense than that obtained in *Standard preparation* prepared with 25 mL of acetone, 2 mL of *Lead standard solution (10 ppm)* and treated similarly to the sample. At most 0.002% (20 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.05%.

DOSAGE

To accurately weigh about 0.5 g of the sample dissolve in 25 mL of ethanol, previously neutralized with 0.1 M sodium hydroxide. To use phenolphthalein SI as an indicator and titrate with 0.1 M SV sodium hydroxide to pink color appears. Each mL of 0.1 M SV sodium hydroxide is equal to 13.812 mg of $C_7H_6O_3$.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

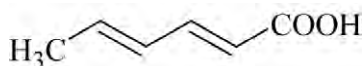
To comply with the legislation in force.

THERAPEUTIC CLASS

Keratolytic.

ACID, SORBIC

Acidum sorbicum



$C_8H_8O_2$; 112.13
sorbic acid; 00346
(2E, 4E) -2,4-hexadienoic acid
[110-44-1]

It contains at least 99.0% and at most 101.0% of $C_8H_8O_2$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. Crystalline, white or almost white powder.

Solubility. Sparingly soluble in water, freely soluble in ethanol and ethyl ether.

Physical and chemical constants.

Melting range (5.2.2): 132°C to 136°C.

IDENTIFICATION

The identification test **A.** may be omitted if the tests **B.** and **C.** are conducted.

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the sorbic acid SQR spectrum similarly prepared.

B. To dissolve 50 mg in water and top up volume to 250 mL. To dilute 2 mL of this solution to 200 mL with 0.1 M hydrochloric acid. The ultraviolet absorption spectrum (5.2.14), ranging from 230 nm to 350 nm, of the resulting solution, exhibits maxima at 256 nm (± 2 nm). The absorbance at 264 nm is from 0.43 to 0.51.

C. To dissolve 0.2 g of the sample in 2 mL of ethanol and add 0.2 mL of bromine water. The solution discolors.

PURITY TESTS

Appearance of solution. The 5% solution in ethanol should be clear (5.2.25) and colorless (5.2.12).

Limit for aldehydes. To dissolve 1 g of the sample in a mixture of 50 mL of isopropyl alcohol and 30 mL of water, adjust the solution to pH 4.0 with 0.1 M hydrochloric acid or 0.1 M sodium hydroxide, and top up to 100 mL with water. To add 1 mL of bleached fuchsin SR to 10 mL of the solution and let it rest for 30 minutes. The color produced should not be more intense than that obtained in solution prepared by adding 1 mL of bleached fuchsin SR in a mixture of 1.5 mL of acetaldehyde standard solution (100 ppm

C_2H_4O), 4 mL of isopropyl alcohol, and 4.5 mL of water. (0.15% calculated as C_2H_4O).

Heavy metals (5.3.2.3). To use *Method III*. At most 0.001% (10 ppm).

Water (5.2.20). To determine in 2 g of the substance. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 0.5 g of the substance. At most 0.2%.

DOSAGE

To dissolve 0.1 g of the sample in 20 mL of ethanol. To titrate with 0.1 M SV sodium hydroxide by using 0.2 mL of phenolphthalein SI as an indicator to change pink color. Each mL of 0.1 M SV sodium hydroxide is equal to 11.213 mg of $C_8H_8O_2$.

PACKAGING AND STORAGE

In tightly closed containers, protected from light and excessive heat.

LABELLING

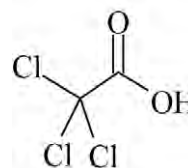
To comply with the legislation in force.

THERAPEUTIC CLASS

Antimicrobial preservative, especially against fungi and yeasts.

ACID, TRICHLOROACETIC

Acidum trichloroaceticum



$C_2HCl_3O_2$; 163.39
trichloroacetic acid; 00366
2,2,2-trichloroacetic acid
[76-03-9]

It contains at least 98.0% and at most 100.5% of trichloroacetic acid.

DESCRIPTION

Physical characteristics. Crystalline, white mass, or colorless crystals, very deliquescent.

Solubility. Very soluble in water, ethanol, and methylene chloride.

IDENTIFICATION

A. To add 2 mL of pyridine and 5 mL of concentrated sodium hydroxide SR solution to 0.5 mL of the solution obtained in *Limit test for chlorides*. To vigorously stir and heat in a water bath at 60-70°C for 5 minutes. The upper phase has an intense red color.

B. The solution obtained in *Limit test for chlorides* is strongly acidic.

PURITY TESTS

Chlorides (5.3.2.1): To dissolve 2.5 g of the sample in water and top up to 25 mL with the same solvent. To pipette 5 mL of this solution and top up to 15 mL with water. This solution meets the *Limit test for chlorides*. At most 0.01% (100 ppm).

Sulfated ashes (5.2.10). At most 0.1% determined in 1 g of the sample.

DOSAGE

To dissolve 0.150 g of the sample in 20 mL of water. To titrate with 0.1 M SV sodium hydroxide by using phenolphthalein SI as an indicator. 1 mL of 0.1 M SV sodium hydroxide corresponds to 16.339 mg of $C_2HCl_3O_2$.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

CATEGORY

Caustic action.

ACID, UNDECYLENIC
Acidum undecylenicum



$C_{11}H_{20}O_2$; 184.28
undecylenic acid; 00367
10-undecylenic acid
[112-38-9]

It contains at least 97.0% and at most 102.0% of $C_{11}H_{20}O_2$.

DESCRIPTION

Physical characteristics. Crystalline, white or pale and yellowish mass; if above the freezing temperature, it is colorless or pale yellow.

Solubility. Practically insoluble in water, freely soluble in ethanol, ethyl ether, fatty oils, and essential oils.

Physical and chemical constants.

Relative density (5.2.5): 0.910 to 0.913.

IDENTIFICATION

A. The freezing temperature (5.2.4) of the sample is between 21 °C and 24 °C.

B. The refractive index (5.2.6) of the sample, determined at $(25.0 \pm 0.5)^\circ\text{C}$, is between 1.447 and 1.450.

C. To accurately dissolve 0.1 g of the sample in mixture of 2 mL of M sulfuric acid and 5 mL of glacial acetic acid. To add dropwise 0.25 mL of potassium permanganate SR. The potassium permanganate solution discolors.

PURITY TESTS

Acids soluble in water. To add 5 mL of water to 5 mL of sample, make it homogeneous, and filter the aqueous layer through filter paper moistened with water. To add a drop of methyl orange SI and titrate with 0.01 M SV sodium hydroxide. No more than 1 mL of 0.01 M SV sodium hydroxide is required to obtain the same color as produced by a drop of methyl orange SI in 5 mL of water.

Index of iodine (5.2.29.10). 131 to 138.

Heavy metals (5.3.2.3). To use *Method III*. At most 0.001% (10 ppm).

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.15%.

DOSAGE

To accurately dissolve about 0.75 g of the sample in 50 mL of ethanol. To titrate with 0.1 M SV sodium hydroxide by using 0.2 mL of phenolphthalein SI as an indicator to change pink color for at least 30 seconds. To perform sample blank correction and adjust it as required. Each mL of 0.1 M SV sodium hydroxide is equal to 18.428 mg of $C_{11}H_{20}O_2$.

PACKAGING AND STORAGE

In tightly closed nonmetallic containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antifungal, topic.

WATER FOR INJECTION

Aqua ad injectabilia

H₂O; 18.02
water for injection; 09320
Water
[7732-18-5]

Water for injection is the material used to prepare parenteral medicaments, as a vehicle or for dissolving or diluting substances or preparations. Other examples of pharmaceutical applications are the manufacture of active parenteral use, for final washing of equipment, piping, and containers used in parenteral preparations and cleaning of certain equipment.

Water for injection is obtained by distillation of water properly treated in equipment whose parts in contact with water are made of neutral glass, quartz or other suitable material. It can also be achieved by a similar or better distillation procedure, in the removal of chemical contaminants, micro-organisms, and bacterial endotoxins. Such procedure must be validated.

In order to ensure that the water meets the requirements of quality, its production should be monitored by means of validated procedures regarding the parameters of electrical conductivity, total organic carbon, microbial counting, and endotoxin.

Sterile water for injection. Sterile water for injection is the *Water for injection* that after sterilization was stored in inert containers, such as stainless steel 316L, kept closed at temperature 80-85° C, under recirculation, for a maximum period of 24 hours, whose conditions ensure that the product complies with the test for bacterial endotoxins. Sterile water is free of any substance.

DESCRIPTION

Physical characteristics. Clear, colorless, tasteless, and odorless liquid.

PURITY TESTS

It complies with the tests described in the monograph *Purified water*.

Sterile water for injection complies with the tests described in the monograph *Purified water* and addition test shown below.

Particle contamination: Sub-visible particulates (5.1.7.1). It complies with the test A or B, as per the volume of containers.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test. To proceed as described for water-soluble substances in the *Membrane filtration* method or any other method that proves

to be similar or better than the validated pharmacopoeial method. To use at least 200 mL of the sample. At most 10 UFC/100 mL.

Bacterial endotoxins (5.5.2.2). At most 0.25 UI of endotoxins per mL.

Sterile water for injection complies with additional tests *Bacterial endotoxins (5.5.2.2)* and *Sterility (5.5.3.2.1)*.

PACKAGING AND STORAGE

Stored and distributed under appropriate conditions to ensure the maintenance of physical and chemical and microbiological requirements.

LABELLING

To comply with the legislation in force.

PURIFIED WATER

Aqua purificata

H₂O; 18,02
purified water; 09879
Water
[7732-18-5]

Purified water is a drinking water that has undergone some treatment to remove possible contaminants and meets the purity requirements set forth in this monograph. It is prepared by distillation, ion exchange, reverse osmosis or other suitable method. It must be free from any added substances dissolved. It is usually used in the preparation of medicines that do not require sterile or pyrogen-free water, for non-parenteral use.

DESCRIPTION

Physical characteristics. Clear, colorless, tasteless, and odorless liquid.

PURITY TESTS

Acidity or alkalinity. To add 0.05 mL of methyl red SI in 10 mL of sample freshly boiled and cooled down in borosilicate glass vial. The solution does not develop red color. To add 0.1 mL of bromothymol blue SI solution to 10 mL of the sample. The solution does not develop blue color.

Oxidizable substances. To boil 100 mL of the sample with 10 mL of *M* sulfuric acid. To add 0.2 mL of 0.02 *M* SV potassium permanganate and boil it for 5 minutes. The remaining solution is faintly pink.

Conductivity of water (5.2.24). At most 1.3 µS/ cm at 25.0°C ± 0.5°C. The user must set the appropriate upper limit for the specific application (**11.vol.1**). Alternatively, it replaces the tests for ammonium, calcium and magnesium, chlorides, nitrates, and sulfates.

Total organic carbon (5.2.30). Alternatively, it replaces the test for oxidizable substances. At most 0.50 mg/L.

Ammonium. To add 1 mL of alkaline mercuric potassium iodide SR1 in 20 mL of the sample. After 5 minutes, examine the solution at vertical axis of the tube. The solution is not more intensely colored than that standard by adding 1 mL of alkaline mercuric potassium iodide SR1 to a mixture of 4 mL of ammonium standard solution (1 ppm NH_4) and 16 mL of ammonia-free water. At most 0.00002% (0.2 ppm).

Calcium and magnesium. To add 2 mL of ammonium chloride buffer pH 10.0, 0.5 mL of eriochrome black T, and 5 μL of 0.05 M sodium edetate in 100 mL of the sample. A clear blue color is produced. At most 1 ppm.

Chlorides. To dissolve 1 mL of nitric acid SR and 2 mL of 0.1 M silver nitrate in 10 mL of the sample. The solution shows no change in appearance for at least 15 minutes.

Nitrates. To transfer 5 mL of the sample to test tube immersed in ice water, add 0.4 mL of 10% potassium chloride solution (w/v) and 0.1 mL of 0.1% diphenylamine (w/v). To drip, under stirring, 5 mL of nitrogen-free sulfuric acid. To transfer the tube to a water bath at 50°C. After 15 minutes, any blue color developed in the solution is not more intense than that of the standard, simultaneously and similarly prepared by using a mixture of 4.5 mL of nitrate-free water and 1 mL of nitrate standard solution 2 ppm in NO_3 freshly prepared. At most 0.00002% (0.2 ppm).

Sulfates. To add 0.1 mL of 2 M hydrochloric acid and 0.1 mL of 6.1% aqueous barium chloride solution (w/v) in 10 mL of the sample. The solution shows no change in appearance for at least a hour.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test. To proceed as described for water-soluble substances in the *Membrane filtration* method or any other method that proves to be similar or better than the validated pharmacopoeial method. To use at least 200 mL of the sample. At most 100 UFC/100 mL.

Another test that can be performed instead of that described above is the counting of heterotrophic bacterial. At most 100 UFC/100 mL.

When purified water is collected from storage reservoir, besides the counting of the total number of mesophilic micro-organisms or heterotrophic bacteria, a survey for pathogenic microorganisms (5.5.1.6.3) should be performed. The absence of total coliforms, *Escherichia coli*, and *Pseudomonas aeruginosa*, especially if the water is used in topical products. To use 100 mL of water in the test.

The modality of sterile purified, which is used in the preparation of eye drops and other processes that can not un-

dergo final heat or filtration sterilization, must additionally meet the sterility test.

PACKAGING AND STORAGE

In inert containers, such as glass or stainless steel 316L, properly identified, that ensure the physical and chemical and microbiological requirements.

Should storage be necessary, the purified water must be stored and distributed under appropriate conditions to prevent microbial growth and avoid any contamination.

LABELLING

To comply with the legislation in force.

ULTRAPURE WATER Aqua ultra purificata

H_2O ; 18.02
ultrapure water; 09880
Water
[7732-18-5]

Ultrapure water is a purified water that has undergone some extra treatment to remove possible contaminants and meets the purity requirements set forth in this monograph. It is prepared by supplementing a set of processes, such as distillation, ion exchange, reverse osmosis, among others. There is no dissolved substance. It is generally used in applications requiring high purity water or in most laboratory test procedures that require readings at low concentrations or purity of the water that may affect the sensitivity, reproducibility, and robustness of the analytical method.

DESCRIPTION

Physical characteristics. Clear, colorless, tasteless, and odorless liquid.

PURITY TESTS

Conductivity of water (5.2.24). At most 0.1 $\mu\text{S}/\text{cm}$ at 25.0 °C $\pm$ 0.5 °C.

Total organic carbon (5.2.30). At most 0.050 mg/L. **Note:** *This test is optional. It should be used if the specific application requires this control.*

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test. To proceed as described for water-soluble substances in the *Membrane filtration* method or any other methodology that proves to be similar or better than the validated pharmacopoeial method. To use at least 200 mL of the sample. At most 1 UFC/100mL.

PACKAGING AND STORAGE

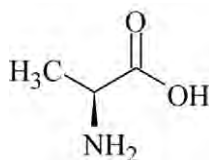
In polymeric or glass containers, depending on the application, that ensure the physical and chemical and microbiological requirements.

Should storage be necessary, the ultrapure water must be stored for 24 hours maximum, under appropriate conditions, to prevent microbial growth and any contamination.

LABELLING

To correctly identify the container for this type of water.

ALANINE
Alaninum



$C_3H_7NO_2$; 89.09
 alanine; 00451
 L-Alanine
 [56-41-7]

It contains at least 98.5% and at most 101.5% of $C_3H_7NO_2$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white or almost white powder, or colorless crystals.

Solubility. Freely soluble in water, very slightly soluble in ethanol, and practically insoluble in ethyl ether.

Physical and chemical constants.

Specific rotation (5.2.8): $+13.7^\circ$ to $+15.1^\circ$, in relation to the desiccated substance. To determine in 10% solution (w/v) in 6 M hydrochloric acid.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the alanine SQR spectrum similarly prepared.

B. The principal spot in the chromatogram of *Solution (2)* obtained in *Ninhydrin-reacting substances* corresponds in position, color, and intensity to that obtained with the *Solution (4)*.

C. The sample responds to the test for *Specific rotation* in *Physical and chemical constants*.

PURITY TESTS

Appearance of solution. To dissolve 2.5 g of the sample in water and top up the volume to 50 mL with the same solvent. To dilute 10 mL of this solution to 20 mL with water. The resulting solution is clear (5.2.25) and is not more intensely colored than the *Reference color solution (5.2.12)* prepared as described below.

Reference color solution: to mix 2.4 mL of the ferric chloride base solution, 1 mL of cobaltous chloride base solution, 0.4 mL of cupric sulfate base solution, and 6.2 mL of 1% hydrochloric acid solution (v/v). To mix 5 mL of the resulting solution with 95 mL of 1% hydrochloric acid (v/v).

pH (5.2.19). 5.5 to 7.0. To determine in 5 % solution (w/v).

Ninhydrin-reacting substances. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using G silica gel as support and a mixture of water, glacial acetic acid, and 1-butanol (20:20:60) as mobile phase. To apply separately to the plate 5 μ l of each of the solutions freshly prepared, as described below.

Solution (1): 10 mg/mL solution of the sample in water.

Solution (2): to dilute 1 mL of *Solution (1)* to 50 mL with water.

Solution (3): to dilute 5 mL of *Solution (2)* to 20 mL with water.

Solution (4): 0.2 mg/mL alanine SQR solution in water.

Solution (5): to dissolve 10 mg of alanine SQR and 10 mg of glycine SQR in water and dilute to 25 mL with the same solvent.

To develop the chromatogram. To remove the plate, and let it drying. To nebulize with ninhydrin SR. To heat the plate at 105°C for 15 minutes and examine immediately. Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (3)* (0.5%). The test is valid only if the chromatogram obtained with *Solution (5)* shows two principal spots clearly separated.

Ammonium. To prepare a small chamber by using 2 watch glasses (60 mm in diameter), placed edge to edge. To adhere to the inner wall of the upper watch glass by means of a few drops of water, a strip of litmus paper red (5 mm x 5 mm). In the bottom glass, suspend 50 mg of the sample finely pulverized in 0.5 mL of water. To add 0.3 g of magnesium oxide, mix rapidly with a glass rod, and close the chamber by joining the 2 watch glasses. To heat at 40°C for 15 minutes. The litmus paper must not have a more intense blue color than a strip of litmus paper red prepared simultaneously, under the same conditions, with 0.1 mL of 0.0296% ammonium chloride solution (w/v), 0.5 mL of water, and 0.3 g of magnesium oxide.

At most 0.02% (200 ppm).

Chlorides (5.3.2.1). At most 0.05% (500 ppm).

Iron (5.3.2.4). To use *Method III*. At most 0.003% (30 ppm).

Heavy metals (5.3.2.3). To use *Method I*. At most 0.0015% (15 ppm).

Sulfates (5.3.2.2). At most 0.03% (300 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample, in an oven at 100-105 °C for 3 hours. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To proceed as described in *Titration in non-aqueous medium (5.3.3.5)*. To accurately weigh about 80 mg of the sample and dissolve in 3 mL of anhydrous formic acid. To add 30 mL of glacial acetic acid and titrate with 0.1 M SV perchloric acid. To potentiometrically determine the endpoint or use 0.1 mL of 1-naphtholbenzein SI to change color to green. To perform sample blank correction and adjust it as required. Each mL of 0.1 M SV perchloric acid is equal to 8.909 mg of $C_3H_7NO_2$.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

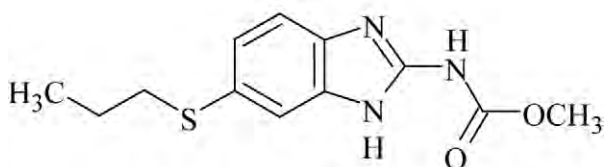
LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Amino acids.

ALBENDAZOLE Albendazolum



$C_{12}H_{15}N_3O_2S$; 265,33
albendazole; 00458

Ester of [6 - (propylthio)-1H-benzimidazole-2-yl]-
carbamic acid
[54965-21-8]

It contains at least 98.0% and at most 102.0% of $C_{12}H_{15}N_3O_2S$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline powder, greasy to the touch, white or almost white, almost odorless.

Solubility. Practically insoluble in water, freely soluble in formic acid, soluble in glacial acetic acid and sulfuric acid, sparingly soluble in chloroform, very slightly soluble in ethyl acetate, acetone, tert-amyl alcohol, benzene, methylene chloride, ethanol, ethyl ether, isopropyl alcohol, methanol, and toluene, insoluble in n-hexane and carbon tetrachloride. Very slightly soluble in 0.1 M hydrochloric acid and insoluble in 0.1 M sodium hydroxide.

Physical and chemical constants.

Melting range (5.2.2): 208 °C to 209 °C.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the albendazole SQR spectrum similarly prepared.

B. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF_{254} silica gel as support and a mixture of chloroform, glacial acetic acid, and ethyl ether (60:10:10) as mobile phase. To apply separately to the plate 10 µL of each of the solutions freshly prepared, as described below.

Solution (1): 1% solution (w/v) in glacial acetic acid.

Solution (2): 1% albendazole SQR solution (w/v) in glacial acetic acid.

To develop the chromatogram. To remove the plate, allow to air dry, and examine under ultraviolet light (254 nm). The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

C. To dissolve in a test tube 10 mg of sample in 5 mL of chloroform. To transfer 1 mL to test tube containing 5 mL of sulfuric acid and 4 drops of formaldehyde solution. Color is developed on the interface. After stirring, the sulfuric layer also develops color.

PURITY TESTS

Loss on drying (5.2.9). To determine in 2 g of the sample. To desiccate in an oven at 105 °C for 4 hours. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.2%.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Titration in non-aqueous medium (5.3.3.5)*. To dissolve 0.4 mg of the sample previ-

ously desiccated in 30 mL of glacial acetic acid. To heat it, if necessary. To cool down and add 5 drops of methylrosanilinium chloride SI. To titrate with 0.1 M SV perchloric acid to obtain emerald green color. To perform sample blank correction and adjust it as required. Each mL of 0.1 M SV perchloric acid is equal to 26.533 mg of $C_{12}H_{15}N_3O_2S$.

B. A. To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To accurately weigh about 25 mg of the sample and dissolve in 25 mL of 2% hydrochloric acid (w/v) in methanol. To top up the volume to 50 mL with water. To transfer 5 mL to 50 mL volumetric flask and top up with 0.1 M hydrochloric acid. To successively dilute with 0.1 M sodium hydroxide to obtain 0.0005% concentration (w/v). To prepare a standard solution at the same concentration by using the same solvents. To measure the absorbance of the resulting solutions at 309 nm, by using 0.1 M sodium hydroxide to adjust zero. To calculate the $C_{12}H_{15}N_3O_2S$ concentration of the sample from the readings obtained.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Anthelmintic.

ALBENDAZOLE TABLETS

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_{12}H_{15}N_3O_2S$.

IDENTIFICATION

A. To weigh and pulverize the tablets. To transfer amount of powder equivalent to 10 mg of albendazole to 50 mL volumetric flask and add 25 mL of 2% hydrochloric acid (v/v) in methanol. To stir for 10 minutes, top up with the same solvent, and filter it. To dilute the filtrate with the same solvent to obtain a 0.001% concentration (w/v). To prepare a standard solution at the same concentration. The ultraviolet absorption spectrum (5.2.14) of the sample solution, ranging from 200 nm to 400 nm, exhibits maxima and minima only in the same wavelength of the standard solution.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method **B.** corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). At most 15 minutes.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 900 mL

Equipment: blades, 50 rpm

Time: 30 minutes

Procedure: after testing, filter, and remove the aliquot of 10 mL from the dissolution medium, transfer to a 250 mL volumetric flask, and top up with 0.1 M sodium hydroxide. To transfer 90 mg of albendazole SQR to a 250 mL volumetric flask, add 10 mL of 2% hydrochloric acid (v/v) in methanol, and make it homogeneous. To dilute with 0.1 M hydrochloric acid to top up to volume. To transfer 5 mL of this solution to a 200 mL volumetric flask and dilute with 0.1 M sodium hydroxide. To measure the absorbances at 308 nm and 350 nm by using the same solvent to adjust zero. To calculate the amount of $C_{12}H_{15}N_3O_2S$ dissolved in the medium, by using the equation: $22.5 C (Aa/AP)$, where C is the concentration in $\mu\text{g/mL}$ of albendazole in the standard solution and Aa and Ap are the difference between the absorbances at 308 nm and 350 nm, obtained for the sample solution and standard solution, respectively.

Tolerance: not less than 80% (Q) of the stated amount of $C_{12}H_{15}N_3O_2S$ dissolves within 30 minutes.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To weigh and spray 20 tablets. To transfer amount of powder equivalent to 10 mg of albendazole to 50 mL volumetric flask and add 25 mL of 2% hydrochloric acid (v/v) in methanol. To stir for 10 minutes, top up with distilled water, and filter it. To successively dilute to 0.0008% concentration (w/v) by using 0.1 M sodium hydroxide as solvent. To prepare a standard solution at the same concentration by using the same solvents. To measure the absorbance of the solutions at 308 nm, by using 0.1 M sodium hydroxide to adjust zero. To calculate the amount of $C_{12}H_{15}N_3O_2S$ in the tablets, from the readings obtained.

B. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 254 nm; 250 mm column and 4.6 mm inner diameter, packed with silica

chemically bonded to the octadecylsilane group (5 μ m), flow of *Mobile phase* of 2.0 mL/min.

Mobile phase: 0.5 g solution of ammonium phosphate monobasic in 1000 mL of water and methanol (4:6).

Internal standard solution: to accurately weigh about 150 mg of parbendazole SQR. To transfer to 50 mL volumetric flask, add 5 mL of 1% sulfuric acid (v/v) in methanol, and top up with methanol.

Sample solution: To weigh and pulverize 20 tablets. To transfer amount of powder equivalent to 100 mg of albendazole to 50 mL volumetric flask and add 5 mL of 1% sulfuric acid (v/v) in methanol and 20 mL of methanol. To stir for 15 minutes, top up with methanol, and filter it. To transfer 5 mL of the filtrate and 5 mL of *Internal standard solution* to 50 mL volumetric flask and top up with methanol.

Standard solution: To accurately weigh about 100 mg of albendazole SQR and transfer to 50 mL volumetric flask, add 5 mL of 1% sulfuric acid (v/v) in methanol, and top up with methanol. To transfer 5 mL of this solution and 5 mL of *Internal standard solution* to 50 mL volumetric flask and top up with methanol.

The column efficiency is greater than 4000 theoretical plates/meter. The resolution between albendazole and parbendazole should not be less than 2.0. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2.0%.

Procedure: to separately inject 20 μ L of *Standard solution* and *Sample solution*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of $C_{12}H_{15}N_3O_2S$ in the sample solution from the responses from the *Standard solution* and *Sample solution* in relation to the *Internal standard solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

ALBENDAZOLE, ORAL SUSPENSION

Albendazole oral suspension is a mixture of albendazole with one or more coloring, flavoring, buffer, sweetener, and preservative agents, in an aqueous vehicle. It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_{12}H_{15}N_3O_2S$.

IDENTIFICATION

To dilute suitable volume of the suspension into mixture of methanol and hydrochloric acid (99:1), to obtain 1 mg/mL concentration. To filter, if necessary, and transfer 10 mL of the filtrate to 100 mL volumetric flask, then top up with

0.1 M sodium hydroxide, and make it homogeneous. The ultraviolet absorption spectrum (5.2.14), for the resulting solution obtained in the range from 200 nm to 400 nm, exhibits maxima and minima only in the same wavelength of a similar albendazole SQR solution.

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

pH (5.2.19). 4.5 to 5.5.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 308 nm; 250 mm column and 4 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μ m), flow of *Mobile phase* of 2 mL/min.

Mobile phase: to dissolve 11 g of monobasic sodium phosphate in 800 mL of water and add 1200 mL of methanol.

Sample solution: to transfer to a 100 mL volumetric flask a volume of suspension corresponding to 0.1 g of albendazole and top up with mixture of methanol and hydrochloric acid (99:1). To successively dilute to obtain 100 μ g/mL concentration by using the *Mobile phase* as solvent. To filter, if necessary.

Standard solution: to accurately weigh about 50 mg of albendazole SQR. To transfer to a 50 mL volumetric flask and top up with mixture of methanol and hydrochloric acid (99:1). To successively dilute to obtain 100 μ g/mL concentration by using the *Mobile phase* as solvent.

The column efficiency is greater than 8000 theoretical plates/meter. The relative standard deviation for the replicate areas for the peaks recorded is not greater than 2.0%.

Procedure: to separately inject 20 μ L of *Sample and standard solutions*, record the chromatograms, and measure the areas of the peaks. To calculate the amount, in mg, of $C_{12}H_{15}N_3O_2S$, in each mL of the oral suspension, from the responses obtained for the *Sample and standard solutions*.

PACKAGING AND STORAGE

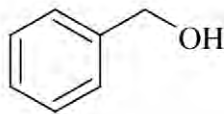
In tightly closed containers, at room temperature.

LABELLING

To comply with the legislation in force.

ALCOHOL, BENZYL

Alcohol benzylicus



C_7H_8O ; 108.14
benzyl alcohol; 00471
Benzenemethanol
[100-51-6]

It contains at least 98.0% and at most 100.5% of C_7H_8O .

DESCRIPTION

Physical characteristics. Oily, colorless, and clear liquid.

Solubility. Slightly soluble in water, miscible with ethanol, ethyl ether, and chloroform.

Physical and chemical constants.

Relative density (5.2.5): 1.042 to 1.047 g/mL.

Refractive index (5.2.6): 1.538 to 1.541. To determine at 20°C.

IDENTIFICATION

The infrared absorption spectrum (5.2.14) of the sample, dispersed among potassium bromide or sodium chloride plates, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the benzyl alcohol SQR spectrum similarly prepared.

PURITY TESTS

Clarity of solution

Hydrazine solution: to transfer 1 g of hydrazine sulfate to 100 mL volumetric flask, dissolve it, and top up with water. To make it homogeneous. Let it rest for 4-6 hours.

Methenamine solution: to transfer 2.5 g of methenamine to a 100 mL volumetric flask, add 25 mL of water, and stir to dissolve it.

Primary opalescent suspension: to transfer 25 mL of *Hydrazine solution* to a 100 mL volumetric flask containing *Methenamine solution*, top up to volume with water, and make it homogeneous. Let it rest for 24 hours. (This suspension is stable for 2 months, if kept in closed glass vial without defects. The suspension may adhere to the glass, so stir it before using it.)

Opalescence standard: To transfer 15 mL of *Primary opalescent suspension* to 1000 mL volumetric flask, top up with water, and make it homogeneous. (Do not use this solution after 24 hours of preparation.)

Reference suspensions: to transfer 5 mL of *Opalescence standard* to a 100 mL volumetric flask, top up with water, and make it homogeneous, to obtain a *Reference suspension A*. To transfer 10 mL of the same standard to another 100 mL flask, top up with water, and make it homogeneous, to obtain the *Reference suspension B*.

Sample solution: to dissolve 2 g of the sample in 60 mL of water.

Procedure: to separately transfer the same amount of *Sample solution*, *Reference suspension A*, *Reference suspension B*, and water to colorless and transparent glass tubes, with 15-25 mm inner diameter, to obtain approximately 40 mm deep. To compare solutions by employing incident light and dark background. The *Sample solution* has the same clarity of the water or it is not more opalescent than the *Reference suspension A*.

Color of solution. to separately transfer the same amount of *Sample solution* obtained in *Clarity of solution*, and water to colorless and transparent glass tubes, with 15-25 mm inner diameter, to obtain approximately 40 mm deep. The *Sample solution* has the same color of the water.

Acidity. To add 1 mL of phenolphthalein SI to a 50 mL of ethanol and neutralize with 0.1 M sodium hydroxide. To dissolve 10 mL of the sample in 10 mL of neutralized ethanol and titrate with 0.1 M sodium hydroxide until a pink color remains up to 30 seconds. Up to 1 mL is spent.

Index of peroxide. To accurately weigh about 5 g of the sample and transfer to a 250 mL erlenmeyer flask. To add 30 mL of a mixture of glacial acetic acid and chloroform (3:2), stir, and add 0.5 mL of saturated potassium iodide solution. To stir for 1 minute and add 30 mL of water. To slowly titrate with 0.01 M SV sodium thiosulfate under constant stirring to yellow color disappears.

To add 5 mL of starch SI and continue titration under vigorous stirring to the blue color disappears. To perform sample blank correction (the volume spent should not exceed 0.1 mL). The peroxide value is equal to the difference between the volumes (mL) of sodium thiosulfate spent in the sample and sample blank correction multiplied by 10 and divided by the mass (g) of the sample. No more than 5.

Limit for nonvolatile residues. To evaporate 10 g of the sample in a water bath and dry the residue at 105 °C for 1 hour. To cool down in desiccator and weigh it. The residue weighs no more than 5 mg: up to 0.05% of non-volatile residues are found.

DOSAGE

To accurately weigh about 0.9 g of the sample, add 15 mL of a mixture of pyridine and acetic anhydride (7:1) freshly prepared and boil it under reflux for 30 minutes. To cool down, add 25 mL of water and 0.25 mL of phenolphthalein SI, and titrate with M SV sodium hydroxide. To perform sample blank correction. To calculate the percentage of C_7H_8O by using the following equation:

10.814 (Vb - Va)/Ma

where *Va* is the volume (mL) of the titrant spent for the sample, *Vb* is the volume (mL) of the titrant spent for sample blank correction, *Ma* is the mass (g) of benzyl alcohol titrated.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

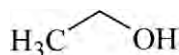
To comply with the legislation in force.

THERAPEUTIC CLASS

Local anesthetic, antimicrobial.

ALCOHOL, ETHYL

Alcohol ethylicus



C₂H₆O; 46,07
ethyl alcohol; 00475
Ethanol [64-17-5]

It contains at least 95.1% (v/v), equivalent to 92.55% (w/w), and at most 96.9% (v/v), equivalent to 95.16% (p/p) of C₂H₆O at 20° C, calculated from the relative density, by using the Alcoholic Strength table (5.2.26). For absolute ethyl alcohol, it contains at least

99.5% (v/v) equivalent to 99.18% (w/w) of C₂H₆O at 20° C, calculated from the relative density, by using the Alcoholic Strength table (5.2.26).

DESCRIPTION

Physical characteristics. Colorless, clear, volatile, flammable, and hygroscopic liquid.

Solubility. Miscible with water and methylene chloride.

Physical and chemical constants

Relative density (5.2.5): 0.805 to 0.812, determined at 20° C. For absolute ethyl alcohol, up to 0.793 determined at 20° C.

IDENTIFICATION

The infrared absorption spectrum (5.2.14) of the sample has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the ethanol SQR spectrum.

PURITY TESTS CLARITY OF SOLUTION (5.2.25).

Hydrazine solution: to transfer 1 g of hydrazine sulfate to 100 mL volumetric flask, dissolve it, top up with water, and stir it. Let it rest for 4-6 hours.

Methenamine solution: to transfer 2.5 g of methenamine to a 100 mL volumetric flask, add 25 mL of water, and stir to dissolve it.

Primary opalescent suspension: to transfer 25 mL of *Hydrazine solution* to a 100 mL volumetric flask containing *Methenamine solution*. To stir and let it rest for 24 hours. (This suspension is stable for 2 months, if kept in closed glass vial without defects. The suspension may adhere to the glass, so stir it before using it.)

Opalescence standard: to transfer 15 mL of *Primary opalescent suspension* to 1000 mL volumetric flask, top up with water, and stir it. (Do not use this solution after 24 hours of preparation.)

Reference suspensions: to transfer 5 mL of *Opalescence standard* to a 100 mL volumetric flask, top up with water, and stir to obtain a *Reference suspension A*. To transfer 10 mL to another 100 mL flask, top up with water, and stir it, to obtain the *Reference suspension B*.

Sample solution A: sample to be examined.

Sample solution B: to dilute 1 mL of the *Sample solution A* to 20 mL of water and let it rest for 5 minutes before using it.

Procedure: to transfer a portion of *Sample solution A* and *Sample solution B* obtained to colorless and transparent glass tubes, with 15-25 mm inner diameter, to obtain approximately 40 mm deep. To transfer to a similar tube the same volume in *Sample solution A*, *Sample solution B*, and water, and to another tube the same amount of water. To compare *Sample solution A*, *Sample solution B*, *Reference suspension A*, *Reference suspension B*, and water, by using dark background and light. The *Sample solution A* and *Sample solution B* have the same clarity of water or have opalescence no higher than *Reference suspension A*.

Color of solution (5.2.12).

Stock standard solution: to mix 3 mL of *Ferric chloride base solution*, 3 mL of *Cobaltous chloride base solution*, 2.4 mL of *Cupric sulfate base solution*, and 1.6 mL of diluted hydrochloric acid (10 mg/mL).

Standard solution: to transfer 1 mL of *Stock standard solution* to a 100 mL volumetric flask, top up with diluted hydrochloric acid (10 mg/mL), and stir it. To use this solution immediately after preparation.

Procedure: to transfer a portion of *Sample solution A* to a colorless and transparent glass tube, with 15-25 mm inner diameter, to obtain approximately 40 mm deep. To transfer to a similar tube the same volume and to another tube the

same amount of water. The *Sample solution A* has no color more intense than that of the *Standard solution*.

Acidity or alkalinity. To add 20 mL of carbon dioxide-free water to 20 mL of the sample and add 0.1 mL of phenolphthalein SI. The solution should be colorless. To add 1.0 mL of 0.01 M sodium hydroxide. The solution turns pink (30 ppm, expressed as acetic acid).

Absorption of light. To record the ultraviolet absorption spectrum of the sample between 200 and 400 nm by using a 1 cm optical cuvette with water as a blank. Maximum absorbance of 0.08 at 240 nm, 0.06 between 250 and 260 nm, and 0.02 between 270 to 340 nm.

Limit for nonvolatile residues. To evaporate 100 mL of the sample in a water bath and dry the residue at 105 °C for 1 hour. To cool down in desiccator and weigh it. The residue weighs no more than 2.5 mg. At most 0.025%.

Volatile organic impurities. To proceed as described in *Gas chromatography (5.2.17.5)*. To use chromatograph equipped with flame ionization detector, 30 m long capillary column and 0.53 mm inner diameter, filled with stationary phase linked to cyanopropyl phenyl (6%) and dimethylpolysiloxane (94%), with a thickness of 1.8 µm; column temperature from 40 °C to 240 °C (40 °C maintained for 12 minutes after injection, increased to 240 °C from 12 to 32 minutes and kept at 240 °C from 32 to 42 minutes), injector temperature at 200 °C and detector temperature at 280 °C; use helium at 35 cm/s as carrier gas and split ratio of 1:20, flow of carrier gas of 1 mL/minute.

Sample solution A: ethyl alcohol sample to be tested.

Sample solution B: to transfer 150 µL of 4-methylpentan-2-ol to a 100 mL volumetric flask and top up with the sample. To make it homogeneous. To transfer 10 mL of this solution to a 50 mL volumetric flask and top up with the sample. To make it homogeneous.

Standard solution A: to transfer 100 µL of methanol to a 50 mL volumetric flask and top up with the sample. To make it homogeneous. To transfer 5 mL of this solution to a 50 mL volumetric flask and top up with the sample. To make it homogeneous.

Standard solution B: to transfer 50 µL of methanol and 50 µL of acetaldehyde to a 50 mL volumetric flask and top up with the sample. To make it homogeneous. To transfer 100 µL of this solution to a 10 mL volumetric flask and top up with the sample. To make it homogeneous.

Standard solution C: to transfer 150 µL of acetal to a 50 mL volumetric flask and top up with the sample. To make it homogeneous. To transfer 100 µL of this solution to a 10 mL volumetric flask and top up with the sample. To make it homogeneous.

Standard solution D: to transfer 100 µL of benzene to a 100 mL volumetric flask and top up with the sample. To make it homogeneous. To transfer 100 µL of this solution

to a 50 mL volumetric flask and top up with the sample. To make it homogeneous.

To separately inject 1 µL of *Sample solution* and *Standard solution* into the gas chromatograph. To obtain the chromatograms and measure the area under the peaks. To calculate the sum of all the amounts of acetaldehyde and acetal, expressed as acetaldehyde, by the following equation:

$$\text{Acetaldehyde (ppm)} = [(10 \times \text{AE})/(\text{AT} - \text{AE})] + [(30 \times \text{CE})/(\text{CT} - \text{CE})]$$

where

AE = area under the acetaldehyde peak obtained from the chromatogram of the *Sample solution A*;

AT = area under the acetaldehyde peak obtained from the chromatogram of the *Standard solution B*;

CE = area under the acetal peak obtained from the chromatogram of the *Sample solution A*;

CT = area under the acetal peak obtained from the chromatogram of the *Standard solution C*;

To calculate the amount of benzene by the following equation: Benzene (ppm) = (2BE)/(BT - BE)

where

BE = area under the benzene peak obtained from the chromatogram of the *Sample solution A*;

BT = area under the benzene peak obtained from the chromatogram of the *Standard solution D*;

To disregard any peak with an area less than 0.03 times the area under the peak corresponding to 4-methylpentan-2-ol in the chromatogram of the *Sample solution B* (9 ppm). The area under the peak corresponding to methanol in the chromatogram of the *Sample solution A* can not be greater than half the area under the corresponding peak in the chromatogram of the *Standard solution A*. The amount of acetaldehyde found in the *Sample solution A* should not be greater than 10 ppm. The amount of benzene found in the *Sample solution A* should not be greater than 2 ppm. The total amount of impurities obtained in the chromatogram of the *Sample solution B* can not be greater than the area corresponding to 4-methylpentan-2-ol peak obtained in the same chromatogram.

DOSAGE

To determine the amount of C₂H₆O at 20 °C, from the relative density, by employing the Alcoholic Strength table (5.2.26).

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

ROSEMARY, VOLATILE OIL

Oleum rosmarini aetheroleum

Rosmarinus officinalis L. - LAMIACEAE

The volatile oil of rosemary is obtained by dragging water vapor from the top of flowers.

CHARACTERISTICS

Organoleptic characteristics. Colorless or slightly greenish-yellow color, strong typical odor and aromatic, bitter, and camphor taste.

IDENTIFICATION

A. To proceed as described in *Chromatographic profile*. To prepare *Sample solution* and *Standard solution* as described below.

Sample solution: to dissolve 0.2 mL of the volatile oil of rosemary in 1 mL of *n*-hexane. To store refrigerated in airtight vial and protected from light.

Standard solution: to dissolve 50 µL of 1,8-cineole (eucalyptol), 30 mg of bornyl acetate, and 10 mg of borneol in 10 mL of *n*-hexane. To store refrigerated in airtight vial and protected from light.

The retention times of the characteristic peaks in the chromatogram of the *Sample solution* should be similar to those obtained in the chromatogram of the *Standard solution* or identification confirmed by using gas chromatography coupled to a mass selective detector (**Figure 1**).

B. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF₂₅₄ silica gel chromatoplate with 250 µm thick as support and methylene chloride as mobile phase. To separately apply to the chromatoplate, as band, 10 µL of each of the solutions, as described below.

Solution (1): to dilute 0.5 mL of the sample to be examined in ethyl acetate and top up with the same solvent to 10 mL.

Solution (2): to dissolve 50 mg of borneol, 50 mg of bornyl acetate, and 100 µL of 1,8-cineole in ethyl acetate and top up with the same solvent to 10 mL.

To develop the chromatogram. To remove the plate, and let it drying. To nebulize with *p*-anisaldehyde solution followed by heating it in an oven at 100 °C - 105 °C for 10 minutes. The chromatogram obtained with *Solution (2)* shall present in the lower third of the plate an intense green spot with yellowish edge (borneol). In the middle third, there are two spots of medium intensity (one is violet (cin-eol) and another one is green with yellowish edge (bornyl acetate)). The chromatogram of *Solution (1)* will present two green spots with yellowish edge, being one with median intensity corresponding to borneol and another one with low intensity, corresponding to bornyl acetate. An intense violet spot corresponds to cineole. An intense red spot should appear in the upper third of the plate.

PURITY TESTS

Relative density (5.2.29.1). At 20°, at least, 0.894 and, at most, 0.912.

Refractive index (5.2.29.4). At 20 °C, at least, 1.460 and, at most, 1.476.

Specific rotation (5.2.29.5). At least, -5° and, at most, +15°.

Index of acidity (5.2.29.7). At most 1%.

CHROMATOGRAPHIC PROFILE

To proceed as described in *Gas chromatography (5.2.17.5)*. To use chromatograph equipped with flame ionization detector, by using mixture of nitrogen, hydrogen, and synthetic air (1:1:10) as the auxiliary gas to flame detector; 60 m long capillary column and 0.25 mm inner diameter, filled with polyethylene glycol, with a film thickness of 0.25 µm. The injector temperature should be adjusted to 200 °C, the detector temperature to 240 °C, and the column temperature set to begin at 50 °C for 10 minutes, by rising from 50 °C to 200 °C at 2° C for minute and keep at 200 °C for 25 minutes (total: 110 min). To use purified helium as carrier gas (1 mL/ minute).

Sample solution: to dissolve 0.2 mL of the volatile oil of rosemary in 1 mL of *n*-hexane. To store refrigerated in airtight vial and protected from light.

Standard solution: To dissolve 10 mg of camphene, 50 µL of 1,8-cineole (eucalyptol), 50 mg of camphor, 30 mg of bornyl acetate, and 10 mg of borneol in 10 mL of *n*-hexane. To store refrigerated in airtight vial and protected from light.

Procedure: to inject volume of 1 µL of *Sample solution* and *Standard solution* into the gas chromatograph, by using 1:50 flow division and the relative concentration obtained by electronic integration (normalization method).

To examine the chromatograms obtained from the chromatographic profile of the *Sample solution*. The characteristic peaks in the chromatogram obtained with the *Sample solution* should have retention times similar to those obtained from the chromatogram of the *Standard solution* or identification confirmed by using gas chromatography coupled to a mass selective detector operating under conditions similar to those of gas chromatography with flame ionization detector (**Figure 1**).

The chromatogram can also have the following compounds: bornyl acetate, borneol, β-pinene, β-myrcene, limonene, *p*-cymene, α-terpineol, and verbenone.

In the chromatogram obtained with *Sample solution*, check the minimum content of the following compounds: α-pinene: 9%; camphene: 2.5%, cineole: 16%, and camphor: 5%.

PACKAGING AND STORAGE

In airtight closed glass containers, protected from light and heat.

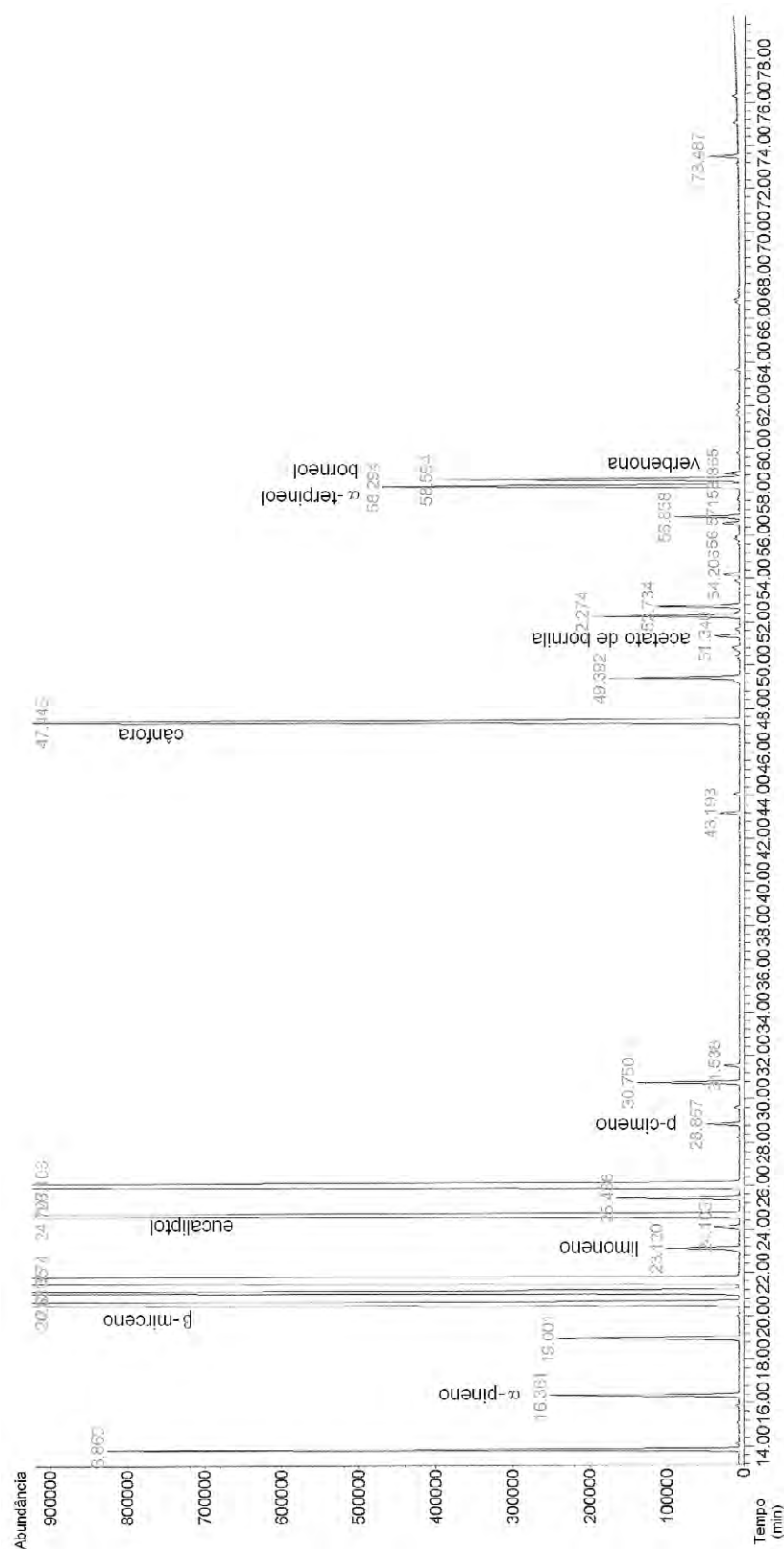


Figure 1 - Illustrative chromatogram obtained with the volatile oil of *Rosmarinus officinalis* L. by gas chromatography with mass detector.

PURIFIED AND STERILE COTTON

Cotton wool. Absorbent cotton.

The purified cotton consists of down of seeds of cultivated varieties of the genus *Gossypium* (Malvaceae), bleached, carded, free of fatty, resinous materials and other impurities that can absorb water.

The purified cotton, when impregnated with medicinal substances, must present concentration uniformly distributed. It shall not contain substances or concentrations capable of causing toxic or reaction accidents.

CHARACTERISTICS

Appearance. Thin and white down, soft to the touch, soft consistency, without lumps or impurities; the purified cotton is odorless and tasteless. Under microscopic examination, it shows only thin, hollow, flattened, twisted, and striated fibers slightly thickened at the edges.

Length of the fiber. To determine the length of the fiber after placing the cotton, free of wraps, for 4 hours in an atmosphere $65\% \pm 2\%$ relative humidity at $21^\circ \text{C} \pm 1^\circ \text{C}$, at least 60% by weight, of the fibers should measure 12.5 mm or more, and up to 10% by weight of fibers measuring 6 mm or less is allowed.

Absorbing capacity. To proceed as indicated in determining the absorbing capacity of the cotton, after placing it for 4 hours under atmospheric conditions described above; the absorption should be complete within 10 seconds, and cotton should retain at least 24 times its weight in water.

Solubility. Insoluble in common solvents and soluble in ammoniacal cupric sulfate SR.

PURITY TESTS

Acidity or alkalinity. To put about 10 g of it in a precipitation container containing 100 mL of distilled water, freshly boiled and cooled without stirring. To compress the cotton with a glass rod, squeeze it, and transfer aliquots of 25 mL for 2 porcelain capsules. To add a drop of methyl orange SI to a capsule, and to another one, add 3 drops of phenolphthalein SI. No pinkish or red color must be produced.

Loss on drying (5.2.9). The purified cotton, desiccated at 100°C , should not lose more than 8% of its weight.

Determination of sulfated ashes (5.2.10). To put about 5 g, accurately weighed, in a tared capsule,

and moisten with dilute sulfuric acid. To cautiously heat until blackening and then increase the heat to total incineration; the residue must not exceed 0.2%.

Dyes. To put 10 g in a percolator with narrow bore to carry out the slow extraction with ethanol until the percolate reaches 50 mL; by observing on a white background, in

a 20 cm high column, the liquid may have slight yellow color, never green or blue.

Greasy substances. To put about 10 g, accurately weighed, in a Soxhlet extractor, to carry out the extraction with ethyl ether, by adjusting the heating to obtain at least 4 siphoning per hour. To proceed extraction for 5 hours. The ether extract must not show any traces of blue, green, or brownish color. To evaporate the extract to dryness, heat at 105°C for 1 hour, cool down in a desiccator and weigh it; the residue should not exceed 0.7%.

Substances soluble in water. To put about 10 g, accurately weighed, in a precipitation container containing 1000 mL of distilled water and gently boil it for 30 minutes, by adding distilled water, when required, to maintain the volume approximately constant. To transfer the content to another container, by removing excess water held by cotton, by compressing with a glass rod. To wash the cotton twice with 250 mL of boiling distilled water, by squeezing after each wash. To filter the liquid from extraction and washing, wash the filter with hot water, and evaporate the filtrate to about 50 mL. To transfer the concentrate to a porcelain capsule, previously tared, wash the container with distilled water, and put the washing in this capsule. To evaporate to dryness; the residue desiccated at 105°C to constant weight must not exceed 0.25%.

Other foreign matter. Portions of cotton wool removed from the original package must not show oil stains, metal particles, or other foreign matter.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). The cotton wool should be sterilized to the consumer. As expressly stated sterile or sterilized, it must meet the requirements specified in the sterility tests for solids.

PACKAGING AND STORAGE

In rolls of a weight not exceeding 500 g in continuous layer on appropriate paper, whose length and width are folded at least 25 mm on the edges of the cotton layer. Rolls must receive a second wrapper that provides complete protection against dust.

The purified cotton when stated sterile or sterilized should be packaged in order to protect its sterility against contamination.

Differently, it may also be packaged in other types of packaging, since the sterile conditions required are maintained.

LABELLING

To comply with the legislation in force. The label must contain the name of the manufacturer, net weight, and, in case of cotton impregnated with medicinal substances, the formula used.

CATEGORY

Adjuvant in general health care.

ALOE

Aloe vera folium

Aloe vera (L.) Burm.f. - ASPHODELACEAE

The drug consists of the fresh leaves, containing colorless gel, mucilaginous, obtained from parenchyma cells, consisting of at least 0.3% of total carbohydrates.

POPULAR NAME

Babosa.

CHARACTERISTICS

Organoleptic characteristics. The drug has slightly bitter taste; it is colorless and odorless.

MACROSCOPIC DESCRIPTION

Succulent leaves, lanceolate, acute, glaucous-green, with whitish spots when young, measuring from 15 cm to 60 cm long and about 7 cm at the base on the adaxial face and 10 cm on the lower face, when they are adults. The adaxial face, in cross-section, is concave, and lower face is convex. The leaf edges are spiny-toothed, with small whitish aculeus, perpendicular to the blade.

MICROSCOPIC DESCRIPTION

The leaf, in cross section, shows a isobilateral structure. It presents a single epidermal layer, covered externally with a thick wavy cuticle. The cells of this layer are tangentially flattened, some of them have greater in length, while in others these parameters are close. In front view, cells are round-polygonal. The leaf is amphistomatic and there are various stomata of tetracytic-type, arranged at the same level as the other epidermal cells, with cuticle showing a slight projection in the region of the ostiole.

The substomatic chamber has size corresponding to one or two layers of chlorenchyma cells. The cross section of the leaf blade shows two distinct zones; the outermost is green and the innermost is colorless and mucilaginous. Below the epidermis, there may be a separate first layer of chlorenchyma cells in the form of palisade and several layers (13 to 18) of chlorenchyma cells, rounded or irregular polyhedral (polygonal), chloroplast and starch-rich, as well as idioblasts containing bundles of raphids of calcium oxalate. Often, no difference of form in the chlorenchyma layers is observed. The number of starch and chloroplasts decreases in the cells near the parenchyma aquifer. In the contact zone between the chlorenchyma and parenchyma aquifer there are vascular bundles, collateral-type, alternating with 3-5 chlorenchyma cells. In the region of the leaf edge, this number of chlorenchyma cells may be higher. The vascular bundles are arranged in parallel to the epidermis and are separated from it by 10 to 16 layers of chlorenchyma cells. The upper portion of each bundle is in contact with the chlorenchyma, and the middle and lower portions penetrate the parenchyma aquifer. The vascular bun-

dles are surrounded by a parenchymal sheath formed of small, hexagonal cells containing starch. Internally to this layer and near the phloem, there is a group of 3 - 5 very large cells and other that are smaller, polyhedral, slightly elongated toward the axis of the leaf, thin walls, called alloetic cells or aloe tissue, filled with yellow latex, viscous, called aloe liquid or aloe juice. At the time the leaf is cut transversally, there is no leakage of aloe liquid from the bundles. The phloem is external and undeveloped, and the xylem is formed by 2-4 tracheary elements with some fibers. In the edge of the blade, some cells can have more thick walls. The fundamental parenchyma is aquifer-type, usually occupying 75% of the thickness of the layer, and being formed by very large cells in relation to those of the chlorenchyma, colorless, thin-walled, full of mucilage, arranged perpendicularly to the epidermis. Cells with raphides of calcium oxalate also occur in this parenchyma.

IDENTIFICATION

To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel chromatoplate with 250 mm thick as stationary phase and a mixture of toluene and ethyl acetate (90:10) as mobile phase. To separately apply to the plate, as bar, 20 µl of *Solution (1)* and 10 µl of *Solution (2)* freshly prepared, as described below.

Solution (1): to transfer 2 mL of liquid aloe gel to 5 mL volumetric flask, top up with methanol, and heat in a water bath (60 °C) by stirring for 10 minutes.

Solution (2): to dissolve 2 mg of β-sitosterol SQR in 1 mL of methanol.

To develop the chromatogram. To remove the plate, and let it drying. To nebulize with anisaldehyde SR and to heat in an oven at 105 °C - 105 °C for 5-10 minutes The chromatogram obtained with *Solution (1)* shows a principal spot of blue color at the same height as that obtained with *Solution (2)* (Rf about 0.31).

DOSAGE

Total carbohydrates

To proceed as described in *Visible absorption spectrophotometry* (5.2.14). To prepare the solutions as described below.

Stock solution: To transfer 3 mL of liquid aloe gel to 100 mL volumetric flask and top up with water. By turbolization, make it homogeneous for 5 minutes.

Sample solution: to transfer 0.2 mL of *Stock solution* to a test tube, top up the volume to 0.5 mL with water, and leave in an ice bath. To add 0.5 mL of 5% phenol solution (w/v) and 2 mL of concentrated sulfuric acid. To stir well and let it rest at room temperature for 30 minutes.

Blank solution: to transfer 0.5 mL of water to a test tube and leave in an ice bath.

To add 0.5 mL of 5% phenol solution (w/v) and 2 mL of concentrated sulfuric acid. To stir well and let it rest at room temperature for 30 minutes.

Solutions for analytical curve: to prepare a 0.2 mg/mL glucose standard solution. To transfer aliquots of 25, 50, 100, 150, 200, and 250 μ L of this solution to test tubes and top up to 0.5 mL with water, thus obtaining the following concentrations: 10, 20, 40, 60, 80, and 100 μ g/mL, and leave in an ice bath. To add 0.5 mL of 5% phenol solution (w/v) and 2 mL of concentrated sulfuric acid. To stir well and let it rest at room temperature for 30 minutes.

To measure the absorbance of *Sample solution* and *Solutions for analytical curve* at 490 nm **(5.2.14)** 30 minutes before preparation, by using *Blank solution* to adjust zero. To calculate the content of total carbohydrate from the sample, by using linear equation obtained with *Solutions for analytical curve* of glucose. The result is expressed as a percentage of total carbohydrate calculated as glucose per 100 mL of the drug.

PACKAGING AND STORAGE

In tightly closed container, protected from light and heat.

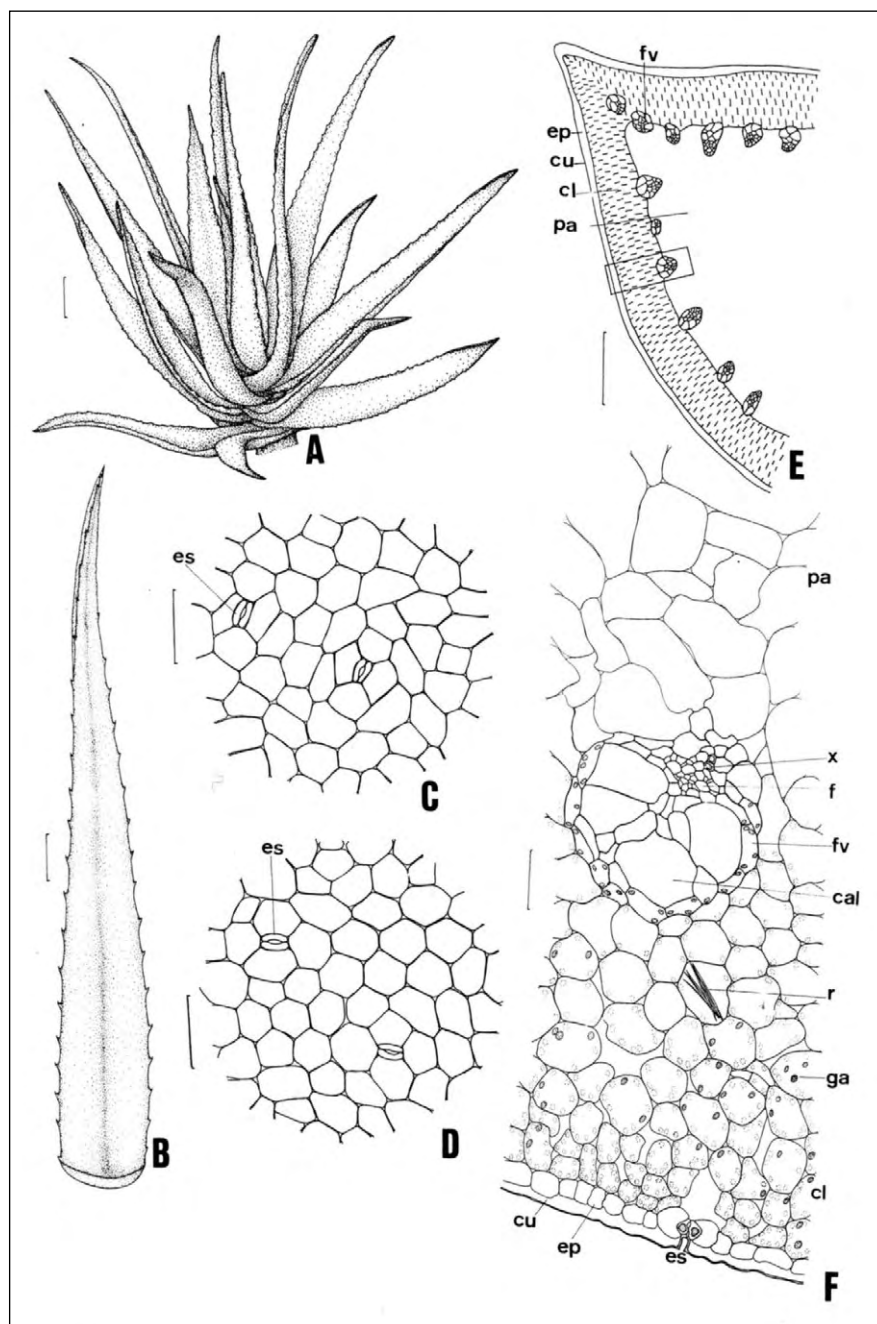


Figure 1 - Macroscopic and microscopic aspects in *Aloe vera* L. Burm. f.

Additional information of **Figure 1**. Scales correspond to 6cm in A; in B, to 2 cm; C, D, and F, to 100 μ m, and 1 mm, in E.

A - general appearance of the plant without inflorescence. B - general appearance of a leaf. C - front view of the epidermis toward the adaxial face; stomata (s). D - front view of the epidermis toward the lower face; stomata (es). E - general appearance of leaf in cross section; chlorenchyma (cl); cuticle (cu); epidermis (ep); parenchyma aquifer (pa); vascular bundle (fv). F - detail of the portion indicated in E; aloe cell (cal); chlorenchyma (cl); cuticle (cu); epidermis (ep); stomata (s); phloem (f); vascular bundle (fv); starch grain (ga); parenchyma aquifer (pa); raphides (r); xylem (x).

ALOE, DRY EXTRACT

Aloe capensis extractum siccum

Aloe ferox Mill., *Aloe africana* Mill. and *Aloe spicata* Baker - ASPHODELACEAE

The vegetable drug consists of thick juice from leaves, desiccated by heat, and belongs to the above species or interspecific hybrids, or even their combination. The dry drug consists of at least 18% of hydroxy anthracene derivatives expressed in barbaloin.

POPULAR NAME

Aloe-do-cabo.

CHARACTERISTICS

Organoleptic characteristics. The drug has pungent, unpleasant, and distinctive odor, and very bitter, nauseating taste.

Solubility. Partially soluble in boiling water, soluble in hot ethanol, and practically insoluble in ethyl ether.

MACROSCOPIC DESCRIPTION

Irregular masses, dark brown color with greenish reflections, smooth and glassy fracture. Its fragments are translucent at the edges, very friable, creating a yellow powder.

IDENTIFICATION

A. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel with 250 µm thick as support and a mixture of water, methanol, ethyl acetate (13:17:100) as mobile phase. To separately apply to the plate, as band, 10 µL of each of the solutions freshly prepared, as described below.

Solution (1): 0.25 g of the pulverized, add 20 mL of methanol, and heat to boiling. To stir for a few minutes, decant the solution, and keep at about 4 °C. This solution can be used within 24 hours later.

Solution (2): to dissolve 25 mg of barbaloin in 10 mL of methanol.

To develop the chromatogram. To remove the plate, and let it drying. To pulverize with 10% potassium hydroxide solution (w/v) in methanol. To examine under ultraviolet light (365nm). The principal spot obtained with *Solution (1)*, with yellow fluorescence, corresponds in position and intensity to that obtained with *Solution (2)* relating to barbaloin. The light blue fluorescent spot obtained with *Solution (1)* at the lower part of the chromatogram refers to aloesina. Then, heat the plate in an oven at 110 °C for 5 minutes.

A violet fluorescence spot is developed; it is located immediately below the spot corresponding to barbaloin.

B. To dissolve the pulverized drug in nitric acid. Effervescence is developed, and a reddish-brown to brown color solution is obtained.

C. In a glass stoppered vial, mix 1 g of the drug finely pulverized with 25 mL of water, and occasionally stir for 2 hours. To filter and wash the filtrate and residue with sufficient water to obtain 100 mL. The color of the filtrate is observed through the body of a 100 mL flask; it is greenish-yellow with aloe-do-cabo. The filtrate darkens.

D. To add 45 mL of water and 20 mL of 5% sodium tetraborate (w/v) to 5 mL of the filtrate obtained in test C. *Identification.* Yellow-greenish fluorescence or yellowish-green is developed later, becomes yellowish-orange (barbaloin).

PURITY TESTS

Substances insoluble in alcohol. To accurately weigh about 1 g of the vegetable drug and transfer to a flask containing 50 mL of ethanol. To heat the mixture and keep it moderately boiling for 15 minutes, by replacing the ethanol evaporated. To cool down and occasionally stir the mixture for 1 hour. To filter through small, desiccated, and tared filter paper; wash the residue with ethanol until the washing liquids become colorless. To desiccate this residue at 105 °C to obtain constant weight, and weigh it. The weight found must be less than 10.0%.

Water (5.4.2.3). At most 12.0%.

Total ashes (5.4.2.4). At most 4.0%.

DOSAGE

Hydroxy anthracene derivatives

To proceed as described in *Visible absorption spectrophotometry* (5.2.14). To prepare the solutions as described below.

Stock solution: to add 0.4 g of the pulverized sample in a 250 mL erlenmeyer flask. To moisten with 2 mL of methanol, add 5 mL of water previously heated at about 60 °C, and mix it. To add 75 mL of water heated to about 60 °C and stir for 30 minutes. To cool down and filter to same volumetric flask. To wash the erlenmeyer flask and filter with 20 mL of water. To pour the washing to a volumetric flask and top up to 1000 mL with water. To put 10 mL of this solution in a 100 mL round bottom flask, containing 1 mL of 60% ferric chloride solution (w/v) and 6 mL of hydrochloric acid. To heat in a water bath under reflux for 4 hours while maintaining the water level above the liquid in the flask and protected from direct light. To cool down and transfer the solution to a separatory funnel. To successively wash the flask with 4 mL of water, 4 mL of *M* sodium hydroxide, and 4 mL of water. To add the washings to the content of the separatory funnel.

To stir 3 times with 20 mL of ethyl ether each time. To gather the ether layers and wash twice with 10 mL of water each time, by discarding the washings. To top up the organic layer up to 100 mL with ethyl ether.

Sample solution: to evaporate 20 mL of *Stock solution* to residue in a water bath. To resuspend the residue in 10 mL of 0.5% magnesium acetate (w/v) in methanol.

To measure the absorbance of the *Sample solution* at 512 nm immediately after preparation, by using methanol to adjust zero. To take into account for barbaloine A (1%, 1 cm) = 255 at 512 nm in methanol. To calculate the content of hydroxy anthracene derivatives expressed in barbaloine, according to the equation:

$$\text{DHC} = \frac{A \times 19,6}{m}$$

where

DHC = hydroxy anthracene derivatives in%;

A = absorbance measured;

m = mass of drug by considering the content of water determined (g).

PACKAGING AND STORAGE

In tightly closed container, protected from light and heat.

ALTHEA *Althaeae radix*

Althaea officinalis L. - MALVACEAE

The drug consists of fragments of desiccated roots, weeded or not, with no side branches.

CHARACTERISTICS

Organoleptic characteristics. Sweet odor and tasteless. Mucilaginous consistency and sweetish taste.

MACROSCOPIC DESCRIPTION

The not weeded root is cylindrical, longitudinally grooved and slightly twisted, with up to 20.0 cm long and up to 2.0 cm thick. The outer surface is grayish-brown and has numerous scars on lateral roots. The fracture is fibrous in the outer portion and granular and irregular in the inner portion. In cross section, concentric layers of the brownish cortex and its stratified structure are visible, separated by a well marked cambium strip, sinuous and dark, followed by white to cream-yellow central cylinder, showing xylem with radial structure, especially after hydration in water and visible by lens. The weeded root is almost cylindrical, and the outer face has dark scars arising from lateral roots and has whitish-yellow color. Usually it is fragmented and shows portions of fibers arranged longitudinally or detached from remaining of cortex and sometimes the three regions described are visible.

MICROSCOPIC DESCRIPTION

The not weeded root, in front view, presents bark with polyhedral cells and straight walls. In cross section, three

regions are distinct: cortex, pale brown; vascular cambium, yellowish color; and central cylinder, whitish color. The cortex has undeveloped bark with cells usually tabular and irregular, of various sizes, with thin and rectilinear walls, arranged in rows and rich in starch grains. In cross section, the cortical parenchyma presents cells in various forms, usually polyhedral and voluminous, with thin and rectilinear walls, full of starch grains. The outer cortical parenchyma has cells with greater volume than those of the inner cortical parenchyma. Irregular groupings of phloem fibers, with varying numbers of cells, showing slightly thickened walls, are arranged randomly in large amounts, in the innermost part of the cortex. Conducting cells of the phloem are rarely observed. The parenchyma beams are arranged from the internal cortex to the central cylinder and consist of a few rows of cells, rarely more, usually small, longitudinally elongated, and straight walls. The cambium has several layers of cells of small size, flattened longitudinally, very thin walls, arranged in rows, fusiform and radial are easily distinguished. The central cylinder is very developed, consisting of xylem with parenchyma cells that have varied cells varying in form and volume, full of starch grains, somewhat regular, with thin and straight walls, and visible intercellular spaces. The conductive elements form irregular grouping regarding the number of elements; they are longitudinally aligned and are often associated with small parenchymal cells. These groupings are less developed and more irregularly distributed along the cambium. They show annular arrangement in the innermost portion, and the number of rings varies. Groups of fibers and sometimes isolated fibers are found throughout the central cylinder in a number much smaller when compared with the cortex. It also occur along the primary xylem, when present. The roots that have solid medulla have primary xylem, formed by elements of small size, associated with rounded and small size parenchymal cells, not occurring groupings of fibers in this tissue. Some roots may present a medullary region filled with parenchyma, composed of large-volume cells, with a smaller amount of starch grain and intercellular spaces smaller than the other parenchyma. In these roots, the residues of arc xylem are visible and are also associated with small parenchymal cells. Simple starch grains, in many forms, often rounded, ovoid or reniform, usually with central and branched hilum, rarely eccentric, or rarely compound grains, often showing lamella, occur in large numbers in all tissues, except the medullary parenchyma. Crystals of calcium oxalate, druze-type, with different sizes are very common in the cortex and central cylinder.

Cells containing mucilage often oval or rounded, and may have greater volume than the other parenchyma, dense and dark protoplast, also occur in the cortex and central cylinder, except the medullary parenchyma. Weeded roots may not have bark and outer cortical parenchyma. Roots in primary growth stage are characterized as “*pentarcas*”. By adding toluidine blue, the vessel elements get intense blue color, the fibers become light blue, and the cells containing mucilage get violet color. Due to the large amount of starch grains and mucilage

cells there is difficulty in making histological slides by using hydrated material.

MICROSCOPIC DESCRIPTION OF POWDER

The powder meets all the requirements for the species, unless the macroscopic characters. Microscopic observation of the powder becomes clearer when chloral hydrate is used. Characteristics: white to yellow-white color from weeded roots or brown -gray from not weeded roots; fragments of bark, in cross section showing rectangular cells and flattened longitudinally; fragments of bark, in cross section, showing square cells; fragments of bark, in cross section, containing crystal idioblasts; fragments of bark, in cross section, with rectangular cells and flattened longitudinally containing crystal idioblasts and starch grains; fragments of bark in front view, containing starch grains, fragments of bark, in cross section, rectangular cells and flattened lengthwise, filled with starch grains, fragments of parenchyma in front view, with cells containing mucilage cells and many starch grains, fragments of parenchyma in front view, showing crystal idioblasts and cells filled with starch grains; fragments of parenchyma in cross section, containing starch grains, fragments of parenchyma in cross section, containing crystal idioblasts; fragments of parenchyma in cross section, containing mucilage cells and large amount of starch grain; fragments of parenchyma beam in longitudinal section, showing fibers and parenchyma cells, isolated parenchymal cells filled with starch grains and/or crystals; fragments of parenchyma beam, in cross section, with cells containing starch grains, fragments of xylem in longitudinal section showing the vessel element with cross-linked thickening associated with fibers and parenchyma, fragments of xylem in longitudinal section showing the vessel elements with crosslinked thickening fibers and parenchyma in cross section and with starch grains; fragments of xylem, in longitudinal section, showing vessel elements with crosslinked thickening and pit thickening associated with parenchyma cells full of starch grains, fragments of xylem, in longitudinal section, showing vessel elements with crosslinked thickening and pit thickening associated with parenchymal cells full of starch grains; portions of vessel element with helicoidal thickening in longitudinal section; vessel elements in cross-section associated with parenchymal cells full of starch grains; portions of vessel elements with crosslinked thickening in longitudinal section; fragments of fiber in longitudinal section associated with the xylem parenchyma cells, fragments of fiber bundles in longitudinal section, containing starch grains, fragments of fiber bundle in longitudinal section associated with the parenchyma beam cells, fragments of grouping of

fibers in cross section; fragments of grouping of fibers in cross section; fibers or their portions in longitudinal section, isolated and/or grouped, starch grains in front view, simple or compound, isolated or grouped in small number; groups forming clumps of starch grains in front view; mucilage detached from cells; isolated cells containing mucilage; crystals of calcium oxalate (druzen-type), isolated.

IDENTIFICATION

To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF₂₅₄ silica gel, with 250 µm thickness as stationary phase and a mixture of ethyl acetate, methyl ethyl ketone, formic acid, and water (50:30:10:10) as mobile phase. To separately apply to the plate, as band, 20 µl of each of the solutions freshly prepared, as described below.

Solution (1): to weigh 1 g of the sample, add 10 mL of methanol, and warm in a water bath for 15 minutes. To filter it.

Solution (2): to dissolve 2.5 mg of rutin and 1 mg of chlorogenic acid in 10 mL of methanol.

To develop the chromatogram. To remove the plate, and let it drying. Then, nebulize it with aminoethanol diphenyl borate SR (Natural Reagent A) and observe under UV light (365 nm). *Solution (1)* when observed under ultraviolet light (365 nm) has 3 blue fluorescent spots with R_f about 0.12, 0.42, and 0.97. The lower spot of the *Solution (1)* appears just below the spot corresponding to rutin (R_f 0.25), orange-colored, and middle spot below the chlorogenic acid (R_f 0.51), green fluorescent color.

PURITY TESTS

Foreign material (5.4.2.2). At most 2.0% of brown color. At most 2.0% of bark elements (weeded root).

Water (5.4.2.3). At most 12.0%. To determine in 1 g of the ground sample (710 µm) in an oven at 100 °C - 105 °C for 2 hours.

Total ashes (5.4.2.4). At most 6.0% in the weeded root. At most 8.0% in the not weeded root.

PACKAGING AND STORAGE

In tightly closed containers, protected from light and heat.

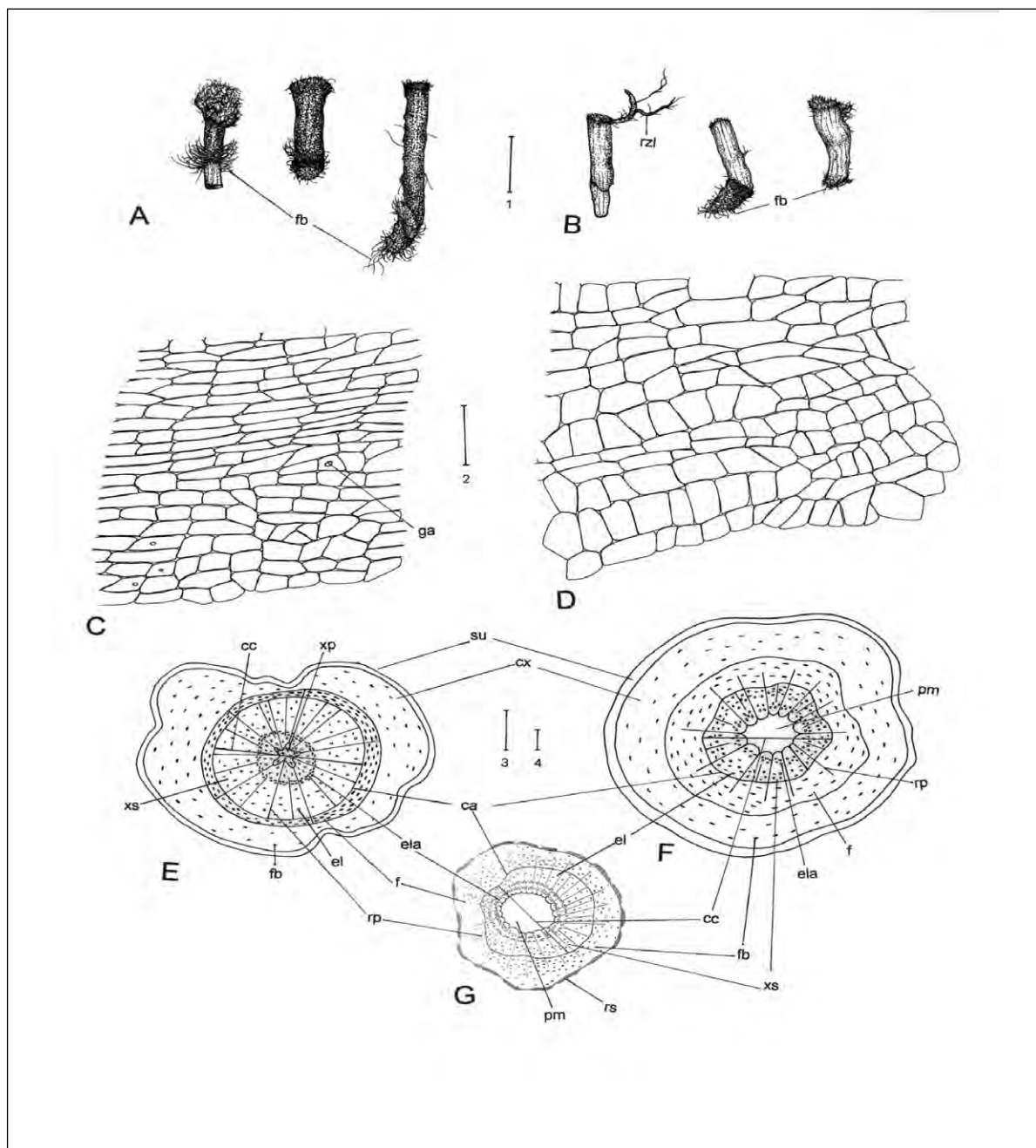


Figure 1 - Macroscopic and microscopic aspects in *Althaea officinalis* L.

Additional information of **Figure 1**. Scales correspond to 2cm in **A** and **B** (rule 1); in **C** and **D**, 100 μ m (rule 2); in **E** and **F**, 1.0 mm (rule 3); in **G**, 1.0 mm (rule 4).

A - General aspects of not weeded roots; fiber (fb). **B** - general aspects of weeded roots; fiber (fb), lateral root (rzi). **C** - front view of external bark of not weeded root; starch grain (ga). **D** - front view of internal bark of a weeded root. **E** - schematic representation of a not weeded root in cross section; cambium (ca); central cylinder (cc); cortex (cx), tracheal element (el); tracheal elements with ring arrangement (ela); phloem (f); fiber (fb); parenchyma beam (rp); bark (su); primary xylem (xp), secondary xylem (xs). **F** - schematic representation of a not weeded root in cross section; cambium (ca); central cylinder (cc); cortex (cx), tracheal element (el); tracheal elements with ring arrangement (ela); phloem (f); fiber (fb); medullary parenchyma (pm); parenchyma beam (rp); bark (su); secondary xylem (xs). **G** - schematic representation of a weeded root in cross section; cambium (ca); central cylinder (cc); cortex (cx), tracheal element (el); tracheal elements with ring arrangement (ela); phloem (f); fiber (fb); medullary parenchyma (pm); parenchyma beam (rp); remaining bark (rs); secondary xylem (xs).

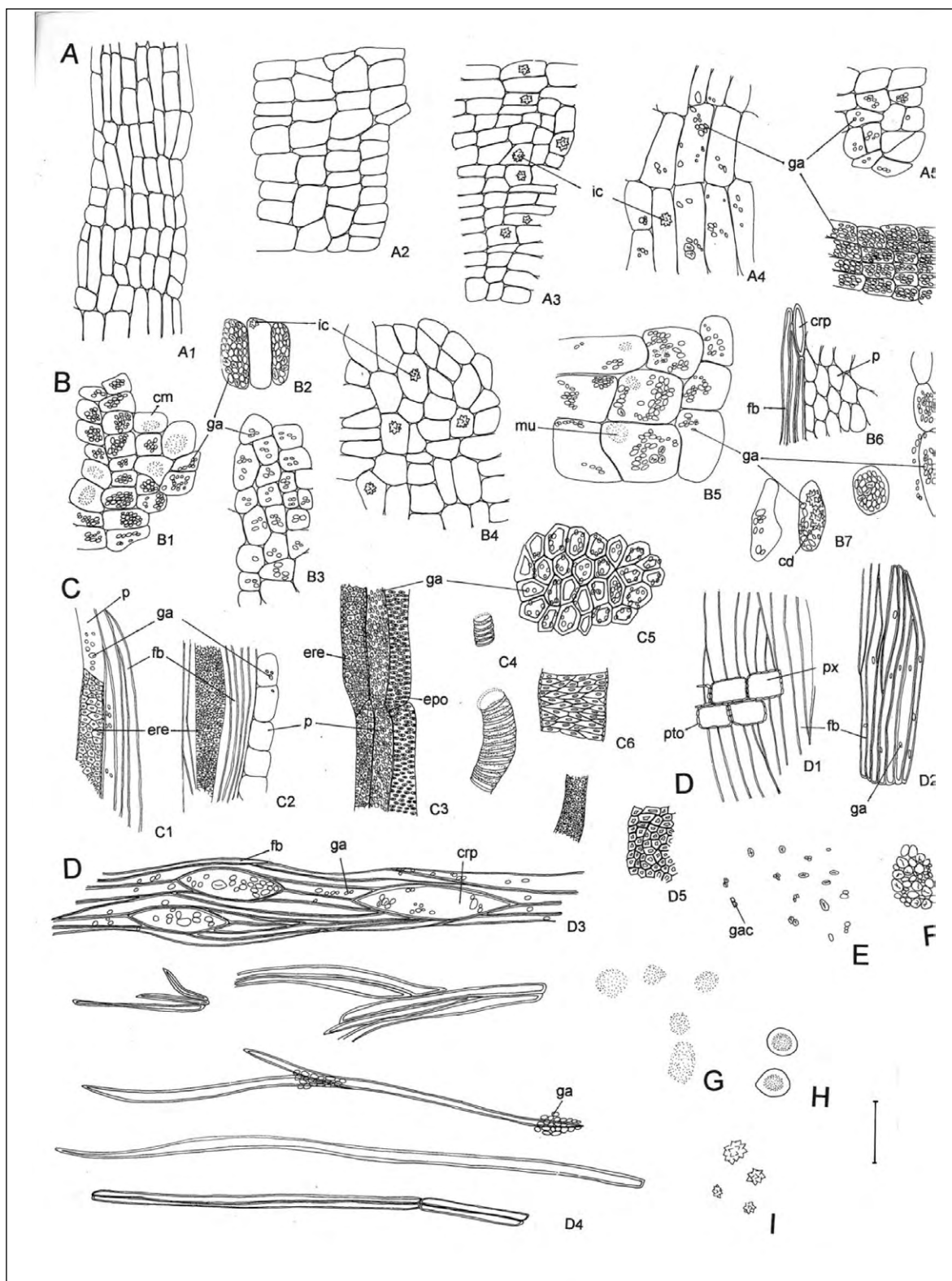


Figure 2 - Microscopic aspects of powder in *Althaea officinalis* L.

Additional information of **Figure 2**. Scales correspond to 100 µm.

A - fragments of bark, A1 - fragment of bark, in cross section, showing rectangular cells, flattened lengthwise; A2 - fragment of bark, in cross section, showing square cells; A3 - fragment of bark, in cross section, containing crystal idioblasts (ic)

A4 - fragment of bark, in cross section, rectangular cells and flattened longitudinally, with crystal idioblasts and starch grains, starch grain (ga); crystal idioblast (ic); A5 - fragment of bark, in front view, containing starch grains (ga); A6 - fragment of bark, in cross section, with rectangular cells flattened longitudinally filled with starch grains (ga). **B** - fragments of parenchyma; B1 - fragment of parenchyma in front view, with cells containing mucilage and many starch grains (ga); cell containing mucilage (cm), B2 - fragment of parenchyma in front view, showing crystal idioblasts and cells filled with starch grain (ga); crystal idioblast (ic); B3 - fragment of parenchyma in cross section, containing starch grains (ga); B4 - fragment of parenchyma in cross section, containing crystal idioblasts (ic); B5 - fragment of parenchyma in cross section, cells with mucilage with many starch grains (ga); mu-

cilage (um); B6 - fragment of parenchyma beam in longitudinal section, showing parenchyma cells and fiber; parenchyma beam cell (crp), fiber (fb); parenchyma (p); B7- isolated parenchyma cells containing starch grains and crystals of calcium oxalate (Druze-type) or full of starch grains; druze crystal (cd); starch grain (ga); B8 - fragment of parenchyma beam, in cross section, with cells containing starch grains (ga). **C** - fragments of xylem; C1 - fragment xylem in longitudinal section, showing vessel element with crosslinked thickening associated with fibers and parenchyma; vessel element with crosslinked thickening (ere); fiber (fb); starch grain (ga); parenchyma ; C2 - fragment of xylem in longitudinal section, showing the vessel element with crosslinked thickening, fibers and parenchyma in cross section and with starch grains; vessel element with crosslinked thickening (ere); fiber (fb); starch granules (ga); parenchyma (p), C3 - fragment of xylem in longitudinal section, showing vessel elements with crosslinked thickening and pit thickening associated with parenchymal cells, full of starch grains; vessel element with crosslinked thickening and pit thickening (epo) ; vessel element with cross-linked thickening (ere); starch grain (ga); parenchyma (p); C4 - portions of vessel element with helicoidal thickening in longitudinal section; C5 - vessel elements in cross section with starch grains (ga); C6 - portions of vessel elements with crosslinked thickening, in longitudinal section. **D** - fragments of fibers, D1 - fragment of fibers, in longitudinal section associated with the parenchyma cells of the xylem; fiber (fb); xylem parenchyma (px); pit (pto); D2 - fragment of the fiber bundles in longitudinal section, containing starch grains (ga); D3- fragments of fiber bundle in longitudinal section, associated with parenchyma beam cells, parenchymal cell beam (crp); fiber (fb); grain starch (g); D4 - fibers or their portions, isolated or grouped, in longitudinal section; grain starch (ga); D5 - fragment of fiber grouping in cross section. **E** - starch grains, in front view, simple or compound, isolated or grouped, in small numbers; compound grain starch (gac), starch grain (ga). **F** - grouping forming lumps of starch grains in front view. **G** - mucilage detached from cells. **H** - isolated cells containing mucilage, mucilage (mu). **I** - crystals of calcium oxalate (Druze-type), isolated.

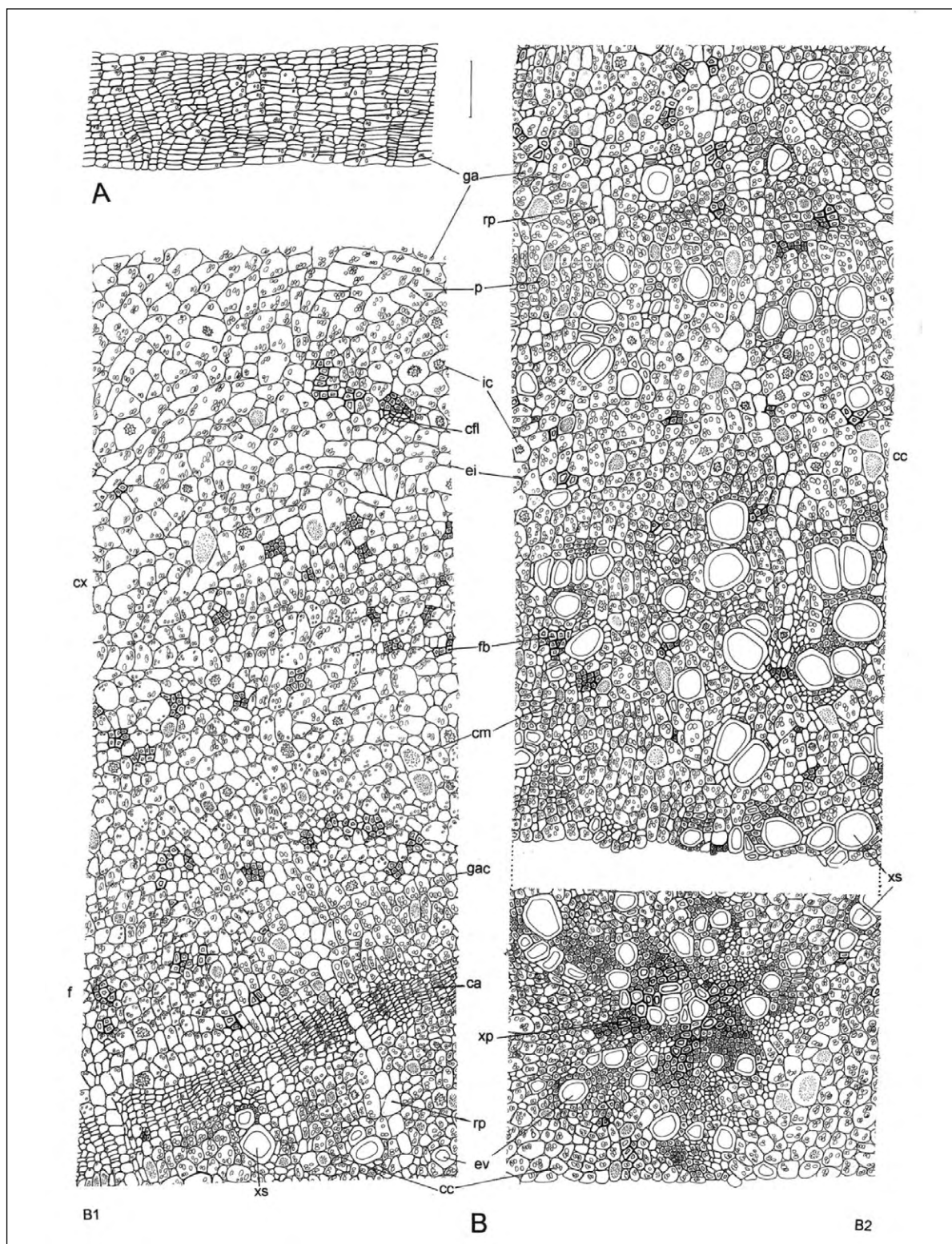
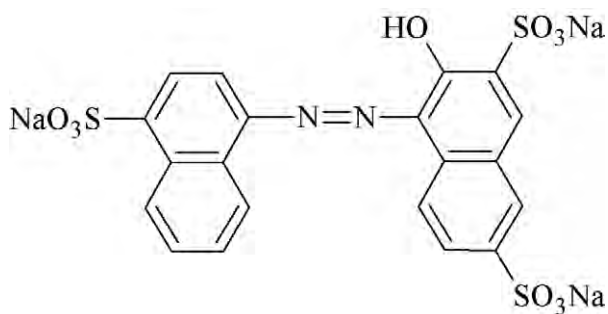


Figure 3 - Microscopic aspects in *Althaea officinalis* L.

Additional information of **Figure 3**. Scales correspond to 100 µm.

A - partial detail of bark, in cross section, of a not weeded root, starch grain (ga). **B** - detail of a partial weeded root, in cross section; **B1**. detail part of the cortex, cambium, and outer portion of central cylinder; cambium (ca); central cylinder (cc); conducting cells of the phloem (cfl); cell containing mucilage (cm); cortex (cx); intercellular space (ei); vessel element (ev); phloem (f); fiber (fb); crystal idioblast (ic); starch grain (ga); compound grain starch (gac); parenchyma beam (rp); parenchyma (p); secondary xylem (xs); **B2**. continuity of partial detail of B1 showing the inner portion of the central cylinder; central cylinder (cc); cell containing mucilage (cm); intercellular space (ei); vessel element (ev); fiber (fb); crystal idioblast (ic), starch grain (ga); parenchyma beam (rp); parenchyma (p); primary xylem (xp), secondary xylem (xs).

AMARANTH



$C_{20}H_{11}N_2Na_3O_{10}S_3$; 604.47
CI16185

Sodium salt of 3-hydroxy-4-[2-(4-sulfo-1-naphthalenyl)diazenyl]-2,7-naphthalenedisulfonic (3:1) acid
[915-67-3]

It contains at least 85.0% of $C_{20}H_{11}N_2Na_3O_{10}S_3$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Fine powder, reddish-brown, hygroscopic. Aqueous burgundy solution.

Solubility. It is soluble in water, methanol, and glycerol; insoluble in ethyl ether and acetone.

IDENTIFICATION

The ultraviolet and visible absorption spectra (5.2.14), from 200 nm to 700 nm, 0.001% solution (w/v) in 0.02 M ammonium acetate (pH 5.6), exhibits maximum at 519 nm, 330 nm, and 217 nm, and minima at 360 nm and 310 nm, similar to those observed in the spectrum of a similar solution of amaranth SQR.

PURITY TESTS

Subsidiary dyes. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using G silica gel as support and a mixture of 1-butanol, ethanol, water, and ammonium hydroxide (50:25:25:10) as mobile phase. To apply separately to the plate 2 μ l of each of the solutions freshly prepared, as described below.

Solution (1): 10 mg/mL solution of the sample in water.

Solution (2) 10 mg/mL solution of amaranth in water.

Solution (3): to dilute 1 mL of *Solution (2)* to 25 mL with water.

To develop the chromatogram. To remove the plate, and let it drying. To examine at ambient light, under ultraviolet light (254 nm). The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*. Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the

principal spot, is not more intense than that obtained with *Solution (3)* (4%).

Lead, copper, tin, zinc. To proceed as described in *Atomic absorption spectrophotometry* (5.2.13). To weigh 2 g of the sample in a silica crucible and burn softly on asbestos canvas ($\pm 350^\circ\text{C}$); take it to the oven for 12 hours, at maximum temperature of 450°C . To remove the crucible and let it cool down. To mix the residue with about 2 mL of water and add two drops of 50% magnesium nitrate (w/v). To dry it over the electric plate and return to oven for 3 - 4 hours, or until the residue is white or yellowish. Then, cool down, drip 1 to 2 mL of nitric acid and 1 mL of water, and heat on a electric plate until almost dry it. To dissolve the metal nitrate with 5 mL of water. If necessary, centrifuge it. To take it to an atomic absorption spectrophotometer, previously calibrated, to read the concentration of the metals. Maximum of 0.001% (10 ppm) lead, 0.002% (20 ppm) copper, 0.025% (250 ppm) tin, and 0.005% (50 ppm) zinc.

Chlorides and sulfates. To weigh 0.5 g of sample, dissolve in 200 mL of water, acidify with 8 mL of 25% nitric acid (v/v), and titrate with 0.1 M SV silver nitrate in a potentiometer pot with combined silver electrode. Each mL of 0.1 M SV silver nitrate is equivalent to 5.85 mg of NaCl.

To weigh 0.5 g of the sample and dissolve it in 100 mL of water, in a water bath. To add 35 g of sulfate-free sodium chloride, and stir it well. To transfer to 200 mL volumetric flask and top up with saturated sodium chloride solution. To make it homogeneous. After 1 hour, filter it by using a filter paper and transfer 100 mL aliquot of the filtrate to 600 mL beaker, dilute to 300 mL with water, and acidify with hydrochloric acid SR, by adding slight excess. To heat to boiling and drip, with stirring, 25 mL of 12% barium chloride (w/v) or until no further precipitation occurs. Let it rest for 4 hours. To separate barium sulfate by filtration, wash with hot water, dry the paper with the residue, transfer to dry crucible previously weighed, and calcined in an oven, at 500°C for 1 hour. To cool down in desiccator and weigh it. The calculate the sulfate content, use the following equation:

$$\frac{N \times 0,6085 \times 100}{p} = \% \text{ sulfates}$$

where

N = barium sulfate in grams;

p = sample used in the precipitation in grams.

Maximum of 5% of chlorides and sulfates.

Substances insoluble in water. To dissolve by stirring 5 g of the sample in 200 mL of hot water ($80-90^\circ\text{C}$). To cool down at room temperature. To filter it by filter plate, previously dried and weighed. To wash with cold water until the washings become colorless. To dry the filter with the residue in oven at 120°C for 4 hours and weigh it. At most 0.5%.

Arsenic (5.3.2.5). To use *Method I*. To determine 1 g of the sample. At most 0.0001% (1 ppm).

Heavy metals (5.3.2.3). To use *Method III*. to determine in 0.5 g of the sample. At most 0.004% (40 ppm).

Loss on drying (5.2.9). To determine in 0.5 g of the sample.

To desiccate in an oven at 120 °C for 4 hours or at 135 °C for 3 hours. At most 10%.

DOSAGE

To proceed as described in *Visible absorption spectrophotometry (5.2.14)*. To prepare sample solution as described in *Identification*. To prepare a standard solution at the same concentration by using the same solvent. To measure the absorbance of the resulting solutions at 519 nm, by using 0.02 M ammonium acetate (pH 5.6) to adjust zero. To calculate the $C_{20}H_{11}N_2Na_3O_{10}S_3$ concentration of the sample from the readings obtained. Alternatively, make the calculations taking into account that $A(1\%, 1\text{ cm}) = 564$ at 481 nm, in anhydrous basis.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

CATEGORY

Dye.

AMARANTH, ALUMINUM LAKE

Dye consists mainly of sodium salt of 3-hydroxy-4-[2 - (4-sulfo-1-naphthalenyl) diazenil] -2,7 - naphthalenedisulfonic (3:1) - amaranth - on the alumina substrate. It contains at least 95% and at most 105% of dye content stated on the label.

DESCRIPTION

Physical characteristics. Fine powder, red. Hygroscopic.

Solubility. It is practically insoluble in water and ethanol. It is soluble in M sodium hydroxide, but the dye decomposes slowly in alkaline pH.

IDENTIFICATION

A. The ultraviolet and visible absorption spectra (5.2.14), from 200 nm to 700 nm, 0.001% solution (w/v) in 0.02 M ammonium acetate (pH 5.6), previously solubilized in M sodium hydroxide, exhibits maximum at 519 nm, 330 nm, and 217 nm, and minima at 360 nm and 310 nm, similar to

those observed in the spectrum of amaranth SQR solution similarly prepared.

B. To transfer 0.15g of the sample to 60 mL beaker and dissolve it in about 20 mL of 30% acetic acid (w/v), hot, until it becomes opalescent. To cool down and divide the solution into two tubes. To add 2 mL of 3 mg/mL morin solution in ethanol, freshly prepared, to one of them. To observe the green fluorescence developing under ultraviolet light (254 nm), by comparing with the non-reactive tube.

PURITY TESTS

Subsidiary dyes. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using G silica gel as support and a mixture of 1-butanol, ethanol, water, and concentrated ammonia solution (50:25:25:10) as mobile phase. To separately apply to the plate 2 µl of each of the solutions freshly prepared, as described below.

Solution (1): 0.25 g of the sample in 10 mL of 0.5 M sodium hydroxide.

Solution (2): 0.05 g of standard amaranth in 10 mL of 0.5 M sodium hydroxide.

Solution (4): to dilute *Solution (2)* so as to obtain a 0.2 mg/mL solution with the same diluent.

Solution (4): to dilute *Solution (1)* so as to obtain a 1 mg/mL solution with the same diluent.

To develop the chromatogram. To remove the plate, and let it drying. To examine at ambient light, under ultraviolet light (254 nm). The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*. The secondary spots obtained with *Solution (1)* must not be more intense than those obtained with *Solution (3)* and *Solution (4)* (4%).

Alternatively, a mixture of 1-butanol, water, glacial acetic acid (20:12:5), as mobile phase, can be used. Instead of G silica gel, chromatographic paper can be used, under the conditions previously described and also observing the spots by transparency.

Chlorides and sulfates. To weigh 10 g of the sample, stir it with 250 mL of water, and leave it in contact for 30 minutes. To filter it. To measure 50 mL of the filtrate, equivalent to 2 g of the sample, dilute to 200 mL with water, acidify with 8 mL of 25% nitric acid (v/v), and titrate with 0.1 M SV silver nitrate in a potentiometer pot with combined silver electrode. Each mL of 0.1 M SV silver nitrate is equivalent to 5.85 mg of NaCl.

To measure remaining 50 mL of the filtrate, dilute to 300 mL with water, acidify with hydrochloric acid SR plus 1 mL of excess. To heat to boiling and by stirring drip 25 mL of 12% barium chloride (w/v). Let it rest for 4 hours. To separate barium sulfate by filtration, wash with hot water, dry the paper with the residue, transfer to dry crucible

previously weighed, and calcined in an oven, at 500 °C for 1 hour.

To cool down in desiccator and weigh it. The calculate the sulfate content, use the following equation:

$$\frac{N \times 0,6085 \times 100}{p} = \% \text{ sulfates}$$

where

N = barium sulfate in grams;

p = sample used in the precipitation in grams.

Maximum of 2% of chlorides and sulfates.

Loss on drying (5.2.9). To weigh about 0.5 g. To desiccate the sample at 120 °C for 4 hours or at 135 °C for 3 hours. At most 20%.

Residue incineration (5.2.10). To weigh about 0.1 of

the sample in previously dried and weighed crucible and incinerated at 800 °C for 2 hours. It should contain between 40 and 55%.

DOSAGE

To dilute as described in Method **A**. in *Identification*, and read the absorbance at the peak maximum at around 519 nm **(5.2.14)**. To calculate the dye content by the following equation:

$$A \times 100/436 \times p = \% \text{ amaranth in the sample at 519 nm}$$

where

p = sample weight in grams in the dilution made.

Alternatively, take into account $A(1\%, 1 \text{ cm}) = 436$ at 519 nm.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

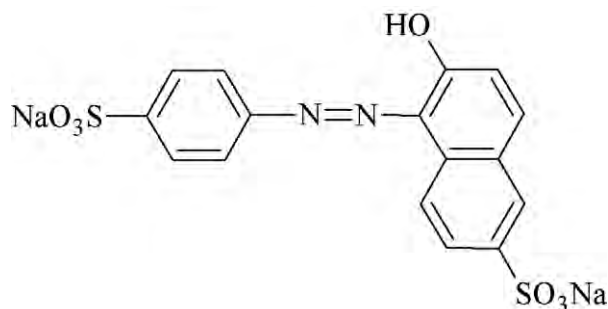
LABELLING

To comply with the legislation in force.

CATEGORY

Dye.

YELLOW TWILIGHT



$C_{16}H_{10}N_2Na_2O_7S_2$; 452.37

CI 15985

Sodium salt of 6-hydroxy-5-[2 - (4-sulfophenyl) diazenil]-2-naphthalenedisulfonic (2:1)
[2783-94-0]

It contains at least 85% of $C_{16}H_{10}N_2Na_2O_7S_2$

DESCRIPTION

Physical characteristics. Fine powder, reddish-orange, hygroscopic. Yellow-orange aqueous solution.

Solubility. It is soluble in water, methanol, ethanol, and glycerol; insoluble in ethyl ether, mineral oil, and acetone. Little stable in the presence of reducing agents.

IDENTIFICATION

The ultraviolet and visible absorption spectra **(5.2.14)**, from 200 nm to 700 nm, 0.001% solution (w/v) in 0.02 *M* ammonium acetate (pH 5.6), exhibits maximum at 481 nm, 312 nm, 234 nm, and 211 nm, and minima at 348 nm, 286 nm, and 218 nm, similar to those observed in the spectrum of a similar solution of yellow twilight SQR.

PURITY TESTS

Subsidiary dyes. To proceed as described in *Thin-layer chromatography (V.2.17.1)* by using G silica gel as support and a mixture of 1-butanol, ethanol, water, and ammonium hydroxide (50:25:25:10) as mobile phase. To separately apply to the plate 2 µl of each of the solutions freshly prepared, as described below.

Solution (1): 0.25 g of the sample in 10 mL of 0.5 *M* sodium hydroxide.

Solution (2): 0.05 g of standard yellow twilight in 10 mL of 0.5 *M* sodium hydroxide.

Solution (3): to dilute *Solution (2)* so as to obtain a 0.25 mg/mL solution with the same diluent.

Solution (4): to dilute *Solution (1)* so as to obtain a 1.25 mg/mL solution with the same diluent.

To develop the chromatogram. To remove the plate, and let it drying. To examine at ambient light, under ultraviolet

light (254 nm). The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*. Any secondary spot in the chromatogram obtained with *Solution (1)* must not be more intense than that obtained with *Solution (3)* and *Solution (4)* (5%).

Alternatively, a mixture of 1-butanol, water, glacial acetic acid (20:12:5), as mobile phase, can be used. Instead of G silica gel, chromatographic paper can be used, under the conditions previously described and also observing the spots by transparency.

Lead, copper, tin, zinc. To proceed as described in *Atomic absorption spectrophotometry (5.2.13)*. To weigh 2 g of the sample in a silica crucible and burn softly on asbestos canvas ($\pm 350^\circ\text{C}$); take it to the oven for 12 hours, at maximum temperature of 450°C . To remove the crucible and let it cool down. To mix the residue with about 2 mL of water and add two drops of 50% magnesium nitrate (w/v). To dry it over the electric plate and return to oven for 3 - 4 hours, or until the residue is white or yellowish. Then, cool down, drip 1 to 2 mL of nitric acid and 1 mL of water, and heat on a electric plate until almost dry it. To dissolve the metal nitrate with 5 mL of water. If necessary, centrifuge it. To take it to an atomic absorption spectrophotometer, previously calibrated, to read the concentration of the metals. Maximum of 0.001% (10 ppm) lead, 0.002% (20 ppm) copper, 0.025% (250 ppm) tin, and 0.005% (50 ppm) zinc.

Chlorides and sulfates. To weigh 0.5 g of sample, dissolve in 200 mL of water, acidify with 8 mL of 25% nitric acid (v/v), and titrate with 0.1 M SV silver nitrate in a potentiometer pot with combined silver electrode. Each mL of 0.1 M SV silver nitrate is equivalent to 5.85 mg of NaCl.

To weigh 0.5 g of the sample and dissolve it in 100 mL of water, in a water bath. To add 35 g of sulfate-free sodium chloride, and stir it well. To transfer to 200 mL volumetric flask and top up with saturated sodium chloride solution. To make it homogeneous. After 1 hour, filter it by using a filter paper and transfer 100 mL aliquot of the filtrate to 600 mL beaker, dilute to 300 mL with water, and acidify with hydrochloric acid SR, by adding slight excess. To heat to boiling and drip, with stirring, 25 mL of 12% barium chloride (w/v) or until no further precipitation occurs. Let it rest for 4 hours. To separate barium sulfate by filtration, wash with hot water, dry the paper with the residue, transfer to dry crucible previously weighed, and calcined in an oven, at 500°C for 1 hour. To cool down in desiccator and weigh it. To calculate the sulfate content, use the following equation:

$$\frac{N \times 0,6085 \times 100}{p} = \% \text{ sulfates}$$

where

N = barium sulfate in grams;

p = sample used in the precipitation in grams.

Maximum of 5% of chlorides and sulfates.

Substances insoluble in water. To dissolve by stirring 5 g of the sample in 200 mL of hot water ($80-90^\circ\text{C}$). To cool down at room temperature. To filter it by filter plate, previously dried and weighed. To wash with cold water until the washings become colorless. To dry the filter with the residue in oven at 120°C for 4 hours and weigh it. At most 0.5%.

Arsenic (5.3.2.5). To use *Method I*. To determine 1 g of the sample. At most 0.0001% (1 ppm).

Heavy metals (5.3.2.3). To use *Method III*. To determine in 0.5 g of the sample. At most 0.004% (40 ppm).

DOSAGE

To dilute as described in *Identification*, and read the absorbance at the peak maximum at around 481 nm (5.2.14). To calculate the dye content by the following equation:

$$\frac{A \times 100}{564 \times p} = \% \text{ amaranth in the sample at } 519\text{nm}$$

where

p = sample weight in grams in the dilution made.

Alternatively, take into account $A(1\%, 1\text{ cm}) = 564$ at 481 nm.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

CATEGORY

Dye.

YELLOW TWILIGHT ALUMINUM LAKE

Dye consists mainly of sodium salt of 6-hydroxy-5-[2 - (4-sulfophenyl) diazenil] -2 - naphthalenedisulfonic (0:1) - yellow twilight - on the alumina substrate. It contains at least 95% and at most 105% of dye content stated on the label.

DESCRIPTION

Physical characteristics. Fine powder, yellow-orange. Hygroscopic.

Solubility. It is practically insoluble in water and ethanol. It is soluble in M sodium hydroxide, but the dye decomposes slowly in alkaline pH.

IDENTIFICATION

A. The ultraviolet and visible absorption spectra (**5.2.14**), from 700 nm to 200 nm, 0.001% solution (w/v) in 0.02 M ammonium acetate (pH 5.6), previously solubilized in M sodium hydroxide, exhibits maximum at about 481 nm, 312 nm, 234 nm, and 211 nm, and minima at 348 nm, 286 nm, and 218 nm, similar to those observed in the spectrum of a similar solution of yellow twilight SQR.

B. To transfer 0.15g of the sample to 60 mL beaker and dissolve it in about 20 mL of 30% acetic acid (w/v), hot, until it becomes opalescent. To cool down and divide the solution into two tubes. To add 2 mL of 3 mg/mL morin solution and ethanol, freshly prepared, to one of them. To observe the green fluorescence developing under ultraviolet light (254 nm), by comparing with the non-reactive tube.

PURITY TESTS

Subsidiary dyes. To proceed as described in *Thin-layer chromatography* (**5.2.17.1**) by using G silica gel as support and a mixture of 1-butanol, ethanol, water, and ammonium hydroxide (50:25:25:10) as mobile phase. To separately apply to the plate 2 µl of each of the solutions freshly prepared, as described below.

Solution (1): 0.25 g of the sample in 10 mL of 0.5 M sodium hydroxide.

Solution (2): 0.05 g of standard yellow twilight in 10 mL of 0.5 M sodium hydroxide.

Solution (3): to dilute *Solution (2)* so as to obtain a 0.25 mg/mL solution with the same diluent.

Solution (4): to dilute *Solution (1)* so as to obtain a 1.25 mg/mL solution with the same diluent.

To develop the chromatogram. To remove the plate, and let it drying. To examine at ambient light, under ultraviolet light (254 nm). The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*. The secondary spots obtained with *Solution (1)* must not be more intense than those obtained with *Solution (3)* and *Solution (4)* (5%).

Alternatively, a mixture of 1-butanol, water, glacial acetic acid (20:12:5), as mobile phase, can be used. Instead of G silica gel, chromatographic paper can be used, under the conditions previously described and also observing the spots by transparency.

Chlorides and sulfates. To weigh 10 g of the sample, stir it with 250 mL of water, and leave it in contact for 30 minutes. To filter it. To measure 50 mL of the filtrate, equivalent to 2 g of the sample, dilute to 200 mL with water, acidify with 8 mL of 25% nitric acid (v/v), and titrate with

0.1 M SV silver nitrate in a potentiometer pot with combined silver electrode. Each mL of 0.1 M SV silver nitrate is equivalent to 5.85 mg of NaCl.

To measure remaining 50 mL of the filtrate, dilute to 300 mL with water, acidify with hydrochloric acid SR plus 1 mL of excess. To heat to boiling and by stirring drip 25 mL of 12% barium chloride (w/v). Let it rest for 4 hours. To separate barium sulfate by filtration, wash with hot water, dry the paper with the residue, transfer to dry crucible previously weighed, and calcined in an oven, at 500 °C for 1 hour. To cool down in desiccator and weigh it. The calculate the sulfate content, use the following equation:

$$\frac{N \times 0,6085 \times 100}{p} = \% \text{ sulfates}$$

where

N = barium sulfate in grams;

p = sample used in the precipitation in grams.

Maximum of 2% of chlorides and sulfates.

Loss on drying (5.2.9). To weigh about 0.5 g. To desiccate the sample at 120 °C for 4 hours or at 135 °C for 3 hours. At most 20 %.

Residue incineration (5.2.10). To weigh about 0.1 of

the sample in previously dried and weighed crucible and incinerated at 800 °C for 2 hours. It should contain between 40 and 55%.

DOSAGE

To dilute as described in Method **A.** in *Identification*, and read the absorbance at the peak maximum at around 481 nm. To calculate the dye content by the following equation:

$$\frac{A \times 100}{564 \times p} = \% \text{ yellow twilight in sample at 481 nm}$$

where

p = sample weight in grams in the dilution made.

Alternatively, take into account *A* (1%, 1 cm) = 564 at 481 nm.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

CATEGORY

Dye.

STARCH

Amylum

starch; 00657
Starch
[9005-25-8]

Starch is obtained from fruits, roots, and other parts of various plants. The starch from maize (*Zea mays* L. Poaceae), rice starch (*Oryza sativa* L., Poaceae), wheat starch (*Triticum aestivum* L. Poaceae), cassava starch (*Manihot utilissima* Pohl, Euphorbiaceae), and potato starch (*Solanum tuberosum* L. Solanaceae) are considered medicinal. Starches from different botanical origins may not have identical properties when used for pharmaceutical purposes. Starch is chemically a polymer blend which corresponds to the formula ($C_6H_{10}O_5$). The maize starch contains about 27% amylose and 73% of amylopectin.

DESCRIPTION

Physical characteristics. White, fine, odorless, and tasteless powder. When examined by thin-layer it must not show visible impurities or dirt.

Solubility. Practically insoluble in cold water, ethanol, and organic solvents.

MICROSCOPIC DESCRIPTION

Rice starch (Figure 1). Very small grains, polyhedral, with sharp corners, and straight edges, usually in groups, 2 μ m - 10 μ m diameter (4 μ m - 6 μ m, on average). The rounded grains are rare, and the hilum is often missing or appears as mini pit.

Potato starch (Figure 2). Simple grains or irregularly ovoid sub spherical, rarely grouped in 2 or 3, characteristic. The ovoid grains are unevenly elongated or triangular, 30 μ m - 100 μ m diameter. Sub spherical grains measure from 10 μ m to 35 μ m. The hilum is round, eccentrically disposed in the narrowest part of the grain, with very clear and concentric striations.

Cassava starch (Figure 3). The grains range from 25 μ m to 35 μ m diameter, irregularly curved, thimble-shaped, truncated sphere on one or more faces, with pits in the hilum, linear or star-shaped, central, and very clear.

Corn starch (Figure 4). A mixture of both form of grains. When they are derived from the periphery of the albumen, they are polyhedral, strongly compressed, showing rounded hilum, cracked or stellar, and measure from 14 μ m to 20 μ m diameter, on average. When they are derived from the most central albumen part, they show contour slightly angular, irregularly rounded, and are elongated, ovoid or pyriform, and with greater hilum, and measure on average from 10 μ m to 35 μ m.

The smaller grains are grouped together, sometimes resembling grain compounds.

Wheat starch (Figure 5). Two forms of grains, clearly differentiated, and almost no intermediate forms: large grains, lenticular, round, oval, and sub-reniform, sometimes cracked lips, exhibit slightly different concentric layers, like the hilum in the form of a central point or a simple line, measuring, on average, from 28 μ m to 35 μ m diameter. Seen in profile, they are elliptical, elongated, almost fusiform, crossed by a crack, sometimes quite large. The smaller grains are rounded, faceted by mutual compression, measuring from 2 μ m to 9 μ m (5 μ m to 7 μ m, on average) diameter. They also found in some groups of 2-4 grains.

IDENTIFICATION

A. To mix 1 g of the sample with 2 mL of cold water. To pour over 15 mL of boiling water. To gently boil for 2 minutes, by stirring. To cool down. Gelatinous, clear, and translucent product is formed.

B. To add a drop of iodine SR to the gelatinous mixture obtained in test A. *Identification.* Blue color is developed, but it disappears when boiled and returns to blue color when cooled.

PURITY TESTS

pH (5.2.19). 4.5 to 7.0 for corn starch and 5.0 to 8.0 for potato starch. To determine in 20 g of the sample. To transfer the sample to a nonmetallic vial and add 100 mL of water. A slurry is formed. To continuously stir for 5 minutes at moderate speed.

Oxidizing substances. To transfer 4 g of the sample to a 125 mL erlenmeyer flask. To add 50 mL of water. To cover and stir for 5 minutes. To transfer to the centrifuge tube with 50 mL capacity and centrifuge. To transfer 30 mL of the clear supernatant to a 125 mL erlenmeyer flask. To add 1 mL of glacial acetic acid and 1 g of potassium iodide. To cover, stir, and let it rest for 30 minutes protected from direct light. To add 1 mL of starch SI and titrate with 0.001 M sodium thiosulphate VS until the blue color disappears. To perform sample blank correction and adjust it as required. Each mL of 0.001 M SV sodium thiosulfate is equal to 17 μ g of oxidant, calculated as hydrogen peroxide. At most 1.4 mL of 0.001 M SV sodium thiosulphate is spent (0.002%).

Sulphur dioxide. To mix 20 g of the sample with 200 mL of water to obtain homogeneous suspension. To filter it. To add 3 mL of starch SI to 100 mL of clear filtrate and titrate with 0.02 M SV iodine to obtain permanent blue color. At most 5.4 mL of 0.02 M SV iodine are spent (0.008%).

Iron (5.3.2.4). To dissolve the residue obtained in *Sulfated ashes* in 8 mL of hydrochloric acid by gently heating.

To dilute to 100 mL with water and make it homogeneous. To transfer 25 mL to a Nessler tube, add 12 mL of water, and proceed as described in *Method I*. At most 0.002% (20 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 105 °C until obtaining constant weight. At most 15.0%.

Sulfated ashes (5.2.10). To determine in 2 g of the sample. At most 0.6%.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). Total aerobic bacteria: At most 100 UFC/g. Fungi and yeasts: At most 100 UFC/g. Severe fungal contamination can cause aflatoxins in the starch.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

PACKAGING AND STORAGE

In tightly closed containers, protected from moisture. The label should indicate the botanical origin.

LABELLING

To comply with the legislation in force.

CATEGORY

Pharmaceutic adjuvant.

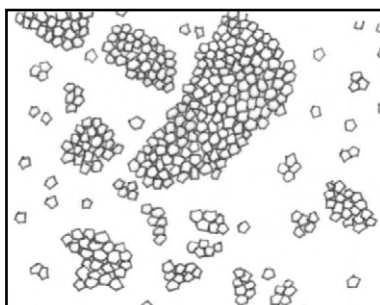


Figure 1 - **Rice starch**

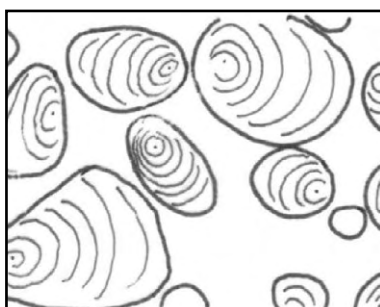


Figure 2 - **potato starch.**

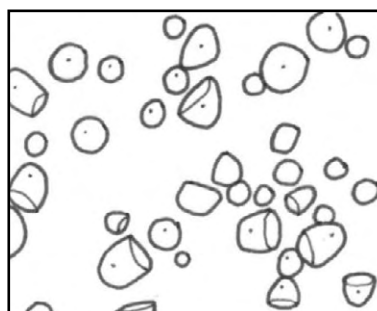


Figure 3 - **cassava starch.**

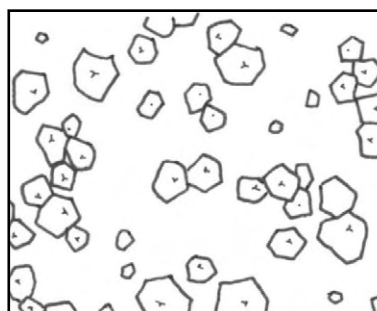


Figure 4 - **maize starch.**

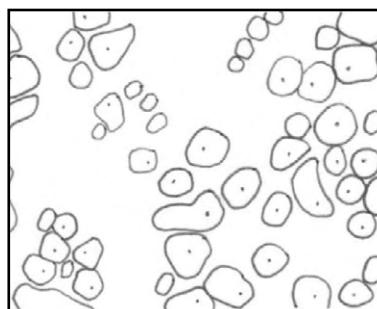
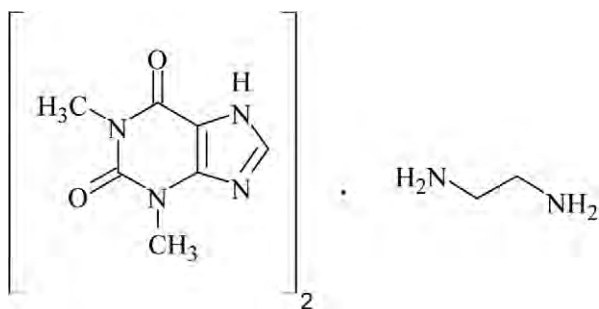


Figure 5 - **wheat starch.**

AMINOPHYLLINE*Aminophyllinum* $(C_7H_8N_4O_2)_2 \cdot C_2H_8N_2$; 420.43 $C_7H_8N_4O_2$; 180.16 $C_2H_8N_2$; 60.10

aminophylline; 00685

3,9-Dihydro-1,3-dimethyl-1*H*-purine-2,6-dione with
1,2-ethanediamine (2:1)

[317-34-0]

Aminophylline is a combination of theophylline and ethylenediamine, which contains at least 84.0% and at most 87.4% of the amount stated of theophylline ($C_7H_8N_4O_2$) and at least 13.5% and at most 15.0% of ethylenediamine ($C_2H_8N_2$) in relation to anhydrous substance.

DESCRIPTION

Physical characteristics. Powder or granules, white or slightly yellowish, with slight ammonia odor and bitter taste.

Solubility. Very soluble in water, practically insoluble in absolute ethanol and ethyl ether.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the precipitate obtained in test **B. Identification**, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the theophylline SQR spectrum similarly prepared.

B. To dissolve 0.5 g of the sample in 10 mL of water, add 1 mL of 3 *M* hydrochloric acid, by constantly stirring. To filter and wash the precipitate with small portions of cold water. To dry at 105°C for 1 hour. The resulting precipitate melts between 270°C and 274°C.

C. To transfer 10 mg of the desiccated precipitate obtained in test **B Identification** for porcelain capsule, add 1 mL of hydrochloric acid and 0.1 g of potassium chloride. To evaporate to dryness in water bath. To invert the capsule over a container containing a few drops of 6 *M* ammonium hydroxide. The residue becomes purple, which disappears with the addition of fixed alkalis.

D. The precipitate obtained in test **B. Identification** responds to xanthine reactions (5.3.1.1).

PURITY TESTS

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using HF_{254} silica gel as support and a mixture of concentrated ammonia solution, acetone, chloroform, and 1-butanol (10:30:30:40) as mobile phase. To separately apply to the plate 10 µL of each of the solutions freshly prepared, as described below.

Solution (1): to dissolve 0.2 g of the pulverized sample in 2 mL of water and dilute to 10 mL with methanol.

Solution (2): to transfer 0.5 mL of the *Solution (1)* to 100 mL volumetric flask and top up with methanol.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). Any spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained in the chromatogram with *Solution (2)* (0.5%).

Heavy metals (5.3.2.3). To use *Method III*. At most 0.002% (20 ppm).

Water (5.2.20.1). To determine in 2 g of the sample. At most 1.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.15%.

DOSAGE**Ethylenediamine**

To dissolve 0.25 g of sample in 30 mL of water. To titrate with 0.1 *M* SV hydrochloric acid by using 0.1 mL of bromocresol green SI as an indicator to change green. Each mL of 0.1 *M* SV hydrochloric acid is equal to 3.005 mg of ethylenediamine ($C_2H_8N_2$).

Theophylline

To employ one of the methods described below.

A. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 254 nm; 150 mm column and 3.9 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), flow of *Mobile phase* of 1 mL/min.

Mobile phase: to mix 200 mL of methanol and 0.96 g of monohydrate sodium 1-pentane sulfonate and top up to 1000 mL with water. To adjust pH to (2.9 ± 0.1) with glacial acetic acid.

Diluent: mixture of water and methanol (4:1).

Sample solution: to transfer accurately weighed 24 mg of the sample to a 250 mL volumetric flask, and top up with *Diluent*, and mix it.

Standard solution: to accurately dissolve weighed amount of theophylline SQR in *Diluent* and properly dilute to obtain a 80 µg/mL solution.

Resolution solution: to prepare 80 µg/mL theobromine SQR solution by using *Standard solution* as diluent. To transfer 20 mL to a 25 mL volumetric flask, top up with the *Diluent*, and make it homogeneous.

To inject replicates of 10 µL of the *Resolution solution*. The relative retention times are 0.65 to theobromine and 1.0 to theophylline. The resolution between theobromine and theophylline peaks should not be less than 3.0. The tailing factor to theophylline peak should not be greater than 2.0. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2 %.

Procedure: to separately inject 10 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of theophylline ($C_7H_8N_4O_2$) from the aminophylline sample, from the responses obtained for theophylline with the *Sample and Standard solutions*.

B. To desiccate the sample at 135 °C until obtaining constant weight. To accurately weigh about 0.2 g of the sample, dissolve in 100 mL of water, and heat it, if necessary. To cool down. To add 20 mL of 0.1 M silver nitrate and stir. To titrate with 0.1 M SV sodium hydroxide, by using 1 mL of bromothymol blue SI as an indicator. Each mL of 0.1 M SV sodium hydroxide is equal to 18.016 mg of theophylline ($C_7H_8N_4O_2$).

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Bronchodilator.

AMINOPHYLLINE, TABLETS

It contains at least 80.6% and at most 90.8% of theophylline ($C_7H_8N_4O_2$) and at least 10.9% of ethylenediamine ($C_2H_8N_2$) in relation to the stated amount of aminophylline.

IDENTIFICATION

A. To weigh and pulverize the tablets. To stir amount of powder equivalent to 0.5 g of aminophylline with 20 mL of water and filter it. To add 1 mL of 2 M hydrochloric acid to the filtrate, under constant stirring, let it rest for a few minutes, and filter it. To set apart the filtrate for test **C. Identification**. To wash the residue with small amounts of cold water, recrystallize in hot water, and dry at 105 °C in an oven to obtain constant weight. The infrared absorption

spectrum (**5.2.14**) of the residue, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the aminophylline SQR spectrum similarly prepared.

B. The residue obtained in test **A. Identification** melts around 271 °C.

C. To add 0.2 mL of benzyl chloride to the filtrate set apart in test **A. Identification**, alkalinize it with 5 M ammonium hydroxide, and vigorously stir it. To filter and wash the residue with cold water, recrystallize in mixture of water and ethanol (10:30), and dry at 100 °C in an oven to obtain constant weight. The resulting crystals melt at around 250 °C.

D. To dissolve 10 mg of the resulting residue obtained in test **A. Identification** in 1 mL of hydrochloric acid. To add 0.1 g of potassium chloride and evaporate to dryness. Reddish residue is obtained, which becomes purple upon exposure to ammonia vapor.

E. To weigh and pulverize the tablets. To mix amount of powder equivalent to 0.25 g of aminophylline with 5 mL

of water and filter it. To add 2 mL of 1% cupric sulfate (w/v) to 2 mL of the filtrate and make it homogeneous. The dark blue color develops.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL

Equipment: blades, 50 rpm

Time: 45 minutes

Procedure: after testing, remove aliquot from the dissolution medium, filter, and dilute in water to obtain suitable concentration. To measure the absorbance of the solutions at 269 nm (**5.2.14**), by using the same solvent to adjust zero. To calculate the amount of anhydrous theophylline ($C_7H_8N_4O_2$) dissolved in the medium, by comparing the readings obtained with 0.001 % theophylline SQR solution (w/v) prepared in the same solvent.

Tolerance: not less than 75% (Q) of the stated amount of theophylline ($C_7H_8N_4O_2$) dissolves within 45 minutes.

DOSAGE

Ethylenediamine

To weigh and pulverize 20 tablets. To transfer amount of powder equivalent to 0.3 g of aminophylline to 150 mL erlenmeyer flask, dissolve in 20 mL of water, and heat at 50 °C for 30 minutes, by occasionally stirring. To titrate with 0.05 M SV sulfuric acid, by using bromocresol green solution as indicator to color change to blue-green. Each mL of 0.05 M SV sulfuric acid is equal to 3.005 mg of ethylenediamine ($C_2H_8N_2$).

Theophylline

To employ one of the methods described below.

A. To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To weigh and spray 20 tablets at fine powder. To transfer amount of powder equivalent to 80 mg of aminophylline [$(C_7H_8N_4O_2)_2 \cdot C_2H_8N_2$] to 200 mL volumetric flask. To add 20 mL of 0.1 M sodium hydroxide and 60 mL of water and mechanically stir for 10 minutes. To top up to volume with water, make it homogeneous, and filter it. To transfer 5 mL of the filtrate to a 200 mL volumetric flask and top up with 0.01 M SV sodium hydroxide, thus obtaining 0.001% concentration (w/v).

To measure the absorbance of the resulting solution at 275 nm, by using 0.01 M SV sodium hydroxide to adjust zero. To calculate the amount of theophylline ($C_7H_8N_4O_2$) in the tablets, by taking into account $A(1\% \text{ 1 cm}) = 650, 250 \text{ nm}$, in 0.01 M SV sodium hydroxide.

B. To weigh and pulverize 20 tablets to fine powder. To transfer amount of powder equivalent to 2 g of aminophylline [$(C_7H_8N_4O_2)_2 \cdot C_2H_8N_2$] to a 200 mL volumetric flask, by using a mixture of 50 mL of water and 15 mL of 6 M ammonium hydroxide. To occasionally stir for 30 minutes, by heating at 50 °C, if necessary, to dissolve the aminophylline. To cool down the mixture at room temperature, if heated, add water, top up to volume, and make it homogeneous. To centrifuge about 50 mL of the mixture, pipette the clear supernatant, equivalent to 250 mg of aminophylline, to a 250 mL erlenmeyer flask, and dilute with water, if necessary, to make up about 40 mL. To add 8 mL of 6 M ammonium hydroxide and 20 mL of 0.1 M SV silver nitrate and heat to boiling for 15 minutes. To cool down from 5 °C to 10 °C for 20 minutes and filter, preferably through a crucible under reduced pressure. To wash the precipitate with three portions of water (10 mL each). To acidify the combined filtrate and washings with nitric acid, then add 3 mL of the acid. To cool down, add 2 mL of ferric ammonium sulfate SR, and titrate the silver nitrate in excess with 0.1 M SV ammonium thiocyanate. Each mL of 0.1 M SV silver nitrate is equivalent to 18.016 mg of theophylline ($C_7H_8N_4O_2$).

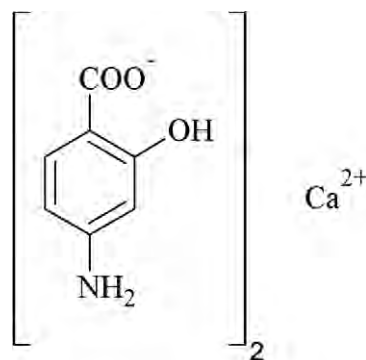
PACKAGING

In tightly closed containers.

LABELLING

To comply with the legislation in force.

AMINOSALICYLATE, CALCIUM
Calcii aminosalicylas



$C_{14}H_{12}CaN_2O_6$; 344.33

calcium aminosalicylate; 00695

Calcium salt of 4-amino-2-hydroxybenzoic acid (1:2)
[133-15-3]

It contains at least 98.0% and at most 100.5% of $C_{14}H_{12}CaN_2O_6$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White to cream crystals or powder. Odorless, alkaline taste slightly bittersweet. It is slightly hygroscopic. Its solutions decompose slowly and darken.

Solubility. Freely soluble in water. Slightly soluble in ethanol.

Additional information. To prepare calcium aminosalicylate solutions within 24 hours before the utilization. Do not use solutions if its color is darker than that of a freshly prepared solution.

IDENTIFICATION

A. To dissolve about 3 g of the sample in 50 mL of water, add glacial acetic acid dropwise until the mixture becomes clearly acidic. To filter with suction, by retaining the filtrate for test C. *Identification.* To wash the precipitate with several small portions of water and dry in vacuum over phosphorus pentoxide. To put about 1 g of the aminosalicylic acid obtained into small round-bottomed flask and add 10 mL of acetic anhydride. To heat such flask in a water bath for 30 minutes, add 40 mL of water, mix, filter, and cool down. Let it rest to diacetyl derivative precipitates. To collect the precipitate in a filter, wash well with water, and dry at 105 °C for 1 hour. The resulting diacetyl derivative melts between 191 °C and 197 °C.

B. To stir 0.1 g of aminosalicylic acid obtained in Test A. *Identification* with 10 mL of water and filter it. To add a

drop of ferric chloride SR to 5 mL of the filtrate. The violet color develops.

C. The filtrate obtained in test **A**, *Identification* responds to the reactions of the calcium ion (**5.3.1.1**).

D. To dissolve 0.25 g of aminosalicic acid obtained in test **A**, *Identification* in 3 mL of sodium hydroxide SR, transfer to a 500 mL volumetric flask, top up with water, and mix it. To transfer 5 mL of this solution to a 250 mL volumetric flask containing 12.5 mL of phosphate buffer pH 7.0, top up to volume with water, and mix it. This solution, when compared in 1 cm cuvettes with suitable spectrophotometer against blank of the same buffer and at the same concentration, presents the maximum absorbance (265 ± 2) nm and (299 ± 2) nm and the ratio between the absorbance values measured at 265 nm and 299 nm is between 1.50 and 1.56.

PURITY TESTS

Appearance of solution. A solution of 1 g of the sample in 50 mL of water has turbidity (**5.2.25**) not more intense than that produced by adding 100 μ L of diluted hydrochloric acid (1:600) to a mixture of 48 mL of water, 1 mL of nitric acid, and 1 mL of silver nitrate SR. The comparisons are made in similar glass beakers, by horizontally examining in a white background and a black background.

Appearance of solution in diluted nitric acid.

1 g sample of the sample is dissolved in 50 mL of diluted nitric acid, thus resulting a clear solution (**5.2.25**), with at most a light color.

pH. (5.2.19). 6.0 to 8.0. To determine in 1:50 aqueous solution.

Hydrogen sulfide and sulfur dioxide. To dissolve 0.5 g of the sample in 5 mL of water, add 5 mL of diluted hydrochloric acid, by vigorously stirring. No odor of hydrogen sulfide or sulfur dioxide is observed. A slight odor of amyl alcohol can be observed. A piece of filter paper moistened with lead acetate SR placed on the mixture does not discolor.

***m*-aminophenol.** To accurately weigh the calculated amount based on *Dosage*, equivalent to 0.562 g of anhydrous calcium aminosalicylate (0.5 g of aminosalicic acid) and put into a 100 mL volumetric flask. To add 1.8 mL of sodium hydroxide SR and dilute with water to approximately 80 mL. To add 10 mL of dilute sulfuric acid (1:10), top up to volume with water, and mix it. 2:30 minutes after adding the acid, transfer 5 mL of this solution to other 100 mL volumetric flask immersed in a ice, containing 50 mL of water at 0°C - 5°C and add 2.5 mL of sodium nitrite (1:100). To mix and let it rest in an ice bath for 3 minutes $\pm$ 5 seconds. To add 25 mL of sodium carbonate SR, mix, and put the flask in a water bath at 25°C for 15 minutes. To top up to volume with water, mix, and let the solution rest at 25°C for 3 hours. To determine the absorbance of the clear supernatant solution in a 1 cm cuvette at

most observed in the spectrum zone between 425 nm and 435 nm, with a suitable spectrophotometer, by using water as a blank. To calculate the percentage of *m*-aminophenol in the sample by using the following equation:

$$(A - 0.320)/1.09$$

where

A = absorbance of the solution;

0.320 = absorbance correction factor that represents the color produced by other factors and not by the reaction of *m*-aminophenol initially present;

1.09 = absorbance conversion factor (%) of *m*-aminophenol.

At most 0.20% of *m*-aminophenol is allowed.

Chlorides (5.3.2.1). 0.5 g of the sample has less chloride than the corresponding at 0.3 mL of 0.02 *M* SV hydrochloric acid. At most 0.04% (400 ppm).

Heavy metals (5.3.2.3). At most 0.003% (30 ppm).

Water (5.2.20.1). 12.5% to 14.5%.

DOSAGE

To accurately dissolve about 0.35 g of the sample in about 25 mL of water and let it rest for 10 minutes by occasionally stirring. To add 25 mL of glacial acetic acid and 20 mL of 1:4 potassium bromide. To cool down to 15°C , add 5 mL of hydrochloric acid, and immediately titrate with 0.1 *M* SV sodium nitrite by vigorously stirring. To potentiometrically determine the color changing, by using suitable electrode system. Each mL of 0.1 *M* SV sodium nitrite is equal to 17.22 mg of $\text{C}_{14}\text{H}_{12}\text{CaN}_2\text{O}_6$.

PACKAGING AND STORAGE

In airtight, opaque containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antibacterial (tuberculostatic).

AMOXICILLIN AND CLAVULANATE POTASSIUM, TABLETS

It contains at least 90.0 % and at most 120.0 % of the amount stated of amoxicillin ($\text{C}_{16}\text{H}_{19}\text{N}_3\text{O}_5\text{S}$) and clavulanate potassium ($\text{C}_8\text{H}_8\text{KNO}_5$).

IDENTIFICATION

The retention times of the main peaks in the chromatogram of the *Sample solution* obtained in the *Dosage* correspond to those of main peaks of *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Disintegration test (5.1.4.1). At most 45 minutes.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL

Equipment: blades, 75 rpm

Time: 30 minutes

Procedure: After testing, remove aliquot from the dissolution medium and dilute, if necessary, with water, to obtain suitable concentration. To proceed as described in *Dosage*.

To calculate the amounts of amoxicillin ($C_{16}H_{19}N_3O_5S$) and clavulanate potassium ($C_8H_8KNO_5$) dissolved in the medium, by comparing the responses obtained with the amoxicillin SQR and lithium clavulanate SQR solutions at 0.05% concentrations (w/v) and 0.02% (w/v), respectively, prepared in the same solvent.

Tolerance: not less than 85% (Q) of the stated amount of amoxicillin ($C_{16}H_{19}N_3O_5S$) and not less than 80% (Q) of the stated amount of clavulanate potassium ($C_8H_8KNO_5$) dissolve within 30 minutes.

The resolution between amoxicillin and clavulanic acid peaks should not be less than 3.5. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2.0%.

Procedure: to separately inject 20 μ L of *Sample and standard solutions*, record the chromatograms, and measure the area under the peaks. To calculate the content of amoxicillin and clavulanate potassium from the sample, from the responses obtained for the *Sample and standard solutions*.

PURITY TESTS

Water (5.2.20.1). At most 7.5% if the stated amount of amoxicillin is up to 250 mg. At most 10.0% if the stated amount of amoxicillin is between 250 mg and 500 mg. At most 11.0% if the labelled amount of amoxicillin is greater than 500 mg.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3).

It complies with the test.

DOSAGE

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 220 nm; 300 mm column and 4 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (3 μ m to 10 μ m), flow of *Mobile phase* of 2.0 mL/min.

Buffer pH 7.4: to dissolve 7.8 g of monobasic sodium phosphate in 900 mL of water. To adjust pH to 4.4 ± 0.1 with sodium hydroxide or phosphoric acid. To top up to 1000 mL with water and make it homogeneous.

Mobile phase: mixture of *Buffer pH 4.4* and methanol (95:5).

Sample solution: To dissolve at least 10 tablets in water, in flask with volume that results in a concentration not exceeding, for amoxicillin, 3 mg/mL, by stirring for 30 minutes. To filter or centrifuge and dilute aliquot from the resulting clear solution, with water, to obtain 0.5 mg/mL amoxicillin concentration. To use this solution within 1 hour.

Standard solution: To dissolve accurately weighed amounts of amoxicillin SQR and lithium clavulanate SQR in water, to obtain a solution containing 0.5 mg/mL and 0.2 mg/mL, respectively.

To inject replicates of 20 μ L of the *Standard solution*. The column efficiency for each analyte is greater than 550 theoretical plates. The relative retention times are about 0.5 to clavulanic acid and 1.0 to amoxicillin. The tailing factor for each analyte peak is not greater than 1.5.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

AMOXICILLIN AND CLAVULANATE POTASSIUM, POWDER FOR INJECTABLE SOLUTION

It contains at least 90.0 % and at most 120.0 % of the amount stated of amoxicillin ($C_{16}H_{19}N_3O_5S$) and clavulanate potassium ($C_8H_8KNO_5$).

IDENTIFICATION

The retention times of the main peaks in the chromatogram of the *Sample solution* obtained in the *Dosage* correspond to those of main peaks of *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Determination of volume (5.1.2). It complies with the test. To determine the reconstituted injectable solution as per label.

Uniformity of dosage units (5.1.6). It complies with the test.

PURITY TESTS

pH (5.2.19). 8.0 to 10.0. To determine in the reconstituted solution containing 10% (w/v) of amoxicillin.

Water (5.2.20.1). To determine 0.5g. At most 3.5%.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

Bacterial endotoxins (5.5.2.2). To dissolve the content of vial in Water to LAL reagent, to obtain a 10 mg/mL amoxicillin solution. At most 2.5 UE/ml of this solution.

DOSAGE

To proceed as described *Dosage*, in the monograph *Amoxicillin and clavulanate potassium, tablets*. To prepare the *Sample solution* as described below.

Sample solution: to mix the contents of 10 units. To transfer amount of powder equivalent to 0.1 mg of amoxicillin to a 200 mL volumetric flask, add water to dissolve, and top up to volume with the same solvent. To make it homogeneous. To use this solution within 1 hour.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of amoxicillin and clavulanate potassium in the sample, from the responses from the *Standard solution* and *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

AMOXICILLIN AND CLAVULANATE POTASSIUM, POWDER FOR ORAL SUSPENSION

It contains at least 90.0 % and at most 120.0 % of the amount stated of amoxicillin ($C_{16}H_{19}N_3O_5S$) and clavulanate potassium ($C_8H_8KNO_5$).

IDENTIFICATION

The retention times of the main peaks in the chromatogram of the *Sample solution* obtained in the *Dosage* correspond to those of main peaks of *Standard solution*.

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test. To determine in the reconstituted oral suspension as informed on the label.

Determination of weight (5.1.1). It complies with the test.

PURITY TESTS

Water (5.2.20.1). At most 7.5% if the stated amount of amoxicillin is up to 40 mg/mL after reconstitution. At most 10.0% if the stated amount of amoxicillin after reconstitution is between 40 mg/mL and 50 mg/mL. At most 11.0% if the stated amount of amoxicillin after reconstitution is between 50 mg/mL and 80 mg/mL.

At most 12.0% if the stated amount of amoxicillin is greater than 80 mg/mL after reconstitution.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To proceed as described *Dosage*, in the monograph *Amoxicillin and clavulanate potassium, tablets*. To prepare the *Sample solution* as described below.

Sample solution: To reconstitute the powder to oral suspension as informed on the label. To quantitatively dilute suspension in water to obtain a 0.5 mg/mL amoxicillin solution. To mechanically stir for 10 minutes and filter. To use this solution within 1 hour.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of amoxicillin and clavulanate potassium in the sample, from the responses from the *Standard solution* and *Sample solution*.

PACKAGING AND STORAGE

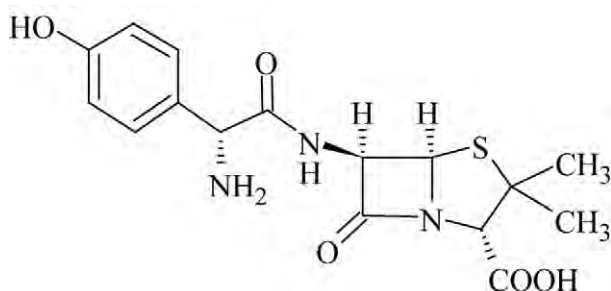
In tightly closed containers.

LABELLING

To comply with the legislation in force.

AMOXYCILLIN, TRIHYDRATE

Amoxicillinum trihydricum



$C_{16}H_{19}N_3O_5S$; 365.40

$C_{16}H_{19}N_3O_5S \cdot 3H_2O$; 419.45

amoxicillin; 00734

amoxycillin trihydrate; 00736

(2*S*,5*R*,6*R*) -6 - [[[(2*R*)-2-amino-2-(4-hydroxyphenyl)acetyl] amino] -3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid
[26787-78-0]

(2*S*, 5*R*, 6*R*) -6 - [[[(2*R*)-2-amino-2-(4-hydroxyphenyl)acetyl] amino] -3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic hydrate acid (1:3)
[61336-70-7]

It shows minimum potency of 900 µg and at most 1050 µg of amoxicillin ($C_{16}H_{19}N_3O_5S$) per milligram, relative to the anhydrous substance.

DESCRIPTION

Physical characteristics. Crystalline, white or almost white powder.

Solubility. Sparingly soluble in water, ethanol, and methanol. Insoluble in benzene, hexane, ethyl acetate, chloroform, ethyl ether, and acetonitrile. Soluble in alkalis.

Physical and chemical constants

Specific rotation (5.2.8): +290° to +315°, in relation to the anhydrous substance. To determine in 0.2% solution (w/v) in carbon dioxide-free water.

IDENTIFICATION

The identification test **A.** may be omitted if the tests **B.** and **C.** are conducted.

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative

intensities to those observed in the amoxycillin trihydrate SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14), from 200 nm to 400 nm, 0.02% solution (w/v), in ethanol, has absorption maximum at 230 nm and 274 nm, similar to those observed in the spectrum of a similar solution of amoxycillin trihydrate SQR.

C. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using G 60 silica gel as support and a mixture of methanol, chloroform, water, and acetone (9:8:3:1) as mobile phase. To separately apply to the plate 5 µl of each of the solutions, as described below, which should be used within 10 minutes after preparation:

Solution (1): 4 mg/mL solution of the sample in 0.1 M hydrochloric acid.

Solution (2): 4 mg/mL trihydrate amoxicillin SQR solution in 0.1 M hydrochloric acid.

To develop the chromatogram. To remove the plate, and let it drying. To nebulize with ninhydrin SR. To heat the plate in an oven for 15 minutes at 110 °C. The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

PURITY TESTS

Crystallinity. To suspend in mineral oil some particles from the sample. Then, transfer it to a microscope slide and examine by polarized light microscope. The particles exhibit birefringence, which is extinguished by micrometric adjustment.

pH (5.2.19). 3.5 to 6.0. To determine in 0.2% aqueous solution (w/v).

Water (5.2.20.1). 11.5% to 14.5%. To determine in 0.3 g of the sample.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 1%.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Microbiological test of antibiotics* (5.5.3.3.1) by using cylinders with the agar diffusion method.

Micro-organism: *Micrococcus luteus* ATCC 9341.

Culture media: culture medium number 1, to maintain the micro-organism; sterile saline solution to the standardization of the inoculum; and culture medium number 11, to the base layer and inoculum layer in the plate.

Sample solution: To weigh amount of the sample equal to 100 g of amoxicillin and transfer to a 100 mL volumetric

flask. To top up with water and stir for about 30 minutes. To transfer 1 mL of this solution to a 100 mL volumetric flask, top up with potassium phosphate buffer, sterile, pH 8.0 (solution 2), and stir. To successively dilute to obtain 0.05 µg/mL, 0.10 µg/mL, and 0.20 µg/mL concentrations, by using the potassium phosphate buffer, sterile, pH 8.0 (solution 2) as diluent.

Standard solution: To weigh amount of amoxycillin trihydrate SQR equal to 25 g of amoxicillin and transfer to a 50 mL volumetric flask. To top up to volume with water. To transfer 1 mL of this solution to a 100 mL volumetric flask, top up with potassium phosphate buffer, sterile, pH 8.0 (solution 2), and stir. To successively dilute to obtain 0.05 µg/mL, 0.10 µg/mL, and 0.20 µg/mL concentrations, by using the potassium phosphate buffer, sterile, pH 8.0 (solution 2) as diluent.

Procedure: to add 21 mL of culture medium number 11 in each plate, wait to solidify, add 4 mL of 0.5% inoculum, and proceed as described in *Microbiological test of antibiotics (5.5.3.3.1)*, by adding 0,2 mL of freshly prepared solutions to the cylinders. To calculate the potency of the sample per µg of amoxicillin from the potency of the standard and responses obtained with the *Standard and sample solutions*.

B. Iodometric method. To dissolve and dilute trihydrate amoxicillin standard and sample in water, to obtain about 1.25 mg/mL concentration. To transfer 2 mL of standard solution to erlenmeyer flask with ground lid and add 2 mL of *M* sodium hydroxide. To stir and let it rest for 15 minutes. To add 2 mL of 1.2 *M* hydrochloric acid and 10 mL of 0.01 *M* SV iodine (w/v). Let it rest for 15 minutes protected from light. To titrate with 0.01 *M* SV sodium thiosulfate. To add 3 drops of starch SI near the endpoint and continue titration until the blue color disappears. To proceed the same test with the sample solution. To perform sample blank correction, including sample and standard, by titration of 2 mL of both solutions by adding 10 mL of 0.01 *M* SV iodine and 0.1 mL of *M* hydrochloric acid. To add 3 drops of starch SI near the endpoint and continue titration until the blue color disappears. To titrate with 0.01 *M* SV sodium thiosulfate. To calculate the potency of the sample according to the following equation:

$$P = \frac{(V_{ba} - V_a) \times P_o \times P_p}{(V_{bp} - V_p) \times P_a}$$

where

P = potency of the sample (µg/mg);

V_{ba} = volume of titrant spent in titration of sample blank (mL),

V_a = volume of titrant spent in titration of sample (mL),

V_{bp} = volume of titrant spent in titration of standard blank (mL),

V_p = volume of titrant spent in titration of standard (mL),

P_o = potency of the standard (µg/mg),

P_p = weight of the standard (mg),

P_a = weight of the sample (mg).

PACKAGING AND STORAGE

In tightly closed containers, at 15 °C - 25 °C.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antibiotic.

AMOXYCILLIN, TRIHYDRATE, CAPSULES

It contains at least 90.0 % and at most 120.0 % of the amount stated of C₁₆H₁₉N₃O₅S. The trihydrate amoxicillin capsules consist of trihydrate amoxicillin with or without one or more lubricating agents, and suitable drying and diluent agents, embedded in gelatin capsules.

IDENTIFICATION

A. The ultraviolet absorption spectrum (5.2.14), from 200 nm to 400 nm, 0.02% solution (w/v), in ethanol, has absorption maximum at 230 nm and 274 nm, similar to those observed in the spectrum of a similar solution of amoxycillin trihydrate SQR.

B. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using G 60 silica gel as support and a mixture of methanol, chloroform, water, and acetone (9:8:3:1) as mobile phase. To separately apply to the plate 5 µl of each of the solutions, as described below, which should be used within 10 minutes after preparation:

Solution (1): 0.4 % solution (w/v) of the sample in 0.1 *M* hydrochloric acid.

Solution (2): 0.4 % trihydrate amoxicillin SQR solution (w/v) in 0.1 *M* hydrochloric acid.

To develop the chromatogram. To remove the plate and allow it to air dry, and nebulize with ninhydrin SR. To heat in an oven for 15 minutes at 110 °C. The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL

Equipment: baskets, 100 rpm

Time: 90 minutes

Procedure: after testing, remove aliquot from the dissolution medium, filter, and dilute in water to obtain suitable concentration. To measure the absorbance of the solutions at 272 nm (5.2.14), by using the same solvent o adjust zero. To calculate the amount of $C_{16}H_{19}N_3O_5S$ dissolved in the medium, by comparing the readings obtained with 0.01 % trihydrate amoxicillin SQR solution (w/v) prepared in the same solvent.

Tolerance: not less than 80% (Q) of the stated amount of $C_{16}H_{19}N_3O_5S$ dissolves within 90 minutes.

PURITY TESTS

Water (5.2.20.1). To determine in 0.3 g of the sample. At most 14.5%.

BIOLOGICAL SAFETY TEST

Counting of the total viable micro-organisms (5.5.3.1.2). It complies with the test.

Survey and identification of pathogens (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Microbiological test of antibiotics (5.5.3.3)* by using cylinders with the agar diffusion method.

Micro-organism: *Micrococcus luteus* ATCC 9341.

Culture media: culture medium number 1, to maintain the micro-organism; sterile saline solution, to the standardization of the inoculum; and culture medium number 11, to the base layer and inoculum layer in the plate.

Sample solution: to remove the content of the capsules and accurately weigh them. To make the content homogeneous. To transfer amount of powder equivalent to 0.1 g of amoxicillin to a 100 mL volumetric flask, top up to volume with water, and stir for 30 minutes. To transfer 1 mL of this solution to a 100 mL volumetric flask, top up with 0.1 M potassium phosphate buffer, sterile, pH 8.0 (solution 2), and stir. To successively dilute to obtain 0.05 µg/mL, 0.10 µg/mL, and 0.20 µg/mL concentrations, by using the 0.1 M potassium phosphate buffer, sterile, pH 8.0 (solution 2) as diluent.

Standard solution: To weigh amount of amoxycillin trihydrate SQR equal to 25 g of amoxicillin, transfer to a 50 mL volumetric flask, and top up with water. To transfer 1 mL of this solution to a 100 mL volumetric flask, top up with 0.1 M potassium phosphate buffer, sterile, pH 8.0 (solution 2), and stir. To successively dilute to obtain 0.05 µg/mL, 0.10 µg/mL, and 0.20 µg/mL concentrations, by using the

0.1 M potassium phosphate buffer, sterile, pH 8.0 (solution 2) as diluent.

Procedure: to add 21 mL of culture medium number 11 in each plate, wait to solidify, add 4 mL of 0.5% inoculum, and proceed as described in *Microbiological test of antibiotics (5.5.3.3.1)*, by adding 0,2 mL of freshly prepared solutions to the cylinders. To calculate the potency of the sample per mg of amoxicillin (capsule) from the potency of the standard and responses obtained with the *Standard and sample solutions*.

B. To proceed as described in *Iodometric test for antibiotics (5.3.3.10)*. To remove the contents of the capsules and weigh them. To make the content of the capsules homogeneous. To transfer amount of powder accurately weighed to volumetric flask and dilute with water, to obtain a 1.25 mg/mL trihydrate amoxicillin solution. To stir 3-5 minutes. To prepare standard solution under the same conditions.

PACKAGING AND STORAGE

In tightly closed containers, at 15 °C - 25 °C.

LABELLING

To comply with the legislation in force.

AMOXYCILLIN TRIHYDRATE, POWDER FOR ORAL SUSPENSION

Amoxicillin trihydrate, powder for oral suspension, is a mixture of one or more agents suitable for suspension, with or without coloring, flavoring, preservative, buffer, sweetener, and stabilizer agents. It contains at least 90.0 % and at most 120 % of the amount stated of $C_{16}H_{19}N_3O_5S$. Amoxicillin trihydrate used in the production meets the specifications outlined in the monograph *Amoxicillin trihydrate*.

IDENTIFICATION

To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using G 60 silica gel as support and a mixture of methanol, chloroform, water, and acetone (9:8:3:1) as mobile phase. To separately apply to the plate 5 µl of each of the solutions, as described below, which should be used within 10 minutes after preparation:

Solution (1): 0.4 % solution (w/v) of the sample in 0.1 M hydrochloric acid.

Solution (2): 0.4 % amoxicillin trihydrate SQR solution (w/v) in 0.1 M hydrochloric acid.

To develop the chromatogram. To remove the plate and allow it to air dry, and nebulize with ninhydrin SR. To heat in an oven for 15 minutes at 110 °C. The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

CHARACTERISTICS

pH (5.2.19). 5.0 to 7.5. To determine in the reconstituted suspension as informed on the label.

Determination of weight (5.1.1). It complies with the test.

Determination volume (5.1.2). It complies with the test for *Liquids in multiple-dose containers*. To determine in the reconstituted suspension as informed on the label.

PURITY TESTS

Water (5.2.20.1). At most 3.0%. To determine in 0.3 g of the sample.

BIOLOGICAL SAFETY TEST

Counting of the total viable micro-organisms (5.5.3.1.2). It complies with the test.

Survey and identification of pathogens (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Microbiological test of antibiotics (5.5.3.3.1)* by using cylinders with the agar diffusion method.

Micro-organism: *Micrococcus luteus* ATCC 9341.

Culture media: culture medium number 1, to maintain the micro-organism; sterile saline solution to the standardization of the inoculum; and culture medium number 11, to the base layer and inoculum layer in the plate.

Sample solution: to transfer the equivalent to 250 mg of amoxicillin to a 250 mL volumetric flask. To top up with water and stir for about 30 minutes. To transfer 1 mL of this solution to a 100 mL volumetric flask, top up with *potassium phosphate buffer, sterile, pH 8.0 (solution 2)*, and stir. To successively dilute to obtain 0.05 µg/mL, 0.10 µg/mL, and 0.20 µg/mL concentrations, by using the *potassium phosphate buffer, sterile, pH 8.0 (solution 2)* as diluent.

Standard solution: To weigh amount of trihydrate amoxicillin SQR equal to 25 g of amoxicillin and transfer to a 50 mL volumetric flask. To top up to volume with water. To transfer 1 mL of this solution to a 100 mL volumetric flask, top up with *potassium phosphate buffer, sterile, pH 8.0 (solution 2)*, and stir. To successively dilute to obtain 0.05 µg/mL, 0.10 µg/mL, and 0.20 µg/mL concentrations, by using the *potassium phosphate buffer, sterile, pH 8.0 (solution 2)* as diluent.

Procedure: to add 21 mL of culture medium number 11 in each plate, wait to solidify, add 4 mL of 0.5% inoculum, and proceed as described in *Microbiological test of antibiotics*, by adding 0.2 mL of freshly prepared solutions to

the cylinders. To calculate the potency of the sample per mg of amoxicillin per mm, from the potency of the standard and responses obtained with the *Standard and sample solutions*.

B. To proceed as described in *Iodometric test for antibiotics (5.3.3.10)*. To reconstitute the contents as indicated by the manufacturer. To transfer amount of powder accurately weighed to volumetric flask and dilute with water, to obtain a 1.25 mg/mL amoxicillin trihydrate solution. To stir 3-5 minutes. To prepare standard solution under the same conditions.

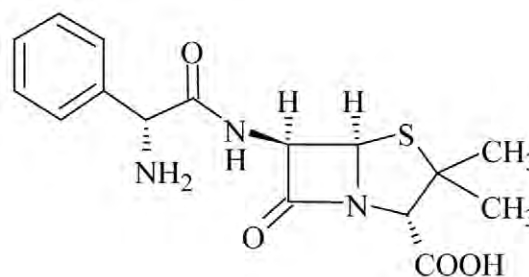
PACKAGING AND STORAGE

In tightly closed containers, at 15 °C - 25 °C.

LABELLING

To comply with the legislation in force.

AMPICILLIN Ampicillinum



C₁₆H₁₉N₃O₄S; 349.40

ampicillin; 00738

(2*S*,5*R*,6*R*)-6-[[*(2R)*-2-amino-2-phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid
[69-53-4]

It shows minimum potency of 900 µg and at most 1050 µg of C₁₆H₁₉N₃O₄S per milligram, relative to the anhydrous substance.

DESCRIPTION

Physical characteristics. Crystalline, white to light yellow powder.

Solubility. Sparingly soluble in water and methanol; practically insoluble in acetone, chloroform, absolute ethanol, and ethyl ether; insoluble in benzene and carbon tetrachloride. Soluble in dilute acid and alkaline solutions.

Physical and chemical constants

Melting range (5.2.2): 199 °C to 202 °C.

Specific rotation (5.2.8): +280° to +305, in relation to the anhydrous substance. To determine at 0.25% solution (w/v).

IDENTIFICATION

The identification test **A.** may be omitted if the tests **B.** and **C.** are conducted. The identification tests **B.** and **C.** can be omitted if the test **A.** is carried out.

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the ampicillin SQR spectrum similarly prepared.

B. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using H silica gel, as support, and a mixture of acetone and 15.4% ammonium acetate (w/v) with pH 5.0 adjusted with glacial acetic acid (10:90) as mobile phase. To apply separately to the plate 2 µL of each of the solutions freshly prepared, as described below.

Solution (1): 2.5 mg/mL solution of the sample in 4.2% sodium bicarbonate (w/v).

Solution (2): 2.5 mg/mL solution of ampicillin SQR in 4.2% sodium bicarbonate (w/v).

To develop the chromatogram. To remove the plate, and let it drying. To expose it to iodine vapor until the spots appear. The principal spot obtained in the chromatogram with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

C. To transfer about 2 mg of the sample to a test tube. To moisten with 0.05 mL of water. To add 2 mL of mixture of sulfuric acid and formaldehyde (2:100) and stir. The solution is almost colorless. To heat in water bath for 1 minute. The dark yellow color develops.

PURITY TESTS

pH (5.2.19). 3.5 to 6.0. To determine in 1% aqueous solution (w/v).

Crystallinity. To suspend some particles of the sample in mineral oil. To transfer to a microscope slide and examine in polarized light microscopy. The particles exhibit birefringence, which is extinguished by micrometric adjustment.

Limit of *N, N*-dimethylaniline. To proceed as described in *Gas Chromatography* (5.2.17.5). To use gas chromatograph equipped with flame ionization detector, 2 m long glass column and 2 mm inner diameter packed with silanized diatomaceous support, impregnated with 3% (w/w) of phenylmethylsilicone (50% phenyl); column temperature at 120 °C; injector and detector temperature 150 °C; nitrogen as carrier gas, flow of 30 mL/min.

Internal standard solution: 0.05 mg/mL naphthalene solution in cyclohexane.

Sample solution: to dissolve 1 g of the sample in 5 mL of *M* sodium hydroxide and add 1 mL of *Internal standard solution*. To vigorously stir for 1 minute, centrifuge, if necessary, and use the supernatant.

Dimethylaniline solution: to dissolve 50 mg of *N, N*-dimethylaniline in a mixture of 2 mL of hydrochloric acid and 20 mL of water by stirring. To top up the volume to 50 mL with water and stir. To transfer 5 mL to 250 mL volumetric flask, top up with water, and stir. To transfer 1 mL to test tube, add 5 mL of *M* sodium hydroxide, 1 mL of *Internal standard solution*, and vigorously stir for 1 minute. To centrifuge, if necessary, and use the supernatant.

Procedure: to separately inject 1 µL of *Dimethylaniline solution* and 1 µL of *Sample solution*, record the chromatograms, and measure the areas under the dimethylaniline and naphthalene peaks.

The area under the dimethylaniline peak obtained with the *Sample solution* is not greater than the area under the main peak obtained with the *Dimethylaniline solution* (0.02%).

Water (5.2.20.1). At most 2.0%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.5%.

BIOLOGICAL SAFETY TEST

Ampicillin for preparing parenteral preparation complies with the following additional tests.

Sterility (5.5.3.2.1). It complies with the test. To dissolve 6 g of the sample in 800 mL of *Fluid II* containing sufficient amount of β-lactamase, to inactivate ampicillin, stir to obtain total solubilization, and proceed as described in *Membrane filtration method*.

Pyrogen (5.5.2.1). It complies with the test. To inject 1 mL/kg of 2 mg/mL of ampicillin in 0.05 *M* sodium hydroxide.

Bacterial endotoxins (5.5.2.2). At most 0.15 UE/mg of ampicillin.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Microbiological test of antibiotics* (5.5.3.3.1) by using agar diffusion method.

Note: dilutions of Standard and sample solutions for the standard curve must be simultaneously prepared.

Sample solution: to accurately dissolve weighed amount of the sample in sterile water to obtain a 0.1 mg/mL solution. To successively dilute with 0.1 *M* potassium phosphate

buffer, sterile, pH 8.0 (Solution 2) to obtain solutions at the concentration range appropriate for the standard curve.

Standard solution: to accurately dissolve weighed amount of ampicillin SQR in sterile water to obtain a 0.1 mg/mL solution. To successively dilute with 0.1 M potassium phosphate buffer, sterile, pH 8.0 (Solution 2) to obtain solutions at the concentration range appropriate for the standard curve.

Procedure: to proceed as described in *Microbiological agar diffusion test (5.5.3.3.1)*. To calculate the potency of the sample in μg of $\text{C}_{16}\text{H}_{19}\text{N}_3\text{O}_4\text{S}$ per milligram from the potency of the standard and the responses obtained with the *Standard and sample solutions*.

B. To proceed as described in *Iodometric test for antibiotics (5.3.3.10)*. To similarly prepare the *Standard solution* and the *Sample solution*.

C. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 254 nm; 300 mm column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μm), flow of *Mobile phase* of 2.0 mL/min.

Mobile phase: mixture of water, acetonitrile, 0.1 M monobasic potassium phosphate, and M acetic acid (90.9:8.0:1.0:0.1).

Diluent: to transfer 10 mL of 0.1 M monobasic potassium phosphate and 1 mL of M glacial acetic acid to 1000 mL volumetric flask and top up to volume with water.

Standard solution: to accurately transfer about 25 mg of ampicillin SQR to a 25 mL volumetric flask, top up with *Diluent*, and make it homogeneous.

Sample solution: to accurately transfer about 100 mg of the sample to a 100 mL volumetric flask, top up with *Diluent*, and make it homogeneous.

Resolution solution: to dissolve sufficient amount of caffeine in *Standard solution* to obtain a solution containing 0.12 mg/mL.

To inject replicates of 20 μL of the *Resolution solution*. The resolution between caffeine and ampicillin peaks should not be less than 2.0. The tailing factor to ampicillin peak should not be greater than 1.4. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2 %.

Procedure: to separately inject 20 μL of *Sample and standard solutions*, record the chromatograms, and measure the area under the peaks. To calculate in μg the content of $\text{C}_{16}\text{H}_{19}\text{N}_3\text{O}_4\text{S}$ per milligram, from the sample, from the responses obtained for the *Sample and Standard solutions*.

PACKAGING AND STORAGE

In airtight closed containers, at temperature below 30 °C.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antibiotic.

AMPICILLIN, CAPSULES

It contains at least 90.0 % and at most 120.0 % of the amount stated of $\text{C}_{16}\text{H}_{19}\text{N}_3\text{O}_4\text{S}$.

IDENTIFICATION

To proceed as described in *Identification test B*, in the monograph *Ampicillin*. To prepare *Solution (1)* as described below.

Solution (1): to weigh capsules, remove the content, and weigh them again. To make the content of the capsules homogeneous. To stir amount of powder equivalent to 0.125 g of ampicillin with 4.2% sodium bicarbonate (w/v) and dilute to 50 mL with the same solvent. To filter it.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL

Equipment: baskets, 100 rpm

Time: 45 minutes

Procedure: after testing, remove aliquot from the dissolution medium, filter, and dilute in cupric sulfate buffer to obtain suitable concentration. To transfer 10 mL to test tube with lid, heat in a water bath at 75 °C for 30 minutes, and cool down quickly. To measure the absorbances of the solutions at 320 nm (5.2.14) by using aliquot from the dissolution medium diluted in cupric sulfate buffer without heating, to adjust zero. To calculate the amount of $\text{C}_{16}\text{H}_{19}\text{N}_3\text{O}_4\text{S}$ dissolved in the medium, by comparing the readings obtained with 0.0022 % ampicillin SQR solution (w/v) similarly prepared.

Tolerance: not less than 75% (Q) of the stated amount of $\text{C}_{16}\text{H}_{19}\text{N}_3\text{O}_4\text{S}$ dissolves within 45 minutes.

PURITY TESTS

Water (5.2.20.1). At most 4%.

BIOLOGICAL SAFETY TEST

Counting of the total viable micro-organisms (5.5.3.1.2).
It complies with the test.

Survey and identification of pathogens (5.5.3.1.3).

It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Microbiological test of antibiotics (5.5.3.3)* by using agar diffusion method.

Note: dilutions of Standard and sample solutions for the standard curve must be simultaneously prepared.

Sample solution: to weigh 20 capsules, remove the content, and weigh them again. To make the content of the capsules homogeneous.

To transfer amount of powder accurately weighed to volumetric flask and dilute with sterile water, to obtain a 0.1 mg/mL ampicillin solution. To stir 3-5 minutes. To successively dilute with 0.1 M potassium phosphate buffer, sterile, pH 8.0 (Solution 2) to obtain solutions at the concentration range appropriate for the standard curve.

Standard solution: to accurately dissolve weighed amount of ampicillin SQR in sterile water to obtain a 0.1 mg/mL solution. To successively dilute with 0.1 M potassium phosphate buffer, sterile, pH 8.0 (Solution 2) to obtain solutions at the concentration range appropriate for the standard curve.

Procedure: to proceed as described in *Microbiological agar diffusion test (5.5.3.3.1)*. To calculate the amount in mg of $C_{16}H_{19}N_3O_4S$ inside the capsules, from the potency of the standard and the responses obtained with the Standard and sample solutions.

B. To proceed as described in *Iodometric test for antibiotics (5.3.3.10)*. To weigh 20 capsules, remove their contents, and weigh them again. To make the content of the capsules homogeneous. To transfer accurately weighed amount of powder to volumetric flask, add water, stir for 3-5 minutes, and adjust the volume with the same solvent, to obtain 1.25 mg/mL ampicillin solution ($C_{16}H_{19}N_3O_4S$). To transfer 2 mL of this solution to 125 mL erlenmeyer flask with lid. To prepare standard solution under the same conditions.

PACKAGING AND STORAGE

In airtight closed containers, at 15 °C - 25 °C.

LABELLING

To comply with the legislation in force.

AMPICILLIN, TABLETS

It contains at least 90.0 % and at most 120.0 % of the amount stated of $C_{16}H_{19}N_3O_4S$.

IDENTIFICATION

To proceed as described in *Identification test B*, in the monograph *Ampicillin*. To prepare *Solution (1)* as described below.

Solution (1): to weigh and pulverize the tablets. To stir amount of powder equivalent to 0.125 g of ampicillin with 4.2% sodium bicarbonate (w/v) and dilute to 50 mL with the same solvent. To filter it.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL

Equipment: baskets, 100 rpm

Time: 45 minutes

Procedure: after testing, remove aliquot from the dissolution medium, filter, and dilute in cupric sulfate buffer to obtain suitable concentration. To transfer 10 mL to test tube with lid, heat in a water bath at 75 °C for 30 minutes, and cool down quickly. To measure the absorbances of the solutions at 320 nm (5.2.14) by using aliquot from the dissolution medium diluted in cupric sulfate buffer without heating, to adjust zero. To calculate the amount of $C_{16}H_{19}N_3O_4S$ dissolved in the medium, by comparing the readings obtained with 0.0022 % ampicillin SQR solution (w/v) similarly prepared.

Tolerance: not less than 75% (Q) of the stated amount of $C_{16}H_{19}N_3O_4S$ dissolves within 45 minutes.

PURITY TESTS

Water (5.2.20.1). At most 4.0%.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Microbiological test of antibiotics (5.5.3.3.1)* by using agar diffusion method.

Note: dilutions of Standard and sample solutions for the standard curve must be simultaneously prepared.

Sample solution: To weigh and pulverize 20 tablets. To transfer amount of powder accurately weighed to volumetric flask and dilute with sterile water, to obtain a 0.1 mg/mL ampicillin solution. To stir for 3-5 minutes. To successively dilute with 0.1 M potassium phosphate buffer, sterile, pH 8.0 (Solution 2) to obtain solutions at the concentration range appropriate for the standard curve.

Standard solution: to accurately dissolve weighed amount of ampicillin SQR in sterile water to obtain a 0.1 mg/mL solution.

To successively dilute with 0.1 M potassium phosphate buffer, sterile, pH 8.0 (Solution 2) to obtain solutions at the concentration range appropriate for the standard curve.

Procedure: to proceed as described in *Microbiological agar diffusion test (5.5.3.3.1)*. To calculate the amount in mg of $C_{16}H_{19}N_3O_4S$ in the tablets, from the potency of the standard and the responses obtained with the Standard and sample solutions.

B. To proceed as described in *Iodometric test for antibiotics (5.5.3.3.10)*. To weigh and pulverize 20 tablets. To transfer accurately weighed amount of powder to volumetric flask, add water, stir for 3-5 minutes, and top up the volume with the same solvent, to obtain 1.25 mg/mL ampicillin solution ($C_{16}H_{19}N_3O_4S$). To transfer 2 mL of this solution to 125 mL erlenmeyer flask with lid. To prepare standard solution under the same conditions.

PACKAGING AND STORAGE

In airtight closed containers, protected from moisture, at temperature below 30 °C.

LABELLING

To comply with the legislation in force.

AMPICILLIN, POWDER FOR ORAL SUSPENSION

Ampicillin powder for oral suspension is a mixture of ampicillin with one or more coloring, flavoring, buffer, sweetener, and preservative agents. It contains at least 90.0 % and at most 120 % of the amount stated of ampicillin ($C_{16}H_{19}N_3O_4S$).

IDENTIFICATION

To proceed as described in *Identification test B*, in the monograph *Ampicillin*. To prepare *Solution (1)* as described below.

Solution (1): to determine the oral suspension as informed on the label. To stir amount of oral suspension equivalent to 0.125 g of ampicillin with 4.2% sodium bicarbonate (w/v) and dilute to 50 mL with the same solvent. To filter it.

CHARACTERISTICS

Determination volume (5.1.2). It complies with the test for *Liquids in multiple-dose containers*. To determine in the reconstituted suspension as informed on the label.

pH (5.2.19). 5.0 to 7.5. To determine in the reconstituted suspension as informed on the label.

Determination of weight (5.1.1). It complies with the test. To determine in the not reconstituted powder.

Uniformity of dosage units (5.1.6). It complies with the test for solid packaged in single-dose containers.

PURITY TESTS

Water (5.2.20.1). At most 2.5%.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Microbiological test of antibiotics (5.5.3.3.1)* by using cylinders with the agar diffusion method.

Note: dilutions of Standard and sample solutions for the standard curve must be simultaneously prepared.

Sample solution: to reconstitute the suspension as informed on the label. To transfer amount of suspension accurately measured to volumetric flask and dilute with sterile water,

to obtain a 0.1 mg/mL ampicillin solution. To stir 3-5 minutes. To successively dilute with 0.1 M potassium phosphate buffer, sterile, pH 8.0 (Solution 2) to obtain solutions at the concentration range appropriate for the analytical curve.

Standard solution: to accurately dissolve weighed amount of ampicillin SQR in sterile water to obtain a 0.1 mg/mL solution. To successively dilute with 0.1 M potassium phosphate buffer, sterile, pH 8.0 (Solution 2) to obtain solutions at the concentration range appropriate for the analytical curve.

Procedure: to proceed as described in *Microbiological agar diffusion test (5.5.3.3.1)*. To calculate the amount in mg of $C_{16}H_{19}N_3O_4S$ in the reconstituted oral suspension, from the potency of the standard and the responses obtained with the *Standard and sample solutions*.

B. To proceed as described in *Iodometric test for antibiotics (5.3.3.10)*. To reconstitute the contents of 10 units according to the label. To mix and make the contents of the vials homogeneous. To transfer accurately measured amount of oral suspension to volumetric flask, add water, stir for 3-5 minutes, and top up with the same solvent, to obtain 1.25 mg/mL ampicillin solution ($C_{16}H_{19}N_3O_4S$). To transfer 2 mL of this solution to 125 mL erlenmeyer flask with lid. To prepare standard solution under the same conditions.

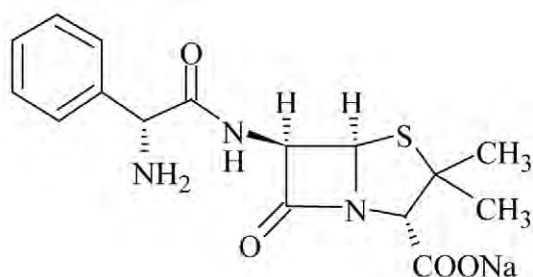
PACKAGING AND STORAGE

In airtight closed containers, protected from moisture, at temperature below 25 °C.

LABELLING

To comply with the legislation in force.

AMPICILLIN, SODIUM Ampicillinum natrium



$C_{16}H_{18}NaN_3O_4S$; 371.39
ampicillin sodium; 00741

Sodium salt of (2*S*, 5*R*, 6*R*)-6-[[[(2*R*)-2-amino-2-phenylacetyl] amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid (1:1) [69-52-3]

It shows minimum potency of 845 µg and at most 988 µg of ampicillin ($C_{16}H_{19}N_3O_4S$) per milligram, relative to the anhydrous substance.

DESCRIPTION

Physical characteristics. White, hygroscopic, crystalline powder.

Solubility. Very insoluble in water, soluble in acetone, sparingly soluble in chloroform, practically insoluble in ethyl ether.

Physical and chemical constants

Melting range (5.2.2): 203 °C to 206 °C.

Specific rotation (5.2.8): 258° to 287°, determined in 0.25% solution (w/v) and 0.4% potassium biphthalate solution (w/v) as solvent, calculated relative to the anhydrous substance.

IDENTIFICATION

The identification test **A.** may be omitted if the tests **B.** and **C.** are conducted. The identification tests **B.** and **C.** can be omitted if the test **A.** is carried out.

A. To dissolve 250 mg of the sample in 5 mL of water, add 0.5 mL of 2 M acetic acid, stir, and let it rest for 10 minutes in an ice bath. To filter through a sintered glass filter under reduced pressure.

To wash with 2 - 3 mL of mixture of 9 parts acetone and 1 part of water and dry it at 60 °C for 30 minutes. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the ampicillin sodium SQR spectrum similarly prepared.

B. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF-254 silica gel, as support, and a mixture of acetone and 15.4% ammonium acetate (w/v) (90:10), with pH 5.0 adjusted with glacial acetic acid. To apply separately to the plate 2 µl of each of the solutions freshly prepared, as described below.

Solution (1): 0.5 % solution (w/v) of the sample in 4.2% sodium bicarbonate (w/v).

Solution (2): 0.5 % ampicillin sodium SQR solution (w/v) in 4.2% sodium bicarbonate (w/v).

To develop the chromatogram. To remove plate, let it air dry, nebulize with 0.3% ninhydrin alcoholic solution (w/v), and warm in dry heat oven at 90°C for 15 minutes. To examine under visible light. The principal spot obtained in the chromatogram with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

C. To transfer about 2 mg of the sample to a test tube. To moisten with 0.05 mL of water and add 2 mL of the mixture of 2 mL of formaldehyde solution with 100 mL of sulfuric acid. To stir the tube and observe the color. It must be nearly colorless. To immerse the tube in a water bath for 1 minute. The reddish-brown color develops.

D. It responds to the reactions of the sodium ion

(5.3.1.1). PURITY TESTS

pH (5.2.19). 8.0 to 10.0. To determine in 1% aqueous solution (w/v).

Water (5.2.20). At most 2.0%.

Crystallinity. To suspend in mineral oil some particles from the sample. Then, transfer it to a microscope slide and examine by polarized light microscope. The particles exhibit birefringence, which is extinguished by micrometric adjustment.

N, N-Dimethylaniline At most 0.02% (200 ppm). To proceed as described in Gas Chromatography (5.2.17.5). To use gas chromatograph equipped with flame ionization detector, (2 m x 2 mm) long glass column packed with silanized diatomaceous support, impregnated with 3% (w/w) of phenylmethylsilicone (50% phenyl); kept at 120 °C; injector and detector at 150 °C; nitrogen as carrier gas for chromatography; flow of 30 mL/min.

Internal standard solution: 0.05 % naphthalene solution (w/v) in cyclohexane.

Dimethylaniline standard solution: to dissolve 50 mg of N, N-dimethylaniline in a mixture of 2 mL of hydrochloric acid and 20 mL of water by stirring. To top up the volume to 50 mL with water and stir. To transfer 5 mL to 250 mL volumetric flask, top up with water, and stir. To transfer 1 mL to test tube, add 5 mL of M sodium hydroxide, 1 mL of Internal standard solution, vigorously stir for 1 minute, centrifuge, if necessary, and use the supernatant.

Sample solution: To dissolve 1 g of the sample in 5 mL of M sodium hydroxide, 1 mL of the sample A, vigorously stir for 1 minute, centrifuge, if necessary, and use the supernatant.

Procedure: to separately inject 1 µL of Sample and standard solutions, record the chromatograms, and measure the areas under the main peaks.

Methylene chloride. To proceed as described in Gas Chromatography (5.2.17.5). To use gas chromatograph equipped with flame ionization detector, (105 m x 4 mm) long glass column packed with silanized diatomaceous support (particles up to 120 µm), washed in acid, coated by 10% macrogol 1000; kept at 100 °C; injector at 100 °C and detector at 150 °C; nitrogen as carrier gas for chromatography; flow of 40 mL/min.

Standard solution: to transfer 1 mL of 0.2% aqueous methylene chloride solution (v/v) to a 100 mL volumetric flask. To add 1 mL of 0.2% aqueous ethylene dichloride solution (v/v) (internal standard), top up to volume with water, and stir.

Sample solution: to dissolve 10 g of the sample in water and transfer to a 100 mL volumetric flask. To add 1.0 mL of 0.2% aqueous ethylene dichloride solution (v/v) (internal standard), top up to volume with water, and stir.

Procedure: to separately inject 1 µL of the Standard solution and Sample solution, record the chromatograms, and calculate the percentage (w/w) of methylene chloride, by considering 1.325 g/mL for density value at 20 °C. No more than 0.2% (w/w).

BIOLOGICAL SAFETY TEST

Ampicillin sodium for preparing parenteral preparation must comply with the following additional tests. When the label states that the substance is sterile, the sample complies with Bacterial endotoxins or Pyrogens tests, as well as Sterility test.

Sterility (5.5.3.2.1). It complies with the test. To use Membrane filtration method. To dissolve 6 g of the sample in 800 mL of Fluid II containing sufficient amount of sterile penicillinase, to inactivate ampicillin, stir to obtain total solubilization, and proceed as described.

Pyrogens (5.5.2.1). It complies with the test. To inject 1 mL/kg, by using 20 mg/mL ampicillin sodium solution in water.

Bacterial endotoxins (5.5.2.2). At most 0.15 UE/ mg of ampicillin.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in Microbiological test of antibiotics (5.5.3.3). To separately dissolve ampicillin sodium SQR sample in sterile water, to obtain the 0.1 mg/mL solution each. To dilute the solutions obtained in 0.1 M potassium phosphate buffer, sterile, pH 8.0 (Solution 2), according to the concentrations used in the standard curve.

B. Iodometric method. To dissolve and dilute sample and ampicillin sodium SQR in water to obtain concentration about 1.25 mg/mL. To transfer 2 mL of standard solution to erlenmeyer flask with ground lid and add 2 mL of M sodium hydroxide. To stir and let it rest for 15 minutes. To add 2 mL of 1.2 M hydrochloric acid and 10 mL of 0.01 M SV iodine (w/v). Let it rest for 15 minutes protected from light. To titrate with 0.01 M SV sodium thiosulfate. To add 3 drops of starch SI near the endpoint and continue titration until the blue color disappears. To proceed the same test with the sample solution. To perform sample blank correction, including sample and standard, by titration of 2 mL of both solutions by adding 10 mL of 0.01 M SV iodine and

0.1 mL of *M* hydrochloric acid. To add 3 drops of starch SI near the endpoint and continue titration until the blue color disappears. To titrate with 0.01 *M* SV sodium thiosulfate.

$$P = \frac{(V_{ba} - V_a) \times P_o \times P_p}{(V_{bp} - V_p) \times P_a}$$

where

P = potency of the sample (µg/mg);

*V*_{ba} = volume of titrant spent in titration of sample blank (mL),

*V*_a = volume of titrant spent in titration of sample (mL),

*V*_{bp} = volume of titrant spent in titration of standard blank (mL),

*V*_p = volume of titrant spent in titration of standard (mL),

*P*_o = potency of standard (µg/mg); *P*_p = weight of the standard (mg),

*P*_a = weight of the sample (mg).

PACKAGING AND STORAGE

In airtight closed containers, at temperature below 30 °C. For injectable purposes, it shall be packaged in sterile containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antibiotic.

AMPICILLIN SODIUM, POWDER FOR INJECTABLE SOLUTION

It contains at least 90.0% and at most 115.0% of the value informed in C₁₆H₁₉N₃O₄S.

IDENTIFICATION

To proceed as described in *Identification test B.*, in the monograph *Ampicillin sodium*.

CHARACTERISTICS

pH (5.2.19). 8.0 to 10.0. To determine in 1% aqueous solution (w/v).

Determination of weight (5.1.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

PURITY TESTS

Water (5.2.20.1). At most 2.0%.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test. To use Membrane filtration method. To dissolve 6 g of the sample in 800 mL of *Fluid II* containing sufficient amount of sterile penicillinase, to inactivate ampicillin, stir to obtain total solubilization, and proceed as described.

Pyrogens (5.5.2.1). It complies with the test. To inject 1 mL/kg, by using 20 mg/mL ampicillin sodium solution in water.

Bacterial endotoxins (5.5.2.2). At most 0.15 UE/ mg of ampicillin.

DOSAGE

To determine the potency of the powder for injectable solution for ampicillin, employ one of the methods described below by using at least 10 vials.

A. To proceed as described in *Microbiological test of antibiotics (5.5.3.3)*. To reconstitute the contents of 10 vials as indicated by the manufacturer. To separately dissolve the standard and sample in sterile water, to obtain the 0.1 mg/mL solution.

To separately dilute the sample and standard solutions in 0.1 *M* potassium phosphate buffer, sterile, pH 8.0 (*Solution 2*), according to the concentrations used in the standard curve.

B. To proceed as described in *Iodometric test for antibiotics (5.3.3.10)*. To reconstitute the contents of 10 vials as indicated by the manufacturer. To dissolve the sample reconstituted in water to obtain concentration about 1.25 mg/mL. To transfer 2 mL of the standard solution to 125 mL erlenmeyer flask with a ground lid. To prepare standard solution under the same conditions.

PACKAGING AND STORAGE

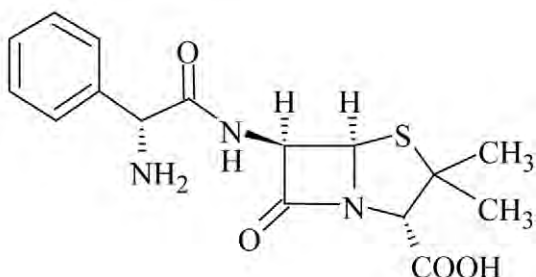
In airtight closed containers, at temperature below 25 °C.

LABELLING

To comply with the legislation in force.

AMPICILLIN, TRIHYDRATE

Ampicillinum trihydricum



$C_{16}H_{19}N_3O_4S \cdot 3H_2O$; 403.45
ampicillin trihydrate; 00742

(2*S*, 5*R*, 6*R*)-6-[[[(2*R*)-2-amino-2-phenylacetyl] amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid hydrate (1:3) [7177-48-2]

It shows minimum potency of 900 µg and at most 1050 µg of ampicillin ($C_{16}H_{19}N_3O_4S$) per milligram, relative to the anhydrous substance.

DESCRIPTION

Physical characteristics. White crystalline powder.

Solubility. Sparingly soluble in water, practically insoluble in ethanol, chloroform, ethyl ether, and fixed oils. Soluble in dilute mineral acid solutions and alkalis.

Physical and chemical constants

Specific rotation (5.2.8): +280° to +305°, in relation to the anhydrous substance. To determine in 0.25% aqueous solution (w/v).

IDENTIFICATION

The identification test **A.** may be omitted if the tests **B.** and **C.** are conducted. The identification tests **B.** and **C.** can be omitted if the test **A.** is carried out.

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the ampicillin trihydrate SQR spectrum similarly prepared.

B. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using H silica gel, as support, and a mixture of acetone and 15.4% ammonium acetate (w/v) (10:90) with pH 5.0 adjusted with glacial acetic acid as mobile phase. To separately apply to the plate 1 µL of each of the solutions freshly prepared, as described below.

Solution (1): sample of the solution containing the equivalent to 2.5 mg of $C_{16}H_{19}N_3O_4S$ per mL in 4.2% sodium bicarbonate (w/v).

Solution (2): 2.5 mg/mL ampicillin SQR solution in 4.2% sodium bicarbonate (w/v).

Solution (3): 2.5 mg/mL ampicillin SQR solution and 2.5 mg/mL amoxycillin trihydrate SQR in 4.2% sodium bicarbonate (w/v).

To develop the chromatogram. To remove the plate, allow to air dry, and expose it to iodine vapor to appear the spots. To examine under visible light. The principal spot obtained in the chromatogram with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*. The test is valid only if the chromatogram obtained with *Solution (3)* has two well-defined spots.

C. To transfer about 2 mg of the sample to a test tube. To moisten with 0.05 mL of water and add 2 mL of the mixture of formaldehyde solution and sulfuric acid (2:100). To stir and observe the color. The solution is almost colorless. To heat in water bath for 1 minute. The dark yellow color develops.

PURITY TESTS

pH (5.2.19). 3.5 to 6.0. To determine in 1% aqueous solution (w/v).

Crystallinity. To suspend in mineral oil some particles from the sample. Then, transfer it to a microscope slide and examine by polarized light microscope. The particles exhibit birefringence, which is extinguished by micrometric adjustment.

Limit of *N*, *N*-dimethylaniline. To proceed as described in *Gas Chromatography* (5.2.17.5). To use gas chromatograph equipped with flame ionization detector, 2 m long glass column and 2 mm inner diameter packed with silyanized diatomaceous support, impregnated with 3% (w/w) of phenylmethylsilicone

(50% phenyl); column temperature at 120 °C; injector and detector temperature at 150 °C; nitrogen as carrier gas, flow of 30 mL/min.

Internal standard solution: 0.05 mg/mL naphthalene solution in cyclohexane.

Sample solution: to dissolve 1 g of the sample in 5 mL of 1 *M* sodium hydroxide and add 1 mL of *Internal standard solution*. To vigorously stir for 1 minute, centrifuge, if necessary, and use the supernatant.

Dimethylaniline solution: to dissolve 50 mg of *N*, *N*-dimethylaniline in a mixture of 2 mL of hydrochloric acid and 20 mL of water by stirring. To top up the volume to 50 mL with water and stir. To transfer 5 mL to 250 mL volumetric flask, top up with water, and stir. To transfer 1 mL to test tube, add 5 mL of 1 *M* sodium hydroxide, 1 mL of *Internal standard solution*, and vigorously stir for 1 minute. To centrifuge, if necessary, and use the supernatant.

Procedure: to separately inject 1 µL of *Dimethylaniline solution* and 1 µL of *Sample solution*, record the chromatograms, and measure the areas under the dimethylaniline and naphthalene peaks. The area under the dimethylaniline

peak obtained with the *Sample solution* is not greater than the area under the main peak obtained with the *Dimethylaniline solution* (0.02%).

Water (5.2.20.1). 12.0% to 15.0%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.5%.

BIOLOGICAL SAFETY TEST

When the label states that the substance is sterile, the sample complies with Bacterial endotoxins or Pyrogens tests, as well as Sterility test. Should the sustenance be indicated to sterilization for sterile preparations, the sample complies with Bacterial endotoxins or Pyrogens tests.

Sterility (5.5.3.2.1). It complies with the test. To dissolve 6 g of the sample in 800 mL of *Fluid II* containing sufficient amount of β -lactamase, to inactivate ampicillin, stir to obtain total solubilization, and proceed as described in *Membrane filtration method*.

Pyrogens (5.5.2.1). It complies with the test. To inject 1 mL/kg of 2 % ampicillin (w/v) in 0.05 M sodium hydroxide.

Bacterial endotoxins (5.5.2.2). At most 0.15 UE/ mg of ampicillin.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Microbiological test of antibiotics (5.5.3.3)* by using agar diffusion method.

To separately dissolve amounts accurately weighed of ampicillin SQR and sample in sterile water, to obtain solutions containing about 0.1 mg of $C_{16}H_{19}N_3O_4S$ per milliliter. To separately dilute the sample and standard solutions in 0.1 M potassium phosphate buffer, sterile, pH 8.0 (*Solution 2*), according to the concentrations used in the standard curve. To calculate the potency of the sample, in μ g, of ampicillin per mg, from the potency of the standard and responses obtained with the Standard and sample solutions.

B. To proceed as described in *Iodometric test for antibiotics (5.3.3.10)*. To prepare the standard by using ampicillin SQR. To prepare the sample as described below.

Sample preparation: to dissolve accurately weighed amount of the sample in water and dilute with the same solvent to obtain a 1.25 mg/mL ampicillin solution ($C_{16}H_{19}N_3O_4S$). To transfer 2 mL of this solution to a 125 mL erlenmeyer flask with lid.

To calculate the potency of the sample, in μ g, of $C_{16}H_{19}N_3O_4S$, per milligram, according to the equation:

$$\frac{F (B_A - I_A)}{2 (C_A)}$$

where

B_A = volume of titrant in mL, spent in the sample blank correction for Sample preparation;

I_A = volume of titrant in mL, spent in *Inactivation and titration of Sample preparation*;

C_A = concentration, in mg/mL, of *Sample preparation*, based on the weighed amount of sample and dilution performed.

C. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 254 nm; 300 mm column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μ m), flow of *Mobile phase* of 2 mL/min.

Mobile phase: mixture of water, acetonitrile, 0.1 M monobasic potassium phosphate, and 1 M acetic acid (90.9:8.0:1.0:0.1).

Diluent: to transfer 10 mL of 0.1 M monobasic potassium phosphate and 1 mL of 1 M glacial acetic acid to 1000 mL volumetric flask and top up to volume with water.

Standard solution: to accurately transfer about 25 mg of ampicillin SQR to a 25 mL volumetric flask, top up with *Diluent*, and make it homogeneous.

Sample solution: to accurately transfer about 100 mg of the sample to a 100 mL volumetric flask, top up with *Diluent*, and make it homogeneous.

Resolution solution: to dissolve sufficient amount of caffeine in *Standard solution* to obtain a solution containing 0.12 mg/mL.

To inject replicates of 20 μ L of the *Resolution solution*. The resolution between caffeine and ampicillin peaks should not be less than 2.0. The tailing factor to ampicillin peak should not be greater than 1.4. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2 %.

Procedure: to separately inject 20 μ L of *Sample and standard solutions*, record the chromatograms, and measure the areas under the peaks. To calculate in μ g the content of ampicillin ($C_{16}H_{19}N_3O_4S$) per milligram, from the content of the standard, from the responses obtained for the *Sample and Standard solutions*.

PACKAGING AND STORAGE

In airtight closed containers, at temperature below 30 °C. Ampicillin trihydrate for preparing parenteral preparations must be packaged in sterile containers.



LABELLING

To comply with the legislation in force. Ampicillin trihydrate for preparing sterile preparations must provide on the label whether the substance is sterile or must be sterilized during the process.

THERAPEUTIC CLASS

Antibiotic.

AMPICILLIN, TRIHYDRATE CAPSULES

It contains ampicillin trihydrate equal to at least 90.0 % and at most 120 % of the amount stated of ampicillin ($C_{16}H_{19}N_3O_4S$).

IDENTIFICATION

A. To proceed as described in *Identification test B.*, in the monograph *Ampicillin trihydrate*. To prepare *Solution (1)* as described below.

Solution (1): to weigh capsules, remove the content, and weigh them again. To make the content of the capsules homogeneous. To stir amount of powder in 4.2% sodium bicarbonate (w/v) and dilute with the same solvent to obtain 2.5 mg/mL ampicillin solution ($C_{16}H_{19}N_3O_4S$). To filter it.

B. To weigh the capsules, remove the content, and weigh them again. To make the content of the capsules homogeneous. To transfer the amount of powder equivalent to 10 mg ampicillin to a beaker, add 1 mL of water and 2 mL of alkaline cupric tartrate mixture SR and water (2:6). Violet color develops immediately.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL

Equipment: baskets, 100 rpm

Time: 45 minutes

Procedure: after testing, remove aliquot from the dissolution medium, filter, and dilute in cupric sulfate buffer to obtain suitable concentration. To transfer aliquot of 10 mL to test tube with lid, heat in a water bath at 75 °C for 30 minutes, and cool down quickly. To measure the absorbance of the solutions at 320 nm (5.2.14), by using *Sample solution* not heated to adjust zero. To calculate the amount of $C_{16}H_{19}N_3O_4S$ dissolved in the medium, by comparing the

readings obtained with 0.002 % ampicillin SQR solution (w/v) similarly prepared.

Tolerance: not less than 75% (Q) of the stated amount of $C_{16}H_{19}N_3O_4S$ dissolves within 45 minutes.

PURITY TESTS

Water (5.2.20.1). 10.0% to 15.0%.

BIOLOGICAL SAFETY TEST

Counting of the total viable micro-organisms (5.5.3.1.2). It complies with the test.

Survey and identification of pathogens (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in Microbiological test of antibiotics (5.5.3.3.1) by using agar diffusion method for Ampicillin.

Sample solution: to weigh 20 capsules, remove the content, and weigh them again. To make the content of the capsules homogeneous. To transfer accurately weighed amount of powder to volumetric flask, add 0.1 M potassium phosphate buffer, sterile, pH 8.0 (*Solution 2*), stir for 3-5 minutes, and top up with the same solvent, to obtain 0.1 mg/mL ampicillin solution ($C_{16}H_{19}N_3O_4S$). To successively dilute with the same solvent to concentrations of analytical curve.

Standard solution: to dissolve accurately weighed amount of ampicillin SQR in sterile water and dilute with the same solvent to obtain a 0.1 mg/mL solution.

To successively dilute in 0.1 M potassium phosphate buffer, sterile, pH 8.0 (*Solution 2*) to the concentrations of analytical curve.

To calculate the amount in mg of ampicillin ($C_{16}H_{19}N_3O_4S$) in the capsules, from the potency of the standard and responses obtained with the *Standard and sample solutions*.

B. To proceed as described in *Iodometric test for antibiotics (5.3.3.10)*. To weigh 20 capsules, remove their contents, and weigh them again. To make the content of the capsules homogeneous. To transfer accurately weighed amount of powder to volumetric flask, add water, stir for 3-5 minutes, and top up the volume with the same solvent, to obtain 1.25 mg/mL ampicillin solution ($C_{16}H_{19}N_3O_4S$). To transfer 2 mL of this solution to a 125 mL erlenmeyer flask with lid. To prepare standard solution under the same conditions.

PACKAGING AND STORAGE

In tightly closed containers, at temperature below 30 °C.

LABELLING

To comply with the legislation in force.

AMPICILLIN, TRIHYDRATE TABLETS

It contains ampicillin trihydrate equal to at least 90.0 % and at most 120 % of the amount stated of ampicillin ($C_{16}H_{19}N_3O_4S$).

IDENTIFICATION

A. To proceed as described in *Identification* test **B.**, in the monograph *Ampicillin trihydrate*. To prepare *Solution (1)* as described below.

Solution (1): to weigh and pulverize the tablets. To stir amount of powder in 4.2% sodium bicarbonate (w/v) and dilute with the same solvent to obtain 2.5 mg/mL ampicillin solution ($C_{16}H_{19}N_3O_4S$). To filter it.

B. To weigh and pulverize the tablets. To transfer amount of powder equivalent to 10 mg of ampicillin to a beaker and continue as described in Test B. *Identification*, in the monograph *Ampicillin trihydrate, capsules*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). At most 15 minutes.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL

Equipment: baskets, 100 rpm

Time: 45 minutes

Procedure: after testing, remove aliquot from the dissolution medium, filter, and dilute in cupric sulfate buffer to obtain suitable concentration. To proceed as described in *Dissolution test*, in the monograph *Ampicillin trihydrate, capsules*.

Tolerance: not less than 75% (Q) of the stated amount of $C_{16}H_{19}N_3O_4S$ dissolves within 45 minutes.

PURITY TESTS

Water (5.2.20.1). 9.5% to 12%.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Microbiological test of antibiotics (5.5.3.3.1)* by using *agar diffusion method for Ampicillin*.

Sample solution: To weigh and pulverize 20 tablets. To transfer accurately weighed amount of powder to volumetric flask, add 0.1 M potassium phosphate buffer, sterile, pH 8.0 (*Solution 2*), stir for 3-5 minutes, and top up with the same solvent, to obtain 0.1 mg/mL ampicillin solution ($C_{16}H_{19}N_3O_4S$). To successively dilute with the same solvent to concentrations of standard curve.

Standard solution: to dissolve accurately weighed amount of ampicillin SQR in sterile water and dilute with the same solvent to obtain a 0.1 mg/mL solution. To successively dilute in 0.1 M potassium phosphate buffer, sterile, pH 8.0 (*Solution 2*) to the concentrations of standard curve.

To calculate the amount in mg of ampicillin ($C_{16}H_{19}N_3O_4S$) in the tablets, from the potency of the standard and responses obtained with the *Standard and sample solutions*.

B. To proceed as described in *Iodometric test for antibiotics (5.3.3.10)*. To weigh and pulverize 20 tablets. To transfer accurately weighed amount of powder to volumetric flask, add water, stir for

3-5 minutes, and top up with the same solvent to obtain a 1.25 mg/mL ampicillin solution ($C_{16}H_{19}N_3O_4S$). To transfer 2 mL of this solution to a 125 mL erlenmeyer flask with lid. To prepare the standard by using ampicillin SQR. To prepare standard solution under the same conditions.

PACKAGING AND STORAGE

In airtight closed containers, protected from moisture, at temperature below 30 °C.

LABELLING

To comply with the legislation in force.

AMPICILLIN TRIHYDRATE, POWDER FOR ORAL SUSPENSION

It contains at least 90.0 % and at most 120.0 % of the amount stated of $C_{16}H_{19}N_3O_4S$. The powder for oral suspension contains one or more coloring, flavoring, buffer, sweetener, and preservative agents.

IDENTIFICATION

To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using G silica gel as support and a mixture of acetone, water, toluene, and glacial acetic acid (650:100:100:25) as mobile phase. To apply separately to the plate 2 μ L of each of the solutions freshly prepared, as described below.

Solution (1): solution containing 5 mg/mL of ampicillin in a mixture of acetone and 0.1 M hydrochloric acid (4:1).

Solution (2): solution containing 5 mg/mL of ampicillin SQR in a mixture of acetone and 0.1 M hydrochloric acid (4:1).

To develop the chromatogram. To remove the plate, and let it drying. To nebulize the plate with 0.3% ninhydrin (w/v) in ethanol. To dry in oven at 90 °C for 15 minutes. The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test. To determine in the reconstituted oral suspension as informed on the label.

pH (5.2.19). 5.0 to 7.5. To determine in the reconstituted oral suspension as informed on the label.

Determination of weight (5.1.1). It complies with the test.

PURITY TESTS

Water (5.2.20.2). At most 2.5% in product containing 50 mg/mL of ampicillin after reconstitution or at most 5.0% in product containing 100 mg/mL of ampicillin.

BIOLOGICAL SAFETY TEST

Counting of the number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph

equipped with an ultraviolet detector at 254 nm; 50 mm pre-column and 4.0 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μ m); 250 mm column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μ m); kept at room temperature; flow of *Mobile phase* of 2.0 mL/min.

Mobile phase: mixture of water, acetonitrile, *M* monobasic potassium phosphate, and *M* acetic acid (909:80:10:1).

Diluent: to mix 10 mL of *M* monobasic potassium phosphate and 1 mL of *M* acetic acid. To dilute with water to 1000 mL.

Sample solution: To reconstitute the suspension as described on the product label. To transfer volume of oral suspension equivalent to 0.1 g of ampicillin to a 100 mL volumetric flask, add 75 mL of *Diluent*, and mix it. If necessary, leave it in ultrasound. To top up the volume with the same solvent.

Standard solution: to dissolve accurately weighed amount of ampicillin SQR in *Diluent* and dilute with the same solvent to obtain a 1 mg/mL solution.

Resolution solution: to dissolve accurately weighed amount of caffeine in *Standard solution* and dilute with the same solvent to obtain a 0.12 mg/mL solution.

To inject replicates of 20 μ L of the *Resolution solution*. The resolution between caffeine and ampicillin is not less than 2.0. To inject replicates of 20 μ L of the *Standard solution*. The tailing factor should not be greater than 1.4. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2 %.

Procedure: to separately inject 20 μ L of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of $C_{16}H_{19}N_3O_4S$ in the powder for oral suspension, from the responses obtained for the *Sample and standard solutions*.

B. To proceed as described in *Microbiological test of antibiotics* (5.5.3.3.1) by using cylinders with the agar diffusion method.

Micro-organism: *Micrococcus luteus* ATCC 9341.

Culture media: culture medium number 1, to maintain the micro-organism; culture medium number 11, to the base layer and inoculum preparation.

Sample solution: to reconstitute the contents as indicated by the manufacturer. To transfer volume of oral suspension for volumetric flask and dilute with 0.1 M potassium phosphate buffer, sterile, pH 8.0 (*Solution 2*), to obtain a 0.1 mg/mL ampicillin solution. To successively dilute to obtain 0.05 μ g/mL, 0.1 μ g/mL, and 0.2 μ g/mL concentrations, by using the 0.1 M potassium phosphate buffer, sterile, pH 8.0 (*Solution 2*) as diluent.

Standard solution: to accurately weigh about 25 mg of ampicillin SQR, transfer to a 250 mL volumetric flask, and top up with sterile water. To successively dilute to obtain 0.05 µg/mL, 0.1 µg/mL, and 0.2 µg/mL concentrations, by using the 0.1 M potassium phosphate buffer, sterile, pH 8.0 (Solution 2) as diluent.

Procedure: to add 20 mL of culture medium number 11 in each plate, wait to solidify, add 5 mL of 0.5% inoculum, and proceed as described in *Microbiological test of antibiotics (5.5.3.3.1)*, by adding 0.2 mL of freshly prepared solutions to the cylinders. To calculate the potency of the sample, in µg, of ampicillin per mL of the reconstituted suspension, from the potency of the standard and responses obtained with the *Standard and sample solutions*.

PACKAGING AND STORAGE

In airtight closed containers, protected from moisture, at temperature below 25 °C.

LABELLING

To comply with the legislation in force.

ANISE *Anisi fructus*

Pimpinella anisum L. — APIACEAE

The vegetable drug consists of fruit, which are dried diachenes containing at least 2.0% of volatile oil, with at least 87% of anethole.

POPULAR NAMES

Fennel.

CHARACTERISTICS

Organoleptic characteristics. The drug has a pleasant smell and sweet and aniseed taste.

MACROSCOPIC DESCRIPTION

The (diachene) fruit is ovoid or pyriform, laterally compressed, broad at the base and narrowed at the apex, which is crowned by a thick stylopodium, with 2 short diverging and reflective stylets, yellowish-brown or greenish-brown color, 3 - 7.0 mm long and 2.0 - 3.0 mm wide, provided with a small fragment of pedicel, thin, rigid, somewhat curved, which extends between each mericarps of each cremocarp by carpophorus (center filament), filamentous, and bifid. The achenes, connected by the apex at the end of carpophorus, have a flat commissural face and a convex dorsal face, the latter covered with simple and short trichomes visible by lens. The fruit is traversed lengthwise by 5 primary filiform edge, straight and smooth, 3 dorsal and 2 commissural slightly protruding and lighter tone. In cross section, the 2 achenes show up most of the time connected by their commissural faces.

MICROSCOPIC DESCRIPTION

In cross section, each achene shows an epicarp of a layer of cells, where several short tector trichomes are found, they are usually unicellular, conical, with thick walls, and verrucous cuticle. In front view, there are sparse stomata and a cuticle strongly striated. The mesocarp is formed by a few layers of parenchyma, which differs, along the dorsal face, a nearly continuous series of schizogenous branched secretory canals (3 - 4 between 2 edges); along the commissural face there are 2 large secretory canals. On the commissural face there are also narrow sclerids, elongated lengthwise, with several pits. Each edge contains a narrow vascular bundle surrounded by fibers. The endocarp is comprised of a layer of cells tangentially elongated, and thin-walled, adhered to the coat; this is formed by a layer of cells of internal thicker walls, yellow or greenish-yellow. The endosperm shows polygonal cells with thickened walls containing oil droplets, aleurone grains, and crystals of calcium oxalate (Druze-type). The carpophorus and pedicel are characterized by the presence of narrow vessels and fibers.

MICROSCOPIC DESCRIPTION OF POWDER

The powder meets all the requirements for the species, unless the macroscopic characters. Characteristics: yellowish-brown or greenish-brown color, irregular fragments of the pericarp, which show portions of secretory canals; intact or fragmented trichomes, unicellular, sometimes curved, with attenuated tips and warty cuticle; fragments of the epicarp with striated cuticle and scarce anomocytic stomata; brown fragments containing branched secretory canals; fragments of vascular tissue, thin-walled coat cells; fragments of endosperm containing aleurone grains and crystals of calcium oxalate; square, rectangular or elongated sclerids, thick-walled, with pits; fiber strands of carpophorus and pedicel.

The powder has no starch grains.

IDENTIFICATION

To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF₂₅₄ silica gel with 250 µm thick as support and toluene as mobile phase. To separately apply to the plate, as band, 2 µL to 3 µL of each of the solutions prepared as described below.

Solution (1) to use 0.1 g of crushed dried fruits, add 2 mL of methylene chloride. To stir for 15 minutes. To filter it. To concentrate the filtrate to dryness in a water bath at temperature below 60 °C. To resuspend the residue in 2 mL of toluene.

Solution (2): to dissolve 3 µL of anethole and 40 µL of olive oil in 1 mL of toluene.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). The chromatogram shows a spot with attenuation of fluorescence obtained with *Solution (2)*, in the upper third of the plate, corresponding to triacylglycerols of olive oil. To

nebulize the plate with anisaldehyde SR and heat at 100 °C - 105 °C for 5 minutes. The light violet spot obtained in the upper third of the chromatogram with *Solution (1)* corresponds in position and intensity to that of anethole obtained with *Solution (2)*. The pink spot obtained in the upper third of chromatogram with *Solution (1)* corresponds in position and intensity to that of triacylglycerols of olive oil obtained with *Solution (2)*. The intense violet color obtained in the central third of the chromatogram with *Solution (1)* corresponds in position and intensity to that referring to compounds from olive oil obtained with *Solution (2)*. The light pink to light violet spots obtained in the lower third of the chromatogram with *Solution (1)* are related to polar fatty compounds.

PURITY TESTS

Foreign matter (5.4.2.2). At most 2%.

Water (5.4.2.3). At most 7%.

Total ashes (5.4.2.4). At most 12%.

DOSAGE

Volatile oils

To proceed as described in *Determination of volatile oils in vegetable drugs (5.4.2.7)*. To use 250 mL flask containing 100 mL of water as distillation liquid. To crush the fruit of the anise to obtain coarse powder. To immediately proceed to determine the volatile oil from 20 g of the powdered drug. To distill for 4 hours.

Anethole

To proceed as described in *Gas chromatography (5.2.17.5)*. To use chromatograph equipped with flame ionization de-

tector, by using mixture of nitrogen, hydrogen, and synthetic air (1:1:10) as the auxiliary gas to flame detector; 60 m long capillary column and 0.25 mm inner diameter, filled with polyethylene glycol, with a film thickness of 0.25 µm. To keep the column temperature at 60 °C for 5 minutes and increase it at a rate of 2 °C per minute to reach 210 °C, to be maintained for 20 minutes (total: 100 minutes). Injector temperature at 200 °C and detector temperature at 220 °C; use purified helium as carrier gas; flow of carrier gas of 1 mL/minute.

Sample solution: volatile oil of anise obtained in xylene, as described in *Determination of volatile oils in vegetable drugs (5.4.2.7)* without dilution. To store refrigerated in airtight vial and protected from light.

Standard solution: to dissolve 60 µL of anethole in 1 mL of *n*-hexane. To store refrigerated in airtight vial and protected from light.

Procedure: to inject 1 µL into the gas chromatograph, by using 1:100 flow division and the relative concentration obtained by electronic integration (normalization method). To examine the chromatogram obtained for the *Sample solution*. The characteristic peaks in the chromatogram obtained with the *Sample solution* should have retention times similar to those obtained from the *Standard solution* or identification confirmed by using gas chromatography coupled to a mass selective detector operating under conditions similar to those of gas chromatography with flame ionization detector.

PACKAGING AND STORAGE

To store refrigerated in airtight vial and protected from light for up to 1 year.

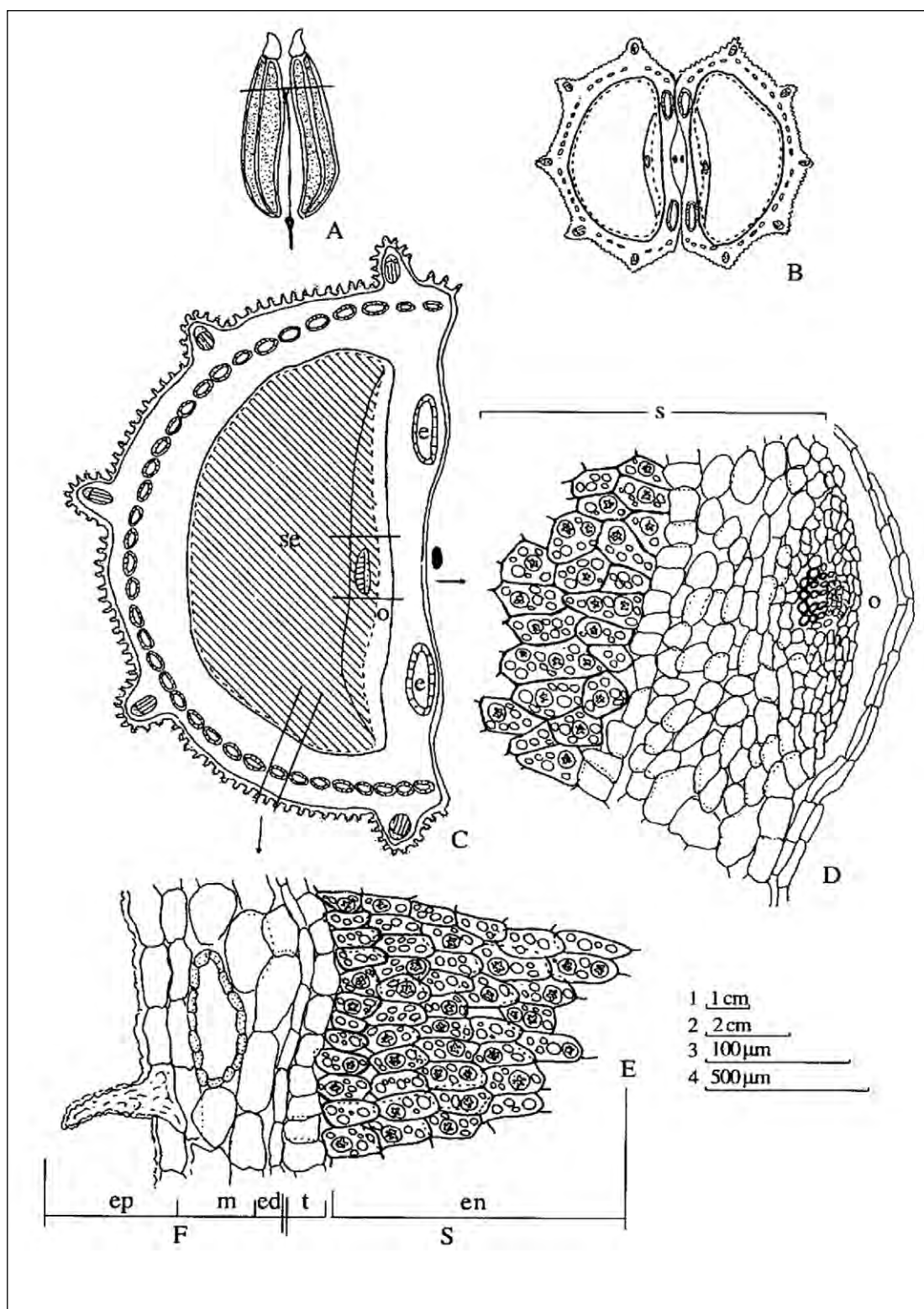


Figure 1 - Macroscopic and microscopic aspects of *Pimpinella anisum* L.

Additional information of **Figure 1**. Scales correspond to 1 cm in **A** (rule 1); in **B**, 0.5 cm (rule 2); in **C**, 500 μ m (rule 4); in **D** and **E**, 100 μ m (rule 3).

A - aspect of diachene (schizocarp). **B** - schematic of cross-section of diachene according to **A**. **C** - schematic of cross-section in one of the mericarps: schizogenous canal (e); hollow (o); seed (se). **D** - detail of commissural region according to **C**. **E** - detail of portion of fruit and seed according to **C**. **F** - section of the pericarp of the fruit: endocarp (ed); epicarp (ep); mesocarp (m). **S** - section of the outer portion of the seed: endosperm (en), tegument (t).

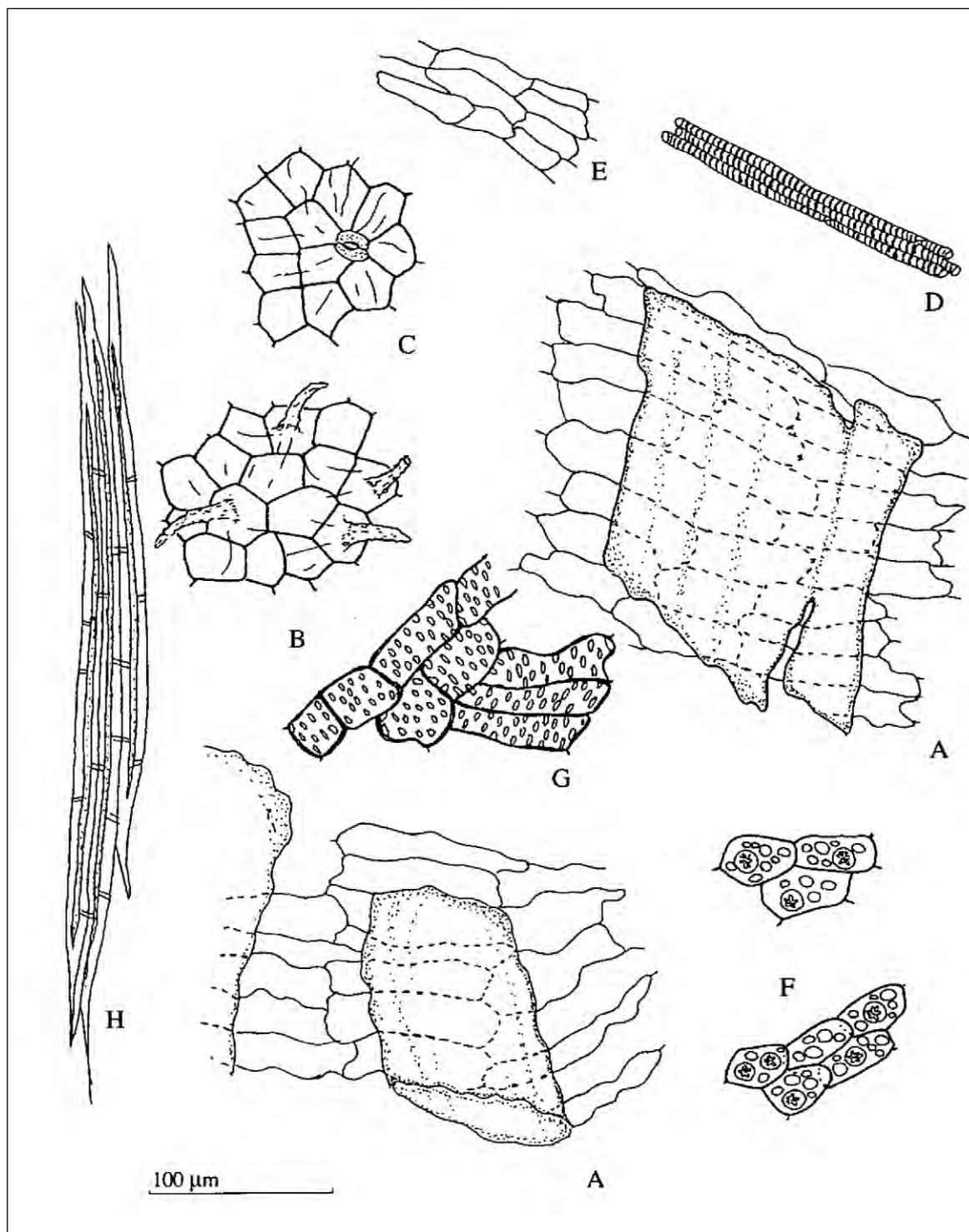


Figure 2 - Microscopic aspects of the powdered fruit of *Pimpinella anisium* L.

Additional information of **Figure 2**. Scales correspond to 100 µm.

A - uneven portions of mesocarp with branched and unbranched brown secretory canals. **B** - portion of the epicarp with intact and fragmented trichomes and striated cuticle. **C** - the same, showing striated cuticle and anomocytic stomata. **D** - fragments of vessel elements with helical thickening. **E** - cells of coat with thin walls. **F** - fragments of the endosperm with polygonal cells containing drops of oil and aleurone grains with 1-2 drusen of calcium oxalate. **G** - sclereids of commissural face. **H** - fiber strands of carpophorus and pedicel.

STAR ANISE

Anisi stellati fructus

Illicium verum Hook. f. - MAGNOLIACEAE

The vegetable drug consists of dried fruit, containing at least 7.0% of volatile oil, with at least 80% of anethole.

POPULAR NAMES

Badian, badian-of-china.

CHARACTERISTICS

Organoleptic characteristics. The pericarp of the drug has pleasant aromatic odor and sweet and aniseed taste; the seed is odorless and has an unpleasant taste.

MACROSCOPIC DESCRIPTION

The fruit is multiple, usually composed of 8 follicles, sometimes up to 11, horizontally arranged in star-shaped around a central axis (columella), ordinarily flattened edges at the height of the carpel. The columella often remains in a small, curved, club-shaped, and fragile pedicel, which seldom is connected to the fruits. The follicles, from 10.0 mm to 20.0 mm long, unevenly developed, woody, careniform, laterally flattened, grayish-brown color, ending in obtuse and curved apex. Each follicle is angled at the base, which is fixed to the central axis; the lower edge of the follicle is thick and rugous; the upper edge is open on two flat and thin lips on each side of the slit; the rugous sides have near the base, a smooth, clear, semi-elliptic portion, whereby the carpels are in contact with each other. In the maturation phase, the follicle becomes dehiscent and opens up to the upper edge (ventral suture), by a wide slit, showing a smooth and shiny inner face, yellowish-brown, and a single oval seed, brown-reddish or yellowish-brown, hard, shiny, truncate at the base, where the hilum and the micropyle are quite close to each other. The seed contains a fragile cover and oily albumen that surrounds a small embryo. It differs from *Illicium anisatum* L. (= *Illicium religiosum* Sieb. et Zucc.) because the latter presents smaller and more oval follicles, wider ventral suture, and straight peduncle, not club-shaped.

MICROSCOPIC DESCRIPTION

The epicarp, in front view, shows polygonal cells, brown, irregular, and slightly thickened walls. The epidermis of the epicarp has large stomata, anomocytic, not very common, and cuticle with irregular, well marked ribs. On the outer portion, the mesocarp is composed of parenchyma cells with reddish-brown walls containing starch,

oil spherical secretory idioblasts can be observed in this tissue, thin-walled, in its inner portion, the mesocarp is composed of smaller cells, thick-walled, in the boundary of these two zones are several vascular bundles. The endocarp is formed by a layer of cells elongated radially in the form of the palisade, 60 µm in length, on average; in the part corresponding to dehiscence (ventral suture), these

cells become smaller, unevenly thick walled and pits, and polygonal cells of mesocarp zone become a massive sclerotic. The central axis (columella), the peduncle (pedicel), and the mesocarp contain several typical sclerotic cells. The astroscleireids of the pedicel and mesocarp are very large and usually are found alone; they may be irregularly branched or have shorter and tapered projections. Other sclereids of the mesocarp are found in groups, but they are elongated, with thickened walls and pits. The seminal integument consists of distinct layers. The outer integument is represented by a hyaline tissue formed by 2-3 layers of cells, followed by a integument comprising a layer of osteosclereids with cells elongated radially thickened walls and pits; there are several layers of cells with lignified walls, thickened and pits, called macrosclereids, and the inner layers have thin walls; the inner integument is bounded by a layer of cells with crystals of calcium oxalate. There are braquisclereids in the micropylar zone. The endosperm is composed of polygonal cells with aleurone grains with crystalloid and oil droplets. The embryo is small. It differs from *Illicium anisatum* L. (= *Illicium religiosum* Sieb. et Zucc.) because the latter presents rare astroscleireids, which are unbranched; the sclereids of the mesocarp are rounded, not elongated.

MICROSCOPIC DESCRIPTION OF POWDER

The powder meets all the requirements for the species, unless the macroscopic characters. Characteristics: reddish-brown color; fragments formed by brown cells of the epicarp, with strongly striated cuticle; fragments of parenchyma cells of the mesocarp, with rounded oil cells; voluminous sclerids, irregularly branched arising from the pedicel; elongated sclereids derived from the mesocarp, with thickened walls and pits; fragments formed by columnar cells of the endocarp, walls slightly thickened, lignified with pigments in the end walls; yellowish masses of small cells with very thick walls and pits, from the suture carpel zone; sclerotic cells (isolated osteosclereids, macrosclereids and braquisclereids) derived from the integument of the seed, arranged in palisades; hyaline fragments of the outer integument of the seed; tabular crystals of calcium oxalate; portions of albumen with aleurone grains with crystalloid.

IDENTIFICATION

A. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF₂₅₄ silica gel chromatoplate with 250 µm thick as stationary phase and toluene as mobile phase. To separately apply, as band, 6 µL of *Solution (1)*, 10 µL of *Solution (2)*, and 4 µL of *Solution (3)*.

Solution (1): to boil 1 g of ground follicles, with no seeds, with 10 ml of 90% ethanol (v/v) for 2 minutes. To filter it.

Solution (2): mixture of volatile oil and ethyl ether (1:30).

Solution (3): to dissolve 3 µL of anethole in 1 mL of toluene.

To develop the chromatogram. After drying the plate, examine it under ultraviolet light (254 nm). The chromato-

gram shows attenuated fluorescence spot at the same height obtained with *Solution (3)*; anethole has R_f of about 0.6. Then, nebulize with anisaldehyde SR and put it in an oven at 100 °C - 105 °C for 5 minutes. The spot corresponding to anethole has slightly violet spot.

B. To boil 1 g of ground follicles, with no seeds, with 10 mL of 90% ethanol (v/v) for 2 minutes. To filter and separate the filtrate into two parts. *Part 1:* in a test tube, add 10 mL of distilled water to the filtrate. Opalescence occurs due to anethole. *Part 2:* to add 25 mL of distilled water to the filtrate. Then, extract twice with 20 mL of petroleum ether. To evaporate the petroleum ether and add 2 mL of acetic acid to the residue. To transfer it to a test tube and add three drops of ferric chloride SR. Then, slowly add 2 mL of sulfuric acid. At the interface between the two liquids, a brown ring is immediately formed due to the presence of anethole.

PURITY TESTS

Foreign matter (5.4.2.2). At most 2.0%.

Water (5.4.2.3). At most 7.0%.

Total ashes (5.4.2.4). At most 6.0%.

DOSAGE

Volatile oils

To proceed as described in *Determination of volatile oils (5.4.2.7)*. To use 250 mL flask containing 100 mL of water as distillation liquid. To crush the fruit to obtain coarse powder. To immediately proceed to determine the volatile oil from 20 g of the pulverized drug. To distill for 2 hours.

Anethole

To proceed as described in *Gas chromatography (5.2.17.5)*. To use chromatograph equipped with 30 m long capillary column and 250 µm inner diameter, filled with poly (dimethyl/diphenyl siloxane), with a film thickness of 0.25 µm. To use flame ionization detector. To use helium as the carrier gas at a pressure of 80 kPa and linear velocity of 1.0 mL/min. To use nitrogen, synthetic air, and hydrogen (1:1:10), respectively, as auxiliary gases to flame detector. To set the column temperature from 60 °C to 300 °C at 3 °C per minute (total: 80 minutes), injector temperature at 220 °C, and the detector temperature at 250 °C.

Sample solution: mixture of volatile oil and ethyl ether (2:100).

Procedure: to inject 1 µL of this solution into the gas chromatograph, by using 1:50 flow division. The anethole shows linear retention time (Kovats index) of 1277. The relative concentration is obtained by manual or electronic integration. The content of anethole is greater than 80.0%.

PACKAGING AND STORAGE

In tightly closed container, protected from light and heat.

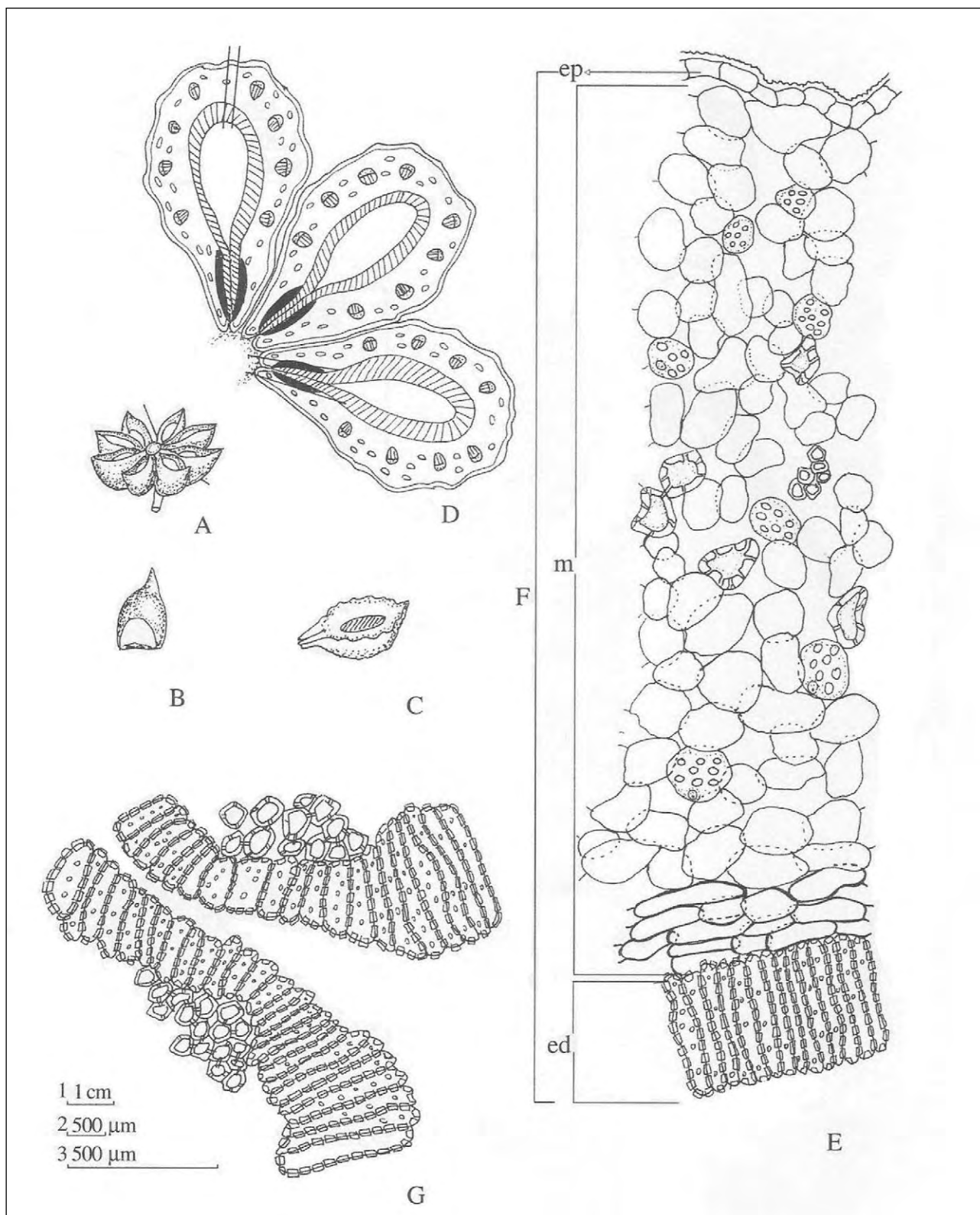


Figure 1 - Macroscopic and microscopic aspects of the fruit in *Illicium verum* Hook. f.

Additional information of **Figure 1**. Scales correspond to: to 1 cm, in **A, B, C** (1), in **D**, (2) 500 μm, in **E, F** (3), 500 μm.

A. aspect of the fruit. **B.** detail of a follicle in dorsal view. **C.** detail of a follicle in ventral view. **D.** detail of three follicles in **A**. **E.** cross section of the pericarp in the portion indicated in **D**. **F.** fruit, ep. epicarp. **G.** detail of the endocarp in the commissural region.

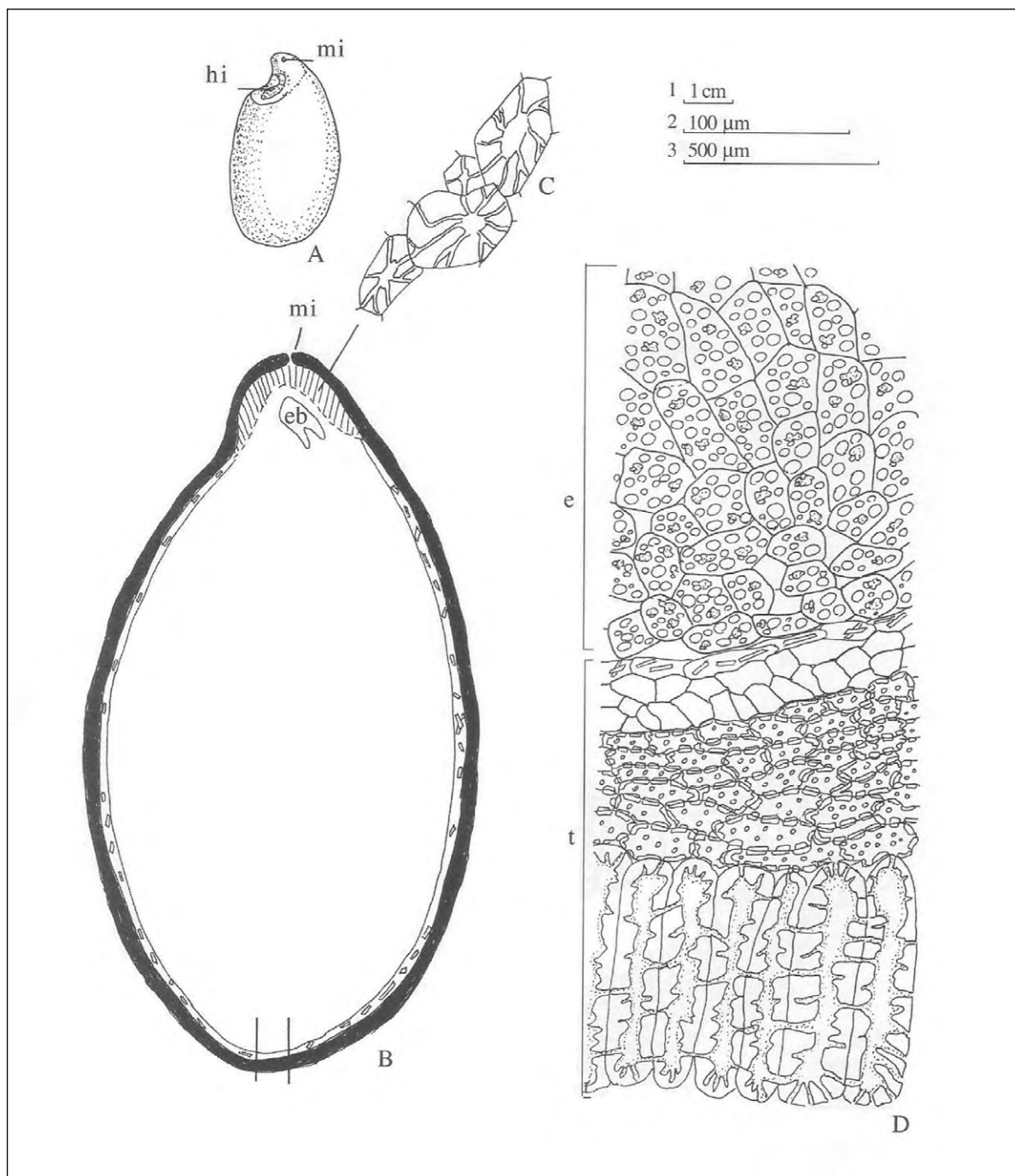


Figure 2 - Macroscopic and microscopic aspects fruit in *Illicium verum* Hook. f.

Additional information of **Figure 2**. Scales correspond to: to 1 cm, in **A**; in **B** (2), to 100 μm; in **C**, **D** (3), to 500 μm.

A. seed in side view. **B.** seed in longitudinal section. **C.** braquisclereids in the micropylar zone. **D.** cross-section of the seed in the portion indicated in **B**. Other details: endosperm (e), embryo (eb); hilum (hi); micropyle (mi), integument (t).

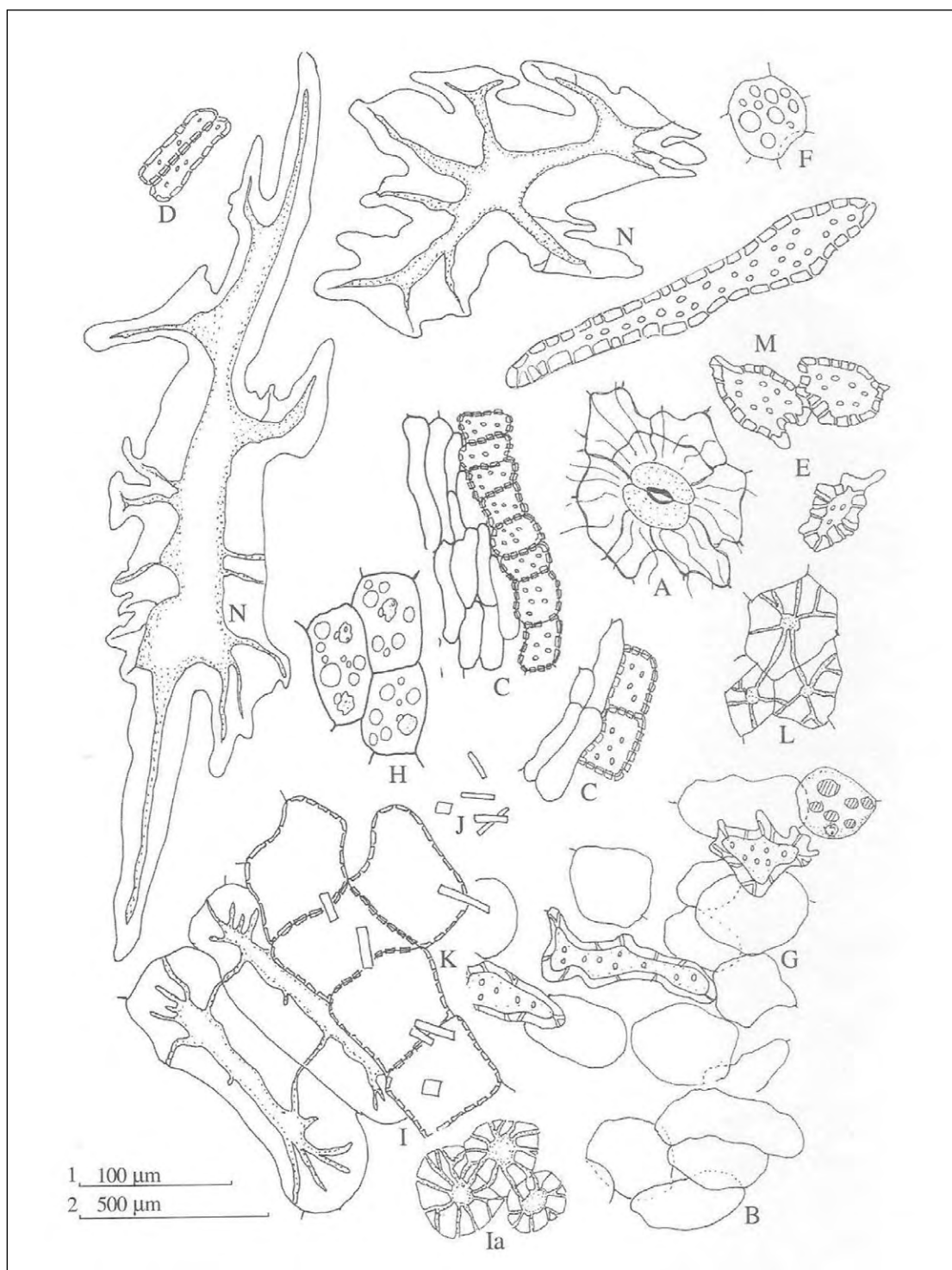
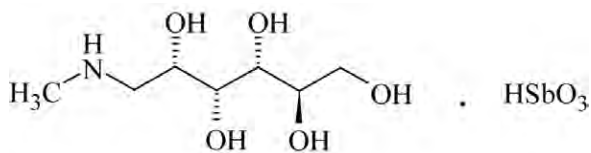


Figure 3 - Microscopic aspects in powder *Illicium verum* Hook. f.

Additional information of **Figure 3**. Scales correspond to: in A-K (1), to 100 µm, L-N (2), to 500 µm.

A - epicarp with anomocytic stomata and striated cuticle. B - parenchyma cells of the mesocarp. C - cells of the commissural zone with thickened walls. D - cell of the endocarp outside the commissural zone. E - sclereid. F - idioblast with oil droplets. G - portion of the mesocarp with oil idioblasts and sclereids. H - endosperm cells with lipid globules and aleurone grains. I - osteosclereids in cross section; Ia - the same in tangential section. J - prismatic crystals of calcium oxalate. K - cells of crystal layer. L - braquisclereids of the commissural region. M - extended macrosclereids of the mesocarp, with thick walls and pits. N - voluminous and branched sclereids of the pedicel.

ANTIMONIATE, MEGLUMINE



$C_7H_{17}NO_5 \cdot HSbO_3$; 365.98

meglumine antimoniate; 05587

Trioxoantimonate (1 -) of 1-deoxy-1-(methylamino)-D-glucitol

[133-51-7]

Meglumine antimoniate consists of pentavalent antimony salt of *N*-methylglucamine. It contains at least 26% and at most 28% of pentavalent antimony (Sb^{5+}) relative to the meglumine antimoniate.

DESCRIPTION

Physical characteristics. White, slightly yellow powder.

Solubility. Soluble in water, practically insoluble in ethanol, chloroform, and ethyl ether.

IDENTIFICATION

A. To dissolve 6 g of the sample in 20 mL of water. To acidify 2 mL of this solution with hydrochloric acid SR and add thioacetamide SR prepared before using it. Orange precipitate is formed.

B. To dissolve 6 g of the sample in 20 mL of water. To dilute 1 mL of this solution with 9 mL of water. To acidify with 5 mL of 0.3% sulfuric acid (v/v) and add 4 mL of alkaline mercuric potassium iodide SR. After a few seconds, yellow color develops.

PURITY TESTS

pH (5.2.19). 5.5 to 7.5. To determine in 30% solution (w/v) in carbon dioxide-free water.

Trivalent antimony. To proceed as described in *Atomic absorption spectrometry with hydride generation* (5.2.13.1.2), batch system, quartz atomization cell, wavelength at 217.6 nm, and resolution of monochromator of 0.20 ± 0.10 nm.

Sample solution: to prepare the 0.3% sample of the solution (w/v) in water and dilute this solution by a factor of 500 times by using the same solvent.

Standard solution: to prepare 0.1% trivalent antimony solution (w/v) by diluting antimony potassium tartrate ($C_4H_4KO_7Sb \cdot 1/2H_2O$) in water.

Reducing solution: to immediately prepare a 1% sodium tetrahydroborate solution (w/v) in 0.1% sodium hydroxide (w/v).

Citric acid solution: to prepare 4% citric acid solution (w/v) in water.

Procedure: to adapt the reaction flask in the hydride generation system, wait 30 seconds to purge the system, and proceed the determination as per manufacturers' specific recommendations to the equipment. The maximum range for mixture of diluted *Sample solution* or the *Standard solution* with the *Citric acid solution* should be 5 seconds before entering the equipment. To construct the analytical curve with aliquots of 0.1 mL of *Antimony standard solution* according to the following concentrations: 0.1 mg/L; 0.2 mg/L; 0.3 mg/L; 0.4 mg/L, and 0.5 mg/L, daily prepared by sequential dilution with water. To put between 0.20 mL and 0.80 mL of the diluted *Sample solution* or *Antimony standard solution* in the reaction flask and add 10 mL of *Citric acid solution*. At most 0.04 mg of trivalent antimony per mL of 0.3% meglumine antimonate solution (w/v) corresponds to 1.33% of trivalent antimony of the substance analyzed.

Heavy metals. Determinations should be made by *Atomic absorption spectrometry* (5.2.13.1) with graphite furnace or hydride generation, by inductively coupled plasma optical emission spectrometry, or inductively coupled plasma mass spectrometry. At most 9 mg/L in the 30% meglumine antimonate solution (w/v), corresponding to 0.003% (30 ppm) of heavy metals in the substance analyzed, to the sum of the concentration for the following elements: aluminum, arsenic, bismuth, cadmium, lead, copper, chromium, manganese, mercury, nickel, and zinc.

DOSAGE

To proceed as described in *Atomic absorption spectrometry* (5.2.13.1), by using *Method I*. To use the following items: air acetylene flame, wavelength at 217.6 nm, monochromator resolution 0.20 ± 0.10 nm.

Sample solution: to prepare 30% meglumine antimonate solution (w/v) in water and dilute by a factor of 2500 times with 6 *M* hydrochloric acid.

Standard solution: to prepare 0.1% trivalent antimony solution (w/v) in water, by using antimony potassium tartrate ($C_4H_4KO_7Sb \cdot 0.5H_2O$).

Procedure: to construct the analytical curve with *Antimony standard solution* according to the following concentrations: 10 mg/L, 20 mg/L, 30 mg/L, 40 mg/L, and 50 mg/L by sequential dilution in 6 *M* hydrochloric acid. From the concentration of Sb determined, calculate the content of Sb in meglumine antimonate.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antiprotozoal.

ANTIMONIATE, MEGLUMINE INJECTABLE SOLUTION

It contains at least 92.0% and at most 108.0% of pentavalent antimony (Sb^{5+}) relative to the amount stated of Sb^{5+} . Each 1.5 g of meglumine antimoniate contains 405 mg of Sb^{5+} .

IDENTIFICATION

A. To acidify 2 mL of injectable solution with hydrochloric acid SR and add thioacetamide SR prepared before using it. An orange precipitate develops.

B. To dilute 1 mL of the injectable solution with 9 mL of water. To acidify this solution with 5 mL of 0.3% sulfuric acid (v/v) and add 4 mL of alkaline mercuric potassium iodide. After a few seconds, yellow color develops.

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test. **pH (5.1.19).** 5.5 to 7.5.

PURITY TESTS

Trivalent antimony. To dilute the injectable solution with water by a factor of 50,000 times and proceed as described in *Trivalent antimony*, in the monograph *Meglumine antimonate*.

Heavy metals. To proceed as described in *Heavy Metals*, in the monograph *Meglumine antimonate*. At most 0.0009% (9 mg/L) of the injectable solution.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

Bacterial endotoxins (5.5.2.2). At most 0.5 UE/mg of meglumine antimoniate.

Toxicity (5.5.2.3). It complies with the test. To intravenously inject the equivalent to 1 mg/g of body weight.

DOSAGE

To dilute the injectable solution by a factor of 2500 times with 6 M hydrochloric acid and proceed as described in *Dosage*, in the monograph *Meglumine antimonate*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

ARNICA *Arnicae flos*

Arnica montana L. - ASTERACEAE; 09894

The drug consists of the dried flowering clusters, whole or partially fragmented. It must contain at least 0.4% w/w of total sesquiterpene lactones expressed in helenalin tiglactate, calculated with reference to the dried drug.

CHARACTERISTICS

Organoleptic characteristics. Pleasant aromatic odor; acid and bitter taste.

MACROSCOPIC DESCRIPTION

The flowers are grouped in inflorescences of heteromorphic cluster, with yellow-orange color. The cluster consists of a peduncle, a receptacle, ligulate radial flowers, and tubular disc flower. The cluster, when closed, measures approximately 2 cm diameter. When radial flowers are expanded, it measures from 5 to 6 cm diameter. The peduncle, when present, measures 2 - 3 cm long. The receptacle, when free of flowers, has a diameter from 6 mm to 10 mm and 15 mm deep. It is of slightly convex, honeycomb, and covered with white, short, and stiff trichomes. The receptacle has a housing consisting of oval-lanceolate 18 - 24 bracts, velvety on the lower face, arranged in 1 or 2 intertwined series. Each involucre bract has an acute apex and entire edge, ciliated, measuring from 8 mm to 10 mm long (rarely 15 mm long). The inner bracts are brown-green and shorter; the outer bracts are green; both of them have the lower face covered with lower yellowish-green trichomes, visible by lens. The radial ligulate flowers are zigomorphic and female, from 14 to 20, and measure from 20 mm to 30 mm long. Each ligulate flower has a reduced calice called pappus, which is formed by a series of whitish-yellow, thick, and rigid bristles, measuring from 4 mm to 8 mm long. The limb of the corolla is oblong, yellow-orange, and 7 to 10 parallel ribs, resulting in 3 small and irregular lobes. The stamens are not fully developed, therefore, staminodes, and have free anthers. The ovary is inferior, narrow, brown color, measures from 4 to 5 mm long, and has 4 or 5 longitudinal edges not clear, in addition to a stylet bifurcated into 2 curved and reflective stigmatic branches.

The tubular flowers of the disc are actinomorphic and perfect, in amount larger than the ligulate flowers, and measure up to 15 mm long. Each tubular flower has a reduced calice called pappus, which is formed by a series of whitish-yellow and rigid bristles, measuring up to 8 mm long. The corolla is short, yellow-orange color, measuring about 8 mm long, and has 5 triangular reflective lobes. There are 5 stamens, fertile, and connected by anthers, forming a tube; teaks are ellipsoidal and the connective extends in a triangular scale. The ovary is inferior, narrow, brown color,

measures from 4 to 8 mm long, and has 4 or 5 longitudinal visible edges, in addition to a stylet bifurcated into 2 curved and reflective stigmatic branches. The fruits, when present, are achenes brown, crowned or not by pappus.

MICROSCOPIC DESCRIPTION

The involucre bracts, in front view, show cells with anticlinal and wavy walls and anomocytic stomata in the lower face of the epidermis; adaxial face with elongate cells, with anticlinal polygonal walls slightly wavy, without stomata. In the lower face, there are different types of trichomes: abundant unicellular or bicellular tector trichomes, sharp, formed by cells with slightly thickened walls, usually straight, and the bicellular trichomes are formed by a short proximal cell and a distal one that is longer, interconnected by an inclined wall; rare multicellular, uniseriate, tector trichomes, 3 - 10 cells formed by 1 to 3 short proximal cells and 2 - 4 long distal cells; multicellular, uniseriate, tector trichomes, especially abundant in the margins of bracts; multicellular tector trichomes with proximal cells with uniform size and longer distal cell; numerous glandular trichomes, with uniseriate or biseriate pedicel, with large glandular head, globose or ovoid, multicellular, abundant on the lower face; glandular trichomes with the same appearance described, but shorter, with uniseriate pedicel, more frequent on the adaxial face; rare glandular trichomes with club-shaped aspect. In cross section, the bract has a loose fundamental parenchyma, with vascular bundles corresponding to the ribs of each bract. The receptacle in front view, presents epidermis similar to those of bracts, with tector trichomes with 2-5 cells. In cross section, there is a loose fundamental parenchyma with vascular bundles and secretory canals. The bristles of the calice, in the form of pappus, are composed of 2 - 3 rows of elongated cells, acute in the distal portion, with a greater number of rows of cells in the proximal portion; these cells resemble cells of twinned trichomes, and their distal ends are acute, exposed, and free, toward the distal end of the bristle. The corolla of ligulate flower, in front view, has the epidermis of adaxial face with cells of polygonal anticlinal walls, papillary, mainly in the distal and median portion of the ligule, with short and rounded papillae, with visible epicuticular ribs and lipid droplets; the epidermis of the lower face has cells of elongated anticlinal walls, almost straight, but noticeable wavy in the distal portion.

The stomata are anomocytic. On the lower face, especially in the region of the tube, there are different types of trichomes: uniseriate and multicellular tector trichomes, consisting of 4 or 5 cells of roughly equal size and slightly thickened walls, with pointed distal cell; uniseriate and multicellular tector trichomes, consisting of 1-3 thick-walled proximal cells and 2-4 distal cells with thin walls; glandular trichomes of uniseriate and multicellular pedicel with unicellular to multicellular globose head; glandular trichomes of biseriate and multicellular pedicel, with biseriate, bicellular to multicellular globose head. In cross section, the mesophyll is formed by a loose parenchyma, ligule axis longitudinally traversed by vascular bundles to an equal number of parallel ribs. The corolla of the tubular flower, in front view, presents epidermis with cells of

slightly wavy anticlinal walls on both sides of the distal portion of the petals, and more polygonal in the middle portion, the cells in the region of the tube have polygonal anticlinal walls; in distal and triangular portion of each petal there are finger-like papillae. Lipid droplets may be present. The flowers of the tubular corolla have the same types of trichomes than those found in flowers of ligulate corolla. The anthers, in cross section, show a thickened endothecium on the side walls. The triangular scale of the distal end of the connective presents, in front view, cells of straight anticlinal thickened-walls. The pollen grains are 3-pore, rounded, with equinate exine, and measure about 30 μm . The ovary, in front view, presents epidermis with elongated cells, covered with glandular trichomes of short pedicel and a club-shaped to globose head, multicellular, with up to 8 cells arranged in 2 rows, and tector trichomes, multicellular, biseriate, with geminate cells, whose adjacent walls have pits, slightly thickened, with acute distal cellular portion and sometimes bifid. The wall of the ovary can display brown or black crosslinked plates, due to the presence of phytomelanin. The stigmatic branches of the stylet present unicellular conical, pointed trichomes in the distal portion. Under the mat formed by these trichomes there are rounded papillae. The fruit, when present, has the same epidermal characteristics as ovary, mainly two types of trichomes and evident phytomelanin plates.

MICROSCOPIC DESCRIPTION OF POWDER

The powder meets all the requirements for the species, unless the macroscopic characters. To perform microscopic examination by using chloral hydrate solution. Characteristics: portions of epidermis of the involucre bracts with stomata and trichomes as described above, more abundant on the lower face; trichomes or their fragments, as described; fragments of ligulate corollas, with trichomes as described; fragments of the distal portion of corolla ligulate covered with rounded papillae; fragments of tubular corolla with trichomes as described; fragments of the distal portion of tubular corolla covered with finger-like papillae; ovarian fragments with two types of typical trichomes, as described above; portions of pappus or fragments of pappus bristles as described; 3-pore, rounded pollen grains with exine equinate.

IDENTIFICATION

To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel, with 250 μm thickness as support and a mixture of anhydrous formic acid, water, methyl ethyl ketone, and ethyl acetate (10:10:30:50) as mobile phase. To separately apply to the plate, as 20 mm band, at 1 cm of distance, 15 μL of each of the solutions described below.

Solution (1): to add 10 mL of methanol to 2 g of sprayed sample, and warm in a water bath (60 °C), by stirring for 5 minutes. To cool down the solution and then filter it.

Solution (2): to dissolve 2 mg of caffeic acid, 2 mg of chlorogenic acid, and 5 mg of rutin in methanol. Then, adjust the volume to 30 mL with methanol.

To develop the chromatogram. To remove the plate, and let it drying. To nebulize with aminoethanol diphenyl borate SR and then with a 5% macrogol 400 solution (w/v) in methanol. To heat the plate for 5 minutes at 100-105 °C. To allow to air dry and then examine under ultraviolet light (365 nm). The chromatogram obtained for the *Solution (2)* presents, at the bottom, a yellow-orange fluorescence (rutin) zone; in the middle part of the chromatogram area is observed a fluorescence zone due to chlorogenic acid and, on the top, a bluish fluorescence zone (caffeic acid). The chromatogram obtained with *Solution (1)* shows at the bottom, just above the zone corresponding to rutin, a blue-green fluorescence band; a bluish fluorescence band (chlorogenic acid) can be viewed a little above; following, from bottom to top, a zone can be observed with fluorescence from yellowish-brown to yellow-orange; three fluorescence zones from yellowish-brown to yellow-orange and just below the zone corresponding to caffeic acid, a blue-green fluorescence band.

PURITY TESTS

Foreign matter (5.4.2.2). Not greater than 5.0% of stems having a diameter greater than 5 mm.

Total ash (5.4.2.4). Not greater than 10.0%.

Loss on drying (5.2.9). Not greater than 10.0% in 1 g of sprayed sample, determined in an oven at 100 - 105 °C, for 2 hours

DOSAGE

Total sesquiterpene lactones

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using santonin as internal standard. To use chromatograph equipped with an ultraviolet detector at 225 nm, 0.12 mm chromatograph column and 4 mm inner diameter, packed with octadecylsilane silica (4µm); flow of *Mobile phase* of 1.2 mL/ min.

Eluent A: methanol.

Eluent B: water.

Mobile phase gradient: to adopt the gradient system described in the following table:

<i>Time (minutes)</i>	<i>Mobile phase A (%)</i>	<i>Mobile phase B (%)</i>	<i>Elution</i>
0-3	62	38	Isocratic
3-20	62→55	38→45	Linear gradient
20-30	55	45	Isocratic
30-55	55→45	45→55	Linear gradient
55-57	45→0	55→100	Linear gradient
57-70	0	100	Isocratic
70-90	62	38	Isocratic

Internal standard solution: just before using it, dissolve 0.01 g of santonin accurately weighed in 10 mL of methanol.

Sample solution: to put 1 g of the pulverized sample into a 250 mL round bottom flask. To add 50 mL of a mixture of methanol and carbon dioxide-free water (equal volumes) and heat it under reflux in a water bath at 50 °C - 60 °C for 30 minutes, by stirring frequently. To allow it to cool down and then filter by using filter paper. To add 50 mL of a mixture of methanol and carbon dioxide-free water (equal volumes) and heat it under reflux in a water bath at 50 °C - 60 °C for 30 minutes, by stirring frequently. To repeat twice the operation. To gather the filtrates, add 3 mL of *Internal standard solution* and evaporate under reduced pressure until obtaining a volume of 18 mL. To wash the round bottom flask with carbon dioxide-free water and top up 20 ml with washing. To transfer the solution to a chromatographic column with about 0.15 m long and about 30 mm inner diameter containing 15 g of kieselguhr silica for chromatography. Let it rest for 15 minutes, then elute with 200 mL of a mixture of ethyl acetate and methylene chloride (equal volumes). To evaporate the eluate to dryness in a 250 mL round bottom flask. To dissolve the residue in 10 mL of methanol, add 10 mL of carbon dioxide-free water and then 7 g of neutral aluminum oxide. To stir for 2 minutes, centrifuge (10 min, 6,000 r/min) and filter it by using filter paper. To evaporate to dryness 10 mL of the filtrate. To dissolve the residue in 3 mL of a mixture (equal volumes) of methanol and carbon dioxide-free water. Then, filter it.

Procedure: to separately inject 20 µL of *Internal standard solution* and *Sample solution*. To calculate the percentage of total sesquiterpene lactones, expressed in helenalin tiglolate, according to the following:

$$\frac{\text{FLS} \times C \times V \times 1,187}{\text{FS} \times m \times 10}$$

where

FLS = total area of the peaks corresponding to the sesquiterpene lactones that appear after the santonin peak in the chromatogram.

FS = area under the peak corresponding to santonin in the chromatogram obtained with *Sample solution*.

m = mass of the test portion in g

C = concentration of santonin in the *Internal standard solution* used in the *Sample solution* (mg/mL)

V = volume (in mL) of the *Internal standard solution* used in the *Sample solution*.

1.187 = correction factor between helenalin tiglolate and santonin.

PACKAGING AND STORAGE

In opaque containers, tightly closed, protected from light and heat.

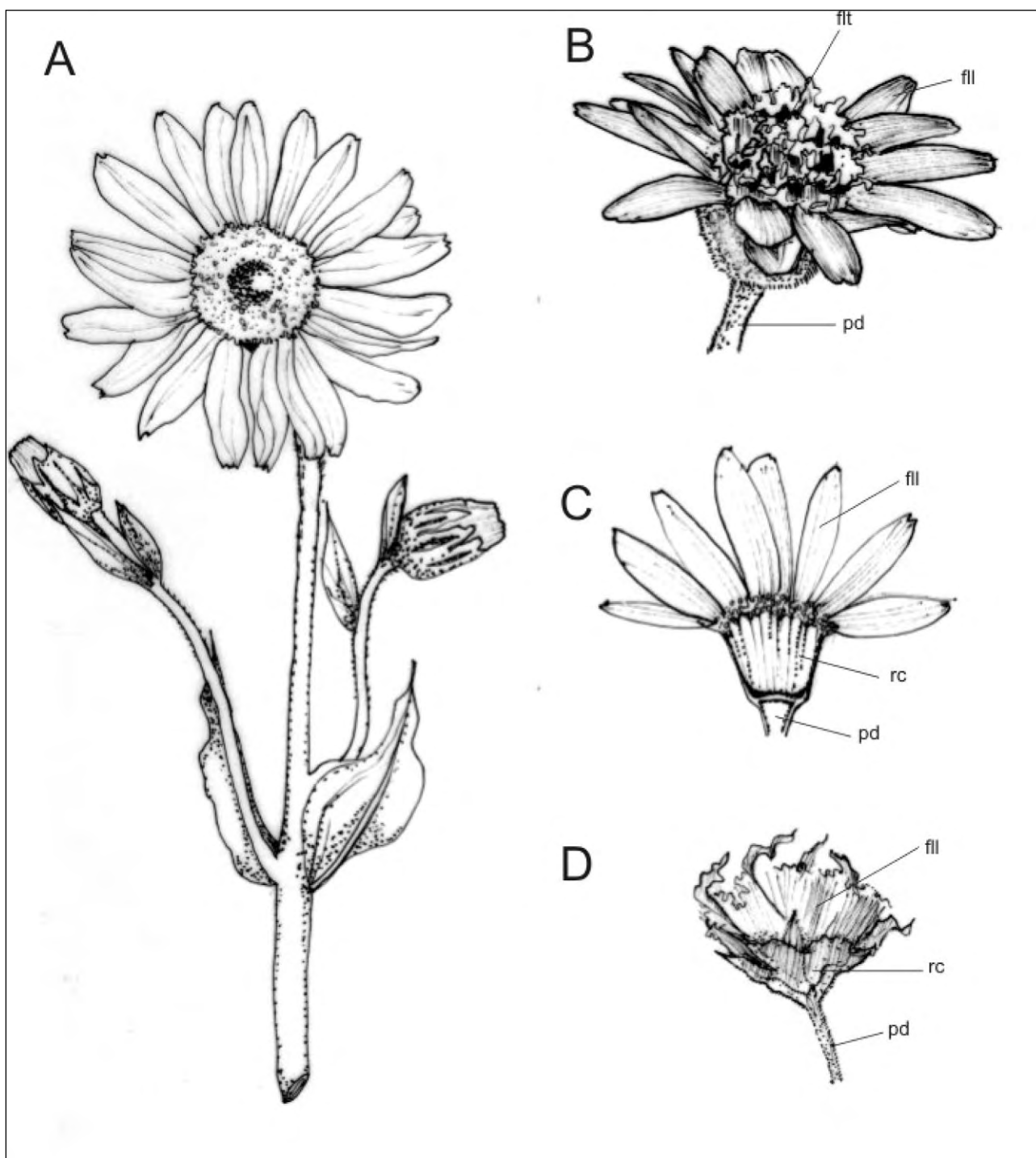


Figure 1 - Macroscopic aspects in *Arnica montana* L.

Additional information of Figure 1.

A - appearance of a branch with inflorescences. **B** - flower cluster: tubular flower (flt); ligulate flower (fil), peduncle (pd). **C** - flower cluster with no tubular flowers: ligulate flower (fil); receptacle (rc); peduncle (pd). **D** - appearance of dry drug: tubular flower (flt); ligulate flower (fil); receptacle (rc); peduncle (pd).

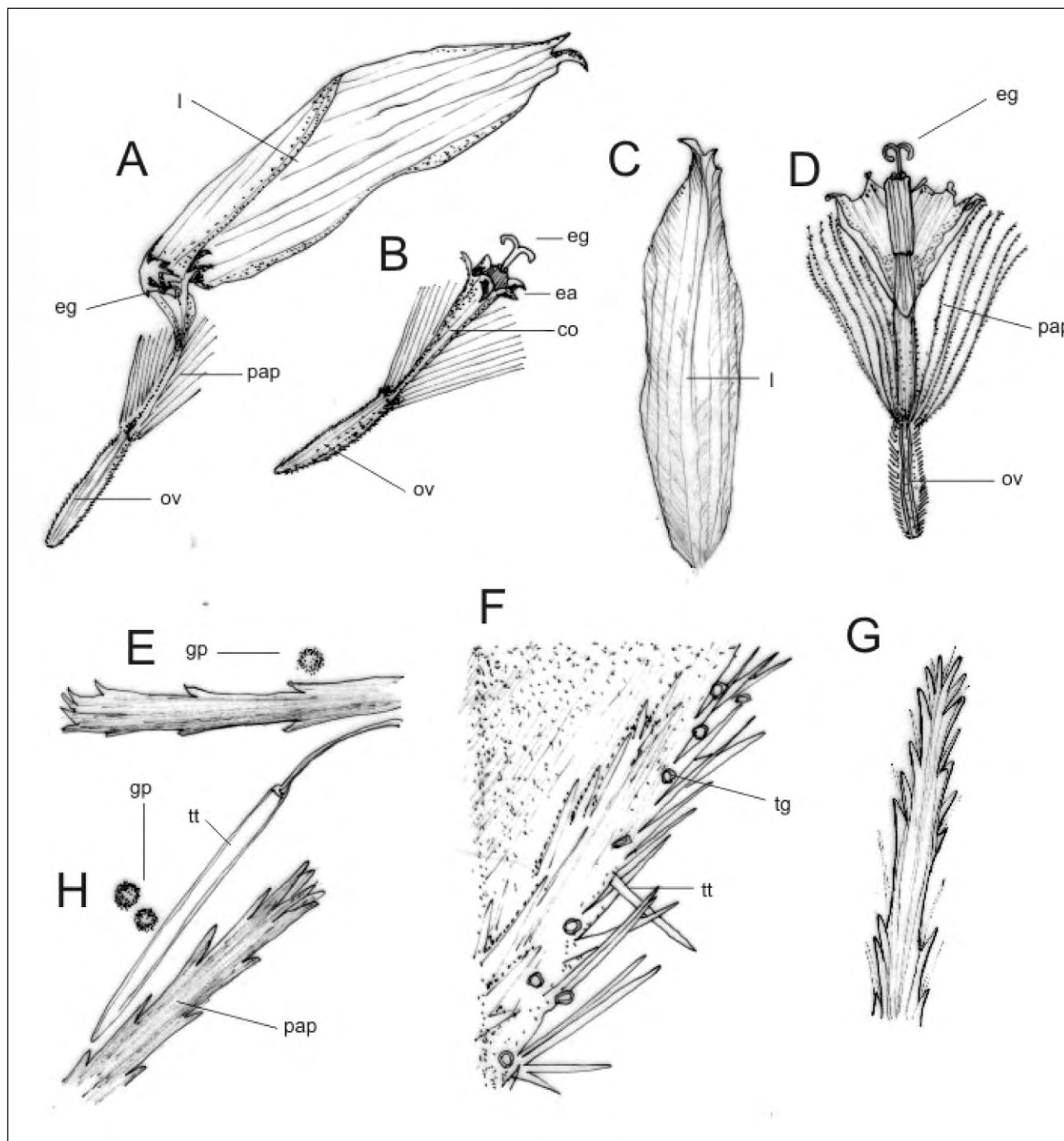


Figure 2 - Macroscopic aspects in *Arnica montana* L.

Additional information of Figure 2.

A - ligulate flower: ovary (ov); pappus (pap); stigma bifid (eg); ligule (l). **B** - tubular flower, ovary (ov); pappus (pap); stamen with anther (ea); stigma bifid (g); corolla (co). **C** - ligulate flower: ligule (l). **D** - tubular flower: ovary (ov); pappus (pap); stigma bifid (eg). **E** - detail of a pappus bristles: pollen grain (gp). **F** - outer surface of the ovary: glandular trichomes (tg); tector trichomes (tt). **G** - fragment of pappus. **H** - detail of a pappus bristles: pollen grain (gp); pappus (pap); tector trichomes (tt).

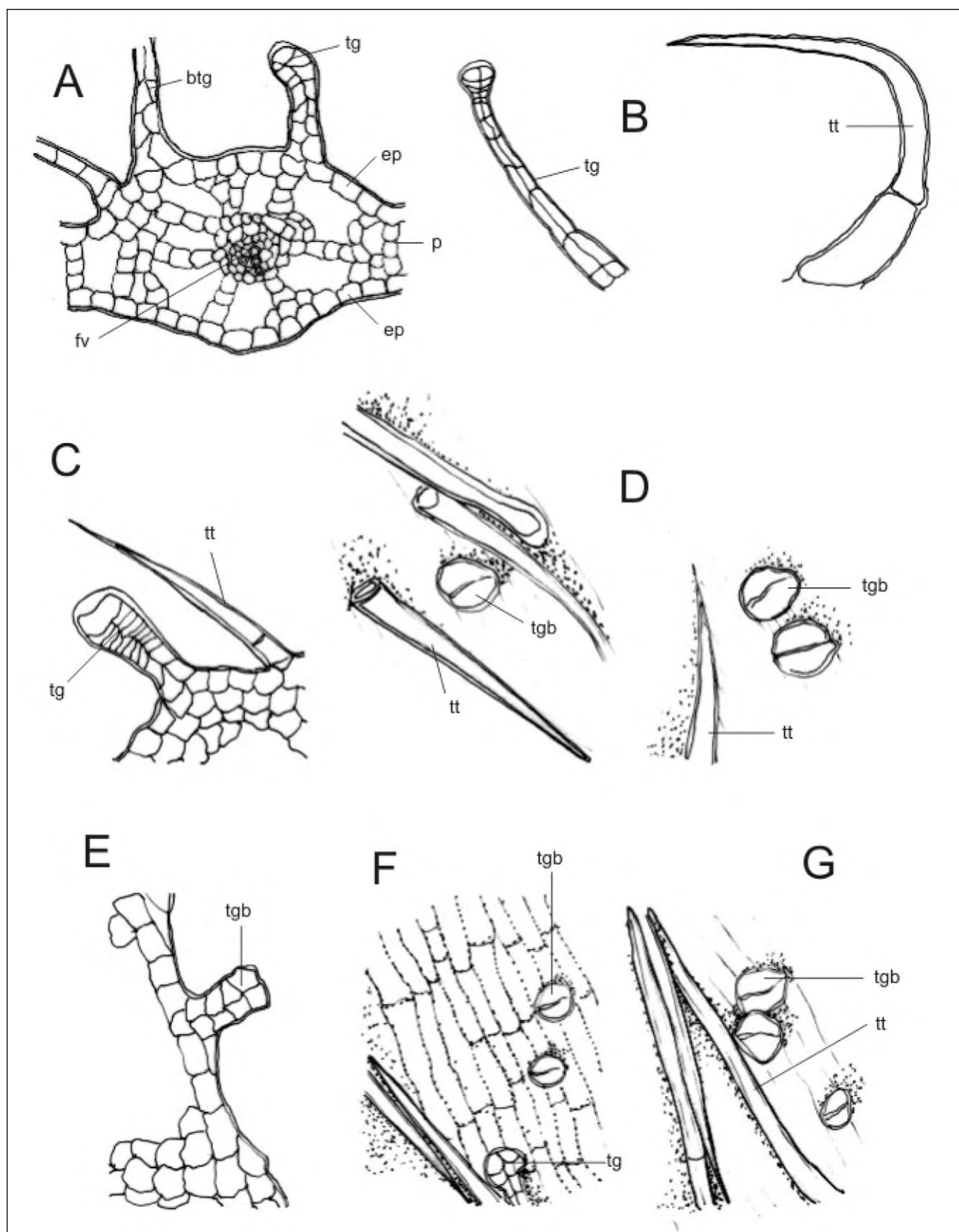


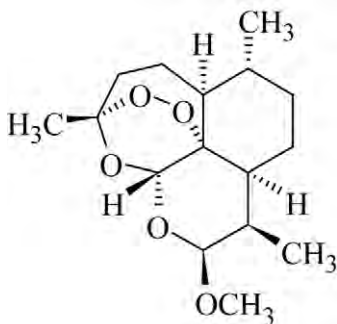
Figure 3 - Macroscopic aspects in *Arnica montana* L.

Additional information of Figure 3.

A - cross-section of bract: epidermis (ep); parenchyma (p); vascular bundle (fv); glandular trichome (tg), base of glandular trichomes (btg). **B** and **C** - details of glandular trichomes and tector: glandular trichomes (tg); tector trichomes (tt). **D** - top view of outer surface of the ovary: glandular trichomes with bicellular head (tgb), with biserial body. **E** - aspects of glandular trichomes. **F** and **G** - fragment of the lower epidermis: glandular trichomes (tg); glandular trichome with bicellular head (tgb).

ARTEMETHER

Artemetherum



$C_{16}H_{26}O_5$; 298.37
artemether; 00885

(3*R*,5*aS*,6*R*,8*aS*,9*R*,10*S*,12*R*,12*aR*)-Deca hydro-10-methoxy-3,6,9-trimethyl-3,12-epoxy-12, ff-pyrano [4,3 -*j*]-1,2-benzodioxepine
[71963-77-4]

It contains at least 98.0% and no more than 102.0% of $C_{16}H_{26}O_5$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Fine, crystal, and white powder.

Solubility. It is practically insoluble in water, very soluble in methylene chloride and acetone and freely soluble in absolute ethanol and ethyl acetate.

Physical and chemical constants.

Melting range (5.2.2): 86 °C to 90 °C.

Specific rotation (5.2.8): +166° to +173°. To determine at 1% solution in absolute ethanol.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide has absorption maximum at the same wavelength, with the same relative intensities to those observed in the artemether SQR spectrum similarly prepared.

B. The principal spot in the chromatogram of *Solution (4)* in the *Related substances* corresponds in position, color, and intensity to that obtained with the *Solution (5)*.

C. To add 1 mL of absolute ethanol and 0.1 g of potassium iodide to 30 mg of the sample. To heat it in water bath. The yellow color develops.

D. To dissolve 10 mg of the sample in 2 mL of absolute ethanol, in a porcelain capsule. To add three drops of vanillin SR. The pink color develops.

PURITY TESTS

Related substances 1. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using G silica gel as support and a mixture of petroleum ether and ethyl acetate (70:30) as mobile phase. To apply separately to the plate 10 µl of each of the solutions freshly prepared, as described below.

Solution (1): 10 mg/mL solution of the sample in acetone.

Solution (2): 0.05 mg/mL solution of the sample in acetone.

Solution (3): 0.025 mg/mL solution of the sample in acetone.

Solution (4): 0.10 mg/mL solution of the sample in acetone.

Solution (5): 0.10 mg/mL solution of artemether SQR in acetone.

Procedure: To develop the chromatogram. To remove the plate, and let it drying. To aerosolize the plate with vanillin SR and examine immediately. Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (2)* (0.5%) and not more than a spot is more intense than that obtained with *solution (3)* (0.25 %).

Related substances 2. To proceed as described in *Dosage*. To prepare *Solutions (1)* and (2) as described below.

Solution (1): 10 mg/mL solution of the sample in mobile phase.

Solution (2): 0.05 mg/mL solution of the sample in mobile phase.

Procedure: To separately inject 20 µL of each solution. To record the chromatograms and measure the area under the peaks. The sum of the areas of all the secondary peaks obtained with *Solution (1)*, except the main peak's one, is not greater than twice the area under the main peak obtained with *Solution (2)* (1.0%), and no peak area is larger than that of the main peak obtained with *Solution (2)* (0.5%). Not more than one peak obtained with *Solution (1)* shows the area greater than half of the area under the main peak obtained with *Solution (2)* (0.25%). To disregard the peaks with an area less than 0.1 times the area under the main peak obtained with *Solution (2)*.

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate with phosphorus pentoxide in an oven at 60°C under reduced pressure for 4 hours. At most 0.5%.

Sulfated ashes (5.2.10). At most 0.1 %. **DOSAGE**

To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 216 nm; 250 mm chromato-

graph column and 4 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at 30 °C; flow of *Mobile phase* of 1.5 mL/min.

Mobile phase: mixture of acetonitrile and water (62:38).

Sample solution: to dissolve accurately weighed amount of the sample in mobile phase to obtain a 4 mg/mL solution.

Standard solution: to dissolve accurately weighed amount of artemether SQR in mobile phase to obtain a 4 mg/mL solution.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_{16}H_{26}O_5$ from the sample, from the responses obtained for the *Sample and standard solutions*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

THERAPEUTIC CLASS

Antimalarial.

ARTEMETHER, INJECTABLE SOLUTION

It contains at least 95.0 % and at most 105.0 % of the amount stated of $C_{16}H_{26}O_5$.

IDENTIFICATION

A. To transfer volume of injectable solution equivalent to 50 mg of artemether to beaker, add 25 mL of acetone, stir, and filter it. To evaporate the filtrate at 40 °C and dry the residue in a desiccator for 24 hours. To proceed as described in *Identification* test A., in the monograph *Artemether*.

B. The principal spot in the chromatogram of *Solution (4)* in the *Related substances* corresponds in position, color, and intensity to that obtained with the *Solution (5)*.

C. To add 6 mL of absolute ethanol to a volume of injection solution equivalent to 30 mg of artemether. To transfer five drops to a porcelain capsule and add a drop of vanillin SR. The pink color develops.

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

PURITY TESTS

Related substances 1. To proceed as described in *Related substances 1*, by *Thin-layer chromatography (5.2.17.1)*, in

the monograph *Artemether*. To prepare the solutions as described below.

Solution (1): to dilute volume of the injectable solution in acetone to obtain a 10 mg/mL solution.

Solution (2): to dilute *Solution (1)* in acetone to obtain a 0.05 mg/mL solution.

Solution (1): to dilute *Solution (2)* in acetone to obtain a 0.025 mg/mL solution.

Solution (4): to dilute *Solution (1)* in acetone to obtain a 0.10 mg/mL solution.

Solution (5): 0.10 mg/mL solution of artemether SQR in acetone.

Related substances 2. To proceed as described in *Related substances 2*, by *High performance liquid chromatography (5.2.17.4)*, in the monograph *Artemether*. To prepare the solutions as described below.

Solution (1): to dilute volume of the injectable solution in mobile phase to obtain a 10 mg/mL solution.

Solution (2): to dilute *Solution (1)* in mobile phase to obtain a 0.05 mg/mL solution.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

Pyrogens (5.5.2.1). It complies with the test.

DOSAGE

To proceed as described in *Dosage*, in the monograph *Artemether*. To prepare the *Sample solution* as described below.

Diluent: mixture of isopropanol and acetonitrile (75:25).

Sample solution: to dilute volume of the injectable solution in *diluent* to obtain a 4 mg/mL solution.

Procedure: to separately inject 20 µL of *Sample and standard solutions*, record the chromatograms, and measure the average area under the peaks. To calculate the content of $C_{16}H_{26}O_5$ in the injectable solution, from the responses obtained for the *Sample and standard solutions*.

PACKAGING AND STORAGE

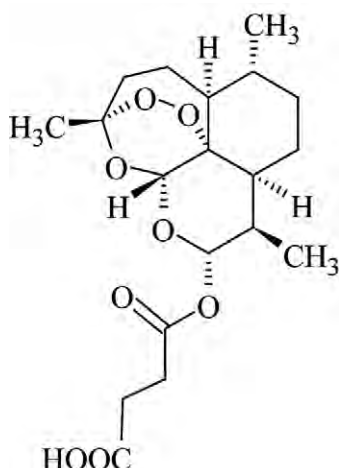
In type I glass containers, protected from light at a temperature below 25 °C.

LABELLING

To comply with the legislation in force.

ARTESUNATE

Artesunatum



$C_{19}H_{28}O_8$; 384.42

artesunate; 09673

Ester, 1 - [(3*R*, 5*aS*, 6*R*, 8*aS*, 9*R*, 10*S*, 12*R*, 12*aR*)- decahydro-3,6,9-trimethyl-3,12-epoxy-12*H*-pyrano [4,3 - *j*] -1,2 - benzodioxepine-10-yl ester] of butanedioic acid [88495-63-0]

It contains at least 98.0% and not more than 102.0% of $C_{19}H_{28}O_8$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. Fine, crystalline, white or almost white powder.

Solubility. Very slightly soluble in water, very soluble in methylene chloride, freely soluble in ethanol and acetone.

Physical and chemical constants.

Melting range (5.2.2): 132 °C to 135 °C.

Specific rotation (5.2.8): +2.5° to +3.5°. To determine at 1% solution (w/v) in methylene chloride.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the artesunate SQR spectrum similarly prepared.

B. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using G silica gel as support and a mixture of toluene and ethyl acetate (95:50) as mobile phase. To separately apply to the plate 2 µl of each of the solutions freshly prepared, as described below.

Solution (1): 0.10 mg/mL solution of the sample in toluene.

Solution (2): 0.10 mg/mL solution of artesunate SQR in toluene.

To develop the chromatogram. To remove the plate, and let it drying. To aerosolize the plate with anisaldehyde SR and heat at 120 °C for 5 minutes. To examine under ultraviolet light (254 nm). The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

C. To dissolve 0.1 g of the sample in 40 mL of absolute ethanol, stir and filter it. To add 0.5 mL of hydroxylamine hydrochloride SR and 0.25 mL of sodium hydroxide SR to 20 mL of the filtrate. To heat in a water bath to boil, cool down, and add two drops of hydrochloric acid SR and two drops of 5% ferric chloride (w/v). The violet color develops.

D. To dissolve 0.1 g of the sample in 40 mL of absolute ethanol, stir and filter it. To evaporate 20 mL of the filtrate in a water bath until obtaining the volume of 5 mL. To transfer five drops to a porcelain capsule and add a drop of vanillin SR. After 30 minutes, the red color develops.

PURITY TESTS

pH (5.2.19). 3.5 to 4.5. To determine in 1% suspension (w/v) in carbon dioxide-free water.

Related substances 1. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using G silica gel as support and a mixture of petroleum ether, ethyl acetate, and glacial acetic acid (48:36:1) as mobile phase. To separately apply to the plate 10 µl of each of the solutions freshly prepared, as described below.

Solution (1): 5 mg/mL solution of the sample in methylene chloride.

Solution (2): 0.05 mg/mL solution of the sample in methylene chloride.

Solution (3): 0.025 mg/mL solution of the sample in methylene chloride.

To develop the chromatogram. To remove the plate, and let it drying. To aerosolize the plate with vanillin SR and examine immediately. Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (2)* (1.0%) and not more than a spot is more intense than that obtained with *Solution (3)* (0.5 %).

Related substances 2. To proceed as described in *Dosage method B*. To prepare the solutions as described below.

Solution (1): 4 mg/mL solution of the sample in acetonitrile.

Solution (2): 0.04 mg/mL solution of the sample in acetonitrile.

Procedure: To separately inject 20 µL of each solution. To record the chromatograms and measure the area under the peaks.

The sum of the areas of all the secondary peaks obtained with *Solution (1)*, except the main peak's one, is not greater than twice the area under the main peak obtained with *Solution (2)* (2.0%), and no peak area is larger than that of the main peak obtained with *Solution (2)* (1.0%). Not more than one peak obtained with *Solution (1)* shows the area greater than half of the area under the main peak obtained with *Solution (2)* (0.5%). To disregard the peaks with an area less than 0.1 times the area under the main peak obtained with *Solution (2)*.

Heavy metals (5.3.2.3). To use the *Method III*. At most 0.002% (20 ppm).

Water (5.2.20.1). To determine in 2 g of the sample. At most 0.5%.

Sulfated ashes. At most 0.1%.

DOSAGE

To employ one of the methods described below.

A. To accurately dissolve about 0.25 g of the sample in 25 mL of ethanol. To titrate with 0.05M SV sodium hydroxide by using two drops of phenolphthalein SI as an indicator. Each mL of 0.05M SV sodium hydroxide is equal to 19.221 mg of $C_{19}H_{28}O_8$.

B. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 216 nm; 125 mm chromatograph column and 3 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at 30 °C; flow of Mobile phase of 0.6 mL/min.

Buffer pH 3.0: to dissolve 1.36 g of monobasic potassium phosphate in 900 mL of water. To adjust pH to 3.0 with phosphoric acid, top up to 1000 mL with water and make it homogeneous.

Mobile phase: mixture of *Buffer pH 3.0* and acetonitrile (50:50).

Sample solution: to accurately dissolve weighed amount of the sample in acetonitrile to obtain a 2 mg/mL solution.

Standard solution: to accurately dissolve weighed amount of artesunate SQR in acetonitrile to obtain a 2 mg/mL solution.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_{19}H_{28}O_8$ from the sample, from the responses obtained for the *Sample* and *standard solutions*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antimalarial.

ARTESUNATE, TABLETS

It contains at least 90.0 % and at most 110 % of the amount stated of artesunate ($C_{19}H_{28}O_8$).

IDENTIFICATION

A. To weigh and pulverize the tablets. To transfer amount of powder equivalent to 50 mg of artesunate to beaker, add 25 mL of acetone, stir, and filter it. To evaporate the filtrate in a water bath and put the residue in the desiccator, under silica gel, for 24 hours. The residue obtained responds to *Identification test A.*, in the monograph *Artesunate*.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage method B.* corresponds to that of main peak of *Standard solution*.

C. To proceed as described in *Identification test B.*, in the monograph *Artesunate*. To prepare *Solutions (1)* and *(2)* as described below.

Solution (1): To weigh and pulverize the tablets. To transfer amount of powder equivalent to 5 mg of artesunate to beaker, add 50 mL of absolute ethanol, stir, and filter it. To evaporate 2 mL of the filtrate in water bath and dissolve the residue in 2 mL of acetone.

D. To weigh and pulverize the tablets. To use the amount of powder equivalent to 0.1 g of artesunate. To proceed as described in *Identification test D.*, in the monograph *Artesunate*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

To proceed as described in *Dosage method B.*

PURITY TESTS

Related substances 1. To proceed as described in *Related substances 1*, in the monograph *Artesunate*. To prepare the solutions as described below.

Solution (1): To weigh and pulverize the tablets. To stir the amount of powder equivalent to 0.1 g of artesunate with 20 mL of methylene chloride and filter it, to obtain a 5 mg/mL solution.

Solution (1): to dilute *Solution (2)* in methylene chloride to obtain a 0.05 mg/mL solution.

Solution (2): to dilute *Solution (2)* in methylene chloride to obtain a 0.025 mg/mL solution.

Related substances 2. To proceed as described in *Related substances 2*, in the monograph *Artesunate*. To prepare the solutions as described below.

Solution (1): To weigh and pulverize the tablets. To transfer amount of powder equivalent to 0.4 mg of artesunate to beaker and add 20 mL of ethanol. To vigorously stir and filter it. To transfer 5 mL of this filtrate to 25 mL volumetric flask and top up with *Mobile phase*.

Solution (2): to transfer 1 mL of *Solution (1)* to 100 mL volumetric flask and top up with *Mobile phase*.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3).

It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To weigh and spray 20 tablets. To accurately transfer amount of powder equivalent to 0.5 mg of artesunate to 50 mL volumetric flask and add 40 mL of ethanol. To mechanically stir for 10 minutes, top up with the same solvent, and filter it. To titrate 25 mL of the filtrate with 0.05 M SV sodium hydroxide by using two drops of phenolphthalein SI as an indicator. Each mL of 0.05 M SV sodium hydroxide is equal to 19.221 mg of $C_{19}H_{28}O_8$.

B. To proceed as described in *Dosage*, in monograph *Artesunate*. To prepare the *Sample solution* as described below.

Sample solution: To weigh and spray 20 tablets. To accurately transfer amount of powder equivalent to 0.25 g of artesunate to 25 mL volumetric flask, add 20 mL of ethanol, and mechanically stir for 10 minutes. To top up with the same solvent, make it homogeneous, and filter it. To transfer 5 mL of this filtrate to 25 mL volumetric flask and top up with *Mobile phase*.

Procedure: to separately inject 20 μ L of *Sample* and *standard solutions*, record the chromatograms, and measure the average area under the peaks. To calculate the amount of $C_{19}H_{28}O_8$ in the tablets, from the responses obtained for the *Sample* and *standard solutions*.

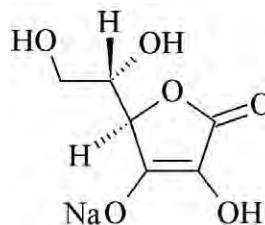
PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

ASCORBATE, SODIUM Natrii ascorbas



$C_6H_7NaO_6$; 198.11
sodium ascorbate; 00107
Sodium salt of the L-ascorbic acid (1:1)
[134-03-2]

It contains at least 99.0% and no more than 101.0% of $C_6H_7NaO_6$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or yellowish crystalline powder.

Solubility. Freely soluble in water, slightly soluble in ethanol and practically insoluble in methylene chloride.

Physical and chemical constants.

Specific rotation (5.2.8): $+103^\circ$ to $+108^\circ$, determined at a 100 mg/mL solution in water.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in mineral oil, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the sodium ascorbate SQR spectrum similarly prepared.

B. To weigh 1 g of the sample and dissolve it in 50 mL of water. To add 1 mL of 0.1 M hydrochloric acid to 4 mL of this solution. The resulting solution slowly reduces alkaline cupric tartrate SR at room temperature and more rapidly under heating.

C. It is related to the reactions of sodium ion (5.3.1.1).

D. To prepare a 10% solution (w/v) of the sample. To add 0.2 mL of nitric acid SR and 0.2 mL of 1.7% silver nitrate (w/v) to 1 mL of this solution. Gray precipitate formation occurs.

PURITY TESTS

Appearance of solution. To dissolve 5 g of the sample in water and top up the volume to 50 mL with the same solvent. This solution is not more intensely colored than the standard solution prepared by diluting 5 mL of the *Reference color solution* described below, in 95 mL of 1% hydrochloric acid (w/v). To proceed as described in *Color of liquids* (5.2.12).

Reference color solution: to mix 1.5 parts of the ferric chloride base solution, 1.2 parts of copper sulfate base solution, 1.5 parts of cobaltous chloride base solution, and 0.8 parts of 1% hydrochloric acid solution (w/v).

pH (5.2.19). 7.0 to 8.0. To determine in 10 % solution (w/v).

Volatile organic impurities. To proceed as described in *Gas chromatography* (5.2.17.5). To use chromatograph equipped with flame ionization detector, by using mixture of nitrogen, synthetic air, and hydrogen (1:1:10) as the auxiliary gas to flame detector; 30 m long capillary column and 0.53 mm inner diameter, filled with stationary linked phase of phenyl and methyl polysiloxane (5:95), with a film thickness of 5 µm; column temperature from 35 °C to 260 °C (35 °C kept for 5 minutes, then rises to 175 °C at 8 °C per minute, and rises to 260 °C at 35 °C and kept at this temperature for at least 16 minutes); injector temperature at 70 °C and the detector temperature at 260 °C; use helium as carrier gas; flow of carrier gas of 1 mL/minute.

Sample solution: To accurately dissolve about 1 g of sample in 50 mL of water, free of organic compounds.

Standard solution: to prepare a solution in water free of organic compounds containing, in each mL, 10 µg of methylene chloride, 1 µg of chloroform, 2 µg of benzene, 2 µg of dioxane, and 2 µg of trichlorethylene.

to separately inject 1 µL of *Sample solution* and *Standard solution* into the gas chromatograph. To obtain the chromatograms and measure the area under the peaks. Based on the retention time, identify any peak in the chromatogram of the sample solution. To compare the chromatograms of the *Sample solution* and *Standard solution* to establish the presence and identification of peaks in the chromatogram. Limits: Benzene (2 ppm), chloroform (50 ppm), dioxane (100 ppm), methylene chloride (500 ppm), and trichlorethylene (100 ppm). It complies with the test.

Oxalic acid. The following preparations must rest for 1 hour. The opalescence of the sample preparation is not greater than that one of the standard preparation.

Sample preparation: To dissolve 0.25 g of sample in 5 mL of water. To add 1 mL of dilute acetic acid and 0.5 mL of calcium chloride SR. At most 0.3% (3000 ppm).

Standard preparation: To dissolve 70 mg of oxalic acid in water and top up the volume to 500 mL with the same solvent. To add 1 mL of dilute acetic acid and 0.5 mL of calcium chloride SR to 5 mL of this solution.

Sulfates (5.3.2.1). To dissolve 1.6 g of the sample in 40 mL of water and proceed as described in *Limit test for sulfates*, by using 0.5 mL of standard solution of 0.005 M sulfuric acid. At most 0.015% (150 ppm).

Copper. To proceed as described in *Atomic spectrometry* (5.2.13.1.1). To use flame spectrophotometer with a mixture of air-acetylene, hollow copper cathode lamp, and select the emission line at 324.8 nm.

Sample solution: To transfer 2 g of the sample to a 25 mL volumetric flask and top up with nitric acid SR. At most 5 ppm of copper.

Iron. To proceed as described in *Atomic spectrometry* (5.2.13.1.1). To use flame spectrophotometer with a mixture of air-acetylene, hollow iron cathode lamp, and select the emission line at 248.3 nm.

Sample solution: To transfer 5 g of the sample to a 25 mL volumetric flask and top up with nitric acid SR. At most 2 ppm of iron.

Nickel. To proceed as described in *Atomic spectrometry* (5.2.13.1.1). To use flame spectrophotometer with a mixture of air-acetylene, hollow nickel cathode lamp, and select the emission line at 248.3 nm.

Sample solution: To transfer 10 g of the sample to a 25 mL volumetric flask and top up with nitric acid SR. At most 1 ppm of nickel.

Heavy metals (5.3.2.3). To dissolve 2 g of the sample in water and top up the volume to 20 mL. To transfer 12 mL of this solution and proceed as described in *Method I*. At most 0.001% (10 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample in oven at 105 °C until reaching constant weight. At most 0.25%.

DOSAGE

To accurately weigh about 0.4 g of the sample and dissolve in a mixture of 100 mL of water and 25 mL of 9.8% sulfuric acid (w/v). To immediately titrate with 0.1 M SV iodine and add 3 mL of starch I near the endpoint. Each mL of 0.1 M SV iodine is equal to 9.905 mg of $C_6H_7NaO_6$.

PACKAGING AND STORAGE

In non-metallic containers, protected from light.

LABELLING

To comply with the legislation in force.

CATEGORY

Excipient.

BANDAGE

The bandage consists of continuous band of purified gauze, type I, tightly rolled, variable width and length, free of lint and yarns.

The gauze bandage, previously unrolled, must meet all the requirements set forth for the hydrophilic purified gauze, in accordance with their techniques and following specifications.

CHARACTERISTICS

Length. To determine by measuring along the midline of the bandage, unrolled, without traction; the length should not be less than 98% of information on the label.

Width. To measure the width at three points evenly spread along the bandage unrolled. The mean of three measurements must have dimensional variation of no more than 2% over the information on the label.

Number of yarns. To determine the number of warp yarns and weft in 5 areas of 1 cm x 1 cm, in center line of the bandage, at regular intervals of at least 30 cm from the end and calculate the number of yarns in an area of 5 x 5 cm.

Weight. To determine the weight of the entire roller bandage, and by using the results of previous measurements, calculate the weight per square meter.

Absorbing capacity. To hold the bandage, properly unrolled horizontally, almost in contact with a surface of distilled water and let it gently touch the liquid; the bandage should completely submerge within 30 seconds.

Medicinal or adhesive substances. The gauze bandage, when impregnated with medicinal or adhesive substances must provide uniform concentration. It shall not contain substances in concentrations capable of causing accidents or toxic reaction.

BIOLOGICAL SAFETY TEST

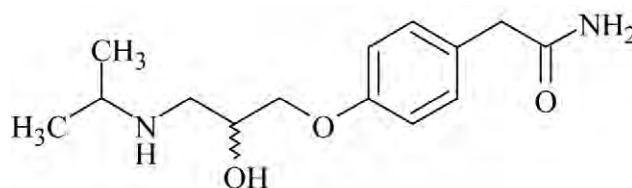
Sterility (5.5.3.2.1). Applicable when the bandage is considered sterile. It complies with the test.

LABELLING

To comply with the legislation in force.

ATENOLOL

Atenololum



$C_{14}H_{22}N_2O_3$; 266.34 atenolol; 00911

4 - [2- Hydroxy-3- [(1-methylethyl) amino] propoxy] benzeneacetamide
[29122-68-7]

It contains at least 98.0% and no more than 102.0% of $C_{14}H_{22}N_2O_3$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white or almost white powder.

Solubility. Very slightly soluble in water, freely soluble in methanol, soluble in ethanol and glacial acetic acid, slightly soluble in methylene chloride, very slightly soluble in acetone, practically insoluble in acetonitrile.

Physical and chemical constants

Melting range (5.2.2): 152 °C to 155 °C.

Specific rotation (5.2.8): +0.10° to -0.10°. To determine at 1% solution (w/v) in water.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the atenolol SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14) ranging from 230 nm to 350 nm, of 0.01% (w/v) in methanol, exhibits maxima and minima only in the same wavelength of a similar atenolol SQR solution. The ratio between the absorbance values measured at 275 nm and 282 nm is between 1.15 and 1.20.

C. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel as support and a mixture of ammonium hydroxide and methanol (3:97) as mobile phase. To apply separately to the plate 10 µl of each of the solutions freshly prepared, as described below.

Solution (1): 10 mg/mL solution of the sample in methanol.

Solution (2) 10 mg/mL solution of atenolol SQR in methanol.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

PURITY TESTS

Chlorides. To dissolve 1 g of the sample in 100 mL of 0.15 M nitric acid, add 1 mL of silver nitrate SR, and make it homogeneous. Any turbidity developed is not more intense than the mixture of 1.4 mL of 0.02 M hydrochloric acid, 98.6 mL of 0.15 M nitric acid, and 1 mL of silver nitrate SR. At most 0.1% (1000 ppm).

Chromatographic purity. To proceed as described in *Dosage method B*. To prepare *Solutions (1)* and *(2)* as described below.

Solution (1): to accurately dissolve weighed amount of the sample in *Mobile phase* to obtain a 0.1 mg/mL solution.

Solution (2): to transfer 0.5 mL of *Solution (1)* to 100 mL volumetric flask and dilute with *Mobile phase*, to obtain a 0.5 µg/mL solution.

Procedure: to separately inject 50 µL of *Solution (1)* and *Solution (2)*, record the chromatograms at least 6 times the retention time of the peak of atenolol, and measure the areas of the peaks. No secondary peak obtained with *Solution (1)* shows the area greater than half of the area under the main peak obtained with *Solution (2)* (0.25%).

The sum of the areas under the secondary peaks obtained with *Solution (1)* shall not be greater than the half of the area under the main peak obtained with *Solution (2)* (0.5%).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 105 °C until obtaining constant weight. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. To accurately weigh about 25 mg of the sample and dissolve in methanol. To top up the volume to 50 mL with the same solvent. To successively dilute in methanol to the 0.01% concentration (w/v). To prepare a standard solution at the same concentration by using the same solvent. To measure the absorbance of the solutions at 275 nm, by using methanol to adjust zero. To calculate the content of $C_{14}H_{22}N_2O_3$ in the sample from the readings obtained.

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 226 nm; 250 mm chromato-

graph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at room temperature; flow of *Mobile phase* of 0.6 mL/min.

Mobile phase: to dissolve 1.1 g of sodium heptane sulphate and 0.71 g of sodium phosphate dibasic anhydrous in 700 mL of water. If necessary, adjust the pH to 3.0 with phosphoric acid MR. To add 300 mL of methanol and 2 mL of dibutylamine and make it homogeneous.

Sample solution: to accurately dissolve weighed amount of the sample in *Mobile phase* to obtain a 10 µg/mL solution.

Standard solution: to accurately dissolve weighed amount of atenolol SQR in *Mobile phase* to obtain a 10 µg/mL solution.

To inject replicates of 10 µL of the *Standard solution*. The column efficiency is greater than 5000 theoretical plates. The tailing should not be greater than 2.0. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2 %.

Procedure: to separately inject 10 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_{14}H_{22}N_2O_3$ from the sample, from the responses obtained for the *Sample* and *standard solutions*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antihypertensive

ATENOLOL, TABLETS

It contains at least 90.0 % and at most 110 % of the amount stated of $C_{14}H_{22}N_2O_3$.

IDENTIFICATION

A. The ultraviolet absorption spectrum (5.2.14) ranging from 230 nm to 350 nm, of *Sample solution* obtained in *Dosage method A.*, exhibits absorption maximum in 275 nm and 282 nm, similar to those observed in the *Standard solution* spectrum.

A. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage method B.* corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

Procedure for content uniformity. To transfer each tablet to a 25 mL volumetric flask containing 15 mL of methanol and to leave it in ultrasound to disintegrate the tablet. To proceed as described in *Dosage Method A.* from “To heat resulting suspension.”

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL

Equipment: blades, 50 rpm

Time: 30 minutes

Procedure: After testing, remove aliquot from the dissolution medium, dilute with 0.1% fosforic acid (v/v) to reach concentration of 10 mg/mL. Then, proceed as described in *Dosage method B.*, in the monograph *Atenolol*. To calculate the amount of $C_{14}H_{22}N_2O_3$ dissolved in the medium, by comparing the areas under the peaks obtained with that one related to the atenolol SQR solution, at a concentration of 10 mg/mL, prepared in the same solvent.

Tolerance: not less than 80% (Q) of the stated amount of $C_{14}H_{22}N_2O_3$ dissolves within 30 minutes.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. By Ultraviolet absorption spectrophotometry (5.2.14). To weigh and pulverize 20 tablets, transfer the amount of powder equivalent to 0.25 g of atenolol to a 250 mL volumetric flask, and add 150 mL of methanol. To heat resulting suspension at 60 °C for 10 minutes, by stirring occasionally. To mechanically stir for 15 minutes, cool down, and top up with methanol. To make it homogenous and filter it. To successively dilute in methanol to the 0.01% concentration (w/v). To prepare a standard solution at the same concentration by using the same solvent. To measure the absorbance of the resulting solutions at 275 nm, by

using methanol to adjust zero. To calculate the amount of $C_{14}H_{22}N_2O_3$ in the tablets, from the responses obtained.

B. By High performance liquid chromatography (5.2.17.4). To proceed as described in *Dosage*, method B., in the monograph *Zidovudine*.

To prepare the *Sample solution* as described below.

Sample solution: to transfer 10 tablets to 1000 mL volumetric flask, add 500 mL of *Mobile phase*, and leave it in ultrasound for 15 minutes, or until total disintegration of the tablets. To top up with the same solvent, make it homogeneous, and centrifuge it. To successively dilute in the same solvent to a 10 mg/mL concentration.

Procedure: to separately inject 10 µL of *Standard solution* and *Sample solution*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of $C_{14}H_{22}N_2O_3$ in the tablets, from the responses obtained for the *Sample* and *standard solutions*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

ATENOLOL AND CHLORTHALIDONE TABLETS

It contains at least 90.0 % and at most 110 % of the amount stated of $C_{14}H_{22}N_2O_3$ and $C_{14}H_{11}ClN_2O_4S$.

IDENTIFICATION

A. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using G silica gel as support and a mixture of ammonium hydroxide *M* and 1-butanol (1:5) as mobile phase. To separately apply to the plate 10 µl of each of the solutions freshly prepared, as described below.

Solution (1): To weigh and pulverize the tablets. To accurately transfer amount of powder equivalent to 10 mg of chlorthalidone to 10 mL volumetric flask and add 8 mL of methanol. To mechanically stir for 15 minutes and top up with the same solvent. To make it homogenous and filter it.

Solution (2): to prepare 1 mg/ml solution of chlorthalidone SQR in methanol.

Solution (3): to prepare 4 mg/ml solution of atenolol SQR in methanol.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). The spots relating to atenolol and chlorthalidone obtained with

Solution (1) correspond in position, color, and intensity to those obtained with *Solution (2)* and *Solution (3)*.

B. The retention times of the main peaks in the chromatogram of the *Sample solution* obtained in the *Dosage* correspond to that of main peaks of *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

Procedure for content uniformity. To transfer each tablet to volumetric flask, thus obtaining chlorthalidone solution at 0.025% concentration (w/v). To add mixture of water and acetonitrile (1:1) equivalent to 50% of the volume of the flask. To mechanically stir for 20 minutes, to fully disintegrate the tablets, and top up with the same solvent. To proceed as described in *Dosage* method, from the *Standard solution*.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.01 M hydrochloric acid, 900 mL

Equipment: blades, 50 rpm

Time: 45 minutes

Procedure: After testing, remove aliquot from the dissolution medium and proceed as described in *Dosage*. To prepare *Sample solution*, *Standard solution*, and *Diluent* as described below.

Diluent: mixture of acetonitrile and 1.8 M sulfuric acid (1000:32).

Sample solution: mixture of 10 mL of sample and 3 mL of *Diluent*.

Standard solution: to prepare solution of atenolol SQR in mixture of water and *Diluent* (750:225), thus obtaining the atenolol solution (0.00085 L) and chlorthalidone solution (0.00085 L'), per milliliter, where L and L' are the amounts informed in tablets in relation to atenolol and chlorthalidone, respectively.

Tolerance: not less than 80% (Q) of the stated amount of $C_{14}H_{22}N_2O_3$ and 70% (Q) of the stated amount of $C_{14}H_{11}ClN_2O_4S$ dissolve within 45 minutes.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 275 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μ m), at room temperature; flow of *Mobile phase* of 1.7 mL/min.

Mobile phase: mixture of water, acetonitrile, and 1.8 M sulfuric acid (740:250:8) and 930 mg of sodium octyl sulfate per liter of mixture.

Sample solution: To weigh and spray 10 tablets. To transfer amounts of powder equivalent to 25 mg of chlorthalidone to 50 mL volumetric flask, add 40 mL of mixture of water and acetonitrile (1:1), and mechanically stir for 20 minutes. To top up with the same solvent, make it homogeneous, and filter it. To transfer 25 mL of the filtrate to 50 mL volumetric flask and top up with water, thus obtaining 0.25 mg/mL solution.

Standard solution: to accurately dissolve weighed amount of atenolol SQR and chlorthalidone SQR in a mixture of water and acetonitrile (3:1), to obtain 1 mg/mL and 0.25 mg/mL solutions, respectively.

To inject replicates of 10 μ L of the *Standard solution*. The relative retention times are about 0.8 for atenolol and 1.0 for chlorthalidone. The resolution between atenolol and chlorthalidone should not be less than 3.0. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2.0%.

Procedure: to separately inject 10 μ L of *Standard solution* and *Sample solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_{14}H_{22}N_2O_3$ and $C_{14}H_{11}ClN_2O_4S$ from the sample, from the responses obtained for the *Sample* and *Standard solutions*.

PACKAGING AND STORAGE

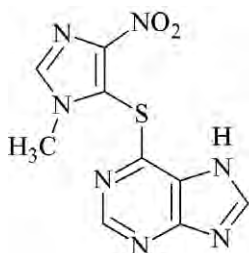
In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

AZATHIOPRINE

Azathioprinum



$C_9H_7N_7O_2S$; 277.26 azathioprine; 00984
6 - [(1-Methyl-4-nitro-1H-imidazole-5-yl) thio] -9
H-purine[446-86-6]

It contains at least 98.0% and no more than 101.5% of $C_9H_7N_7O_2S$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Pale yellow powder.

Solubility. Practically insoluble in water, ethanol, and chloroform. Soluble in dilute solutions of alkali hydroxides and slightly soluble in dilute acid solutions.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the azathioprine SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14), from 230 nm to 350 nm, 0.00075% solution (w/v) prepared in 0.1 M hydrochloric acid, has absorption maximum at 280 nm, similar to those observed in the spectrum of a similar solution of azathioprine SQR.

C. To heat 20 mg of the sample with 100 mL of water and filter it. To add 1 mL of hydrochloric acid and 10 mg of zinc powder to 5 mL of the filtrate, and let it rest for 5 minutes. The yellow color develops. To filter it. To add 0.1 mL of sodium nitrite SR and 0.1 g of sulfamic acid. To stir until bubbles disappear. To add 1 mL of 2-naphthol SR. Pink precipitate is produced.

PURITY TESTS

Acidity or alkalinity. To accurately stir 2 g of the sample with 100 mL of water for 15 minutes. To filter it. A maximum of 0.1 mL of 0.02 M sodium hydroxide is spent to neutralize 20 mL of the filtrate, by using methyl red SI

as an indicator. At most 0.1 mL of 0.02 M hydrochloric acid is spent to neutralize 20 mL of the filtrate, by using methyl red SI as an indicator.

Limit of mercaptopurine. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ sil-

ica gel as support and a mixture of 1-butanol, ethanol, and water (4:1:1) as mobile phase. To apply separately to the plate 5 µl of each of the solutions freshly prepared, as described below.

Solution (1): 20 mg/mL solution of the sample in ammonia SR.

Solution (2): 0.2 mg/ml mercaptopurine solution in ammonia SR.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). Then, submit it to iodine vapor. Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (2)* (1.0%).

Loss on drying (5.2.9). To determine in 0.5 g of the sample. To desiccate in an oven at 105 °C for 5 hours. At most 1.0%.

Sulfated ashes (5.2.10). At most 0.1 %.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Titrimetric methods in non-aqueous medium* (5.3.3.5). To accurately weigh about 0.25 mg of the sample and dissolve in 25 mL of dimethylformamide. To titrate with 0.1 M SV tetrabutylammonium hydroxide, to potentiometrically determine the endpoint. Each mL of 0.1 M SV tetrabutylammonium hydroxide is equal to 27.726 mg of $C_9H_7N_7O_2S$.

B. By *Ultraviolet absorption spectrophotometry* (5.2.14). To exactly transfer about 0.1 g of azathioprine to a 500 mL volumetric flask and add 300 mL of 0.1 M hydrochloric acid. Let it in water bath for 30 minutes. To cool down and top up the volume with the same solvent. To make it homogeneous. To dilute to 0.001% concentration (w/v) by using the same solvent. To prepare standard solution under the same conditions. To measure the absorbance of the solutions at 280 nm, by using 0.1 M hydrochloric acid to adjust zero. To calculate the content of $C_9H_7N_7O_2S$ in the sample from the readings obtained. Alternatively, make the calculations taking into account that $A(1\%, 1\text{ cm}) = 628$ at 280 nm, in 0.1 M hydrochloric acid.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

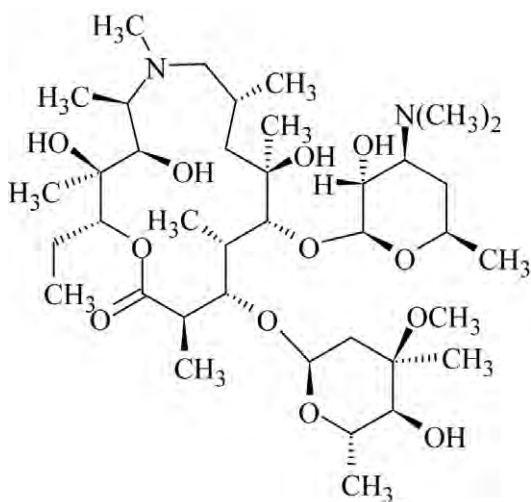
To comply with the legislation in force.

THERAPEUTIC CLASS

Immunosuppressant.

AZITHROMYCIN

Azithromycinum



$C_{38}H_{72}N_2O_{12}$; 748.98

$C_{38}H_{72}N_2O_{12} \cdot 2H_2O$; 785.02

azithromycin; 00997

azithromycin, di-hidratado; 00998

(2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl- α -L-ribo-hexopyranosyl oxy]-2-ethyl-3, 4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11 - [[3,4,6-trideoxy-3-(dimethylamino)- β -D-xylohexopyranosyl oxy]-1-oxa-6-azaciclopentadecan-15-one [83905-01-5]

(2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl- α -L-ribo-hexopyranosyl oxy]-2-ethyl-3, 4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11 - [[3,4,6-trideoxy-3-(dimethylamino)- β -D-xylohexopyranosyl oxy]-1-oxa-6-azaciclopentadecan-15-one di-hidratado [117772-70-0]

It shows minimum potency of 945 μ g and at most 1030 μ g of azithromycin ($C_{38}H_{72}N_2O_{12}$) per milligram, relative to the anhydrous substance.

DESCRIPTION

Physical characteristics. White crystalline powder.

Solubility. It is very slightly soluble in water, soluble in chloroform, freely soluble in ethanol and methanol. Very slightly soluble in alkalis. Slightly soluble in acidic solutions.

Physical and chemical constants

Specific rotation (5.2.8): -45° to -49° in relation to the anhydrous substance. To determine at 2% solution (w/v) in ethanol at 20 °C.

IDENTIFICATION

The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the azithromycin SQR spectrum similarly prepared.

PURITY TESTS

pH (5.2.19). To determine at 0.2% solution (w/v) in mixture of water and ethanol (1:1).

Heavy metals (5.3.2.3). To use *Method IV*. At most 0.0025% (25 ppm).

Water (5.2.20.1). At most 5.0%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. To moisten the sample with 2 mL of nitric acid and five drops of sulfuric acid. At most 0.3%.

Crystallinity. To suspend in mineral oil some particle from the sample. Then, transfer it to a microscope slide and examine by polarized light microscope. The particles exhibit birefringence, which is extinguished by micrometric adjustment.

DOSAGE

To proceed as described in *Microbiological test of antibiotics (5.5.3.1)* by using cylinders with the agar diffusion method.

Micro-organism: *Micrococcus luteus* ATCC 9341.

Culture media: culture medium number 1, to maintain the micro-organism; culture medium number 3, to standardize the inoculum; and culture medium number 11, to the base layer and inoculum preparation.

Sample solution: to accurately weigh about 25 mg of the sample, transfer to a 25 mL volumetric flask by using 10 mL of methanol. To mechanically stir for 15 minutes and top up with methanol. To filter it. To successively dilute the resulting solution in 0.1 M Potassium phosphate buffer, sterile, pH 8.0 (solution 2) to obtain 0.1 μ g/mL, 0.2 μ g/mL, and 0.4 μ g/mL solutions.

Standard solution: to accurately weigh about 25 mg of azithromycin SQR, transfer to a 25 mL volumetric flask, and top up with methanol. To successively dilute the resulting solution in 0.1 M Potassium phosphate buffer, sterile, pH 8.0 (solution 2) to obtain 0.1 μ g/mL, 0.2 μ g/mL, and 0.4 μ g/mL solutions.

Procedure: to add 20 mL of culture medium number 11 to each plate, wait to solidify it, add 5 mL of 1% inoculum and add 0.2 mL of Sample and standard solutions prepared freshly to the cylinders. To calculate the potency of the sample, in μ g of azithromycin per milligram, from the

standard potency and the responses obtained for the *Sample and standard solutions*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antibacterial.

AZITHROMYCIN, CAPSULES

It presents potency of at least 90.0% and at most 110.0% of the value informed in $C_{38}H_{72}N_2O_{12}$.

IDENTIFICATION

To weigh the capsules, remove the content, and weigh them again. To transfer amount equal to 0,25 mg of azithromycin to a 50 mL volumetric flask, dissolve in methanol, and top up with the same solvent. To filter it. To evaporate the filtrate in a water bath and weigh 1.5 mg of the residue. To proceed as described in *Identification*, in the monograph *Azithromycin*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

PURITY TESTS

Water (5.2.20.1). At most 5.0%.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To proceed as described in *Dosage*, in the monograph *Azithromycin*. To prepare the *Sample solution* as described below.

Sample solution: to weigh 20 capsules, remove the content, and weigh them again.

To transfer amount of powder equal to 25 mg of azithromycin to a 25 mL volumetric flask, and top up with methanol. To stir for 15 minutes and filter it. To successively dilute the resulting solution in *0.1 M Potassium phosphate buffer, sterile, pH 8.0 (solution 2)* to obtain 0.1 µg/mL and 0.4 µg/mL solutions.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

AZITHROMYCIN, POWDER FOR ORAL SUSPENSION

Azithromycin powder for oral suspension is a mixture of azithromycin with one or more flavoring agents, buffers, sweeteners, and suspending agents. It presents potency of at least 90.0% and at most 110.0% of the value informed in azithromycin ($C_{38}H_{72}N_2O_{12}$).

IDENTIFICATION

To transfer amount of powder equal to 0.2 mg of azithromycin to a 50 mL volumetric flask, and top up with methanol. To make it homogenous and filter it. To evaporate the filtrate in a water bath to obtain the residue. To proceed as described in *Identification*, in the monograph *Azithromycin*.

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test. To determine in the diluent vial.

pH (5.2.19). 8.5 to 11.0. To reconstitute the suspension as described on the product label.

Determination of weight (5.1.1). It complies with the test. To determine in the not reconstituted powder.

Uniformity of dosage units (5.1.6). Applied to powder packed in single dose. It complies with the test. To reconstitute the suspension as described on the product label.

PURITY TESTS

Water (5.2.20.1). At most 1.5%.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To proceed as described in *Dosage*, in the monograph *Azithromycin*. To prepare the *Sample solution* as described below.

Sample solution: To reconstitute the suspension as described on the product label. To transfer the volume of oral suspension freshly homogenized and free of bubbles, containing the equivalent to 0.2 g of azithromycin to a 20 mL volumetric flask with 10 mL of methanol. To stir and top up the volume with the same solvent. To filter it. To transfer 5 mL of the filtrate to 50 mL volumetric flask and top

up with 0.1 M Potassium phosphate buffer, sterile, pH 8.0 (solution 2). To dilute to obtain 0.1 µg/mL, 0.2 µg/mL, and 0.4 µg/mL concentrations, by using the 0.1 M Potassium phosphate buffer, sterile, pH 8.0 as diluent.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

BALSAM, PERU

Balsamum peruvianum

Myroxylon balsamum (L.) Harms var. *pereirae* (Royle) Harms - FABACEAE

The vegetable drug consists of balsam from the trunk to the chiseled hot. It contains at least 45% and at most 70% of esters, especially benzyl benzoate and benzyl cinnamate.

CHARACTERISTICS

Organoleptic characteristics. Viscous, clear, and dark brown to reddish-brown liquid. When examined in thin-layer, it shows yellowish brown color. It has a characteristic and aromatic odor, typical of vanilla, and bitter and acid flavor. It does not solidify on exposure to air, or prolonged period or by heating, and does not produce filaments.

Solubility. Practically insoluble in water, very soluble in ethanol, soluble in chloroform and acetic acid, slightly soluble in diethyl ether and petroleum ether, immiscible in fatty oils, except castor oil.

IDENTIFICATION

A. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel, with 250 µm thickness as support and a mixture of glacial acetic acid, ethyl acetate, and hexane (0.5:10:90) as mobile phase. To separately apply to the plate, as band, 10 µl of each of the solutions freshly prepared, as described below.

Solution (1): to dissolve 0.5 g of the sample in 10 mL of ethyl acetate.

Solution (2): to dissolve 4 mg of thymol, 30 mg of benzyl cinnamate, and 80 µL of benzyl benzoate in 5 mL of ethyl acetate.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). The principal spots in the *Solution (4)* correspond in position, color, and intensity to those obtained with the *Solution (2)*. When examined under ultraviolet light (254 nm) chromatogram presents, in its upper third, two spots of fluorescence quenching obtained with *Solution (2)* corresponding to benzyl benzoate (upper) and benzyl cinnamate (lower) that correspond to position to those obtained with *Solution (1)*. Two other intense spots, one above and another one below the reference spots, can be seen. To aerosolize the plate with freshly prepared 20% phosphomolybdic acid solution (w/v) in ethanol, by using 10 mL for a 20 mm x 20 mm plate. To heat at 100 °C - 105 °C for 5 - 10 minutes,

and examine the chromatogram in daylight. The spots corresponding to the benzyl benzoate and benzyl cinnamate are blue on yellow background. The chromatogram shows in its middle parte a gray-violet spot (thymol) obtained with *Solution (2)*. The chromatogram shows a blue spot (nerolidol) obtained with *Solution (1)*, just below the spot

corresponding to the thymol obtained with *Solution (2)*. To examine it under ultraviolet light (254 nm). Just below the spot corresponding to nerolidol, no blue spot should appear or have fluorescence quenching upon examined under ultraviolet light (254 nm) corresponding to the rosin.

B. To dissolve 0.20 g of the sample in 10 mL of ethanol. To add 0.2 mL of ferric chloride SR. Green to olive green color develops.

C. To mix two or three drops of concentrated sulfuric acid approximately 5 times larger. Dark red color develops. After the addition of 40 mL of water, it becomes violet. No brown hue (tampering) should appear.

PURITY TESTS

Relative density (5.2.5). 1.14 to 1.17.

Index of acidity (5.2.29.7). 56 to 84. To dissolve 1 g of the sample in 100 mL of neutralized ethanol, add 1 mL of phenolphthalein SI, and titrate with 0.5 M SV ethanolic potassium hydroxide.

Index of saponification (5.2.29.8). 230 to 255. To determine in the residue obtained in *Dosage*.

Artificial balsams. To vigorously stir 0.2 g of the sample with 6 mL of petroleum ether. The petroleum ether solution shall remain clear and colorless, and all the insoluble parts of the balsam are adhered to the walls of the test tube.

Turpentine. To evaporate 4 mL of the solution obtained in *Artificial balsams*. The residue presents no turpentine odor.

Fatty oils. To stir 1 g of the sample with 3 mL of a 1000 g/L chloral hydrate solution. The resulting solution and 1000 g/L chloral hydrate solution are both clear.

DOSAGE

Esters

To add 2.5 g of the sample, 7.5 ml of sodium hydroxide solution diluted to 8.5% (w/v), and 40 ml of peroxide-free ethyl ether into a dropping funnel. To vigorously stir for 10 minutes. To separate the ether phase and stir the base phase for 1 minute with three portions of 15 mL of peroxide-free ethyl ether. To gather ether phases, desiccate with 10 g of anhydrous sodium sulfate, and filter it. To wash twice the sodium sulfate residue with 10 mL of peroxide-free ethyl ether.

To gather ether phases and evaporate to dryness. To desiccate the residue (ester) from 100 °C - 105 °C for 30 minutes, cool down in a desiccator, and weigh it.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

BALSAM, TOLU

Balsamum tolutanum

Myroxylon balsamum (L.) Harms and Myroxylon balsamum var. pereirae (Royale) Harms - FABACEAE.

The tolu balsam consists of oleoresin from *Myroxylon balsamum* (L.) Harms and *Myroxylon balsamum* var. *pereirae* (Royale) Harms. It contains at least 25% and at most 50% of free or combined acids, expressed in cinnamic acid ($C_9H_8O_2$, M 148.16).

CHARACTERISTICS

Organoleptic characteristics. Brown to reddish-brown, hard, and brittle mass, whose fine fragments have brownish-yellow color for transparency. Vanilla-like smell and taste slightly bitter.

Solubility. Practically insoluble in water and petroleum ether, very soluble in ethanol, and soluble in acetone and chloroform.

IDENTIFICATION

A. To gently add a drop of concentrated sulfuric acid on a fragment of the sample. Red-wine color develops.

B. To heat 1 g of the sample with 5 mL of water to boiling, filter it with pleated filter paper. To boil the filtrate with 1 mL of 3% potassium permanganate (w/v). It produces strong odor of benzoic aldehyde.

C. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF_{254} silica gel, with 250 μ m thickness as stationary phase and a mixture of petroleum ether and toluene (5:95) as mobile phase. To separately apply to the plate, as band, 20 μ L of *Solution* (1) and 10 μ L of *Solution* (2) freshly prepared, as described below.

Solution (1): to stir 0.4 g of fragmented sample with 10 mL of methylene chloride for 5 minutes. To filter it by using pleated filter paper.

Solution (2): to dissolve 50 mg of benzyl cinnamate in 1 mL of methylene chloride, add 50 μ L of benzyl benzoate and top up to 10 mL with methylene chloride.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). The principal spots in the *Solution* (1) correspond in position, color, and intensity to those obtained with the *Solution* (2). When examined under ultraviolet light (254 nm), the chromatogram presents, in its upper third, two spots of fluorescence quenching: the upper one corresponds to benzyl benzoate, and lower one to benzyl cinnamate. In *Solution* (1), spots with fluorescence quenching can also be observed: one on the front and two spots just below the spot corresponding to methyl cinnamate. Then, aerosolize the plate with vanillin sulfuric SR and put it in an oven at 100 °C - 105 °C for 5 minutes. The spots corresponding

to the benzyl benzoate and benzyl cinnamate are blue on yellow background. Two other spots of purple color are observed above the spot of benzyl benzoate. At the bottom of the chromatogram, there are several spots of blue and purple color. Among them, there is a yellow one.

PURITY TESTS

Index of acidity (5.2.29.7). 100 to 160. To dissolve 1 g of fragmented sample in 50 mL of neutralized ethanol. To add 1 mL of phenolphthalein SI and titrate with 0.5 M SV ethanolic potassium hydroxide.

Index of saponification (5.2.29.8). 154 to 220.

Limit of substances insoluble in alcohol. To heat to boiling 2 g of fragmented sample with 25 mL of 90% ethanol (v/v). To filter by porous glass filter previously weighed. To wash the container and the residue inside funnel with 90% hot ethanol (v/v) until complete extraction. To heat the glass funnel and its contents in an oven at 105 °C for 2 hours. To cool down in desiccator and weigh it. At most 5.0%.

Rosin. To crush 1 g of the sample with 10 mL of petroleum ether for 1 - 2 minutes. To filter to test tube and add 10 mL of a 0.5% copper acetate solution (w/v) freshly prepared. To vigorously stir and allow the separation process. The ethereal layer should not show green color.

Water (5.4.2.3). At most 5.0%. To spread 2 g of a fragmented sample on the surface of a plan crystallizer of 9 cm diameter and let it to dry under reduced pressure for 4 hours.

Total ashes (5.4.2.4). At most 0.3%.

DOSAGE

Free or combined acids expressed as cinnamic acid

To heat under reflux in a water bath 1.5 g of sample with 25 mL of 0.5 M SV ethanolic potassium hydroxide for 1 hour. To evaporate the ethanol and heat the residue with 50 mL water until the solution becomes homogeneous.

After cooling down to room temperature, add 80 mL of water and 1.5 g of magnesium sulfate solution in 50 mL of water. To mix it and let it rest for 10 minutes. To filter and wash the residue with 20 mL of water. To gather the filtrate and washing, acidify with concentrated hydrochloric acid, and extract 4 times with 40 mL of ethyl ether. To discard the aqueous phase. To gather the organic extracts and extract twice with 20 mL and three times with 10 mL of 5% sodium bicarbonate solution (w/v). To discard the ether. To gather the aqueous extracts, acidify with concentrated hydrochloric acid and extract once with 30 mL, twice with 20 mL, and once with 10 mL of methylene chloride. To gather the methylene chloride extracts and dry out with 10 g of anhydrous sodium sulfate. To filter and wash the residue with 10 mL of methylene chloride. To concentrate the

extracts combined under reduced pressure to 10 mL and remove the remaining methylene chloride in a stream of air in a fume hood. To hotly dissolve the residue in 10 mL of ethanol previously neutralized in the presence of phenol red SI solution. After cooling, titrate with 0.1 M SV sodium hydroxide by using the same indicator. Each mL of 0.1 M SV sodium hydroxide is equal to 14.816 mg of cinnamic acid ($C_9H_8O_2$).

PACKAGING AND STORAGE

In tightly closed container. Do not store as powder.

BARBATIMÃO Barbadetimani cortex

Stryphnodendron adstringens (Mart.) Coville - FABACEAE

The vegetable drug consists of dried stem bark containing at least 8% of total tannins, expressed as pyrogallol ($C_6H_6O_3$; 126.11), from which at least 0.2 mg/g is equivalent to gallic acid ($C_7H_6O_5$; 170.1) and 0.3 mg/g correspond to galocatechin ($C_{15}H_{14}O_7$; 306.27) in relation to the dry drug. Stem bark is the tissue located externally to the vascular cambium of the body.

SCIENTIFIC SYNONYM

Stryphnodendron barbatimam Mart.

CHARACTERISTICS

Organoleptic characteristics. Odorless dry barks with strongly astringent taste.

innermost, brownish lighter color in comparison with the bark region, outer, and intense reddish brown color. In young stems, bark has in front view a dark color, grainy, smooth, with narrow and deep fissures in the transverse direction. In older stem portions, it has dark-brown or brown-gray, upon the presence of lichens, always with deep crevices, predominant in the transverse direction, or consecutive waistlines, detaching as plates of variable sizes and shapes, irregular, with deep depressions on site. The fracture of the bark is grainy-type in relation to the bark region, fibrous, and longitudinally striated, with chips in the phloematic region.

MICROSCOPIC DESCRIPTION

The outer portion of the bark has 20-30 layers of tabular cells radially lined with thin walls and brown contents, followed by many layers of parenchyma cells of isodiametric shape or slightly periclinal elongated also with thin walls. Most of these cells have reddish-brown content, which does not easily discolor with 30% sodium hypochlorite (w/v) and does not change color in the presence of ferric chloride SR. In this parenchyma portion, there are stone cells (the majority) and macrosclereids positioned at various levels,

grouped or isolated, with very thick lignin walls, with clear lamella and simple pits sometimes branched. In the outer portions of the bark, both parenchymal cells and sclereids can be viewed compressed and deformed by mechanical action in the internal tissues. In the phloem region, there are few sets of gelatinous fiber elements, relatively narrow, always with adjunct idioblasts containing a large prismatic crystal of calcium oxalate, with a varying number of sides, in whole or superficially eroded. The sets of fibers, when viewed in longitudinal sections, attached parenchyma beams of the phloem, which are generally uniseriate, but become bi-multiseriate in the outer portions. The sieve tube elements have combined sieve plates, being collapsed in the outer regions of the phloem. Isolated stone cells, similar to those of bark, and spherical starch grains are abundant in the parenchyma tissue of the phloem. The cells around the parenchyma beam react positively to the presence of ferric chloride SR, and become dark green. In the phloem region, we can also find bulky cells with hyaline content arranged in sets of 5 to 7 elements.

MACROSCOPIC DESCRIPTION

The stem bark, when dried, are presented in arched fragments, with varied sizes and shapes. In cross-section, they have, on average, 0.6 mm thick when dry, and 10 to 12 mm thick when hydrated. The phloematic region is

MICROSCOPIC DESCRIPTION OF POWDER

The powder meets all the requirements for the species, unless the macroscopic characters. Characteristics: fragments of bark with tabular cell; groups of parenchyma cells with reddish-brown contents, juxtaposed with stone cells

or macrosclereids, in groups or isolated, strongly lignified walls with simple pits, sometimes branched; sets of fibers with adjunct crystal idioblasts, enclosing fragments of parenchyma beams of the phloem, parenchyma cells with spherical starch grains.

IDENTIFICATION

A. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel, with 250 µm thickness as support and a mixture of ethyl acetate, formic acid, and water (75:5:5) as mobile phase. To separately apply to the plate, as band, 10 mL of *Solution* (1) and 3mL of *Solution* (2) and *Solution* (3) freshly prepared, as described below.

Solution (1): to exactly extract by turbolysis about 10 g of the vegetable drug grinded in 90 mL of acetone and water (7:3) for 15 minutes, with 5-minute intervals so that the temperature does not exceed 40 °C. To filter and discard the acetone in a rotary evaporator under reduced pressure. To extract the resulting aqueous phase with 3 portions of 20 mL of ethyl acetate in a separatory funnel (125 mL). To let it rest at -18 °C for 15 minutes, to obtain complete separation of the phases. To gather and filter the organic fractions with 5 g of anhydrous sodium sulfate. To evaporate the organic fraction

in a rotary evaporator under reduced pressure to obtain the residue, by resuspending it in 1 mL of methanol.

Solution (2): to weigh about 1 mg of epigallocatechin SQR and dissolve in 1 mL of methanol.

Solution (3): to weigh about 1 mg of 4'-O methyl gallo catechin SQR and dissolve in 1 mL of methanol.

To develop the chromatogram. To remove the plate and dry in fume hood. To examine under ultraviolet light (254 nm). The chromatogram obtained with *Solution (1)* has attenuated fluorescence spots at the same height as that obtained with *Solutions (2)* and *Solution (3)* (R_f approximately 0.75 and 0.82, respectively). Then, nebulize the plate with 1% ferric chloride (w/v) in methanol. After nebulize it, the chromatogram of *Solution (1)* should present the same color bands and R_f of *Solutions (2)* and *Solution (3)*.

B. To heat under reflux about 3 g of grinded vegetable drug with 60 mL of water for 15 minutes To cool down and filter it. To add 2 drops of hydrochloric acid SR to 2 mL of extract and drip gelatin SR to precipitation. The appearance of clear precipitate indicates a positive reaction for total tannins.

C. To add 10 mL of water and 2-4 drops of 1% ferric chloride solution (w/v), in methanol, to 2 mL of the extract obtained in *Identification*, test B. The development of dark-gray color indicates a positive reaction for total tannins.

D. To add 0.5 mL of 1% vanillin (w/v), in methanol, and 1 mL of hydrochloric acid SR to 2 mL of the extract Obtained in *Identification*, test B.

The development of dark-gray color indicates a positive reaction for condensed tannins.

E. To add 10 mL of 2 M acetic acid and 5 mL of lead acetate SR to 5 mL of the extract obtained in *Identification*, test B. The appearance of whitish precipitate indicates the presence of tannins.

PURITY TESTS

Foreign matter (5.4.2.2). At most 2.0%.

Water (5.4.2.3). At most 14.0%.

Total ashes (5.4.2.3). At most 2.0%.

Sulfated ashes (5.4.2.6). At most 3.0 %.

DOSAGE

Total tannins

Note: to perform all the extraction and dilution operations protected from the light.

To prepare the solutions as described below.

Stock solution: to weigh 0.750 g of the pulverized drug (250 μ m) and transfer to a 250 mL erlenmeyer flask with standard ground mouth. To add 150 mL of distilled water. To heat it in water bath at 30 °C for 30 minutes. To cool down with running water and transfer to a 250 mL volumetric flask. To wash the erlenmeyer flask and transfer the washings with all the content of vegetable drug to the same volumetric flask. To top up to volume with distilled water. To decant and filter the supernatant liquid by using filter paper. To discard the first 50 mL of the filtrate.

Sample solution for total polyphenols: to dilute 5 mL of the filtrate in 25 mL volumetric flask with distilled water. To volumetrically transfer 2 mL of this solution, 1 mL of phosphotungstic molybdate reagent, and 10 mL of distilled water to a 25 mL volumetric flask and dilute with a 29% sodium carbonate solution (w/v). To determine the absorbance at 760 nm (A_1) after 30 minutes by using distilled water to adjust zero.

Solution sample for polyphenols not adsorbed by hide powder: to obtain 10 mL of the filtrate, add 0.1 g of hide powder SQR and mechanically stir in 125 mL erlenmeyer flask for 60 minutes. To use filter paper. To dilute 5 mL of the filtrate in 25 mL volumetric flask with distilled water. To volumetrically transfer 2 mL of this solution, 1 mL of phosphotungstic molybdate reagent, and 10 mL of distilled water to a 25 mL volumetric flask and dilute with a 29% sodium carbonate solution (w/v). To determine the absorbance at 760 nm (A_2) after 30 minutes by using distilled water to adjust zero.

Standard solution: just before using it, dissolve 50 mg of pyrogallol in a 100 mL volumetric flask with distilled water. To volumetrically transfer 5 mL of this solution to a 100 mL volumetric flask and top up with distilled water.

To volumetrically transfer 2 mL of this solution, 1 mL of phosphotungstic molybdate reagent, and 10 mL of distilled water to a 25 mL volumetric flask and top up with a 29% sodium carbonate solution (w/v). To determine the absorbance at 760 nm (A_3) after 30 minutes by using distilled water to adjust zero.

To calculate the content of dry tannin (in percentage) expressed as pyrogallol, according to the equation:

$$TT = \frac{62,5 \times (A_1 - A_2) \times m_2}{A_3 \times m_1}$$

where

A_1 = absorbance of *Sample solution for total polyphenols*;

A_2 = absorbance of *Sample solution for polyphenols not adsorbed by hide powder*;

A_3 = absorbance of *Standard solution*;

m_1 = mass of the sample used in the test, in grams;

by considering the determination of water;

m_2 = mass of pyrogallol, in grams.

Gallic acid and gallo catechin

To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 210 nm; pre-column packed with silica chemically bonded to the octadecylsilane group; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), flow of *Mobile phase* of 0.8 mL/min.

Eluent A: mixture of water and 0.05% trifluoroacetic acid (v/v).

Eluent B: mixture of acetonitrile and 0.05% trifluoroacetic acid (v/v).

Mobile phase gradient: to adopt the gradient system described in the following table:

Time (minutes)	Eluent A (%)	Eluent B (%)	Elution
0 - 10	95 → 80.7	5 → 19.3	linear gradient
10 - 13.5	80.7 → 75	19.3 → 25	linear gradient
13.5 - 23	75 → 62	25 → 38	linear gradient
23 - 25	62 → 25	38 → 75	linear gradient
25 - 28	25 → 95	75 → 5	linear gradient
28 - 32	95	5	isocratic

Sample solution: to extract by turbolysis 10 g of the pulverized vegetable drug (250 µm) in 90 mL of acetone and water (7:3) for 15 minutes, with 5-minute intervals so that the temperature does not exceed 40 °C. To filter in cotton and discard the acetone in a rotary evaporator under reduced pressure. To extract the resulting aqueous phase with 3 portions of 20 mL of ethyl acetate in a separatory funnel (125 mL). To let it rest at -18 °C for 15 minutes, to obtain complete separation of the phases. To gather the organic phases and filter by using filter paper with 5 g of anhydrous sodium sulfate. To evaporate the organic fraction obtained in a rotary evaporator under reduced pressure to obtain the residue. To take the residue with 5 mL of methanol:water (2:8). To extract in extraction cartridge in solid phase, packed with silica chemically bonded to the octadecylsilane group (55 mm, 70 Å), previously packed with 10 mL of methanol and water (2:8) to a 100 mL flask. To elute 10 mL of methanol and water (2:8) to the same flask and top up the volume (S_1) with methanol and water (2:8). To volumetrically transfer 5 mL of S_1 to 25 mL volumetric flask and top up with methanol and water (1:1) (S_2). To filter S_2 (PTFE membrane, porosity 0.5 µm) and inject it into the chromatograph.

Galocatechin standard solution: to dissolve accurately weighed amount of galocatechin SQR in mixture of methanol and water (1:1) to obtain 0.152 mg/mL solution.

Gallic acid standard solution: to dissolve accurately weighed amount of gallic acid SQR in mixture of methanol and water (1:1) to obtain 0.100 mg/mL solution.

Solutions for calibration curve of galocatechin: To dilute an aliquot of 600 µL of *Galocatechin standard solution* into 5 mL volumetric flask, with methanol and water (1:1). To dilute to obtain 1.14 mg/mL; 2.28 mg/mL; 4.56 mg/mL; 9.12 mg/mL; 18.24 mg/mL concentrations.

Solutions for calibration curve of gallic acid: To dilute an aliquot of 800 µL of *Gallic acid standard solution* into 5 mL volumetric flask, with methanol and water (1:1). To dilute to obtain 2 µg/mL; 4 µg/mL; 8 µg/mL; 14 µg/mL, and 16 mg/mL concentrations.

Procedure: to separately inject 20 µL of solutions to build calibration curves and *Sample solution* in quintuplicate, record the chromatograms, and measure the areas under the peaks. The relative retention time to gallic acid and galocatechin is about 8.4 and 10.8 minutes, respectively. To calculate the content of gallic acid and galocatechin in the sample, from the linear equation obtained from the calibration curves of the standards. The result is the mean of the determinations in mg/g of vegetable drug, by considering the water content according to the equation:

$$\text{SQR} = \frac{\text{VLR} \times 500}{1000 \times m}$$

where

SQR = reference chemical substance;

VLR = value obtained in (µg/mL) of SQR/mL in S_2 , from the linear equation;

500 = dilution factor;

1000 = conversion factor from µg to mg;

m = mass (g) of vegetable drug, by considering the water determination.

PACKAGING AND STORAGE

In tightly closed container, protected from light and heat.

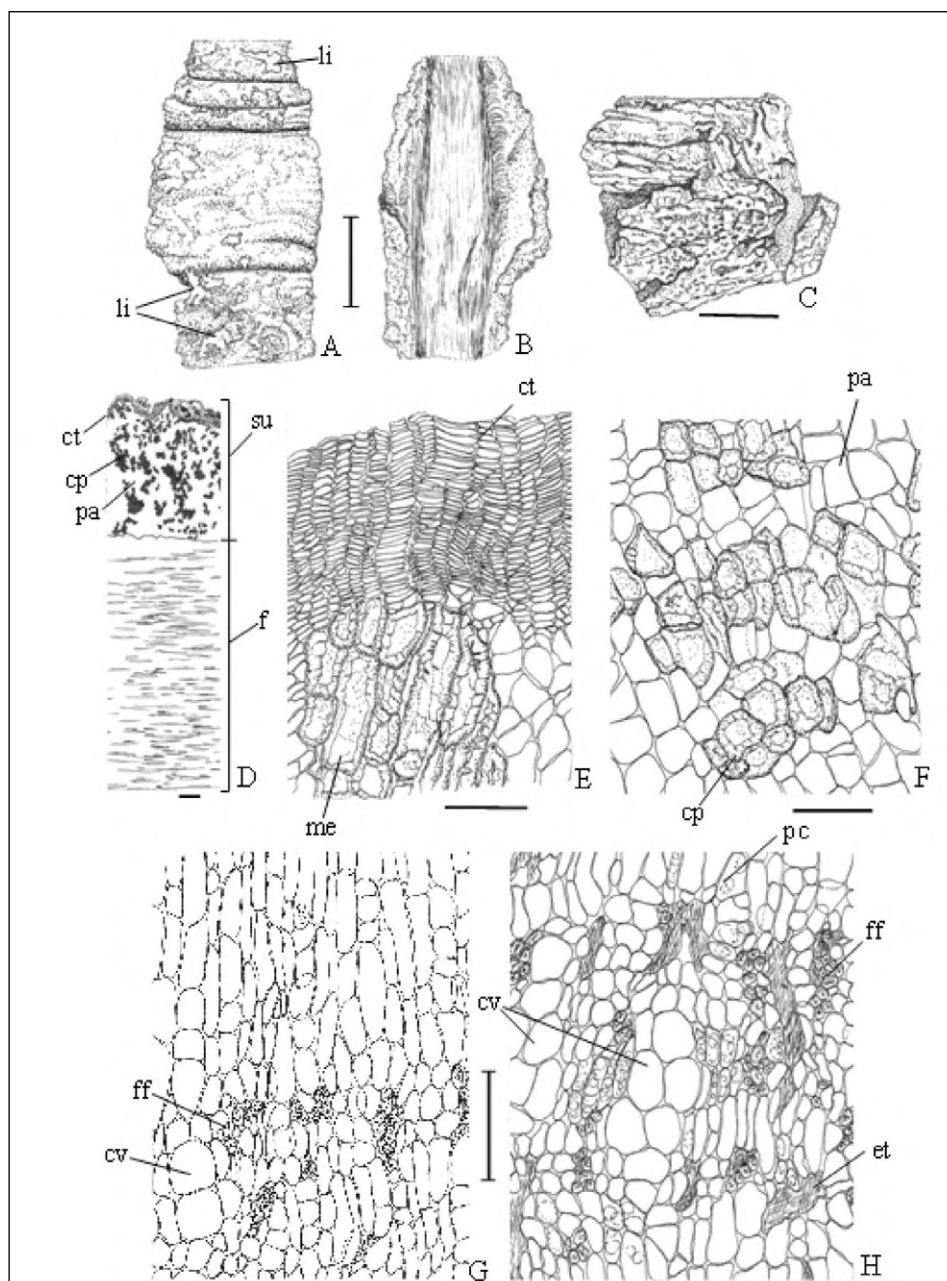


Figure 1 - Macroscopic and microscopic aspects in *Stryphnodendron adstringens* (Mart.) Coville

Additional information of **Figure 1**. Scales correspond to 1 cm in **A**, **B**, and **C**; in **D**, 2 mm, and **E**, **F**, **G**, and **H**, 100 μ m.

A and **B** - partial aspect of the outer and inner surface of the bark with newest branch, respectively: lichens (li). **C** - partial aspect of the outer surface of the elder branch. **D** - diagram of the distribution of bark tissue: tabular cells (ct), stone cell (cp); parenchyma (pa); bark (su); phloem (f). **E** and **F** - partial details of the bark, in cross-sections: tabular cells (ct); macrosclereids (me); parenchyma (pa); stone cell (cp). **G** and **H** - partial details of the phloem, in cross-sections: phloem fibers (ff); voluminous cells (cv); sieve plate (cp); obliterated sieve tube element (et).

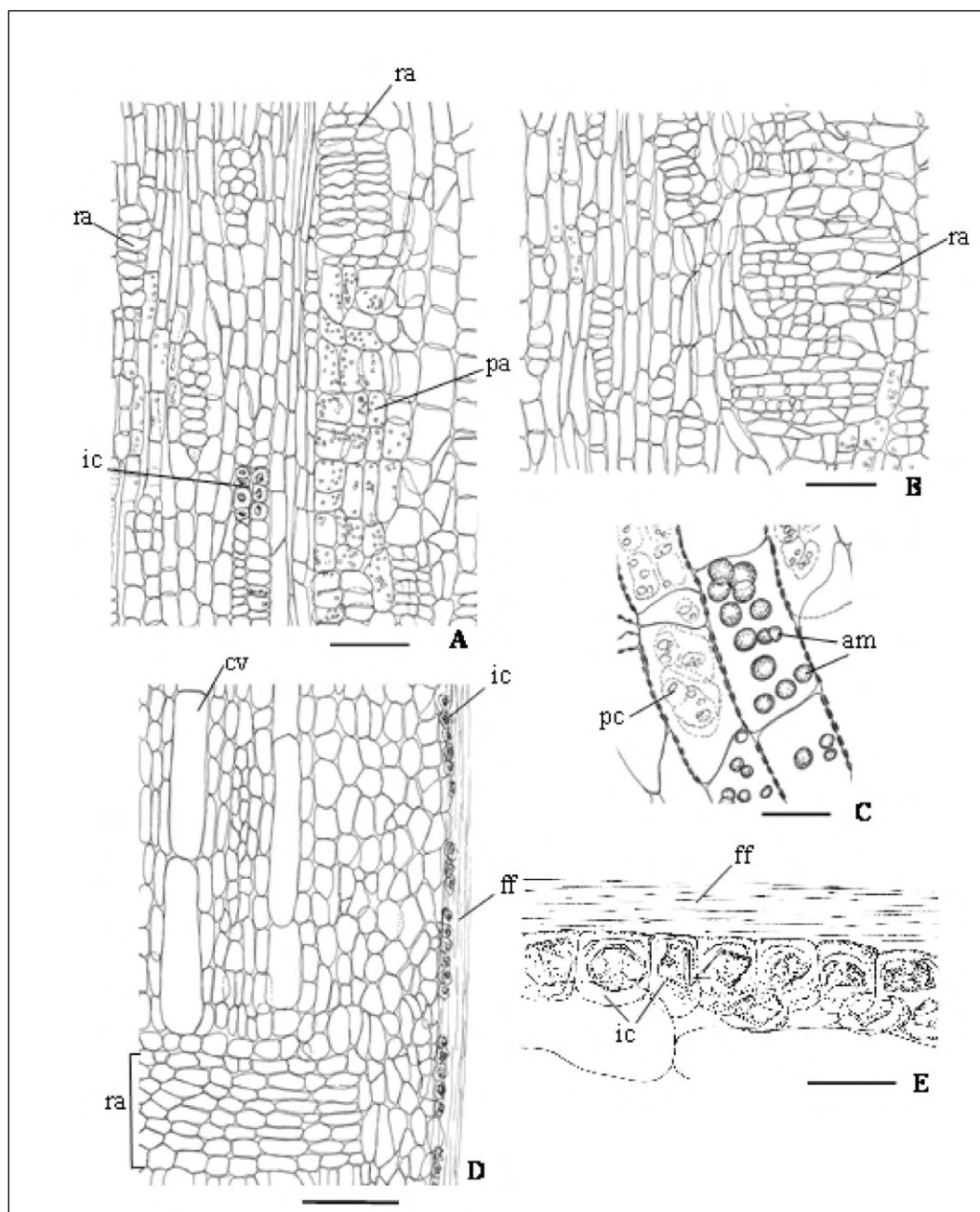


Figure 2 — Microscopic aspects in *Stryphnodendron adstringens* (Mart.) Coville

Additional information of **Figure 2**. Scales correspond to 100 µm in **A**, **B**, and **D**, and **C** and **E**, 25 µm.

A and **B** - partial details of phloem in tangential longitudinal sections: parenchyma beam (ra); parenchyma cell (pa); crystal idioblasts (ic). **C** - partial detail of phloematic parenchyma with starch grains: starch grains (am); sieve plate (cp). **D** - partial detail of phloem in radial longitudinal section: voluminous cells (cv); crystal idioblast (ic); phloem fibers (ff); parenchyma beam (ra). **E** - detail of crystal idioblasts of the phloem: phloem fibers (FF); crystal idioblast (ic).

VANILLA

Vanillae fructus

Vanilla planifolia Andrews- ORCHIDACEAE

The vegetable drug consists of dried and immature fruit, and containing at least 12% of dry hydroalcoholic extract.

CHARACTERISTICS

Organoleptic characteristics. The drug has a pleasant and floral smell that reminds vanillin, which, however, is much more subtle and full bodied than the substance isolated.

MACROSCOPIC DESCRIPTION

The fruits are capsules polyspermic derived from ovarian superoinferior, tricarpellate unilocular. The fruit shape in cross section is variable, depending on the storage mode; the non-compact ripe fruit has triangular outline in cross section. The ripe fruit is dark brown, has longitudinal ribs, is flexible, and measure from 20 cm to 25 cm long, and about from 1 cm to 1.5 cm diameter at its middle region.

MICROSCOPIC DESCRIPTION

In general, the pericarp has a single cell layer exocarp and multicellular mesocarp, predominantly parenchymatous, with distinct regions; endocarp with a single specialized cell layer. In storage function, the shape of the cells is not characterized and counting of layers is also constrained, particularly in the inner portion of mesocarp. Idioblasts with raphides longitudinally oriented to the pericarp are common; prismatic crystals are also observed. The exocarp has tangentially elongated cells, whose external periclinal walls are thick and cutinized, and the inner periclinal walls are also thick and pectin; cells usually accumulate phenolic compounds. The exocarp is "stomata-type" and glabrous. The outer mesocarp has 2-4 layers similar to an angular not vascularized collenchyma; the middle mesocarp has voluminous cells with great accumulation of phenolic compounds. Collateral vascular bundles in groups of 2 or 3, usually with larger diameter than the individual bundles of small diameter, surrounded by a sclerenchymatic sheath with 2-5 cell layers thick; the sclerenchyma is comprised of vacuolated voluminous cells, with lignified walls and slightly thickened; the inner mesocarp has flattened cells containing phenolic compounds. The endocarp is differentiated into a pilose dense stratum, whose base has a compact arrangement of cells and locular portion projected into locular space; cells have thin and pectic walls with dense cytoplasm with secretory aspect. In cross-section, it is possible to distinguish three placental regions, with placenta profusely branched, their seeds are inserted into the ends.

The seeds, black or dark brown color, anatropous and slightly platispermic, have enlarged chalazal region, and the conical micropylar region with obtuse apex; they have sclerenchymatic coat, being classified as coated; the seminal coat consists of a single layer of brachysclereids with very thick walls, lignified, whose flame is barely discern-

ible, the cell walls present *linea lucida*. The inner integument or tegmen is compressed, and the structure of its cells is barely discernible. The endosperm has voluminous cells with reservations; differentiated embryos are not observed.

MICROSCOPIC DESCRIPTION OF POWDER

The powder meets all the requirements for the species, unless the macroscopic characters. Characteristics: fragments in the form of lumps, due to nature of the fruit, leading to a greater difficulty in observing dissociated elements; lumps composed of amalgamated fragments from different tissues; elements such as crystals and sclereids, referred to in the microscopic description are not easily viewed; only prismatic crystals are observed, although it is not possible to determine with clarity the nature of the tissue that covers them; fragments with exocarp cells have clear thickened walls; fibers can be easily recognized in groups of 2 or 3 elements, and often appear to be separated from other tissue, the xylem vessels elements are easily recognized due to the characteristics of its cells; reinforcements of lignin and pits walls are even observed in the most dense clumps, where the cells that make up the tissue keep together, thus offering the visualization of tissue structure. Seeds are the element that stands out, which remain virtually intact in its structure.

IDENTIFICATION

A. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF₂₅₄ silica gel with 250 µm thick as support and a mixture of methylene chloride and acetone (95:5) as mobile phase. To separately apply to the plate, as band, 20 mL of *Solution (1)* and 10 mL of *Solution (2)*.

Solution (1): to use the hydroalcoholic extract obtained in *Dosage*.

Solution (2): to dissolve 1 mg of vanillin in 10 mL of ethanol.

To develop the chromatogram. To remove the plate and let it dry. To examine under ultraviolet light (254 nm). The blue-violet fluorescence spot obtained with *Solution (1)* with R_f of approximately 0.5 corresponds in position to that obtained with *Solution (2)*, referring to vanillin.

B. To place some seeds of the fruit on the watch glass, add a drop of phloroglucin SR and one drop of hydrochloric acid. The solution becomes immediately red.

PURITY TESTS

Sulfated ashes (5.2.10). At most 7.0 %.

DOSAGE

Extractable substances

To determine the content of extractable substances by calculating the yield of the hydroalcoholic extract. To accu-

rately weigh about 2 g of vanilla previously cut into small pieces or crushed to coarse powder. To transfer the powder to an erlenmeyer flask, with ground lid, and add 70 mL of diluted ethanol (prepared with 263 mL of ethanol in 250 mL of distilled water). To tightly close it and use a mechanical stirrer to stir well the container for 2 hours, or leave in contact overnight and stir often for a further 8 hours.

To decant the liquid and filter, by collecting the filtrate in a 100 mL volumetric flask. To successively wash 4 times the flask and the residue, with portions of 8 mL of diluted ethanol solution. To filter the washing through the same filter and mix with the filtrate obtained above. With suffi-

cient amount of diluted ethanol, top up to 100 mL, make it homogeneous and evaporate 50 mL exactly measured in a tared porcelain capsule, in a water bath. To desiccate the residue in an oven at 105 °C for 4 hours. To cool down the capsule in a desiccator and weigh it. The weight of the residue is the dried hydroalcoholic extract regarding 1 g of the drug.

PACKAGING AND STORAGE

In tightly closed containers, and store in dry and cool place protected from light.



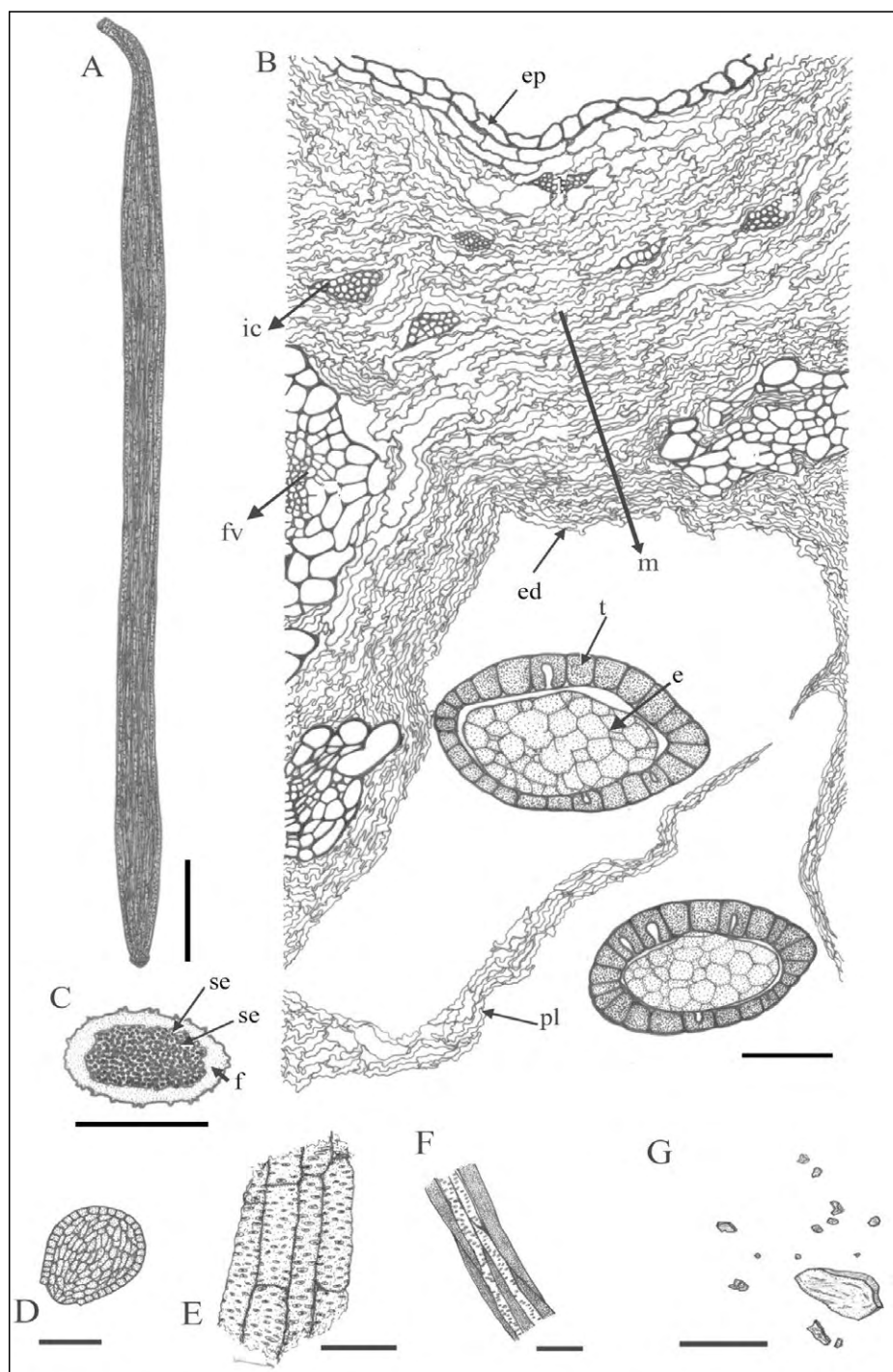


Figure 1 - Microscopic aspects of *Vanilla planifolia* Andrews

Additional information of **Figure 1**. Scales correspond to: **A**, 20 mm; **B**, 5 mm; **C**, 100 μ m; **D**, 160 μ m; **E**, 74 μ m; **F**, 9 μ m; **G**, 37 μ m.

A - Schematic representation of the capsule, in side view. **B** - representation of the histology of the pericarp and seed, in cross-section: endocarp (ed), endosperm (e); exocarp (ep); crystal idioblasts with raphides (ic); vascular bundle (fv), mesocarp (m); placental tissue (pl), seed integument (t). **C** - Schematic representation of the capsule in cross-section: pericarp (f); seed (se). **D** - seed in side view. **E** - fragment of xylem vessels elements. **F** - fragment group of the bundle sheath fibers. **G** - crystals of calcium oxalate.

BELLADONNA

Belladonnae folium

Atropa belladonna L. - SOLANACEAE

The drug consists of dried leaves and must have at least 0.3% of total alkaloids, expressed as hyoscyamine with reference to the dried material at 100 °C - 105 °C. Among these alkaloids, the hyoscyamine is clearly predominant and accompanied by small amounts of scopolamine.

CHARACTERISTICS

Organoleptic characteristics. The drug has a bitter taste and unpleasant and faintly nauseous odor, resembling the smoke.

MACROSCOPIC DESCRIPTION

The leaves are elliptical, oval-lanceolate to broadly ovate, entire, acuminate apex, attenuated base, symmetrical and somewhat decurrent, and full edge. Their measurement ranges from 5.0 cm to 25.0 cm long and 3.0 cm to 12.0 cm wide, with petioles from 0.5 cm to 4.0 cm long. The color varies from green to greenish brown, being darker on the adaxial face. The dried leaves are wrinkled, brittle, and thin. The young leaves are pubescent, but the old ones are only slightly pubescent along the ribs and petiole. The nervation is of "peninérvea"-type, and the secondary ribs run from the main rib at an angle of about 60° to anastomose near the edge. The blade surface is dry and rough to the touch due to the presence of cells with microcrystalline content of calcium oxalate in the mesophyll. These cells appear as tiny bright spots when the surface is illuminated; other cells shrink more during drying. The microscope investigation reveals the same dark spots by transparency and bright ones by reflection.

MICROSCOPIC DESCRIPTION

The leaf blade is amphistomatic and dorsiventral symmetry. The epidermis, in front view, shows fundamental cells with sinuous anticlinal walls and cuticle finely striated; on the area of midrib, cells are elongated with thin walls. There are several tector and glandular trichomes throughout the blade. The tector trichomes have 2-5 cells, uniseriate and conical, smooth and thin walls; the glandular trichomes have multicellular pedicel composed of 2-4 cells, with club-shaped terminal cell or have multicellular pedicel and multicellular head, formed by 4-7 cells with ovoid to pyriform aspect. The stomata, anisocytic-type, are more frequent in the lower epidermis. In cross section, the epidermis is uniseriate and the cuticle is thin. The mesophyll is composed of unistratified palisade parenchyma and spongy parenchyma with large idioblasts containing prismatic crystals of calcium oxalate and microcrystalline sand.

The midrib is prominent on both sides and has bicollateral vascular bundles in open arc, and the phloem is discontin-

uous intraaxillary. Angular collenchyma occurs below the epidermis on both sides of the midrib.

MICROSCOPIC DESCRIPTION OF POWDER

The powder meets all the requirements for the species, unless the macroscopic characters. Characteristics: dark green; fragments of the blade in front view, with epidermal cells with sinuous anticlinal walls and striated cuticle; fragments of mesophyll, in cross section showing epidermis with few stomata and unistratified palisade parenchyma; fragments of epidermis facing the lower face, in front view, showing anisocytic stomata and rare tector and glandular trichomes; fragments of epidermis on the ribs, in front view, showing elongated cells with thin walls, fragments of parenchyma, in cross section, containing crystal idioblasts; isolated prismatic crystals as those described, glandular trichomes, as those described, isolated, fragmented or epidermal debris; isolated tector trichomes or their fragments.

IDENTIFICATION

A. To stir 3 g of pulverized drug with 30 mL of 0.05 *M* sulfuric acid for 2 minutes, and filter. To alkalize the filtrate with 3 mL of ammonium hydroxide and add 15 mL of water through the filter. To transfer the alkaline solution to a separatory funnel and successively extract with three aliquots of chloroform (15 mL). To gather chloroform phases and add anhydrous sodium sulfate. To filter and divide the filtrate into two porcelain capsules, by proceeding to solvent evaporation. In a porcelain capsule, add 0.5 mL of fuming nitric acid and evaporate to dryness in a water bath. To add 2 mL of acetone to the residue and drip a 10% potassium hydroxide solution (w/v) in ethanol. An intense violet color develops. To use another capsule to run the *Identification test B*.

B. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF₂₅₄ silica gel with 250 µm thick as support and a mixture of toluene, ethyl acetate, and diethylamine (7:2:1) as mobile phase. To separately apply to the plate, as band, 20 µl of each of the solutions freshly prepared, as described below.

Solution (1): to dissolve the residue in 0.25 mL of methanol in the capsule for this purpose, as described in *Identification Test A*.

Solution (2): to dissolve 24 mg of atropine sulfate in 9 mL of methanol and 7.5 mg of scopolamine hydrobromide in 10 mL of methanol. To mix 9 mL of atropine sulfate solution and 1 mL of scopolamine hydrobromide solution.

To develop the chromatogram. To dry the plate at 100 °C - 105 °C for 15 minutes. To cool down and successively nebulize with potassium iodide and bismuth subnitrate SR and 5% sulfuric acid ethanolic solution (w/v) (or aqueous 5% sodium nitrite solution (w/v)) to obtain red or orange-red spots on a yellow gray background. When examined under visible light, *Solution (2)* has spots with R_f ranging from 0.3 to 0.45, corresponding to hyoscyamine/atropine and

b

spots with Rf ranging from 0.55 to 0.65 corresponding to scopolamine. The spots of *Solution (1)* should be similar in position and color to those obtained for the *Solution (2)*.

PURITY TESTS

Foreign matter (5.4.2.2). Not greater than 5.0% of stems having a diameter greater than 5 mm. No fragments of leaves with raphids in the mesophyll (*Phytolacca americana* L.) or twinned layers of cells with calcium oxalate along the ribs (*Ailanthus altissima* Swingle) should appear.

Total ashes (5.4.2.4). At most 10.0%.

Insoluble ashes (5.4.2.5). At most 4.0%.

DOSAGE

Total alkaloids

To weigh about 10 g of pulverized sample (180 µm) and moisten with 5 mL of ammonium hydroxide. To add 10 mL of ethanol and 30 mL of peroxide-free ethyl ether carefully mixed. To transfer the mixture to a percolator, if necessary, by using an extraction solution. To macerate for 4 hours and percolate the mixture with a mixture of chloroform and peroxide-free ethyl ether (1:3) until complete extraction of alkaloids. To evaporate to dryness 1 mL of leachate and dissolve the residue in 0.25 M sulfuric acid. Then, check the absence of alkaloids with mercury potassium iodide SR. To reduce the volume of leachate to 50 mL and transfer to a separatory funnel with the aid of peroxide-free ethyl ether. To add peroxide-free ethyl ether to the liquid obtained 2.5 times the volume of the percolator

to obtain a liquid of density less than water. To extract the solution at least three times, by using 20 mL of 0.25 M sulfuric acid solution each time. To separate by centrifugation the phases, if necessary, and transfer the acid phase to another separatory funnel. To alkalize the acid phase with ammonium hydroxide to pH between 8.0 and 9.0 and extract with chloroform three times, with aliquots of 30 mL. To gather the chloroform phases and remove the residual water, by adding 4 g of anhydrous sodium sulfate. Let it rest for 30 minutes by occasionally stirring. To remove the chloroform phase and wash the remaining sodium sulfate with three aliquots of 10 mL of chloroform. To gather the chloroform extracts and evaporate to dryness in a water bath. To heat the residue in oven at 100 °C - 105 °C for 15 minutes. To dissolve the residue in 5 mL of chloroform, add 20 mL of 0.01 M SV sulfuric acid, and remove the chloroform by evaporation in a water bath. To titrate the acid in excess with a solution of 0.02 M SV sodium hydroxide by using methyl red as indicator. To calculate the percentage of total alkaloids, expressed as hyoscyamine, according to the equation:

$$\% \text{ alkaloids} = 57.88 \times (20 - n) / (100 - d) \times m$$

where

d = loss on drying in%;

n = volume of 0.02 M sodium hydroxide solution used (mL);

m = mass of the drug (g).

PACKAGING AND STORAGE

In tightly closed containers, protected from light and heat.

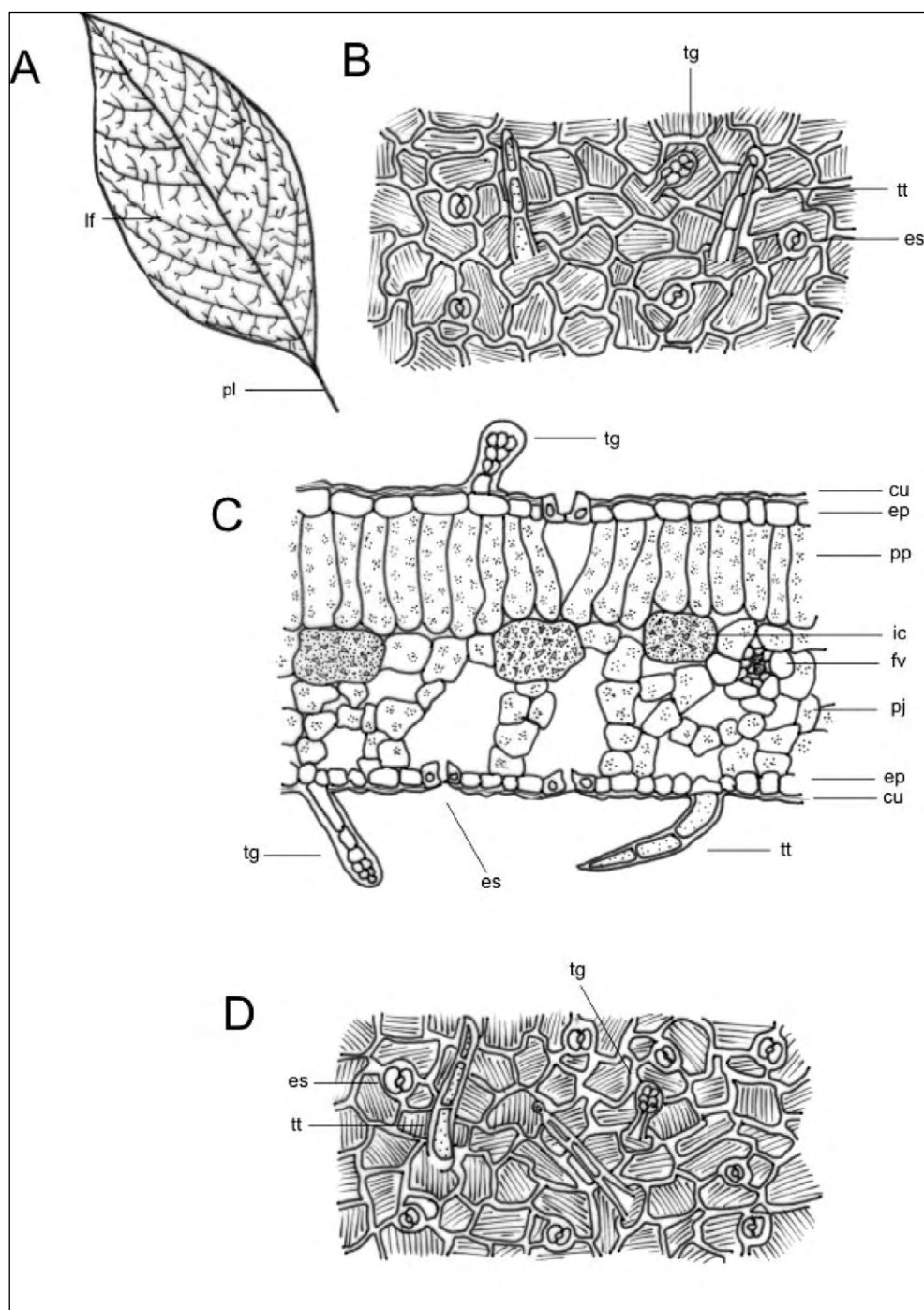


Figure 1 - Macroscopic and microscopic aspects in *Atropa belladonna* L.

Additional information of **Figure 1**.

A — Schematic representation of the leaf: leaf blade (lf); petiole (pl). **B** — detail of the portion of the epidermis facing the adaxial side, in front view: glandular trichomes (tg); tector trichomes (tt); stomata (es). **C** — detail of portion of the mesophyll, in cross-section: glandular trichomes (tg); cuticle (cu); epidermis (ep); palisade parenchyma (pp); idioblast containing microcrystals of calcium oxalate (ic); vascular bundle (fv); spongy parenchyma (pj); epidermis (ep); tector trichomes (tt); stomata (es). **D** — detail of the portion of the epidermis facing the lower side, in front view: glandular trichomes (tg); tector trichomes (tt); stomata (es).

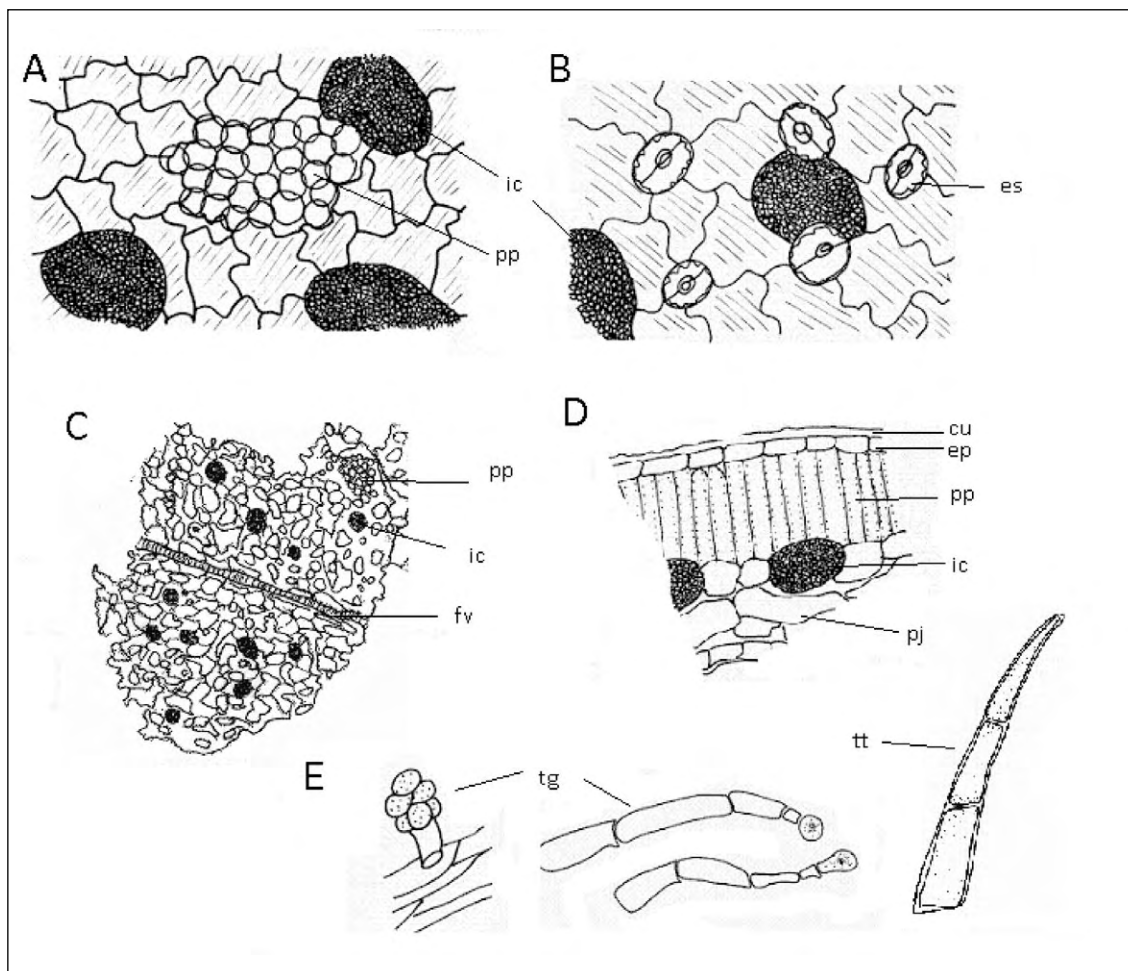


Figure 2 - Aspects of microscopy of powder in *Atropa belladonna* L.

Additional information of Figure 2.

A and C — fragments of the epidermis facing the adaxial side, in front view, showing crystal idioblasts by transparency: crystal idioblast (ic); palisade parenchyma (pp); vascular bundle (fv). B — fragments of the epidermis facing the lower side, in front view, showing crystal idioblasts by transparency: crystal idioblast (ic); stomata (es). D — fragment of the leaf blade in cross section: cuticle (cu); epidermis (ep); palisade parenchyma (pp); crystal idioblast (ic); spongy parenchyma (pj). E — trichomes or their parts, isolated: glandular trichomes (tg); tector trichomes (tt).

BENJOIM Benzoe sumatranus

Styrax benzoin Dryander or *Styrax paralleloneuron* Perkins - STYRACACEAE

Benzoin is a balsamic resin obtained by incisions in the trunk of *Styrax benzoin* Dryander or *Styrax paralleloneuron* Perkins. It contains at least 25% and at most 50% of total acid, calculated as benzoic acid ($C_7H_6O_2$).

CHARACTERISTICS

Organoleptic characteristics. It has rounded or ovoid fragments, irregular,

cream-white color, which may be coated with a resinous material of grayish-brown or red-brown color. They are hard and brittle, and the fracture surface is rough and ir-

regular. Mild odor and balsamic and sweet taste at first, becoming slightly spicy and pungent.

Solubility. It is practically insoluble in water, sparingly soluble in ethanol, xylene, and carbon disulfide.

IDENTIFICATION

A. To slowly heat 0.5 g of the sample in a dry test tube. The material melts and emits white smoke, acrimonious and irritant, which condenses on the upper part of the tube, in blades, and small crystals.

B. To gently heat 1 g of the ground sample with 10 mL of 3% potassium permanganate (w/v). It produces strong odor of benzoic aldehyde.

C. To add 0.2 g of finely pulverized sample to 10 mL of ethanol. To vigorously stir to obtain complete dissolution. To filter it. To put in a test tube 5 mL of the filtrate and 0.5

mL of 5% ferric chloride solution (w/v) in ethanol and stir. Green color is not developed.

D. To add 5 ml of ethanol to 0.5 g of the ground sample and put it in ultrasound for 2 minutes. To filter it. To add 10 mL of water to the filtrate. There is formation of turbid mixture with a milky appearance. It has acid reaction to litmus paper.

E. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF₂₅₄ silica gel, with 250 µm thickness as stationary phase and a mixture of glacial acetic acid, isopropyl ether, and hexane (10:40:60) as mobile phase. To separately apply as band, 10 µl of each of the solutions freshly prepared, as described below.

Solution (1): to take 0.2 g of the sample finely pulverized, add 5 mL of ethanol, and put into ultrasonic bath for 2 minutes. To centrifuge and use the supernatant solution.

Solution (2): to dissolve 20 mg of benzoic acid, 10 mg of cinnamic acid, 4 mg of vanillin, and 20 mg of methyl cinnamate in 10 mL of ethanol.

The principal spots obtained with *Solution (1)* corresponds in position, color, and intensity to those obtained with *Solution (2)*. The chromatogram obtained with *Solution (1)*, when examined under ultraviolet light (254 nm), has, in its upper third, spots of fluorescence quenching in the same positions corresponding to methyl cinnamate (intense dark spot), benzoic acid (spot dark), cinnamic acid (intense dark spot), and a spot of very low intensity in the middle of the plate referring to vanillin.

PURITY TESTS

Dammar gum. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using aluminum oxide G, with 250 µm thickness as support and a mixture of petroleum ether and ethyl ether (40:60) as mobile phase. To separately apply, as band, 5 µl of each of the solution freshly prepared, as described below.

Solution (1): to heat 0.2 g of ground sample with 10 mL of 90% ethanol (v/v). To centrifuge it.

To develop the chromatogram. To remove the plate, and let it drying. To nebulize with anisaldehyde SR1. To heat in an oven at 100 °C - 105 °C for 5 minutes The chromatogram should not show any clear spot with R_f between 0.4 and 1.0.

Styrax tonkinensis. To proceed as described in *Identification, test E. Solution (1)* shows 2 low intensity spots and does not exhibit intense spots, respectively in the same position as the dark spots corresponding to benzoic acid and vanillin in the chromatogram obtained with *Solution (2)*.

Rosin. To take 1 g of the sample with 10 ml of xylene and put in ultrasound for 1 minute. To filter it. To add 10 mL of 1% copper acetate (w/v) to the filtrate. To stir well

and separate the phases. The xylene layer should not show green color.

Limit of substances insoluble in ethanol. To weigh 2 g of the pulverized sample and add 25 mL of 90% ethanol (v/v). To heat to boiling to almost complete dissolution. To use porous filter sinter previously tared to filter it, wash 3 times with 5 mL of hot 90% ethanol (v/v). To heat the glass funnel and its contents in an oven at 100 °C - 105 °C for 2 hours. To cool down in desiccator and weigh it. At most 25.0%.

Water (5.4.2.3). At most 5.0%. To determine in 2 g of the sample coarsely pulverized under reduced pressure for 4 hours.

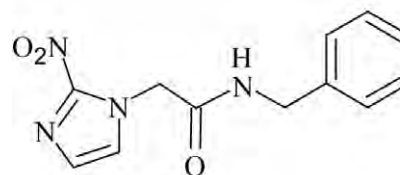
Total ashes (5.4.2.4). At most 2.0%. DOSAGE

To put 0.75 g of the finely pulverized sample and 15 mL of 0.5 M SV ethanolic potassium hydroxide in 250 mL flask with ground mouth. To heat under reflux in water bath for 30 minutes. To wait to cool down and wash the capacitor with 20 mL of ethanol. To titrate the potassium hydroxide in excess with 0.5 M SV hydrochloric acid. To potentiometrically determine the endpoint. To perform sample blank correction and adjust it as required. Each mL of 0.5 M SV ethanolic potassium hydroxide is equal to 61.050 mg of benzoic acid (C₇H₆O₂).

PACKAGING AND STORAGE

In tightly closed containers, protected from light and heat.

BENZNIDAZOLE Benznidazolium



C₁₂H₁₂N₄O₃; 260.25
benznidazole; 01153

2-Nitro-N-(phenylmethyl)-1, H-imidazole-1-acetamide
[22994-85-0]

It contains at least 99% and at most 101.0% of

C₁₂H₁₂N₄O₃ in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline powder, slightly yellowish, odorless, tasteless, and stable in air.

Solubility. Very slightly soluble in water, very soluble in dimethylsulfoxide, freely soluble in dimethylformamide, soluble in hexane, slightly soluble in ethanol, methanol,

ethyl acetate, and methylene chloride, slightly soluble in acetone, very slightly soluble in chloroform, isopropyl alcohol, glycerol, and practically insoluble in petroleum ether. Very slightly soluble in 0.1 M sodium hydroxide and 0.1 M hydrochloric acid.

Physical and chemical constants.

Melting range (5.2.2): 188 °C to 190 °C.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the benznidazole SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14), ranging from 200 nm to 400 nm, of Sample solution obtained in Dosage exhibits absorption maximum in 316 nm, similar to those observed in the Standard solution spectrum. The absorbance at 316 nm is approximately 0.352.

C. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel as support and a mixture of ethyl acetate and methanol (85:15) as mobile phase. To previously saturate the tank with the mobile phase. To separately apply to the plate 5 mL of each of the solutions freshly prepared, as described below.

Solution (1): 10 mg/mL solution of the sample in methanol.

Solution (2): 10 mg/mL solution of benznidazole SQR in methanol.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). To nebulize with extemporaneous tin chloride SR solution, let it drying, and put in a container with nitrous gases for 10 minutes. To remove the nitrous gases in excess with the cold air flow and nebulize with dihydrochloride of *N*-(1-naphthyl) ethylenediamine SR. The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

D. To dissolve about 30 mg of sample in 3 mL of methanol in a test tube, by slightly heating. To add 1 mL of 2 M hydroxylamine hydrochloride in water. To slightly heat in a water bath at 70 °C - 90 °C for about 1 minute. To cool down and add 1 mL of 0.1 M hydrochloric acid and 1 mL of 5% ferric chloride (w/v). It produces violet-brown color.

PURITY TESTS

Chlorides. To dissolve 30 mg of sample in 3 mL of methanol in a test tube and add 5 mL of 12% nitric acid (v/v) and 5 mL of silver nitrate SR. No turbidity occurs.

Loss on drying (5.2.9). To determine in an oven at 105 °C for 2 hours. At most 0.5%.

DOSAGE

To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To exactly transfer about 0.12 g of the sample to a 200 mL volumetric flask and add 150 mL of methanol. To mechanically stir until obtaining solubilization. To top up with the same solvent and make it homogeneous. To successively dilute in 0.1 M hydrochloric acid to the 0.0012% concentration (w/v). To prepare a standard solution at the same concentration by using the same solvents. To determine the absorbance of the solutions at 316 nm, by using 0.1 M hydrochloric acid to adjust zero. To calculate the C₁₂H₁₂N₄O₃ concentration of the sample from the readings obtained.

PACKAGING AND STORAGE

In airtight closed containers, protected from light.

LABELLING

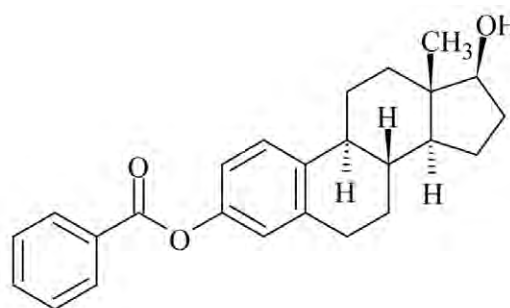
To comply with the legislation in force.

THERAPEUTIC CLASS

Anti-chagas disease.

BENZOATE, ESTRADIOL

Estradioli benzoas



C₂₅H₂₈O₃; 376.49

estradiol benzoate; 03597

3-benzoate of (17β)-estra-1, 3,5 (10)-triene-3,17-diol [50-50-0]

It contains at least 97.0% and at most 103.0% of C₂₅H₂₈O₃, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white or yellowish-white powder, odorless and stable in air.

Solubility. Practically insoluble in water, slightly soluble in acetone, very slightly soluble in ethanol and vegetable oils.

Physical and chemical constants.

Melting range (5.2.2): 191 °C to 196 °C.

Specific rotation (5.2.8): +57° to +63°, in relation to the desiccated substance. To determine at 1% solution (w/v) in dioxane.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the estradiol benzoate SQR spectrum similarly prepared.

B. The principal spot in the chromatogram of *Solution (2)* in the *Related substances* corresponds in position, color, fluorescence, and dimension to that obtained with the *Solution (3)*.

C. To dissolve 2 mg in 2 mL of sulfuric acid. The solution is yellow-green with blue fluorescence. After adding 2 mL of distilled water, the color changes to orange.

PURITY TESTS

Related substances. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF₂₅₄ silica gel as support and a mixture of toluene and ethanol (90:10) as mobile phase. To separately apply to the plate 5 µL of each of the solutions freshly prepared, as described below.

Solution (1): to dissolve 0.2 g of the sample in a mixture of methanol and chloroform (1:9) and top up to 10 mL with the same mixture.

Solution (2): to dissolve 2.5 mL of the *Solution (1)* and top up to 50 mL with a mixture of methanol and chloroform (1:9).

Solution (3): to dissolve 25 g of estradiol benzoate SQR in a mixture of methanol and chloroform (1:9) and top up to 25 mL with the same mixture.

Solution (4): to dissolve 2 mL of the *Solution (3)* and top up to 10 mL with a mixture of methanol and chloroform (1:9).

To develop the chromatogram. To remove the plate, and let it drying. To heat at 100 °C for 10 minutes. To nebulize the hot plate with ethanolic sulfuric acid solution. To heat it again at 110 °C for 10 minutes. To examine under ultraviolet light (365 nm). Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (4)* (1.0%).

Loss on drying (5.2.9). To determine in 0.5 g of the sample. To desiccate in an oven at 105 °C for 3 hours. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 0.5 g of the sample. At most 0.2%.

DOSAGE

To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. To accurately weigh about 25 mg of the sample and dissolve in ethanol. To dilute to 250 mL with the same solvent. To transfer 10 mL of the solution to 100 mL volumetric flask, dilute, and top up with ethanol. To measure the absorbance of the resulting solutions at 231 nm, by using ethanol to adjust zero. To calculate the content of C₂₅H₂₈O in the sample by considering that A (1%, 1 cm) = 500, at 231 nm, in ethanol.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

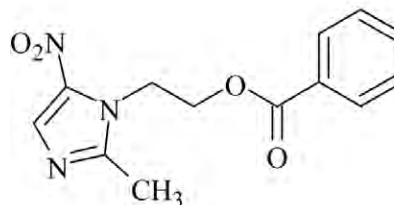
LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Estrogen.

BENZOYL METRONIDAZOLE
Metronidazoli benzoas



C₁₃H₁₃N₃O₄; 275.26

benzoyl metronidazole; 01166

1-Benzoate of 2-methyl-5-nitro-1H-imidazole-1-ethanol
[13182-89-3]

It contains at least 98.5% and at most 101.0% of C₁₃H₁₃N₃O₄, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline powder or flakes, white to yellowish-white.

Solubility. Practically insoluble in water, freely soluble in chloroform, soluble in acetone, slightly soluble in ethanol.

Physical and chemical constants.

Melting range (5.2.2): 99 °C to 102 °C.

IDENTIFICATION

The identification tests C. and D. may be omitted if the tests A. and B. are conducted. The identification test A. can be omitted if the tests B., C., and D. are carried out.

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the benzoyl metronidazole SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14), from 200 nm to 400 nm, 0.001% solution (w/v) prepared in 1 M hydrochloric acid, has absorption maximum at 232 nm and 275 nm, similar to those observed in the spectrum of a similar solution of benzoyl metronidazole SQR. The absorbance at 232 nm is between 0.525 and 0.575.

C. The principal spot in the chromatogram of Solution (2) in the *Related substances* corresponds in position, color, and intensity to that obtained with the Solution (3).

D. To add 20 mg of zinc powder, 2 mL of water, and 1 mL of hydrochloric acid to a 20 mg of the sample. To heat in water bath for 5 minutes and cool down to 0 °C. The resulting solution responds to the reaction of primary aromatic amine (5.3.1.1).

PURITY TESTS

Acidity. To dissolve 2 g of sample in 40 mL of mixture of dimethylformamide and water (1:1), previously neutralized with 0.02 M hydrochloric acid or 0.02 M sodium hydroxide by using 0.2 mL of methyl red SI as an indicator. No more than 0.25 mL of 0.02 M sodium hydroxide VS is required to change the color of the indicator.

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel as support and ethyl acetate as mobile phase. To separately apply to the plate 10 µL of each of the solutions freshly prepared, as described below.

Solution (1): 20 mg/mL solution of the sample in acetone.

Solution (2): to quantitatively dilute the Solution (1) in acetone to obtain a 0.1 mg/mL benzoyl metronidazole solution.

Solution (3): 0.1 mg/mL benzoyl metronidazole SQR solution in acetone.

Solution (4): to dilute 4 mL of Solution (3) to 10 mL with acetone.

Solution (5): solution containing 0.2 mg/mL metronidazole SQR and 0.2 mg/mL 2-methyl-5-nitroimidazole SQR (*Impurity A*) in acetone.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). Any secondary spot in the chromatogram obtained with Solution (1), other than the principal spot, is not more intense than that obtained with Solution (3) (0.5%) and not more than three secondary spots are more intense than that obtained with Solution (4) (0.2 %). The test is valid only if the chromatogram obtained with Solution (5) shows two principal spots clearly separated.

Heavy metals (5.3.2.3). To use *Method IV*. At most 0.002% (20 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample, in an oven at 80 °C for 3 hours. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To proceed as described in *Titrations in non-aqueous medium* (5.3.3.5). To dissolve 0.25 mg of the sample in 50 mL of acetic anhydride. To titrate with 0.1 M SV perchloric acid, to potentiometrically determine the endpoint or use methyl "rosalinio" chloride SI (crystal violet) until color changes to blue-green. Each mL of 0.1 M SV perchloric acid is equal to 27.526 mg of C₁₃H₁₃N₃O₄.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antiprotozoal, antibacterial.

BENZOYL METRONIDAZOLE, ORAL SUSPENSION

It contains at least 95.0 % and at most 105 % of the amount stated of C₁₃H₁₃N₃O₄.

IDENTIFICATION

The test A. may be omitted if the tests B. and C. are conducted. The identification test B. can be omitted if the tests A. and C. are carried out.

A. The ultraviolet absorption spectrum (5.2.14) ranging from 250 nm to 400 nm, of sample solution obtained in *Dosage* method A., exhibits absorption maximum in 308 nm similar to that observed in the standard solution spectrum.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method **B.** corresponds to that of main peak of *Standard solution*.

C. To add 20 mg of zinc powder, 1 mL of water, and 1 mL of hydrochloric acid to an oral suspension equivalent to 20 mg of benzoyl metronidazole. To heat in water bath for 5 minutes. To cool down at 0 °C. The resulting solution responds to the reaction of primary aromatic amine (**5.3.1.1**).

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

pH (5.2.19). 5.5 to 6.5. To determine in the reconstituted oral suspension as informed on the label.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3).

It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. To transfer the volume of oral suspension equivalent to 0.4 g of benzoyl metronidazole to a 100 mL volumetric flask, and add 10 mL of dimethylformamide and 60 mL of ethanol. To leave it in ultrasound for 15 minutes, mechanically stir for 15 minutes, top up with ethanol, make it homogeneous, and filter it. To successively dilute in ethanol to the 0.002% concentration (w/v). To prepare a standard solution at the same concentration by using the same solvents. To measure the absorbance of the resulting solutions at 308 nm, by using ethanol to adjust zero. To calculate the amount of $C_{13}H_{13}N_3O_4$ in the oral suspension, from the readings obtained.

B. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 254 nm; 150 mm chromatograph column and 3.9 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at room temperature; flow of *Mobile phase* of 1 mL/min.

Mobile phase: mixture of methanol and water (50:50).

Sample solution: to transfer volume of oral suspension equivalent to 0.2 g of benzoyl metronidazole to a 50 mL volumetric flask, add 35 mL of methanol, and

leave it in ultrasound for 15 minutes. To cool down at room temperature, top up with the same solvent, make it homo-

geneous, and filter it. To transfer 5 mL of the filtrate to 100 mL volumetric flask and top up with *Mobile phase*, thus obtaining 0.2 mg/mL solution.

Standard solution: to transfer 50 mg of benzoyl metronidazole SQR to 25 mL volumetric flask, add 20 mL of methanol, and leave it in ultrasound for 15 minutes. To cool down at room temperature, top up with the same solvent, and make it homogeneous. To transfer 5 mL to 50 mL volumetric flask and top up with *Mobile phase*, thus obtaining 0.2 mg/mL solution.

To inject replicates of 20 µL of the *Standard solution*. The relative standard deviation for the replicate areas for the peaks recorded is not greater than 2.0%.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of $C_{13}H_{13}N_3O_4$ in the oral suspension, from the responses obtained for the *Sample and standard solutions*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

BICARBONATE, POTASSIUM

Kalli hydrogenocarbonas

$KHCO_3$; 100,12
potassium bicarbonate; 01248
Potassium salt, carbonic acid (1:1)
[298-14-6]

It contains at least 99.0% and at most 101.0% of $KHCO_3$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White, crystalline powder, or colorless crystals. When heated, dry substance or in solution, it gradually converts into potassium carbonate.

Solubility. Freely soluble in water and practically insoluble in ethanol.

IDENTIFICATION

A. To add 0.1 mL of phenolphthalein SI to 5 mL of the solution obtained in *Appearance of solution*. The solution becomes pale pink color. To heat it. The gas evaporates, and color becomes red.

B. It responds to the reactions of carbonate ion (**5.3.1.1**).

C. It responds to the reactions of bicarbonate ion (**5.3.1.1**).

D. The solution obtained in *Appearance of solution* responds to the reactions of the potassium ion (5.3.1.1).

PURITY TESTS

Appearance of solution. To dissolve 5 g of the sample in carbon dioxide-free water and top up the volume to 100 mL with the same solvent. The solution is clear (5.2.25) and colorless (5.2.12).

pH (5.2.19). At most 8.6. To determine in the solution obtained, in *Appearance of solution*.

Sodium. To proceed as described in *Atomic absorption spectrometry with flame* (5.2.13.1.1). To use flame spectrophotometer with a mixture of air-acetylene, with light emission at 589.6 nm. To prepare the solutions as described below.

Solution (1): to dissolve 1 g of the sample in water and top up to 100 mL with the same solvent.

Solution (2): to prepare 0.1% solution (w/v) in water, by using analytical grade sodium chloride. To prepare analytical curve solutions by sequential dilution in water.

To add amount equivalent to 0.5% (v/v) of a 1% cesium chloride solution (w/v) to *Solution (1)* and *Solution (2)*. At most 0.5% of sodium (5000 ppm).

Ammonia (5.3.2.6). To use 10 mL of the solution obtained in *Appearance of solution*. At most 0.002% (20 ppm).

Calcium (5.3.2.7). To use 10 mL of the solution obtained in *Appearance of solution*. At most 0.001% (10 ppm).

Chlorides (5.3.2.1). To determine in 2.4 g of the sample. At most 0.015% (150 ppm).

Iron (5.3.2.4). To determine in 10 g of the sample. At most 0.002% (20 ppm).

Heavy metals (5.3.2.3). To use the *Method I*. To dissolve 2 g of the sample in 25 mL of water and continue as described in *Limit test for heavy metals*. There is no need to adjust the pH. At most 0.001% (10 ppm).

Sulfates (5.3.2.2). To determine in 8 g of the sample. At most 0.015% (150 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample, in silica gel, for 4 hours. At most 0.3%.

DOSAGE

To dissolve 0.8 g of the sample in 50 mL of carbon dioxide-free water. To add 0.1 mL of methyl orange SI. To titrate with *M* SV hydrochloric acid to the yellow color starts to change to pink-yellow. To carefully heat and boil it for 2 minutes. The solution becomes yellow. To cool down and titrate to obtain pink-yellow color.

Each mL of *M* SV hydrochloric acid is equal to 100.100 mg of KHCO_3 .

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

CATEGORY

Pharmaceutic adjuvant.

BICARBONATE, SODIUM

Natrii hydrogenocarbonas

NaHCO_3 ; 84.01
sodium bicarbonate; 01249
Sodium salt, carbonic acid (1:1)
[144-55-8]

It contains at least 99% and at most 101.0% of NaHCO_3 .

DESCRIPTION

Physical characteristics. White, crystalline, and odorless powder. When heated, dry or in solution, it gradually becomes into sodium carbonate.

Solubility. Soluble in water, practically insoluble in ethanol.

IDENTIFICATION

A. To prepare 5% sodium bicarbonate solution (w/v) in carbon dioxide-free water. To add 0.1 mL of phenolphthalein SI to 5 mL of this solution. The pinkish color develops. Upon heating, gas release occurs and the color of the solution changes to red.

B. It responds to the reactions of bicarbonate and carbonate ions (5.3.1.1).

C. It responds to the reactions of sodium ion (5.3.1.1).

PURITY TESTS

Appearance of solution. The 5% solution (w/v) in carbon dioxide-free water is clear (5.2.25) and colorless (5.2.12).

Ammonia (5.3.2.6): to dilute 10 mL of solution described in *Appearance of solution* to 15 mL with water. To proceed as described in *Limit test for ammonia*. At most 0.002% (20 ppm).

Arsenic (5.3.2.5). To add sulfuric acid 3.5 *M* to 0.5 g of the sample until effervescence stops.

To proceed as described in *Limit test for arsenic*. At most 0.0002% (2 ppm).

Carbonates. The pH (5.2.19) of the solution described in *Appearance of solution*, freshly prepared is not greater than 8.6.

Calcium (5.3.2.7). To neutralize the suspension (1 g) in 10 mL of water with hydrochloric acid and dilute to 15 mL with water. To proceed as described in *Limit test for calcium*. At most 0.01% (100 ppm).

Chlorides (5.3.2.1): to add 2 mL of nitric acid to 7 mL of solution described in *Appearance of solution* and dilute to 15 mL with water. To proceed as described in *Limit test for chlorides*. At most 0.015% (150 ppm).

Iron (5.3.2.4). To dissolve 0.5 g in 5 mL of hydrochloric acid. To proceed as described in *Limit test for iron*. At most 0.002% (20 ppm).

Heavy metals (5.3.2.3). To use *Method III*. To dissolve 2 g of the sample in the mixture of 2 mL of hydrochloric acid and 18 mL of water. To use 12 mL of the solution and proceed as described in *Limit test for heavy metals*. At most 0.001% (10 ppm).

Sulfates (5.3.2.2). To suspend 1 g of sample in 10 mL of water and add hydrochloric acid to neutrality. To proceed as described in *Limit test for sulfates*. At most 0.015% (150 ppm).

DOSAGE

To dissolve 1.5 g of the sample in 50 mL of carbon dioxide-free water. To titrate with *M* SV hydrochloric acid, by using 0.2 mL of methyl orange SI as an indicator. Each mL of *M* SV hydrochloric acid is equal to 84.010 mg of NaHCO_3 .

PACKAGING AND STORAGE

In tightly closed containers.

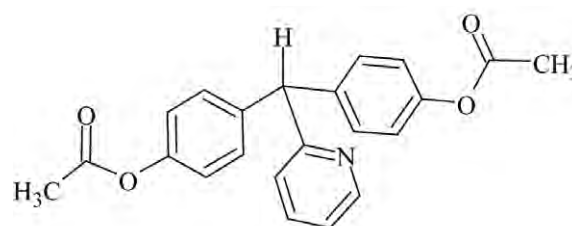
LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antacid.

BISACODYL Bisacodylum



$\text{C}_{22}\text{H}_{19}\text{NO}_4$; 361.39

bisacodyl; 01287

1,1'-diacetate 4,4' - (2-pyridinyl methylene) bis-phenol
[603-50-9]

It contains at least 98.0% and at most 101.0% of $\text{C}_{22}\text{H}_{19}\text{NO}_4$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white or almost white powder.

Solubility. Practically insoluble in water, soluble in acetone, slightly soluble in ethanol, very slightly soluble in ethyl ether. Soluble in dilute mineral acids.

Physical and chemical constants

Melting range (5.2.2): 131 °C to 135 °C.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, desiccated at 105 °C to constant weight and dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the bisacodyl SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14) ranging from 200 nm to 350 nm, of solution of the 0.001% solution of the sample (w/v) obtained in 0.6% methanolic potassium hydroxide (w/v), exhibits absorption maximum in 248 nm and shoulder in 290 nm. The absorbance at 248 nm is approximately between 0.632 and 0.672.

C. To nebulize the chromatograms obtained in *Related Substances* with a mixture of 0.05 *M* iodine solution and *M* sulfuric acid (50:50). The principal spot in the chromatogram of *Solution (2)*, in the *Related substances*, corresponds in position, color, and intensity to that obtained with the *Solution (3)*.

PURITY TESTS

Acidity or alkalinity. To stir 1.0 g of the sample with 20 mL of carbon dioxide-free water. To heat to boiling, cool down, and filter it. A maximum of 0.2 mL of 0.01 *M* sodi-

um hydroxide is spent to neutralize the filtrate, by using methyl red SI as an indicator.

At most 0.4 mL of 0.01 M hydrochloric acid is spent to neutralize the filtrate, by using the same indicator.

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel as support and a mixture of xylene and methyl ethyl ketone (50:50) as mobile phase. To separately apply to the plate 10 mL of each of the solutions freshly prepared, as described below.

Solution (1): to dissolve 0,2 g of the sample in acetone and top up to 10 mL with the same solvent.

Solution (2): to dilute 1.0 mL of *Solution (1)* to 10 mL with acetone.

Solution (3): to dissolve 20 mg of bisacodyl SQR in acetone and top up to 10 mL with the same solvent.

Solution (4): to dilute 1.0 mL of *Solution (1)* to 100 mL with acetone.

Solution (5): to dilute 5.0 mL of *Solution (4)* to 10 mL with acetone.

To develop the chromatogram. To remove the plate, air dry; if necessary, heat the plate at 105 °C. To examine under ultraviolet light (254 nm). Any secondary spot obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (4)* (1.0%) and no other spot is more intense than that obtained in the chromatogram with *Solution (5)* (0.5 %).

Loss on drying (5.2.9). To determine in 0.5 g of the sample.

To desiccate in an oven at 105 °C until obtaining constant weight. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To proceed as described in *Titrations in non-aqueous medium* (5.3.4.5). To dissolve 0.250 g of the sample in 70 mL of glacial acetic acid, add two drops of 1-naphtholbenzein SI, and titrate with 0.1 M SV perchloric acid. To perform sample blank correction and adjust it as required. Each mL of 0.1 M SV perchloric acid is equal to 36.139 mg of C₂₂H₁₉NO₄.

PACKAGING AND STORAGE

In tightly closed containers, protected from light, at room temperature.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Cathartic.

BISACODYL, TABLETS

It contains at least 90.0 % and at most 110 % of the amount stated of C₂₂H₁₉NO₄. Tablets must be coated.

IDENTIFICATION

A. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

B. To weigh and spray the tablets. To extract amount of powder equivalent to 50 mg of bisacodyl with chloroform, filter, evaporate the filtrate to dryness, and dissolve the residue in 10 mL of 0.5% sulfuric acid (v/v). To add 50 µL of mercury potassium iodide SR to 2 mL of the obtained solution. A white precipitate is formed.

C. To add sulfuric acid to 2 mL of the solution obtained in the *Identification* test B. The violet color develops.

D. To boil 2 mL of the solution obtained in *Identification* test B with a little amount of nitric acid. The yellow color develops. To cool down and add 5 M sodium hydroxide. The brown-yellow color develops.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test. To perform the acid step in 0.1 M hydrochloric acid for 120 minutes. The second step must be carried out with 1.5% sodium bicarbonate solution (w/v) for 60 minutes.

Uniformity of dosage units (5.1.6). It complies with the test. To proceed as described in *Dosage*. To prepare solution with a final concentration of 0.5 mg/mL.

PURITY TESTS

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel as support and a mixture of xylene and methyl ethyl ketone (50:50) as mobile phase. To separately apply to the plate 10 mL of each of the solutions freshly prepared, as described below.

Solution (1): to stir amount of powder equivalent to 20 mg of bisacodyl with 2 mL of acetone for 10 minutes, centrifuge it, and use the supernatant liquid.

Solution (2): to dilute 3 mL of *Solution (1)* to 100 volumes with acetone.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, not related to excipients, is not more intense than that obtained with *Solution (2)* (3%).

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3).

It complies with the test.

DOSAGE

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 265 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica gel chemically bonded to the octadecylsilane group (4.6 µm), kept at room temperature; flow of *Mobile phase* of 2.0 mL/min.

0.074 M Sodium acetate buffer: it contains 10.06 g of sodium acetate trihydrate in water to make 1000 mL. To adjust pH to 7.4 with 2.5% acetic acid (v/v).

Mobile phase: mixture of *0.074 M Sodium acetate buffer* and acetonitrile (50:50).

Sample solution: To weigh and spray 20 tablets. To transfer amount of powder equivalent to 50 mg of bisacodyl to 100 mL volumetric flask and add 12 mL of water. To mechanically stir for 15 minutes and put it in ultrasound bath at room temperature for 15 minutes. To add 50 mL of acetonitrile, mechanically stir, and sonicate for 15-minute intervals. To top up to volume with acetonitrile, to make it homogeneous, and centrifuge for 15 minutes. To filter the supernatant and use the filtrate in the determinations.

Standard solution: to accurately dissolve weighed amount of bisacodyl SQR in acetonitrile and properly dilute to obtain a 0.5 mg/mL solution.

Procedure: to separately inject 10 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_{22}H_{19}NO_4$ in the tablets, from the responses obtained for the *Sample and standard solutions*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light, at room temperature.

LABELLING

To comply with the legislation in force.

BISACODYL, SUPPOSITORIES

It contains at least 90.0 % and at most 110 % of the amount stated of $C_{22}H_{19}NO_4$.

IDENTIFICATION

A. To proceed as described in *Related substances*. To apply 2 µL of each solution to the chromatographic plate and use 1% bisacodyl SQR (w/v) in acetone as *Solution (2)*. The principal spot in the chromatogram of *Solution (1)* corresponds to that obtained with *Solution (2)*.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method **B**, corresponds to that of main peak of *Standard solution*.

C. To dissolve amount of suppositories containing the equivalent to 0.15 g of bisacodyl in 150 mL of petroleum ether. To filter and wash the residue with petroleum ether until it be free of oily material, and dry it at 100 °C. To dissolve the residue in minimal amount of chloroform gently warmed and solubilize in 10 mL of 0.5% sulfuric acid (v/v). To add 50 µL of mercury potassium iodide SR to 2 mL of the obtained solution. A white precipitate is formed.

D. C. To add sulfuric acid to 2 mL of the solution obtained in the *Identification* test C. The violet color develops.

E. To boil 2 mL of the solution obtained in *Identification* test C with a little amount of nitric acid. The yellow color develops. To cool down and add 5 M sodium hydroxide. The brown-yellow color develops.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test. To proceed as described in *Dosage* method **B**.

PURITY TESTS

Related substances. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF_{254} silica gel as support and a mixture of xylene and methyl ethyl ketone (50:50) as mobile phase. To separately apply to the plate 10 mL of each of the solutions freshly prepared, as described below.

b

Solution (1): to dissolve amount of suppositories containing the equivalent to 20 g of bisacodyl with 20 mL of petroleum ether, and filter it. To wash the residue with petroleum ether until it be free of oily material, and dissolve it in 2 mL of acetone.

Solution (2): to dilute 3 mL of *Solution (1)* to 100 volumes with acetone.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (2)* (3%).

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. To dissolve amount of suppositories containing the equivalent of 0.1 g of bisacodyl in 80 mL of glacial acetic acid previously neutralized with *M* SV 0.02 perchloric acid, by using 1-naphtholbenzein SI to verify neutralization. To titrate with 0.02 *M* SV perchloric acid, to potentiometrically determine the endpoint. To perform sample blank correction and adjust it as required. Each mL of 0.02 *M* SV perchloric acid is equal to 7.228 mg of $C_{22}H_{19}NO_4$.

B. To proceed as described in *Dosage*, in the monograph *Bisacodyl, tablets*. To prepare the *Sample solution* as described below.

Sample solution: to transfer amount of suppositories containing the equivalent to 0.1 g of bisacodyl to a 500 mL separatory funnel, and add 150 mL of n-hexane. To mechanically stir until the suppositories are dissolved. To add 50 mL of acetonitrile, stir for 1 minute, and wait for phase separation. To transfer the lower layer to a 200 mL volumetric flask. To extract the contents remaining in the separatory funnel with two portions of 50 mL of acetonitrile. To gather the lower layers in the 200 mL volumetric flask and top up with acetonitrile. To stir and filter it.

Procedure: to separately inject 10 μ L of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_{22}H_{19}NO_4$ from the sample, from the responses obtained for the *Sample and standard solutions*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light, at room temperature.

LABELLING

To comply with the legislation in force.

BOLDO Boldus folium

Peumus boldus Molina - MONIMIACEAE

The vegetable drug consists of dried leaves containing at least 1.5% of volatile oil and at least 0.1% of total alkaloids expressed in boldine.

POPULAR NAMES

Boldo-do-chile.

CHARACTERISTICS

Organoleptic characteristics. The drug has a typical aromatic odor, camphor, and slightly acre, which increases with the crushing. Bitter and somewhat acrid taste.

MACROSCOPIC DESCRIPTION

Single leaf, entire, elliptic, elliptic ovate, elliptic obovate or obovate, obtuse, acute or retuse apex or and rounded, obtuse or conical base, apex and base are symmetric or asymmetric, margin is slightly revolute, coriaceous blade, brittle, gray-green to gray silver, slightly translucent pits corresponding to secretory cavities, visible to the naked eye or magnifying glass six times from 1.2 cm to 7.0 cm long and 0.6 cm to 5.0 cm wide; pilose blade with star trichomes visible with magnifying glass, commonly obsolete in adaxial face, this face being rough to the touch due to the prominence of the base of trichomes; camptodrome-brochidodromous venation. Short petiole, pilose, measuring from 0.1 cm to 0.5 cm long and 0.1 cm to 0.2 cm wide, concave on the adaxial face with two small lateral ribs, and convex on the lower surface with higher density of trichomes on that face.

MICROSCOPIC DESCRIPTION

Leaf blade of dorsiventral symmetry, hypostomatic with anomocytic stomata. In front view, the cuticle is smooth and the epidermis faces the adaxial side, in the region between the ribs, shows polygonal cells of anticlinal thick walls, slightly sinuous, and in lower surface has cells of different forms, with thick sinuous walls; the stomata are above the other epidermal cells and are accompanied by 4-8 cells; in the midrib area, cells facing the adaxial side have different shapes and are slightly elongated, with homogeneous size and straight walls, while those facing the lower surface are more elongated and have different sizes;

between the ribs by transparency, secretory cells are visible; trichomes are star-type are more frequent on the adaxial face and formed by different numbers of long cells with thickened walls; in general, the epidermal cells have radial arrangement around the basal portion of the trichome. In cross section, the cuticle is thicker on the adaxial face, the epidermis is uniseriate with elongated cells and thick walls; the hypodermis also has thick walls, unistratified, rarely bistratified, occurs in both sides, exclusively in the region of midrib, on the lower surface; the epidermis and hypodermis, in general, are prominent around the base of each trichome, the palisade parenchyma is unistratified or bistratified, more elongated columnar cells, whereas the second layer is loose, with smaller cells and greater concentration of starch grains; the spongy parenchyma has several layers of cells of different shapes and large intercellular spaces;

secondary collateral bundles are distributed in the mesophyll, surrounded by full or not sheath of fiber, or by endoderm, or xylem groups occur surrounded by endoderm. In the main ribs, in cross section, the cuticle is thicker, especially in the lower surface, where epidermal cells are small and hypodermis usually has two layers of cells on both sides, the collenchyma is angle and is further developed by the lower surface; the parenchyma is formed by thick-walled polygonal cells, the vascular system is formed by a single collateral bundle, covered by endoderm and fiber sheath very sclerified; other two smaller bundles facing the adaxial face may occur, the whole being surrounded by fiber sheath. On any blade, in the hypodermis, collenchyma and parenchyma cells containing phenolic compounds occur; in the parenchyma there is higher concentration of starch grains and spherical secreting cells are common, unicellular and large volume, suberized walls; crystals of calcium oxalate, usually in the form of single crystals or prismatic crystals are found in the epidermis and in the form of rod-shaped, very small, thin and grouped in the parenchyma; lipid droplets occur in all tissues. The petiole, in front view, has slightly wavy cuticle, epidermis consists of small cells, quadrangular, with anticlinal thick walls, most containing phenolic compounds and many star trichomes, equal to the blade one; various spherical secretory cells, large volume and suberized walls are visible by transparency. In cross section, the petiole has two side ribs facing the adaxial side; the cuticle is thick, the epidermal cells are small, the trichomes are more common in the lower surface and its insertion can reach the cortical parenchyma; the hypodermis is unistratified, rarely bistratified, formed by small cells with thick walls; the collenchyma is angular and cortical parenchyma is composed of polygonal cells with too thick walls, small crystals of calcium oxalate, normally isolated crystal or rod-shaped groups, as well as lipid droplets and secretory cells with large volume and suberized walls; the endoderm is continuous, formed by rounded to elliptical cells, with large amounts of starch grains; the vascular system is represented by a central and open collateral bundle with phloem with or without a fiber cap or scattered fibers, isolated or grouped; the procambium is evident and has large amount of starch grains; the xylem has distribution in beams and may present isolated fibers

or in small groups, with their conducting cells, as well as a significant grouping of fibers together to protoxylem elements.

MICROSCOPIC DESCRIPTION OF POWDER

The powder meets all the requirements for the species, unless the macroscopic characters. To microscopically examine the powder, the use of chloral hydrate is required. Characteristics: greenish yellow to brownish yellow color; star trichomes, intact and isolated or part of them, in front and/or side view; portions of the epidermis in the region of the mesophyll, cells with thickened walls and visible pits, in front view;

portions of epidermis with stomata in front view; portions of epidermis with cells with thick walls showing the star trichome base in front view; fragments of epidermis with portions of ribs in front view; portions of epidermis of the petioles, with secretory cells visible by transparency in frontal view; portions of mesophyll with secretory cells in front view; portions of mesophyll with crystal idioblast and cell with phenolic compounds, in front view; groups of fibers, in longitudinal section; fragments of the vascular system with portions of fiber, tracheal elements, parenchyma with portions of fibers, in longitudinal section; fragments of the blade with portions of epidermis, hypodermis, and palisade parenchyma in cross section; fragments of epidermis and hypodermis, in cross section; portions of palisade parenchyma with secretory cells and cells containing rod-shaped crystals, in cross section; fragments of the region of mesophyll in, cross section.

IDENTIFICATION

A. To crush a few leaves with ethanol. To evaporate the ethanol in water bath. To add few drops of 1% vanillin solution (w/v) in hydrochloric acid SR to the resulting residue. Reddish brown or deep red color develops.

B. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF₂₅₄ silica gel as support and a mixture of methanol, diethylamine, and toluene (10:10:80) as mobile phase. To separately apply to the plate, as band, 40 µL of (or 6 µL) of *Solution (1)* and 20 µL (or 2 µL) of *Solution (2)*, freshly prepared, as described below.

Solution (1): to transfer 0.5 g of the pulverized drug to a 50 mL flask, add a mixture of 1 mL of 2 M hydrochloric acid and 20 mL of water. To make it homogeneous. To heat under reflux in water bath for 10 minutes. To cool down and filter it. To add 2 mL of 6 M ammonium hydroxide to the filtrate. To extract twice the filtrate in a separatory funnel with 20 mL of ethyl ether each time, with moderate stirring to avoid formation of an emulsion. To gather the organic phases and evaporate the solvent under reduced pressure. To dissolve the residue in 1 mL of methanol.

Solution (2): to dissolve 2 mg of boldine SQR and 5 mL of methanol.

b

To develop the chromatogram. To remove the plate, and let it drying. To observe the plate under ultraviolet light (365 nm). The chromatogram obtained with *Solution (2)* presents a violet blue spot. The chromatogram obtained with *Solution (1)* shows spot similar in position and color to the spot in the chromatogram obtained from the *Solution (2)*. To nebulize the plate with aqueous acetic potassium iodobismuthate. To allow to air dry for 5 minutes. To nebulize the plate with sodium nitrite SR. To observe after 30 minutes under the light. Boldine has brown color.

PURITY TESTS

Foreign matter (5.4.2.2). At most 3.0%.

Water (5.2.20.2). At most 10.0%.

Total ashes (5.4.2.4). At most 10.0%.

Ashes insoluble in acid (5.4.2.5). At most 6.0%.

DOSAGE

Total alkaloids

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 304 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at room temperature; flow of *Mobile phase* of 1.5 mL/min.

Mobile phase: To prepare a mixture of *Solution A* and *Solution B*, as described below.

Solution A: mixture of 0.2 mL of diethylamine and 99.8 mL of acetonitrile.

Solution B: mixture of 0.2 mL of diethylamine and 99.8 mL of water, and adjust the pH to 3.0 by using anhydrous formic acid.

Sample solution: to accurately weigh about 1 g of the pulverized drug to erlenmeyer flask, add 50 mL of 2 M hydrochloric acid, and by stirring heat it in a water bath at 80 °C for 30 minutes. To filter and dissolve the residue with 50 mL of 2 M hydrochloric acid and by stirring heat it in a water bath at 80 °C for 30 minutes. To filter again and repeat the operation with the resulting residue. To filter it. To combine the cooled filtrates in a separatory funnel and stir with 100 mL of a mixture of n-hexane and ethyl acetate (1:1). To discard the organic phase. To adjust the pH of the aqueous phase to 9.0 with 6 M ammonium hydroxide To extract the aqueous phase with a portion of 100 mL and two portions methylene chloride (50 mL). To combine the organic phases and evaporate in a rotary evaporator to dry-

ness. To transfer the residue to a 10 mL volumetric flask by using the *Mobile phase* as diluent. To top up with *Mobile phase* and mix it.

Standard solution: To accurately weigh about 12 mg of boldine SQR. To dissolve the weighed amount in 100 mL volumetric flask by using the *Mobile phase* as diluent. To top up with *Mobile phase* and mix it.

To transfer 1 mL of the solution obtained, by using a volumetric pipette, to a 10 mL volumetric flask. To top up with *Mobile phase* and mix it.

Resolution solution: to use the *Sample solution*.

To inject 20 µL of *Resolution solution*. The retention times for boldine, whose retention time is about 6 minutes, are about 0.9 to isoboldine, 1.0 to boldine, 1.8 to isocorydine N-oxide, 2.2 to laurotetanin, 2.8 to isocorydine, and 3.2 to N-methyl laurotetanin. Other peaks may be present. The resolution between the isoboldine and boldine peaks is greater than 1.0.

Procedure: To separately inject 20 µL of the *Standard solution* and *Sample solution*, record the chromatograms, and measure the areas under the peaks relating to the boldine standard and 6 alkaloids described and identified in the *Resolution solution*, that is, in the *Sample solution*. To calculate the content (percentage) of total alkaloids, expressed in boldine, according to the equation:

$$\% \text{ de boldine} = \frac{(\sum A_i) \times m_2}{A_2 \times m_1}$$

where

m_1 = mass of drug (g);

m_2 = mass of boldine SQR in *Standard solution* (g);

$\sum A_i$ = sum of the area under the peaks for the 6 alkaloids identified in the chromatogram obtained from the *Sample solution*;

A_2 = area under the boldine peak in the chromatogram obtained with the *Standard solution*.

Volatile oils

To proceed as described in *Determination of volatile oils in vegetable drugs (5.4.2.7)*. To use 1000 mL flask containing 500 mL of water as distillation liquid. To use 0.5 mL of xylene. The drug previously ground must be turbolized with 100 mL of water. To immediately transfer to the flask and make hydrodistillation from 50 g of the drug. To distill for 4 hours.

PACKAGING AND STORAGE

In tightly closed containers, protected from light and heat.

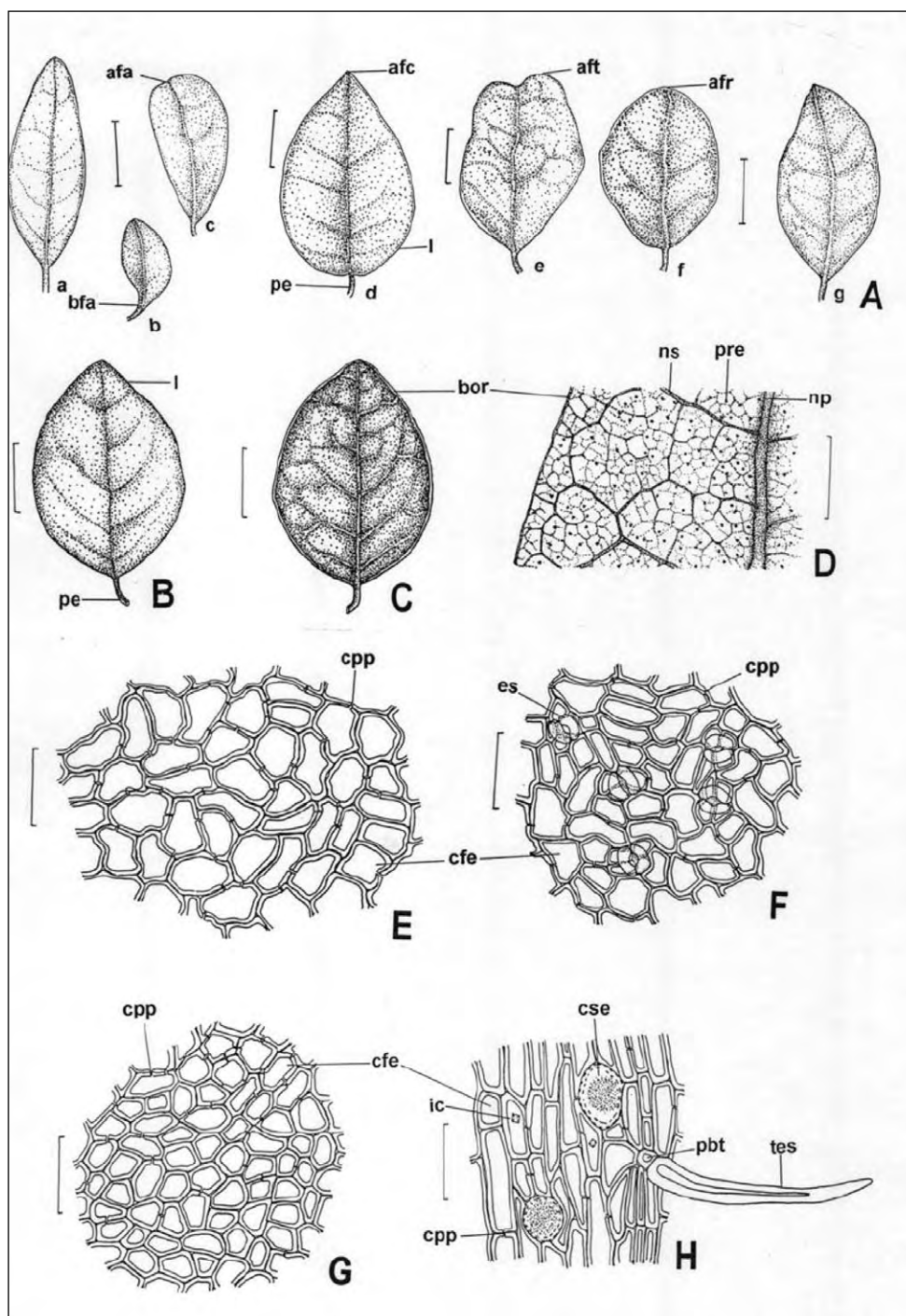


Figure 1 - Macroscopic aspects in *Peumus boldus* Molina

Additional information of **Figure 1**. Scales correspond to 10 mm, in **A** (a, b, c, e, f, and g); 15 mm, in **A** (d); 14 mm, in **B** and **C**; 5 mm, in **D**; 100 μ m, in **E**, **F**, **G**, and **H**. **A** - general appearance of different leaf forms: asymmetrical leaf base (bfa); asymmetrical leaf apex (afa); acuminate leaf apex (afc); petiole (pe); blade (l); retuse leaf apex (aft); rounded leaf apex (afr). **B** - general appearance of the adaxial leaf: pedicle (pe); blade (l). **C** - general appearance of lower leaf: edge (bor). **D** - Detail of portion of the lower surface of leaf blade in front view showing the nervation of the midrib to the edge: edge (bor), secondary rib (ns); prominence formed by the basal region of star trichome (pre); midrib (np). **E** - detail of the portion of the epidermis facing the adaxial side, in the region of the mesophyll, in front view: primary field of pit (cpp); fundamental cell of the epidermis (cfe). **F** - detail of the portion of the epidermis facing the lower side, in the region of the mesophyll, in front view: stomata (es); primary field of pit (cpp); fundamental cell of the epidermis (cfe). **G** - detail of the portion of the epidermis, in the region of the midrib, facing the adaxial side, in front view: primary field of pit (cpp); fundamental cell of the epidermis (cfe). **H** - detail of the portion of the epidermis, in the region of the midrib, facing the lower side, in front view: fundamental cell of the epidermis (cfe); secretory cell (cse); crystal idioblast (ic); primary field of pit (cpp), basal portion of fractured trichome cell (pbt); star trichomes (tes).

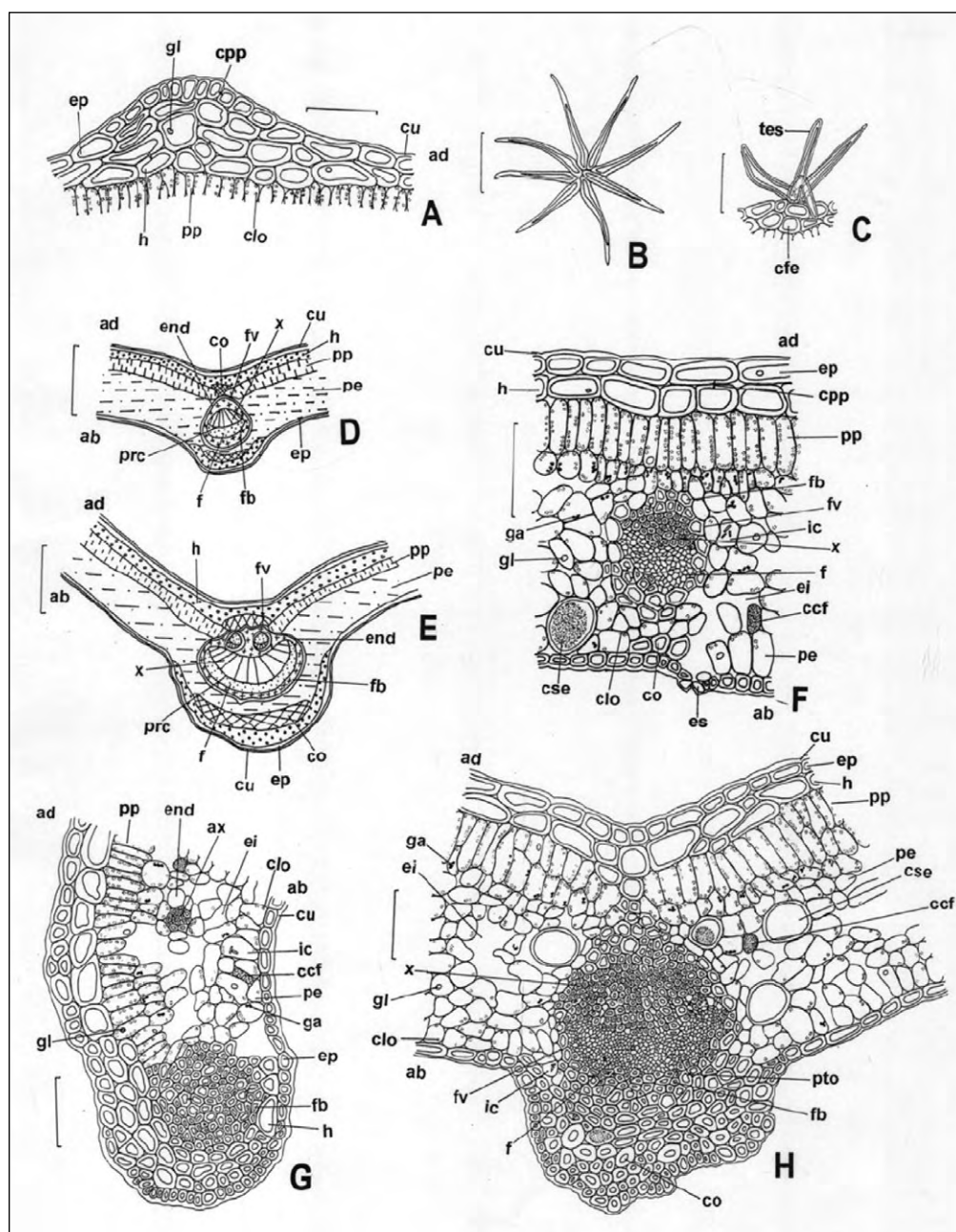


Figure 2 - Macroscopic aspects in *Peumus boldus* Molina

Additional information of **Figure 2**. Scales correspond to 100 µm in **A**, **C**, **F**, **G**, and **E**; 400 µm, in **B**; and 400 µm, in **D** and **H**.

A - detail of portion of the leaf blade, in cross section, with the adaxial side, showing prominence of the basal region of star trichome: chloroplast (clo), lipid droplet (gl); primary field of pit (cpp); cuticle (cu); adaxial face (ad); hypodermis (h); palisade parenchyma (pp); epidermis (ep).

B - detail of star trichomes in frontal view. **C** - detail of star trichomes in side view: star trichoma (tes); fundamental cell of the epidermis (cfe). **D** - partial scheme of midrib of the leaf blade, in cross section, showing a single vascular bundle: adaxial face (ad); lower face (ab); endoderm (end); collenchyma (co); vascular bundle (fv); xylem (x), cuticle (cu), hypodermis (h); palisade parenchyma (pp); spongy parenchyma (pe); epidermis (ep), fibers (fb); phloem (f); procambium (prc). **E** - partial scheme of midrib of the leaf blade, in cross section, showing 3 vascular bundles: adaxial face (ad); lower face (ab), hypodermis (h); vascular bundle (fv); palisade parenchyma (pp); spongy parenchyma (pe); endoderm (end); fibers (fb), collenchyma (co); epidermis (p); cuticle (cu), phloem (f); procambium (prc), xylem (x). **F** - detail of portion of the leaf blade, in the mesophyll, in cross section, showing secondary vascular bundle: adaxial face (ad); lower face (ab); epidermis (ep), cuticle (cu); primary field of pit (cpp), hypodermis (h); palisade parenchyma (pp); fibers (fb); vascular bundle (fv); crystal idioblast (ic); xylem (x), phloem (f); starch grain (ga), lipid droplet (gl); intercellular space (ei); cell with phenolic compounds (ccf); spongy parenchyma (pe), stomata (es), collenchyma (co); chloroplast (clo); secretory cell (cse). **G** - detail of the edge in the middle region of the leaf blade, in cross section: adaxial face (ad); lower face (ab); palisade parenchyma (pp); xylem grouping (ax), intercellular space (ei); chloroplast (clo); cuticle (cu); crystal idioblast (ic); cell with phenolic compounds (ccf); spongy parenchyma (pe); starch grain (ga); lipid droplet (gl); epidermis (ep), fibers (fb); hypodermis (h). **H** - detail of portion of the middle region of the leaf blade in cross section, in the midrib: adaxial face (ad); lower face (ab); starch grain (ga), intercellular space (ei); xylem (x), lipid droplet (gl); chloroplast (clo); vascular bundle (fv); crystal idioblast (ic); phloem (f); collenchyma (co); fibers (fb); pit (pto); cell with phenolic compounds (ccf); secretory cell (cse); spongy parenchyma (pe); palisade parenchyma (pp); hypodermis (h), epidermis (p); cuticle (cu).

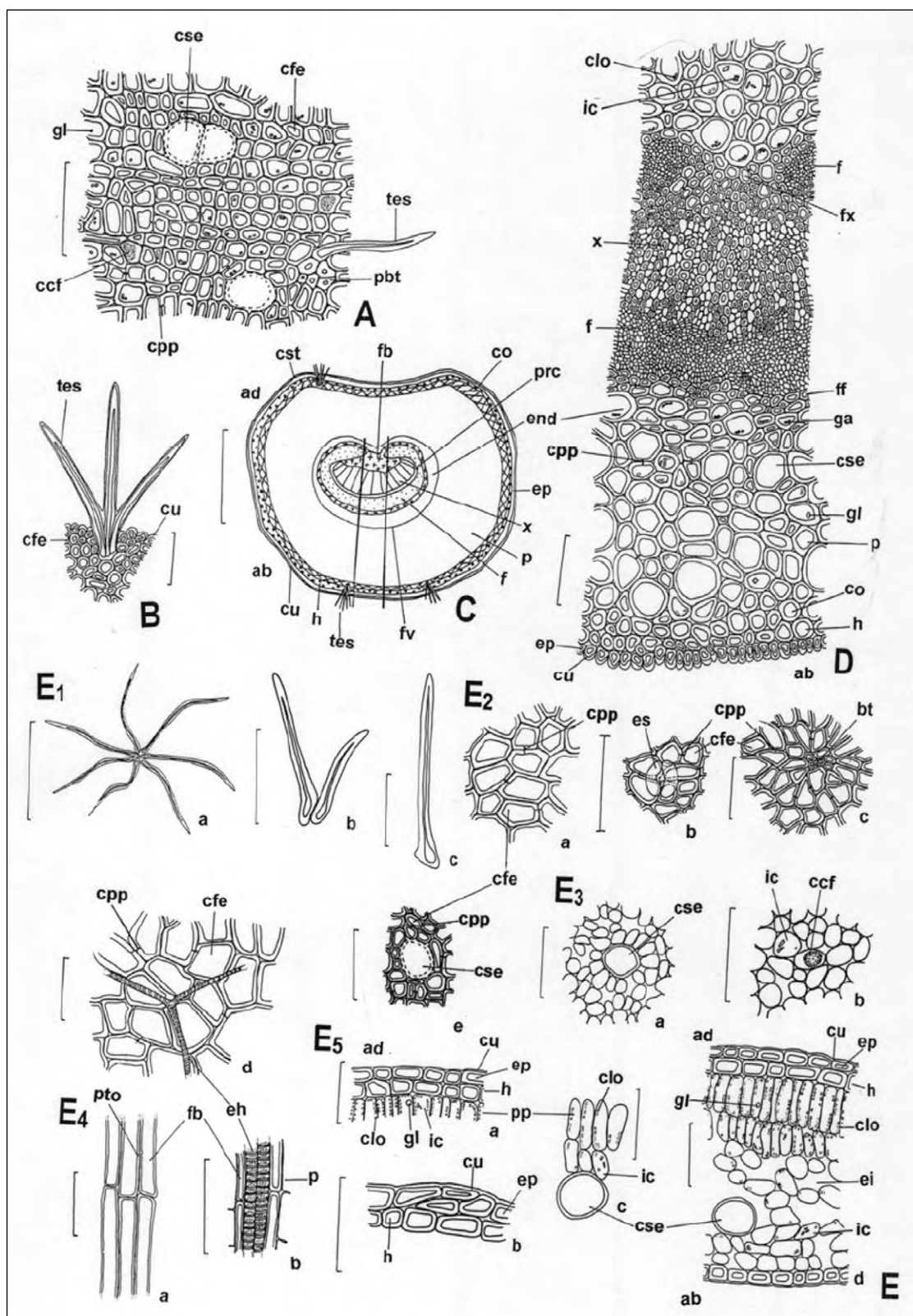


Figure 3 - Microscopic aspects and microscopy of powder in *Peumus boldus* Molina

Additional information of **Figure 3**. Scales correspond to 100 μm , in **A**, **B**, **D**, and **E** (**E**₂ to **E**₅); 400 μm , in **C**; and 400 μm , in **E** (**E**₁).

A - detail of portion of the epidermis, in front view: lipid droplet (gl); cell with phenolic compounds (ccf); secretory cell (cse); primary field of pit (cpp); fundamental cell of the epidermis (cfe); star trichomes (tes), basal portion of star trichome cells (pbt). **B** - detail of portion of the epidermis, in side view: star trichoma (tes); fundamental cell of the epidermis (cfe); cuticle (cu). **C** - general scheme

of petiole in cross-section: adaxial face (ad); lower face (ab); rib (cst); fibers (fb), collenchyma (co), procambium (prc); endoderm (end); epidermis (ep), xylem (x), phloem (f); parenchyma (p); star trichomes (h); cuticle (cu). **D** - detail of portion of the petiole, in cross section, as highlighted in **C**: lower face (ab), hypodermis (h); cuticle (cu); epidermis (ep), collenchyma (co); parenchyma (p), lipid droplet (gl); secretory cell (cse); primary field of pit (cpp), starch grain (ga); endoderm (end); xylem (x), phloem (f); xylem fibers (fx); phloem (F); crystal idioblast (ic); chloroplast (clo). **E** - details of powder: fundamental cell of the epidermis (cfe); primary field of pit (cpp); stomata (es), base of trichomes (bt);

secretory cell (cse); cell with phenolic compounds (ccf); crystal idioblast (ic); pit (pto); fibers (fb); vessel element with helical thickening (eh); parenchyma (p); adaxial face (ad); lower face (ab); chloroplast (clo); lipid droplet (gl); cuticle (cu); epidermis (ep); hypodermis (h); palisade parenchyma (pp); intercellular space (ei). **E₁** - details of trichomes: star tricoma in front view (a), portion of star trichomes in side view (b), isolated star trichome cell in side view (c). **E₂** - details of the epidermis: portion of the epidermis in the region of the mesophyll, in front view (a); portion of the epidermis with stomata, in front view (b); portion of the epidermis with cells with thick walls showing the base of star trichomes in front view (c); fragment of the epidermis with rib portion, in front view (d); portion of the epidermis of the petiole, in front view (e). **E₃** — detail of the mesophyll, in cross-section: portion of the mesophyll with secretory cell (a); portion of the mesophyll with crystals of calcium oxalate and cell with phenolic compounds (b). **E₄** - details of portions of the vascular system, in longitudinal section: grouping of fibers (a), fragment of the vascular system with portions of fibers, tracheary and parenchyma elements (b). **E₅** - details of leaf blade tissues in cross-section: fragment of the blade with portion of epidermis, hypodermis, and palisade parenchyma (a); fragment of the epidermis and hypodermis (b); portion of palisade parenchyma with secretory cell and cell containing crystals (c); fragment of the mesophyll (d).

BOLDO, TINCTURE

Boldus tinctura

The tincture is prepared from the 10.0% dried leaves of *Peumus boldus* Molina - Monimiaceae (w/v), by maceration or percolation and using 60.0% ethanol (v/v) as the extraction liquid. It contains at least 0.01% of total alkaloids expressed in boldine.

CHARACTERISTICS

Organoleptic characteristics. Clear, dark greenish brown liquid with typical odor and flavor.

IDENTIFICATION

A. To evaporate to dryness 10 mL of the tincture in water bath. To add few drops of 1% vanillin solution (w/v) in hydrochloric acid SR to the resulting residue. Reddish brown or deep red color develops.

B. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel as support and a mixture of methanol, diethylamine, and toluene (10:10:80) as mobile phase. To separately apply to the plate, as band, 10 µL of *Solution (1)* and 5 µL of *Solution (2)* freshly prepared, as described below.

Solution (1): to evaporate 25 mL of the tincture in water bath to obtain consistency of soft extract. To triturate the hot residue twice with 10 mL of 2 M hydrochloric acid each time. To filter and alkalize the filtrate at pH 9.0 with 6 M ammonium hydroxide. To extract twice the filtrate in a separatory funnel with 20 mL of ethyl ether each time, with moderate stirring to avoid formation of an emulsion. To gather the organic phases and evaporate the solvent in a water bath. To dissolve the residue in 0.5 mL of methanol.

Solution (2): to dissolve 2 mg of boldine SQR and 5 mL of methanol.

To develop the chromatogram. To remove the plate, and let it drying. To observe the plate under ultraviolet light (365 nm). The chromatogram obtained with *Solution (2)* presents a violet blue spot.

The chromatogram obtained with *Solution (1)* shows spot similar in position and color to the spot in the chromatogram obtained from the *Solution (2)*. To nebulize the plate with aqueous acetic potassium iodobismuthate. To allow

to air dry for 5 minutes. To nebulize the plate with sodium nitrite SR. To observe after 30 minutes under the light. Boldine has brown color.

PURITY TESTS

Ethanol (5.3.3.8.1). 60 ± 5% (w/v). To proceed as described in *Method by distillation, Special treatments, Liquids with more than 30% of alcohol*.

Dry residue (5.4.3.2.3). At least 2.0%.

DOSAGE

Total alkaloids

To employ one of the methods described below.

A. To accurately weigh about 100 mg of tincture. To evaporate in a rotary evaporator to obtain consistency of soft extract. To quantitatively transfer the sample to a separatory funnel, by using a few milliliters of water. To add 6 mL of 6 M ammonium hydroxide. To stir with consecutive fractions of 40 mL, 25 mL, and 25 mL of methylene chloride. To check the complete extraction of alkaloids by adding a drop of mercuric potassium iodide SR to a few drops of aqueous phase. In case of a positive reaction, stir the aqueous phase with successive fractions of 20 mL of methylene chloride to obtain negative Mayer reaction. To gather the organic phases in separatory funnel and wash with water until neutrality. To add 2 g of anhydrous sodium sulfate to the organic solution, and leave it in contact for a few minutes by occasionally stirring. The organic solution should be clear. To decant and wash 3 times the sodium sulfate with 10 mL of methylene chloride. To gather the organic fractions and evaporate in a rotary evaporator. To transfer the residue with the smallest possible amount of methylene chloride to a erlenmeyer flask and add 20 mL of 0.005 M SV sulfuric acid. To titrate the acid in excess with a 0.01 M SV sodium hydroxide by using methyl red SI as indicator.

To calculate the content in percentage of total alkaloids, expressed in boldine, according to the equation:

$$\% \text{ de boldine} = \frac{32,74 \times (20 - n)}{100 \times m}$$

where

n = number of ml of 0.01 M SV sodium hydroxide spent;

m = mass of the tincture (g).

B. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 304 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μ m), kept at room temperature; flow of *Mobile phase* of 1.5 mL/min.

Mobile phase: To prepare a mixture of *Solution A* and *Solution B* (16:84), as described below.

Solution A: mixture of 0.2 mL of diethylamine and 99.8 mL of acetonitrile.

Solution B: mixture of 0.2 mL of diethylamine and 99.8 mL of water, and adjust the pH to 3.0 by using anhydrous formic acid.

Sample solution: To pipette an aliquot of 10 mL of the tincture, which is equivalent to 1 g of the vegetable drug. To evaporate in a water bath at 80 °C to obtain the consistency of soft extract. To triturate the hot residue with 50 mL of 2 *M* hydrochloric acid for 5 minutes. To filter and repeat the procedure again with the resulting residue. To filter it. To combine the cooled filtrates in a separatory funnel and stir with 100 mL of a mixture of hexane and ethyl acetate (1:1). To discard the organic phase. To adjust the pH of the aqueous phase to 9.0 with 6 *M* ammonium hydroxide. To extract the aqueous phase with portions of 100 mL, 50 mL, and 50 mL of methylene chloride. To combine the organic phases and evaporate in a rotary evaporator to dryness. To transfer the residue to a 10 mL volumetric flask by using the *Mobile phase* as diluent. To top up with *Mobile phase* and mix it.

Standard solution: To accurately weigh about 12 mg of boldine SQR. To dissolve the weighed amount in 100 mL volumetric flask by using the *Mobile phase* as diluent. To top up with *Mobile phase* and mix it. To transfer 1 mL of the solution obtained, by using a volumetric pipette, to a 10 mL volumetric flask. To top up with *Mobile phase* and mix it.

Resolution solution: to use the *Sample solution*.

To inject 20 μ L of *Resolution solution*. The retention times for boldine, whose retention time is about 6 minutes, are about 0.9 to isoboldine, 1.0 to boldine, 1.8 to isocorydine N-oxide, 2.2 to laurotetanin, 2.8 to isocorydine, and 3.2 to N-methyl laurotetanin. Other peaks may be present. The resolution between the isoboldine and boldine peaks is greater than 1.0.

Procedure: To separately inject 20 μ L of the *Standard solution* and *Sample solution*, record the chromatograms, and measure the areas under the peaks relating to the boldine standard and 6 alkaloids described and identified in the *Resolution solution*, that is, in the *Sample solution*. To calculate the content in percentage of total alkaloids, expressed in boldine, according to the equation:

$$\% \text{ boldine} = (\sum A_1) \times m_b / A_2$$

where

$\sum A_1$ = sum of the area under the peaks for the 6 alkaloids identified in the chromatogram obtained with the *Sample solution*;

m_b = mass of boldine SQR in *Standard solution* (g);

A_2 = area under the boldine peak in the chromatogram obtained with the *Standard solution*.

PACKAGING AND STORAGE

In tightly closed amber glass containers, protected from light and heat.

BORATE, SODIUM Natrii boras

$\text{Na}_2\text{B}_4\text{O}_7$; 201,22

$\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$; 381.37

sodium borate; 00117

Boron sodium oxide

[1330-43-4]

Borax

[1303-96-4]

It contains at least 99.0% and at most 105.0% of $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$.

DESCRIPTION

Physical characteristics. White, crystalline powder, or colorless crystals.

Solubility. Soluble in water, very soluble in boiling water, freely soluble in glycerol, insoluble in ethanol.

IDENTIFICATION

A. To dissolve 0.2 g of the sample in carbon dioxide-free water and top up the volume to 5 mL with the same solvent. To add 0.1 mL of phenolphthalein SI. The red color develops. To add 5 mL of 85% glycerol (v/v). The color disappears.

B. The solution similarly prepared to that one in *Identification test A*. responds to reactions of borate ion (5.3.1.1).

C. The solution similarly prepared to that one in *Identification test A*. responds to reactions of sodium ion (5.3.1.1).

PURITY TESTS

Appearance of solution. To dissolve 4 g of the sample in carbon dioxide-free water and top up the volume to 100 mL with the same solvent. The solution is clear (5.2.25) and colorless (5.2.12).

pH (5.2.19). 9.0 to 9.6. To determine in the solution obtained, in *Appearance of solution*.

Carbonate and bicarbonate. In a test tube To add 5 mL of aqueous solution for 5% sample (w/v) and 1 mL of 3 M hydrochloric acid. No effervescence occurs.

Sulfates (5.3.2.2). To use 15 mL of the solution obtained in *Appearance of solution* and proceed as described in *Limit test for sulfates*. To prepare the standard solution with mixture of 3 mL of standard sulfate solution (10 ppm SO_4) and 12 mL of water. At most 0.005% (50 ppm).

Heavy metals (5.3.2.3). To use 12 mL of the solution obtained in *Appearance of solution* and proceed as described in *Method I*. To prepare standard solution by using *Standard lead solution (1 ppm)*. At most 0.0025% (25 ppm).

Arsenic (5.3.2.5). To use *Method I*. To use 15 mL of the solution obtained in *Appearance of solution* and proceed as described in *Limit test for arsenic*. At most 0.0005% (5 ppm).

Ammonia (5.3.2.2). To use 6 mL of the solution obtained in *Appearance of solution* to 14 mL with water and proceed as described in *Limit test for ammonia*. To prepare the standard solution with mixture of 2.5 mL of *Standard ammonia solution (1 ppm)* and 7.5 mL of water. At most 0.001% (10 ppm).

Calcium (5.3.2.7). To use 15 mL of the solution obtained in *Appearance of solution* and proceed as described in *Limit test for calcium*. To prepare the standard solution with mixture of 6 mL of *Standard calcium solution (10 ppm)* and 9 mL of water. At most 0.01% (100 ppm).

DOSAGE

To accurately weigh about 0.3 g of the sample and dissolve in 50 mL of water. To add a few drops of methyl red SI and titrate with 0.1 M SV hydrochloric acid. Each mL of 0.1 M SV hydrochloric acid is equal to 19.069 mg of $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

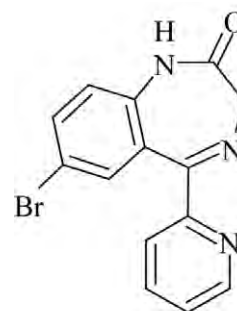
To comply with the legislation in force.

THERAPEUTIC CLASS

Antiseptic agent, detergent, astringent to mucous membranes.

BROMAZEPAM

Bromazepamum



$\text{C}_{14}\text{H}_{10}\text{BrN}_3\text{O}$; 316.15

bromazepam; 01366

7-Bromine-1,3-dihydro- 5 - (2-pyridinyl)-2H-1,4-benzodiazepin-2-one
[1812-30-2]

It contains at least 99.0% and no more than 101.0% of $\text{C}_{14}\text{H}_{10}\text{BrN}_3\text{O}$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white or slightly yellowish, and odorless powder.

Solubility. Insoluble in water, slightly soluble in ethanol and methylene chloride.

Physical and chemical constants.

Melting range (5.2.2): 237 °C to 238.5 °C with decomposition.

IDENTIFICATION

The identification tests C. and D. may be omitted if the tests A. and B. are conducted.

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated to constant weight, and dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the bromazepam SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14) ranging from 220 nm to 350 nm, of 0.0005% (w/v) in methanol, exhibits maxima and minima only in the same wavelength of a similar bromazepam SQR solution. The ratio between the absorbance values measured at 233 nm and 325 nm is between 980 and 1080.

C. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF_{254} silica gel as support and a mixture of diethylamine and ethyl ether (30:70) as mobile phase.

To apply separately to the plate 5 µl of each of the solutions freshly prepared, as described below.

Solution (1): 1 mg/mL solution of the sample in mixture of methanol and methylene chloride (1:9).

Solution (2): 1 mg/mL solution of bromazepam SQR in mixture of methanol and methylene chloride (1:9).

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

D. To dissolve about 20 mg of sample in 5 mL of methanol. To add 5 mL of water and 1 mL of 1% ammonium ferrous sulfate (w/v). The violet color develops.

PURITY TESTS

Related substances. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF₂₅₄ silica gel as support and a mixture of ethanol, triethylamine, methylene chloride, and petroleum ether (5:5:20:70) as mobile phase. To separately apply to the plate 5 µl of each of the solutions freshly prepared, as described below. The test shall be protected from light, to be conducted.

Solution (1): 10 mg/mL solution of the sample in mixture of methanol and methylene chloride (1:9).

Solution (2): to dilute *Solution (1)* in mixture of methanol and methylene chloride (1:9), to obtain a 20 mg/mL solution of the sample.

To develop the chromatogram. To remove the plate, and allow it to dry in a current of air for 20 minutes. To examine under ultraviolet light (254 nm). Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (2)* (0.2%).

Loss on drying (5.2.9). To determine in 1 g of the sample, in a vacuum oven at 80 °C for 4 hours. At most 0.2%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To accurately weigh about 0.25 g of the sample, dissolve in 20 mL of glacial acetic acid, and add 50 mL of acetic anhydride. To titrate with 0.1 M SV perchloric acid, to potentiometrically determine the endpoint. To perform sample blank correction and adjust it as required. Each mL of 0.1 M SV perchloric acid is equal to 31.615 mg of C₁₄H₁₀BrN₃O.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Anxiolytic

BROMAZEPAM TABLETS

It contains at least 93.0 % and at most 110.0 % of the amount stated of C₁₄H₁₀BrN₃O.

IDENTIFICATION

A. The ultraviolet absorption spectrum (5.2.14) ranging from 200 nm to 400 nm, of sample solution obtained in *Dosage*, exhibits maxima and minima similar to those observed in the standard solution spectrum.

B. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF₂₅₄ silica gel as support and a mixture of ethyl acetate and 25% ammonium hydroxide (v/v) (100:1) as mobile phase. To apply separately to the plate 10 µl of each of the solutions freshly prepared, as described below.

Solution (1): to weigh and pulverize the tablets. To use amount of powder equivalent to 25 mg of bromazepam and add 10 mL of methanol. To make it homogenous and filter it.

Solution (2): 2.5 mg/mL bromazepam SQR solution in methanol.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

Procedure for content uniformity. To protect from light. To individually weigh and transfer each tablet to a 100 mL

volumetric flask. To proceed as described in Dosage, from "Add 70 mL of 0.1 M methanolic sulfuric acid.."To add 70 mL of 0.1 M methanolic sulfuric acid..."

DISSOLUTION TEST (5.1.5)

Dissolution medium: simulated gastric fluid (no enzyme), 900 mL

Equipment: blades, 50 rpm

Time: 20 minutes

Procedure: After testing, remove aliquot from the dissolution medium, filter, cool down at 20 °C, and dilute, if necessary, in simulated gastric fluid (no enzyme), to proper concentration. To measure the absorbance of the solutions at 239 nm (5.2.14), by using simulated gastric fluid (no enzyme) to adjust zero. To calculate the amount of $C_{14}H_{10}BrN_3O$ dissolved in the medium, by comparing the readings obtained with 0.00033 % bromazepam SQR solution (w/v) prepared in the same solvent.

Tolerance: not less than 80% (Q) of the stated amount of $C_{14}H_{10}BrN_3O$ dissolves within 20 minutes.

DOSAGE

Note: to carry out the preparation of the solutions protected from light.

To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To weigh and spray 20 tablets. To transfer amount of powder accurately weighed equivalent to 0.6 g of bromazepam to 100 mL volumetric flask. To add 70 mL of 0.1 M methanolic sulfuric acid and leave it in ultrasound for 20 minutes. To top up with the same solvent, centrifuge, and filter, if necessary. To successively dilute to 0.0006% concentration (w/v) by using the same solvent. To prepare standard solution under the same conditions. To measure the absorbance of the resulting solutions at 239 nm, by using 0.1 M methanolic sulfuric acid to adjust zero. To calculate the amount of $C_{14}H_{10}BrN_3O$ in the tablets, from the responses obtained.

PACKAGING AND STORAGE

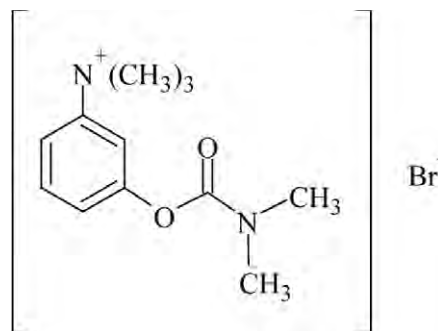
In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

BROMIDE, NEOSTIGMINE

Neostigmini bromidum



$C_{12}H_{19}BrN_2O_2$; 303.20

neostigmine bromide; 06287

Bromide 3 - [[(dimethylamino) carbonyl] oxy]-*N,N,N*-trimethylbenzeneminium
[114-80-7]

It contains at least 98.0% and at most 102.0% of $C_{12}H_{19}BrN_2O_2$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White crystalline powder. It is colorless and has a bitter taste. Its solutions are neutral to litmus paper.

Solubility. Very soluble in water, and soluble in ethanol.

Physical and chemical constants.

Melting range (5.2.2): 171 °C to 176 °C, with decomposition.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated at 105 °C for 3 hours, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the neostigmine bromide SQR spectrum similarly prepared.

B. The solution 1:50 responds to the reactions of the bromide (5.3.1.1).

PURITY TESTS

Sulfate. To dissolve 0.25 g of the sample in 10 mL of water, 1 mL of hydrochloric acid, and 1 mL of barium chloride. Turbidity does not occur immediately.

Loss on drying (5.2.9). To desiccate the sample at 105 °C for 3 hours. At most 2.0%.

Sulfated ashes (5.2.10). At most 0.15 %.

DOSAGE

To accurately dissolve about 0.75 g of the sample in mixture of 70 mL of glacial acetic acid and 20 mL of mercury acetate SR. To add 4 drops of methylrosanilinium chloride SI and titrate with 0.1 M SV perchloric acid to obtain blue color. To perform sample blank correction and adjust it as required. Each mL of 0.1 M SV perchloric acid is equal to 30.32 mg of $C_{12}H_{19}BrN_2O_2$.

PACKAGING AND STORAGE

In airtight containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Cholinergic.

BROMIDE, SODIUM

Natrii bromidum

NaBr; 10289
sodium bromide; 01445
Sodium bromide
[7647-15-6]

It contains at least 98.0% and at most 100.5% of NaBr, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White powder or colorless or opaque crystals, slightly hygroscopic.

Solubility. Freely soluble in water and soluble in ethanol.

IDENTIFICATION

A. It responds to the reactions of the bromide ion (5.3.1.1).

B. The 10% solution (w/v) responds to the reactions of sodium ion (5.3.1.1).

PURITY TESTS

Appearance of solution. To transfer 10 g of the sample to 100 mL volumetric flask, dissolve in carbon dioxide-free water, and top up the volume with the same solvent. The solution is clear (5.2.25) and colorless (5.2.12).

Acidity or alkalinity. To add 0.1 mL of bromothymol blue SI to 10 mL of the solution obtained in *Appearance of solution*. More than 0.5 mL of 0.01 M hydrochloric acid or 0.01 M sodium hydroxide is not necessary to change the indicator.

Bromides. To add 1 mL of starch solution SI, 0.1 mL of 10% potassium iodide solution (w/v), and 0.25 mL of 0.5 M sulfuric acid to 10 mL of the solution obtained in *Appearance of solution*. No blue or violet color should develop.

Chlorides. To transfer 1 g of the sample to erlenmeyer flask and dissolve in 20 mL of 20% nitric acid (w/v). To add 5 mL of concentrated hydrogen peroxide and heat in a water bath until the solution is completely colorless. To wash the walls of the flask with a little amount of water and heat it in a water bath for 15 minutes. To cool down, dilute to 50 mL with water, add 5 mL of 0.1 M SV silver nitrate, and 1 mL of dibutylphthalate. To make it homogeneous and titrate with 0.1 M SV ammonium thiocyanate solution, by using 5 mL of ferric ammonium sulfate SR as an indicator. More than 1.7 mL of 0.1 M SV silver nitrate solution is not necessary to change the indicator (0.6%). To record the volume of 0.1 M SV silver nitrate solution used.

Iodides. To add 0.15 mL of ferric chloride SR and 2 mL of chloroform to 5 mL of the solution obtained in *Appearance of solution*. To stir and observe the phases. The chloroform phase is colorless.

Sulfates (5.3.2.2). To use 15 mL of the solution obtained in *Appearance of solution* and proceed as described in *Limit test for sulfates*. At most 0.01% (100 ppm).

Barium. To add 5 mL of distilled water and 1 mL of dilute sulfuric acid SR to 5 mL of the solution obtained in *Appearance of solution*. After 15 minutes, any opalescence observed is not more intense than the mixture of 5 mL of the solution obtained in *Appearance of solution* and 6 mL of water.

Heavy metals (5.3.2.3). To use *Method III*. To use 12 mL of the solution obtained in *Appearance of solution* and proceed as described in *Limit test for heavy metals*. To prepare a reference solution by using *lead solution (1 ppm Pb)*. At most 0.001% (10 ppm).

Iron (5.3.2.4). To dilute 5 mL of the solution obtained in *Appearance of solution* to 10 mL with water and proceed as described in *Limit test for iron*. At most 0.002% (20 ppm).

Magnesium and alkaline earth metals (5.3.2.9). To use 10 g of the sample mL and proceed as described in *Limit test for magnesium and alkaline earth metals*. The volume of 0.01 M SV disodium edetate used does not exceed 5 mL. At most 0.02% (200 ppm) calculated as calcium.

Loss on drying (5.2.9). To determine in 1 g of the sample, in an oven at 100 °C - 105 °C for 3 hours. At most 3.0%.

DOSAGE

To exactly transfer 2 g of the sample to 100 mL volumetric flask, dissolve in water, and top up the volume with the same solvent.

To add 50 mL of water, and 5 mL of 20% nitric acid (w/v), 25 mL of 0.1 M SV silver nitrate, and 2 mL of dibutylphthalate to 10 mL of this solution. To make it homogeneous. To titrate with 0.1 M SV ammonium thiocyanate, by using 2 mL of ammonium ferric sulfate SR as an indicator and vigorously stirring to change color. To correct the volume by subtracting the volume of 0.1 M silver nitrate SV spent in the *Chlorides* test, in *Purity tests*. Each mL of 0.1 M SV silver nitrate is equivalent to 10.289 mg of NaBr.

PACKAGING AND STORAGE

In tightly closed containers.

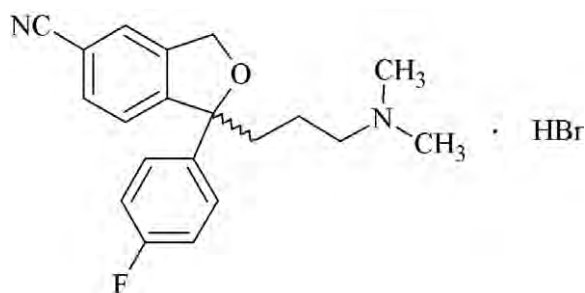
LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Sedative, hypnotic, anticonvulsant.

HYDROBROMIDE, CITALOPRAM Citaloprami hydrobromidum



$C_{20}H_{21}FN_2O \cdot HBr$; 405.30
citalopram hydrobromide; 02162
Hydrobromide 1 - [3 - (dimethylamino) propyl]
- 1 - (4-fluorophenyl) - 1,3-dihydro-5-isobenzofuran
carbonitrile (1:1)
[59729-32-7]

It contains at least 98.0% and at most 101.0% of $C_{20}H_{21}FN_2O \cdot HBr$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white or almost white powder.

Solubility. Slightly soluble in water, soluble in chloroform, methanol, and ethanol, practically insoluble in ethyl ether.

Physical and chemical constants.

Melting range (5.2.2): 182 °C to 189 °C.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, desiccated in a vacuum desiccator to constant weight, and dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the citalopram hydrobromide SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14), from 200 nm to 400 nm, 0.001% solution (w/v) in 0.1 M hydrochloric acid, has maxima at 239 nm, similar to that observed in the citalopram hydrobromide SQR spectrum of a similar solution.

C. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF_{254} silica gel as support and a mixture of water, 1-butanol, and acetic acid (15:12:3) as mobile phase.

D. To prepare the mobile phase with 24 hours in advance and discard the organic layer. To apply separately to the plate 5 µL of each of the solutions freshly prepared, as described below.

Solution (1): 1 mg/mL solution of the sample in water.

Solution (2): 1 mg/mL citalopram hydrobromide SQR solution in water.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

E. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method **B**, corresponds to that of main peak of *Standard solution*.

F. It responds to the reactions of the bromide ion (5.3.1.1).

PURITY TESTS

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate under vacuum at room temperature to constant weight. At most 0.5 %.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To exactly transfer 10 mg of the sample to a 100 mL volumetric flask, dissolve in 0.1 M hydrochloric acid, and top up with the same solvent. To successively dilute with the same solvent to a 0.001% concentration (w/v). To prepare a standard solution at the same concentration by using the same solvent. To measure the absorbance of the resulting solutions at 239 nm, by using 0.1 M hydrochloric acid to adjust zero. To calculate

the $C_{20}H_{21}FN_2O \cdot HBr$ concentration of the sample from the readings obtained.

B. To proceed as described in High performance liquid chromatography (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 239 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μ m), kept at room temperature; flow of *Mobile phase* of 1.0 mL/min

Mobile phase: mixture of 0.3% triethylamine (v/v), adjust with phosphoric acid (pH 6.6) and acetonitrile (55:45).

Sample solution: to transfer amount equal to 10 mg of the sample to a 50 mL volumetric flask, and top up with water. To transfer 5 mL to 25 mL volumetric flask and top up with the same solvent, thus obtaining 40 μ g/mL solution.

Standard solution: to transfer amount equal to 10 mg of citalopram hydrobromide SQR to a 50 mL volumetric flask, and top up with water. To transfer 5 mL to 25 mL volumetric flask and top up with the same solvent, thus obtaining 40 μ g/mL solution.

Procedure: to separately inject 20 μ L of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_{20}H_{21}FN_2O \cdot HBr$ in the sample, from the responses obtained for the *Sample and Standard solutions*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light, at room temperature.

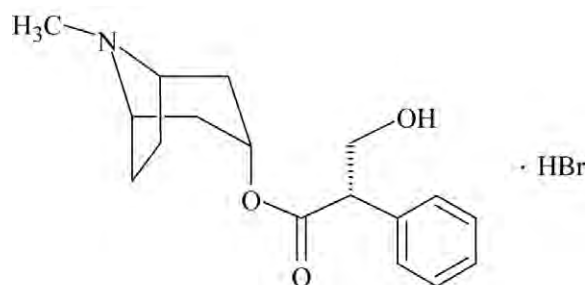
LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antidepressant.

HYDROBROMIDE, HYOSCYAMINE Hyoscyamini hydrobromidum



$NO_3 \cdot HBr$; 370.28

hyoscyamine hydrobromide; 04727

Ester hydrobromide (αS)- (3-endo)-8-methyl-8-azabicyclo [3.2.1]

octahydro-3-yl acid ester α -(hydroxymethyl)-benzeneacetic

[306-03-6]

It contains at least 98.5% and no more than 100.5% of $C_{17}H_{23}NO_3 \cdot HBr$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white, and odorless powder with bitter taste. Air deliquescent and light sensitive.

Solubility. Very soluble in water, ethanol, and chloroform. Very slightly soluble in ethyl ether.

IDENTIFICATION

A. To put 10 mg of the sample in a porcelain capsule, add 5 drops of nitric acid, and heat in a water bath to full evaporation. To add a few drops of 0.5 M ethanolic potassium hydroxide to the residue, after cooling. The violet color develops.

B. To add gold chloride SR drop by drop to 1 mL of 5% aqueous solution (w/v) of the sample, until it precipitates. To add a small amount of dilute hydrochloric acid and heat to dissolve the precipitate. After cooling, small, reddish brown glossy blades must be formed. They may be followed by needles of the same color (atropine vs. scopolamine).

C. To add silver nitrate SR to 5% aqueous solution (w/v) of the sample. A white-yellowish precipitate is formed. It is insoluble in nitric acid.

PURITY TESTS

Other alkaloids. To dissolve 250 mg of the sample in 1 mL of 0.1 M hydrochloric acid, dilute with water to 15 mL, and divide into two portions. To add a few drops of platinum chloride SR to a portion of 5 mL of the solution. Precipitate should not be formed immediately. To add 2 mL of

ammonia SR to the other portion of 5 mL of the solution. The mixture may develop slight opalescence, but should present no immediate precipitation or turbidity.

Loss on drying (5.2.9). To desiccate in an oven at 105 °C for 2 hours. At most 1.0%.

Sulfated ashes (5.2.10). At most 0.2 %. **DOSAGE**

To dissolve about accurately weighed 700 mg of the sample in mixture of 50 mL of glacial acetic acid and 10 mL of mercury acetate SR. To add a drop of methylrosanilinium chloride SI and titrate with 0.1 M SV perchloric acid to obtain blue-green color. To perform sample blank correction and adjust it as required. Each mL of 0.1 M SV perchloric acid is equal to 37.028 mg of $C_{17}H_{23}NO_3 \cdot HBr$.

PACKAGING AND STORAGE

In airtight, opaque containers.

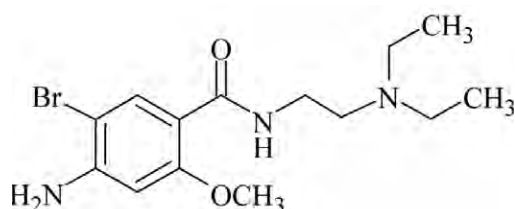
LABELLING

To comply with the legislation in force.

CATEGORY

Anticholinergic.

BROMOPRIDE Bromopridum



$C_{14}H_{22}BrN_3O_2$; 344.25
bromopride; 01471

4-Amino-5-bromine-*N*-[2 - (diethylamino) ethyl]-2-methoxybenzamide
[4093-35-0]

It contains at least 99.0% and at most 102.0% of $C_{14}H_{22}BrN_3O_2$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white to ivory white powder; it is practically odorless.

Solubility. It is practically insoluble in water. Slightly soluble in acetone, ethanol, and ethyl ether. Slightly soluble in acetonitrile. Soluble in dilute mineral acid solutions.

Physical and chemical constants.

Melting range (5.2.2): 151 °C to 155 °C.

IDENTIFICATION

The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the bromopride SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14), ranging from 230 nm to 350 nm, of sample solution obtained in Dosage method B, exhibits absorption maximum in 274 nm, similar to those observed in the bromopride SQR spectrum.

PURITY TESTS

Appearance of solution. To dissolve 1 g of the sample in 10 mL of 0.5 M hydrochloric acid. The solution is clear (5.2.25).

Heavy metals (5.3.2.3). To use Method III. At most 0.003% (30 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample, in an oven at 105 °C for 4 hours. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Titrimetric methods in non-aqueous medium* (5.3.3.5). To accurately weigh about 0.17 g of the sample, transfer to a 150 mL erlenmeyer flask, and dissolve in 80 mL of glacial acetic acid. To add 2 mL of acetic anhydride. To titrate with 0.02 M SV perchloric acid, to potentiometrically determine the endpoint. Each mL of 0.1 M perchloric acid VS is equal to 34.425 mg of $C_{14}H_{22}BrN_3O_2$.

B. To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To accurately weigh about 0.1 g of the sample to a 100 mL volumetric flask. To add 50 mL of 0.1 M hydrochloric acid, leave it in ultrasound for 10 minutes, and top up with the same solvent. To successively dilute in 0.1 M hydrochloric acid to the 0.001% concentration (w/v). To prepare a standard solution at the same concentration by using the same solvent. To measure the absorbance of the solutions at 274 nm, by using 0.1 M hydrochloric acid to adjust zero. To calculate the $C_{14}H_{22}BrN_3O_2$ concentration of the sample from the readings obtained.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antiemetic.

BROMOPRIDE, TABLETS

It contains at least 90.0 % and at most 110 % of the amount stated of $C_{14}H_{22}BrN_3O_2$.

IDENTIFICATION

The ultraviolet absorption spectrum (5.2.14) ranging from 200 nm to 400 nm, of sample solution obtained in *Dosage*, exhibits maxima in 274 nm, similar to those observed in the standard solution spectrum.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

Procedure for content uniformity. to crush each tablet to fine powder, quantitatively transfer to a 100 mL volumetric flask, add 50 mL of 0.1 M hydrochloric acid, and leave it in ultrasound for 15 minutes. To successively dilute in 0.1 M hydrochloric acid to the 0.001% concentration (w/v) and proceed as described in *Dosage*.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 500 mL

Equipment: baskets, 50 rpm

Time: 30 minutes

Procedure: After testing, remove aliquot from the dissolution medium and filter it. To measure the absorbance of the solutions at 274 nm (5.2.14), by using the same solvent to adjust zero. To calculate the amount of $C_{14}H_{22}BrN_3O_2$ dissolved in the medium, by comparing the readings obtained with 0.002 % bromopride SQR solution (w/v) prepared in the same solvent.

Tolerance: not less than 80% (Q) of the stated amount of $C_{14}H_{22}BrN_3O_2$ dissolves within 30 minutes.

DOSAGE

To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To weigh and spray 20 tablets. To transfer amount of powder equivalent to approximately 10 mg of bromopride to a 100 mL volumetric flask, add 50 mL of 0.1 M hydrochloric acid, and leave it in ultrasound for 10 minutes. To top up to volume with 0.1 M hydrochloric acid, make it homogeneous, and filter it. To successively dilute in 0.1 M hydrochloric acid to the 0.001% concentration (w/v). To prepare a standard solution at the same concentration by using the same solvent. To measure the absorbance of the solutions at 274 nm, by using 0.1 M hydrochloric acid to adjust zero. To calculate the amount of $C_{14}H_{22}BrN_3O_2$ in the tablets, from the readings obtained.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

BROMOPRIDE, ORAL SOLUTION

It contains at least 90.0 % and at most 110 % of the amount stated of $C_{14}H_{22}BrN_3O_2$.

IDENTIFICATION

The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

pH (5.2.19). 2.8 to 3.7.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 310 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), flow of *Mobile phase* of 1 mL/min.

Buffer pH 7.0: to dissolve 1.361 g of monobasic potassium phosphate in 900 mL of water, add 2 mL of triethylamine,

adjust the pH to 7.0 ± 0.05 with phosphoric acid, and dilute to 1000 mL with water.

Mobile phase: mixture of *Buffer pH 7.0* and acetonitrile (60:40).

Diluent: mixture of water and acetonitrile (3:2).

Sample solution: to transfer volume of the sample equal to 8 mg of bromopride to a 100 mL volumetric flask, and top up with *Diluent*.

Standard solution: To transfer 40 g of bromopride SQR to 50 mL volumetric flask, dissolve in acetonitrile, and top up the volume with the same solvent. To transfer 1 mL to 10 mL volumetric flask and top up with *Diluent*, thus obtaining 80 mg/mL solution.

To inject replicates of 10 μ L of the *Standard solution*. The column efficiency is greater than 3500 theoretical plates/meter. The relative standard deviation for the replicate areas for the peaks recorded is not greater than 2.0%.

Procedure: to separately inject 10 μ L of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks.

To calculate the amount of $C_{14}H_{22}BrN_3O_2$ in oral solution from the responses from the *Standard solution* and the *Sample solution*.

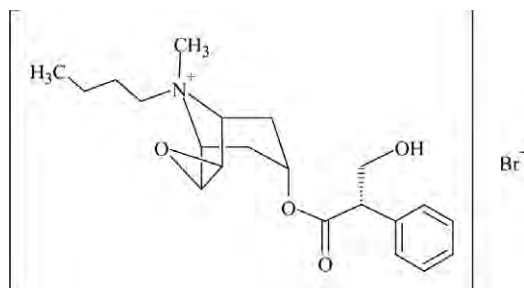
PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

BUTYLBROMIDE, SCOPOLAMINE Scopolamini butylbromidum



$C_{21}H_{30}BrNO_4$; 440.37
scopolamine butylbromide; 03517
Bromide (1 α ,2 β ,4 β ,5 α ,7 β)-9-butyl-7-[(2*S*)-3-hydroxy-1-oxo-2-phenylpropoxy]-9-methyl-3-oxa-9-azoniatriciclo [3.3.1.0^{2,4}] nonane
[149-64-4]

It contains at least 98.0% and at most 101.0% of $C_{21}H_{30}BrNO_4$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white or almost white powder.

Solubility. Freely soluble in water and methylene chloride, sparingly soluble in ethanol.

Physical and chemical constants

Melting range (5.2.2): 139 °C to 141 °C.

Specific rotation (5.2.8): -18° to -20°, in relation to the desiccated substance. To determine at 10% solution (w/v) in water.

IDENTIFICATION

Tests B., C., and D. may be omitted if the tests A. and E. are conducted.

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the scopolamine butylbromide SQR spectrum.

B. To dissolve by stirring 1 mg of the sample with 0.2 mL of nitric acid and evaporate to dryness in a water bath. To dissolve the residue in 2 mL of acetone and add 0.1 mL of 3% potassium hydroxide (w/v) in methanol. The violet color develops.

C. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method **B.** corresponds to that of main peak of *Standard solution*.

D. To dissolve by stirring 0.5 g of the sample with 5 mL of water and add 2 mL of sodium hydroxide SR. No precipitate should be formed.

E. It responds to the reactions of the bromide ion (5.3.1.1).

PURITY TESTS

pH (5.2.19). 5.5 to 6.5. To determine in 10% solution (w/v) in carbon dioxide-free water.

Related substances. To proceed as described in *Dosage* method **B.** To prepare the solutions as described below.

Solution (1): to accurately weigh about 0.1 mg of the sample and transfer to a 10 mL volumetric flask by using the *Mobile phase*. To top up the volume with the same solvent.

Solution (2): to accurately weigh about 10 mg of scopolamine hydrobromide SQR, transfer to a 100 mL volumetric

flask, and top up with the *Mobile phase*. To transfer 10 mL to 50 mL volumetric flask and top up with *Mobile phase*.

Solution (3): to transfer 5 mL of *Solution (2)* to 10 mL volumetric flask and top up with *Mobile phase*.

Resolution Solution: To add 10 µL of *Solution (1)* to 10 mL of *Solution (2)*.

To inject 20 µL of *Resolution solution*. The resolution between scopolamine and butylscopolamine should not be less than 3.0. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2.0%.

Procedure: to separately inject 20 µL of *Solution (1)*, *Solution (2)*, and *Solution (3)*. Then, record the chromatograms at least 6 times the retention time of the main peak and measure the areas of the peaks. The area under the peak corresponds to scopolamine eventually present in the chromatogram obtained with *Solution (1)* is not greater than the area under the main peak obtained with *Solution (3)* (0.1%). The area of other secondary peak obtained with *Solution (1)*, except the main peak's one and the peak corresponding to scopolamine, is not greater than the area under the main peak obtained with *Solution (2)* (0.2%). To disregard the solvent peaks and bromide ion that appear at the beginning of the chromatogram.

Loss on drying (5.2.9). To determine in 0.5 g of the sample. To desiccate in an oven at 105 °C until obtaining constant weight. At most 2.5%.

Sulfated ashes (5.2.10). To determine in 0.5 g of the sample. At most 0.1%.

DOSAGE

To employ one of the methods described below.

A. To accurately weigh about 0.4 g of the sample and dissolve in 50 mL of water. To titrate with 0.1 M SV silver nitrate, to potentiometrically determine the endpoint. To use the silver indicator electrode and silver/silver chloride reference electrode. To perform sample blank correction and adjust it as required. Each mL of 0.1 M SV silver nitrate is equivalent to 44.037 mg of $C_{21}H_{30}BrNO_4$.

B. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 210 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 mm - 10 mm), kept at room temperature; flow of *Mobile phase* of 2 mL/minute.

Mobile phase: 2 g of sodium lauryl sulfate in a mixture of 0.001 M hydrochloric acid and methanol (37:68).

Sample solution: to dissolve accurately weighed amount of the sample in 0.001 M hydrochloric acid to obtain a 0.4 mg/mL.

Standard solution: to dissolve accurately weighed amount of scopolamine butylbromide SQR in 0.001 M hydrochloric acid to obtain a 0.4 mg/mL solution.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_{21}H_{30}BrNO_4$ from the sample, from the responses obtained for the *Sample and standard solutions*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antispasmodic.

BUTYLBROMIDE, SCOPOLAMINE TABLETS

It contains at least 92.5 % and at most 107.5 % of the amount stated of $C_{21}H_{30}BrNO_4$. Tablets must be sugar-coated.

IDENTIFICATION

A. To weigh and pulverize the tablets. To stir amount of powder equivalent to 50 mg of scopolamine butylbromide with 20 mL of chloroform. To filter, evaporate to dryness, and dissolve the residue with 5 mL of acetonitrile. To evaporate to dryness at 50 °C under reduced pressure for 1 hour. A. The infrared absorption spectrum (5.2.14) of the residue, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the scopolamine butylbromide SQR spectrum.

B. To weigh and pulverize the tablets. To stir amount of powder equivalent to 50 mg of scopolamine butylbromide with 20 mL of chloroform. To filter, evaporate to dryness, resuspend the residue with 50 mL of water, and filter it. The ultraviolet absorption spectrum (5.2.14), ranging from 230 nm to 350 nm, of the filtered solution, exhibits maxima at 252 nm, 257 nm, and 264 nm.

C. To use 1 mg of the residue obtained in *Identification method A.* of this monograph, and proceed as described in *Identification method B.*, in the monograph *Scopolamine butylbromide*.

D. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

Procedure for content uniformity. To transfer each tablet to a 25 mL volumetric flask and add 15 mL of 0.001 M hydrochloric acid. To mechanically stir for 15 minutes to disintegrate the tablet. To leave it in ultrasound for 15 minutes, centrifuge it, and top up with the same solvent. If necessary, filter the supernatant. To proceed as described in *Dosage* method.

PURITY TESTS

Limit for scopolamine. To proceed as described in *Dosage* method **B.**, in the monograph *Scopolamine butylbromide*. To prepare the solutions as described below.

Solution (1): to weigh and pulverize 20 tablets. To use the amount of powder equivalent to about 0.1 g of scopolamine butylbromide. To add 10 mL of 0.001 M hydrochloric acid, leave it in ultrasound for 15 minutes, and centrifuge for 15 minutes. If necessary, filter the supernatant.

Solution (2): To accurately weigh about 10 mg of scopolamine hydrobromide SQR, transfer to a 100 mL volumetric flask, and top up with 0.001 M hydrochloric acid. To transfer 5 mL to 50 mL volumetric flask and top up with the same solvent.

Resolution solution: To add 10 µL of *Solution (1)* to 10 mL of *Solution (2)*.

To inject replicates of 20 µL of the *Resolution solution*. The resolution between scopolamine and butylscopolamine peaks should be greater than 5. The relative standard deviation for replicate areas of the peaks recorded is greater than 2.0%.

Procedure: to separately inject 20 µL of each solution, record the chromatograms, and measure the areas under the peaks. The area under the peak corresponding to scopolamine obtained with *Solution (1)* should not be greater than the area under the main peak obtained with *Solution (2)* (0.1%).

Related substances. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using 60F₂₅₄ silica gel as support and a mixture of formic acid, water, and methylene chloride (0.5:1.5:9:9) as mobile phase. To allow the mobile phase to migrate around 4 cm above the point of application of the chromatographic plate and separately 2 µL of each of the solutions freshly prepared as described below.

Solution (1): to weigh and pulverize 20 tablets. To use the amount of powder equivalent to about 20 mg of scopolamine butylbromide. To add 5 mL of 0.01 M hydrochloric acid, leave it in ultrasound for 15 minutes, and centrifuge for 15 minutes. If necessary, filter the supernatant.

Solution (2): to dilute 3 mL of *Solution (1)* to 100 mL with 0.01 M hydrochloric acid.

Solution (3): to dilute 1 mL of *Solution (1)* to 50 mL with 0.01 M hydrochloric acid.

Solution (4): to dilute 1 mL of *Solution (1)* to 400 mL with 0.01 M hydrochloric acid.

To develop the chromatogram. To remove the plate, dry it in an oven at 60 °C for 15 minutes, and nebulize with potassium iodide and bismuth subnitrate SR. To allow the plate to dry, nebulize it with 5% sodium nitrite (w/v), and examine immediately. The principal spot obtained in the chromatogram of the *Solution (1)* shows R_f of approximately 0.45. Any secondary spot obtained with *Solution (1)*, other than the principal spot, is not more intense than that one obtained with *Solution (2)* (3.0%) and not more than 2 spots are more intense than that spot obtained in the chromatogram with *Solution (4)* (0.25 %). Any secondary spot with R_f greater than the principal spot is not more intense than that obtained with *Solution (3)* (2%) and not more than one spot is more intense than that obtained with *Solution (4)* (0.25 %).

DOSAGE

To proceed as described in *Dosage* method **B.**, in the monograph *Scopolamine butylbromide*. To prepare the Sample solution as described below.

Sample solution: To weigh and spray 20 tablets. To transfer amount of powder equivalent to 40 mg of scopolamine butylbromide to a 100 mL volumetric flask, add 60 mL of 0.001 M hydrochloric acid. Then, leave it in ultrasound for 15 minutes, top up the volume with the same solvent, and centrifuge it for 15 minutes. If necessary, filter the supernatant.

Procedure: to separately inject 20 µL of *Sample and standard solutions*, record the chromatograms, and measure the average area under the peaks. To calculate the amount of C₂₁H₃₀BrNO₄ in the tablets, from the responses obtained for the *Sample and standard solutions*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

BUTYLBROMIDE, SCOPOLAMINE INJECTABLE SOLUTION

It contains at least 92.5 % and at most 107.5 % of the amount stated of $C_{21}H_{30}BrNO_4$. It can be prepared in water for injection or another suitable solvent.

IDENTIFICATION

A. To use volume of injectable solution equivalent to 0.1 g of scopolamine butylbromide. To evaporate to dryness and resuspend the residue with chloroform. To evaporate to dryness and resuspend the residue with 5 mL of acetonitrile. To evaporate to dryness at 50 °C under reduced pressure for 1 hour. The infrared absorption spectrum (5.2.14) of the residue, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the scopolamine butylbromide SQR spectrum.

B. The ultraviolet absorption spectrum (5.2.14) ranging from 230 nm to 350 nm, of *Sample solution* obtained in *Dosage*, exhibits maxima in 252 nm, 257 nm, and 264 nm.

C. To use 1 mg of the residue obtained in *Identification* method **A.** of this monograph, and proceed as described in

Identification method **B.**, in the monograph *Scopolamine butylbromide*.

D. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

pH (5.2.19). 3.7 to 5.5.

Determination of volume (5.1.2). It complies with the test.

PURITY TESTS

Limit for scopolamine. To proceed as described in *Dosage* method **B.**, in the monograph *Scopolamine butylbromide*.

To prepare the solutions as described below.

Solution (1): to dilute, if necessary, volume of the injectable solution in 0.001 M hydrochloric acid to prepare a 10 mg/mL solution.

Solution (2): to accurately weigh about 10 mg of scopolamine hydrobromide SQR, transfer to a 100 mL volumetric flask, and top up with 0.001 M hydrochloric acid. To transfer 5 mL of this solution to 50 mL volumetric flask and top up with the same solvent.

Resolution solution: To add 10 µL of *Solution (1)* to 10 mL of *Solution (2)*.

To inject replicates of 20 µL of the *Resolution solution*. The resolution between scopolamine and butylscopolamine peaks should be greater than 5. The relative standard deviation for replicate areas of the peaks recorded is greater than 2.0%.

Procedure: to separately inject 20 µL of each solution, record the chromatograms, and measure the areas under the peaks. The area under the peak corresponding to scopolamine obtained with *Solution (1)* should not be greater than the area under the main peak obtained with *Solution (2)* (0.1%).

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using 60F₂₅₄ silica gel as support and a mixture of formic acid, water, and methylene chloride (0.5:1.5:9:9) as mobile phase. To allow the mobile phase to migrate around 4 cm above the point of application of the chromatographic plate and separately 2 µL of each of the solutions freshly prepared as described below.

Solution (1): to dilute, if necessary, volume of the sample to prepare 20 mg/mL scopolamine butylbromide solution in 0.01 M hydrochloric acid.

Solution (2): to dilute 3 mL of *Solution (1)* to 100 mL with 0.01 M hydrochloric acid.

Solution (3): to dilute 1 mL of *Solution (1)* to 50 mL with 0.01 M hydrochloric acid.

Solution (4): to dilute 1 mL of *Solution (1)* to 400 mL with 0.01 M hydrochloric acid.

To develop the chromatogram. To remove the plate, dry it in [sic] at 60 °C for 15 minutes, and nebulize with potassium iodide and bismuth subnitrate SR. To allow the plate to dry, nebulize it with 5% sodium nitrite (w/v), and examine immediately. The principal spot obtained in the chromatogram of the *Solution (1)* shows R_f of approximately 0.45. Any secondary spot obtained in the chromatogram with *Solution (1)* with R_f lower than one of the principal spot is not more intense than that one obtained with *Solution (2)* (3%) and not more than 2 spots are more intense than that spot obtained in the *Solution (4)* (0.25 %). Any secondary spot with R_f greater than the principal spot is not more intense than spot obtained with *Solution (3)* (2%) and not more than one spot is more intense than that obtained with *Solution (4)* (0.25 %).

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

Bacterial endotoxins (5.5.2.2). At most 555 UE/mg of scopolamine butylbromide.

DOSAGE

To proceed as described in *Dosage* method **B.**, in the monograph *Scopolamine butylbromide*. To prepare the *Sample solution* as described below.

b

Sample solution: to transfer volume of injectable solution equivalent to 40 mg of scopolamine butylbromide to 100 mL volumetric flask, and top up with 0.001 M hydrochloric acid.

Procedure: to separately inject 20 µL of *Sample and standard solutions*, record the chromatograms, and measure the average area under the peaks. To calculate the content of $C_{21}H_{30}BrNO_4$ in the injectable solution, from the responses obtained for the *Sample and standard solutions*.

PACKAGING AND STORAGE

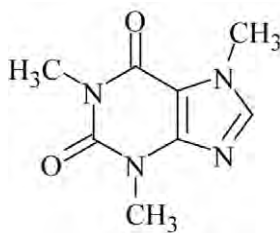
In type I glass containers, protected from light.

LABELLING

To comply with the legislation in force.

CAFFEINE

Coffeinum



$C_8H_{10}N_4O_2$; 194.19

caffeine; 01642

3,7-dihydro-1,3,7-trimethyl-1*H*-purine-2,6-dione
[58-08-2]

It contains at least 98.5% and at most 101.0% of $C_8H_{10}N_4O_2$ in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White powder, or white acicular and bright crystals. It sublimates easily under heat. Odorless and bitter tasting. The hydrate form is efflorescent in air.

Solubility. It is easily soluble in water and ethanol, freely soluble in chloroform, sparingly soluble in ethyl ether.

Physical and chemical constants.

Melting range (5.2.2): 235 °C to 239 °C.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the caffeine SQR spectrum similarly prepared.

B. To dissolve about 5 mg of sample in 1 mL of hydrochloric acid in watch glass or porcelain capsule, add 50 mg of potassium chlorate, and evaporate in a water bath to dryness. To invert the watch glass over another containing a small amount of 6 *M* ammonium hydroxide. The residue becomes purple, but such color will disappear by adding *M* sodium hydroxide.

C. To add 0.1 mL of iodine SR to 2 mL of a saturated aqueous solution of the sample. The solution is clear. To add 0.1 mL of dilute hydrochloric acid. Brown precipitate is formed. This precipitate dissolves after neutralization with dilute sodium hydroxide.

PURITY TESTS

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel as support and a mixture of ammonia, acetone, chloroform,

and 1-butanol (10:30:30:40) as mobile phase. To apply separately to the plate 10 µl of each of the solutions as described below.

Solution (1): to dissolve 0.2 g of the sample in a mixture of methanol and chloroform (4:6) and top up to 10 mL volumetric flask.

Solution (2): to dissolve 0.5 g of the *Solution (1)* to 100 mL volumetric flask and top up with a mixture of methanol and chloroform (4:6).

To develop the chromatogram in the path length of 15 cm. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). In case of other spots, in addition to the principal one, in the chromatogram obtained with *Solution (1)*, no one is more intense than the spot in the chromatogram obtained with *Solution (2)* (0.5%).

Other alkaloids. To add drops of mercury potassium iodide SR to 5 mL of a 0.02% solution. It should not precipitate.

Arsenic (5.3.2.5). To use *Method I*. At most 0.0003% (3 ppm).

Lead (5.3.2.12). At most 0.001% (10 ppm).

Heavy Metals (5.3.2.3). To mix 2 g of the sample with 5 mL of 0.1 *M* hydrochloric acid and 45 mL of water and heat to dissolve. After cooling down, use 25 mL of this solution used for the heavy metals test. To proceed as described in *Method I*. At most 0.002% (20 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 115 °C to constant weight, or by Karl Fischer method. At most 0.5% for caffeine anhydrous. At most 8.5% for hydrated caffeine.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To proceed as described in *Titrations in non-aqueous medium* (5.3.3.5). To dissolve by heating 0.4 mg of the sample accurately weighed in 40 mL of acetic anhydride. To cool down and add 80 mL of benzene. To titrate with 0.1 *M* SV perchloric acid, to potentiometrically determine the endpoint. Each mL of 0.1 *M* SV perchloric acid is equal to 19.47 mg of $C_8H_{10}N_4O_2$.

PACKAGING AND STORAGE

In airtight containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Central stimulant.

CALAMINE

ZnO; 81,41
calamine; 01646
Calamine
[8011-96-9]

Calamine is a zinc oxide with a small proportion of iron oxide. It contains, after ignition, at least 98.0% and not more than 100.5% of zinc oxide (ZnO).

DESCRIPTION

Physical characteristics. Amorphous powder, not palpable, pinkish or reddish brown, depending on the color of the variety and amount of ferric oxide, as well as the process by which it is combined.

Solubility. It is practically insoluble in water. It dissolves with effervescence in hydrochloric acid.

IDENTIFICATION

A. To dissolve 1 g of the sample in the mixture of 10 mL of 3 M hydrochloric acid and filter it. The filtrate responds to the reactions of zinc (5.3.1.1).

B. To dissolve 1 g of the sample in 10 mL of 3 M hydrochloric acid, heat to boiling, and filter it. The filtrate becomes reddish after adding ammonium thiocyanate SR.

PURITY TESTS

Calcium. To digest 1 g of the sample in 25 mL of 3 M hydrochloric acid for 30 minutes. To filter it to remove insoluble ferric oxide, add 6 M sodium hydroxide to the filtrate until the first precipitate formed is redissolved. Then, add extra 5 mL of 6 M sodium hydroxide. To add 2 mL of 3.5% ammonium oxalate (w/v) to 10 mL of this solution. No more than a slight turbidity is produced.

Calcium or magnesium. To add 2 mL of 12% heptahydrate dibasic sodium phosphate (w/v) to the other portion of 10 mL prepared for test for *Calcium*. No more than a slight turbidity is produced.

Lead. For 1 g of the sample, add 15 mL of water, stir, and add 3 mL of glacial acetic acid. To heat it in a water bath to dissolve. To filter it and add 5 drops of potassium chromate SR. No turbidity is formed.

Substances insoluble in acid. To weigh 2 g and add 50 mL of 3 M hydrochloric acid. In case of remaining insoluble residue, collect it and put into a tared filter, and wash with water and dry at 105 °C for 1 hour. After that, cool down and weigh it. The weight of the residue does not exceed 40 mg (2.0%).

Alkaline substances. To digest 1 g with 20 mL of water in a water bath for 15 minutes, filter it, and add 2 drops of phe-

nolphthalein SI. If a red color is produced, no more than 0.2 mL of 0.05 M sulfuric acid is required to remove it.

Arsenic (5.3.2.5). To use *Method 1*. To use 3.5 M sulfuric acid and 40% stannous chloride solution (w/v) in hydrochloric acid. The limit is 0.0008% (8 ppm).

Sulfated ashes (5.2.10). To weigh about 2 g of the sample and calcine at 500 °C to constant weight. At most 2.0%.

BIOLOGICAL SAFETY TEST

Survey and identification of pathogens (5.5.3.1.3).

It complies with the test to detect the absence of *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

DOSAGE

To exactly calcine about 1.5 g of calamine. To this freshly calcined sample, digest with 50 mL of 0.5 M sulfuric acid SV by applying gentle heat to avoid solubilization. To filter the mixture and wash the residue inside the filter with hot water. The last washing must be neutral to litmus paper. To add 2.5 g of ammonium chloride to the combined filtrate. Then, cool it down, add methyl orange, and titrate with sodium hydroxide M SV. Each mL of 0.5 M SV sulfuric acid is equivalent to 40.69 mg of zinc oxide.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Astringent, antipruritic.

CALENDULA Calendulae flos

Calendula officinalis L. - ASTERACEAE

The vegetable drug consists of whole or ground ligulate flowers, accompanied by sparse tubular flowers separate from receptacle and involucre dried bracts. It must contain at least 0.4% of total flavonoid, calculated as hyperoside (C₂₁H₂₀O₁₂, 464.4) in relation to the desiccated material.

CHARACTERISTICS

Organoleptic characteristics. The drug has faint odor and slightly bitter taste.

MACROSCOPIC DESCRIPTION

Flowers arranged in clusters from 3 cm to 7 cm diameter, covered by two series of bracts. The peripheral flowers are ligulate, pistillate, ranging from 1.5 cm to 3.0 cm long and 0.5 cm to 0.7 cm wide, at the middle portion of ligule. Yellow or orange corollas, with tridentate limbo, with 4 or 5 ribs, and short tube covered by trichomes, occasionally accompanied by a filamentous stylet and a stigma bifid. The flowers in the center are slim, tubular, small, short, approximately 0.5 cm long, hermaphrodite, yellow or orange, rarely almost reddish, corolla 5-dentate; anthers sagittate, and undivided stylet. No pappus.

MICROSCOPIC DESCRIPTION

In front view, the adaxial face of the epidermis of the ligulate corolla shows rectangular, elongated cells with slightly sinuous contour and striated cuticle, and has no stomata. In the apical region of this same face, cells are smaller and less regularly arranged; in the basal end of the ligule there is a layer of cells with outer thickened-wall, with prisms and small bunch of crystals. The lower face of the epidermis is similar to that of adaxial, by differing from it by presenting a few anomocytic stomata, which are relatively large in the apical region of the ligule, when compared with other epidermal cells of this portion. In the basal region of the lower face, there are long tector trichomes, multicellular, biseriate, conical, rounded apex, and multicellular glandular trichomes, uniseriate pedicel, with 3-5 cells, or biseriate, with 3-4 cells in each row, both with oval head, multicellular, generally biseriate. The parenchyma cells underlying the ligulate corolla have numerous yellow-orange to light yellow oil droplets. The parenchyma of the ligule is traversed lengthwise by 4 or 5 vascular bundles, with vessel elements presenting ringed and helical thickening. There are 5 forked vascular bundles below the weld zone of the petals. Such bundles are close to the parenchyma cells of the tubular corolla. When involucre bracts are present, there are long tector trichomes, multicellular, biseriate, conical, rounded apex, and tector trichomes with 4 or 5 cells, uniseriate, of which the apical cell is much longer than the others and often folded and flattened, as well as rarer glandular trichomes, multicellular, biseriate pedicel, conical, basal cell longer and more irregular than the others.

In the anther, we observe the endothecium, composed of cells slightly elongated, in front view, showing typical thickening restricted to the transverse walls (anticlines). There are small orange sclereids associated with endothecium, with slightly thickened walls and numerous pits. The pollen grains are echinate, three colpi, measuring around 45 µm in diameter. The epidermal cells of the stigmas are polygonal to slightly elongated in front view and show short papillae, bulbous, while those of the ovaries are small, polygonal, in front view, containing brown pigments. In the ovaries there are glandular trichomes equal to the ligulate corollas. The achenes, when present, are navicular shaped, with toothed ornamentation on the dorsal surface.

MICROSCOPIC DESCRIPTION OF POWDER

The powder must meet all the requirements for the species, unless the macroscopic characters. Characteristics: yellowish-brown color; presence of tubes of ligulate flowers; parts of ligules; fragments of ligule epidermis with striated cuticle; fragments of subepidermal parenchyma with oil droplets; fragments of epidermis with large anomocytic stomata; corolla basal cells containing crystals; fragments of vascular tissue, corollas of tubular flowers; anthers of tubular flowers; fragments of anthers most often with portions of conductive bundles; echinate, three colpi pollen grains; fragments of epidermal cells of the stigma with bulbous papillae; fragments in the ovaries' walls with pigmented cells: achenes and trichomes same as described above.

IDENTIFICATION

To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel, with 250 µm thickness as support and a mixture of anhydrous formic acid, water, and ethyl acetate (10:10:80) as mobile phase. To separately apply to the plate, as band, 20 µl of *Solution (1)* and 10 µL of *Solution (2)*, as described below.

Solution (1): to boil under reflux 1 g of the pulverized drug with 10 mL of methanol for 10 minutes, and filter it.

Solution (2): to dissolve 2.5 mg of rutin, 1 mg of caffeic acid, and 1 mg of chlorogenic acid in methanol. Then, top up the volume to 10 mL with the same solvent.

To develop the chromatogram. To remove the plate, dry it in an oven at 100 °C - 105 °C, and nebulized it if still warm with 1% aminoethanol diphenyl borate solution (w/v) in methanol, followed by a 5% macrogol 400 solution (w/v) in methanol. Plate must air dry for 30 minutes. To examine under ultraviolet light (365 nm). The chromatogram obtained with *Solution (2)* must show in the lower third of the plate 2 fluorescent spots (brownish-yellow (rutin) and light blue color (chlorogenic acid)), and the upper third a light blue fluorescent spot

(caffeic acid). The chromatogram of the *Solution (1)* must show yellowish-brown fluorescent spot corresponding in position to the spot obtained with rutin in the chromatogram of *Solution (2)*; yellowish green and light blue fluorescent spots corresponding in position to the spot obtained with chlorogenic acid in chromatogram of the *Solution (2)*; yellowish green fluorescent spots and light blue corresponding in position to the spot obtained with caffeic acid in the chromatogram of *Solution (2)*. Other spots may be present.

PURITY TESTS

Foreign matter (5.4.2.2). At most 3.0%.

Water (5.4.2.3). At most 12.0%.

Total ashes (5.4.2.4). At most 10.0%.

DOSAGE

Total flavonoids

To proceed as described in Visible *absorption spectrophotometry* (5.2.14). To prepare the solutions as described below.

Stock solution: to accurately weigh about 0.4 g of pulverized drug (800 µm) and transfer to a 100 mL round bottom flask. To add 1 mL of 0.5% aqueous methenamine (w/v), 20 mL of acetone, and 2 mL of hydrochloric acid. To heat under reflux in water bath for 30 minutes. To filter the mixture in cotton to a 100 mL volumetric flask. Then, the residue of the drug and cotton should be back to the same round bottom flask. To add 20 mL of acetone. To place under reflux for 10 minutes. After cooling to room temperature, filter the solution to a 100 mL volumetric flask. To repeat the operation. Then, top up the volume of the flask with acetone.

In a separatory funnel, add 20 mL of this solution and 20 mL of distilled water, and after extracting with 15 mL of ethyl acetate, repeat 3 times with portions of 10 mL of ethyl acetate each time. To gather the ethyl acetate phases and wash them in a separatory funnel with 2 portions of 50 mL of distilled water. To transfer the ethyl acetate phase to 50 mL volumetric flask and top up with ethyl acetate.

Sample solution: To add 1 mL of 2% aluminum chloride solution (w/v), in 5% acetic acid solution (v/v), in methanol, to a 10 mL of stock solution. To dilute in 25 mL volumetric flask with 5% acetic acid solution (v/v) in methanol.

Blank solution: To add 10 mL of *Stock solution* to 25 mL volumetric flask and top up with 5% acetic acid solution (v/v) in methanol.

To measure right after 30 minutes the absorbance of *Sample solution* at 425 nm in a 1 cm cuvette, by using *the Blank solution* to adjust zero. To calculate the percentage of total flavonoid according to the equation:

$$\text{TFT} = \frac{A \times 1,25}{(m - \text{PD})}$$

where

A = absorbance of the *Sample solution* measured;

m = mass of the drug (g);

PD = loss on drying (% w/w).

The result is given in percent (w/w) of total flavonoids calculated as hyperoside (C₂₁H₂₀O₁₂). To calculate alternatively by considering A (1%, 1cm) = 500.

PACKAGING AND STORAGE

In tightly closed glass or metal container, protected from light and heat.

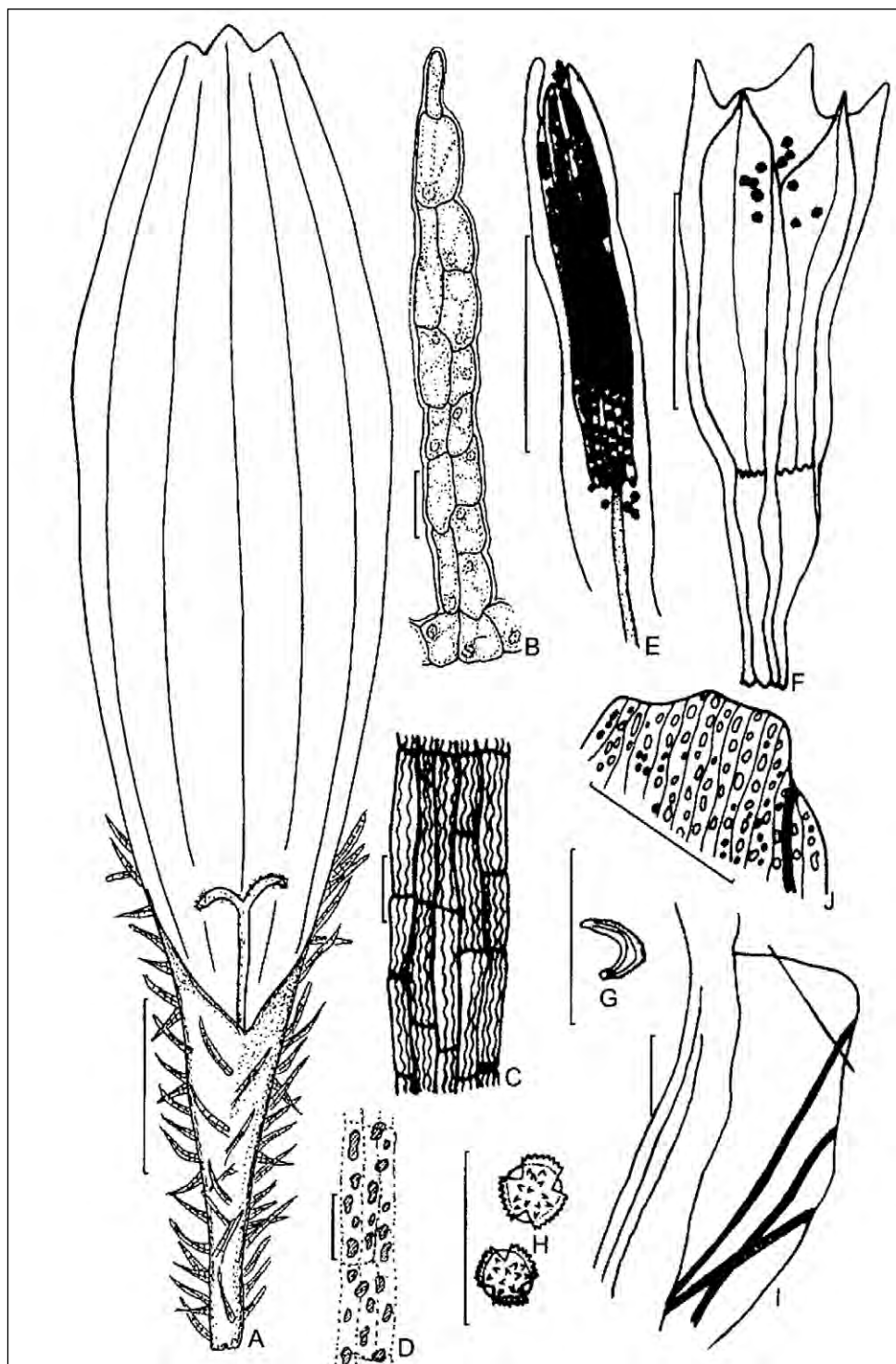


Figure 1 - Macroscopic and microscopic aspects in *Calendula officinalis* L.

Additional information of **Figure 1**. Scales correspond to 100 µm in A, B, C, D, G, H, and J ; 500 µm, in E, F, and I.

A — ligulate pistillate flower. B — multicellular biseriate trichomes of the corolla tube of the ligulate flower. C — ligule epidermis with striated cuticle. D - ligule parenchyma containing oil droplets. E — anthers of the tubular flower. F — corolla of tubular flower of the disc. G — fruit. H — three colpi pollen grains. I — fragment of ligule. J — detail of the parenchyma with oil droplets in the portion shown in I.

CINNAMON, CHINESE

Cinnamomi cortex

Cinnamomum cassia (L.) J. Presl - LAURACEAE

The vegetable drug corresponds to the dry bark containing at least 1.0% of volatile oil, comprising 70.0% - 90.0% of *trans*-cinnamaldehyde.

SCIENTIFIC SYNONYM

Cinnamomum aromaticum Nees.

CHARACTERISTICS

Organoleptic characteristics. It has a typical aromatic odor and its taste is less sweet, slightly mucilaginous, and less aromatic than cinnamon-of-ceylon.

MACROSCOPIC DESCRIPTION

The drug has the bark in fragments ranging from 3.0 cm to 7.0 cm long, 1.0 cm to 2.0 cm wide, and 1.0 mm to 2.0 mm thick. The outer surface corresponding to the remains of bark has brownish, brown, or grayish color, with spots or ribs and lenticels; texture is rugose, not rough. The inner surface corresponding to the region of the phloem has light brown to brown color and smooth and homogeneous texture.

The secondary phloem has, in addition to the sieve tube elements and companion cells, large amount of parenchyma, including seriate parenchyma and sparse libriform fibers usually isolated. The beams are predominantly heterocellular, and homocellular beams may occur with 2 cells in width, rare three, and 5 - 18 cells height, where it is usual to find phenolic idioblasts with high concentration of acicular crystals of calcium oxalate similar to raphids, in addition to prismatic crystals; the better observation of the crystals is performed in 1000x magnification. Idioblasts containing oils and mucilaginous cells are also observed. The phloem is not stratified; the sieve plates have a sieve single area, and they are straight or with various types of tilt. In the external portion of the phloem, the tissue dilation occurs through proliferation of beams and tangential growth of its cells. This expansion tissue does not accumulate phenolic compounds.

MICROSCOPIC DESCRIPTION OF POWDER

The powder meets all the requirements for the species, unless the macroscopic characters. Characteristics: brown color; isolated and/or grouped starch grains, simple or compound; fragments of parenchyma tissue containing starch grains and lipid droplets; large amount of separated crystals, acicular and/or prismatic of truncated apex; isolated and/or grouped stone cells, dissociated or within fragments of parenchymal tissue; rare columnar sclerids, isolated; fibers of 600 µm in length, on average, and 35 µm in width, on average, thick-walled, narrow lumen, isolated or associated with fragments of parenchyma.

MICROSCOPIC DESCRIPTION

The bark has primary tissues, mainly cortical tissue, and secondary tissue derived from vascular cambium and phellogen. The phellogen differs slightly, and its cells are tangentially elongated, vacuolated, and contains phenolic compounds. The felema in its inner portion has cells with suberized walls, and the external periclinal are thick. Externally the newly formed felema, we observe 2-3 layers of rhytidome in scaling. Lenticels are common. Internally to the phellogen prevails the parenchymal tissue, where we find stone cells, which may occur isolated or grouped; it may present uneven thickening. The middle portion of the primary cortical tissue consists of parenchyma with intercellular schizogenous spaces and accumulation of mucilaginous material in some of these spaces. Some oil idioblasts are observed, in addition to the large number of cells containing starch grains, mostly simple or compound. Phenolic idioblasts prevail in the cortical region. In the region of the inner cortical parenchyma, proximal to the secondary phloem, there is a continuous and irregular band of thick-walled stone cells with 2-10 layers of thick cell.

IDENTIFICATION

To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel with 250 µm thick as support and a mixture of methanol and toluene (10:90) as mobile phase. To separately apply to the plate, as band, 10 µl of *Solution (1)* and *Solution (2)* freshly prepared, as described below.

Solution (1): to dissolve 0.5 mL of the volatile oil being examined in acetone and dilute to 10 mL with the same solvent.

Solution (2): to dissolve 10 µL of eugenol and dilute to 10 mL with the same solvent.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (365 nm). A blue fluorescent spot is assigned to coumarin (Rf about 0.55). To nebulize the chromatoplate with anisaldehyde SR and put in an oven at 100 °C - 105 °C for 5 minutes. The chromatogram obtained with *Solution (2)* has a dark gray color zone (eugenol) with Rf about 0.5. The chromatogram obtained with *Solution (1)* presents violet zone, immediately above the standard spot of eugenol, Rf 0.6, corresponding to *trans*-cinnamaldehyde. Other thin areas may be present.

PURITY TESTS

Sulfated ashes (5.4.2.6). At most 5.0 %.

DOSAGE

Volatile oils

To proceed as described in *Determination of volatile oils in vegetable drugs* (5.4.2.7). To use 1000 mL flask containing

500 mL of water as distillation liquid and 0.5 mL of xylene. To use 50 g of crushed drug and distill at speed of 3 - 4 mL per minute for 4 hours. The volatile oil content should not be lower than 1.0%.

***trans*-cinnamaldehyde**

To proceed as described in *Gas chromatography* (5.2.17.5). To use gas chromatograph equipped with flame ionization detector, 30 m long capillary column and 0.25 mm inner diameter, filled with poly (dimethyl/diphenyl siloxane), with a film thickness of 0.25 µm; column temperature from 60 °C to 300 °C at 3 °C per minute (total 80 minutes); injector temperature at 220 °C, and the detector temperature at 250 °C; use helium at 80 kPa as carrier gas and flow of 1 mL/minute. To use mixture of nitrogen, synthetic air, and hydrogen (1:1:10) as auxiliary gases.

Sample solution: to dilute the volatile oil in diethyl ether (2:100).

Procedure: to inject 1 µL of *Sample solution* into the gas chromatograph, by using 1:50 flow division. The *trans*-cin-

namaldehyde and *cis*-cinnamaldehyde have linear retention times (Relative Retention Index) of 1270 and 1219, respectively. The relative concentrations are obtained by integrating manually or electronically. To calculate the Relative Retention Index (RRI), according to the equation:

$$IRR = 100 \times n + \frac{100 \times (tr_x - tr_z)}{(tr_{z+1} - tr_z)}$$

where

n = number of carbon atoms in the alkane with lower molecular weight,

tr_x = retention time of compound "x" (intermediate to tr_z and tr_{z+1});

tr_z = retention time of the alkane with 'n' carbons;

tr_{z+1} = retention time of the alkane with 'n+1' carbons;

PACKAGING AND STORAGE

In tightly closed container, protected from light and heat.

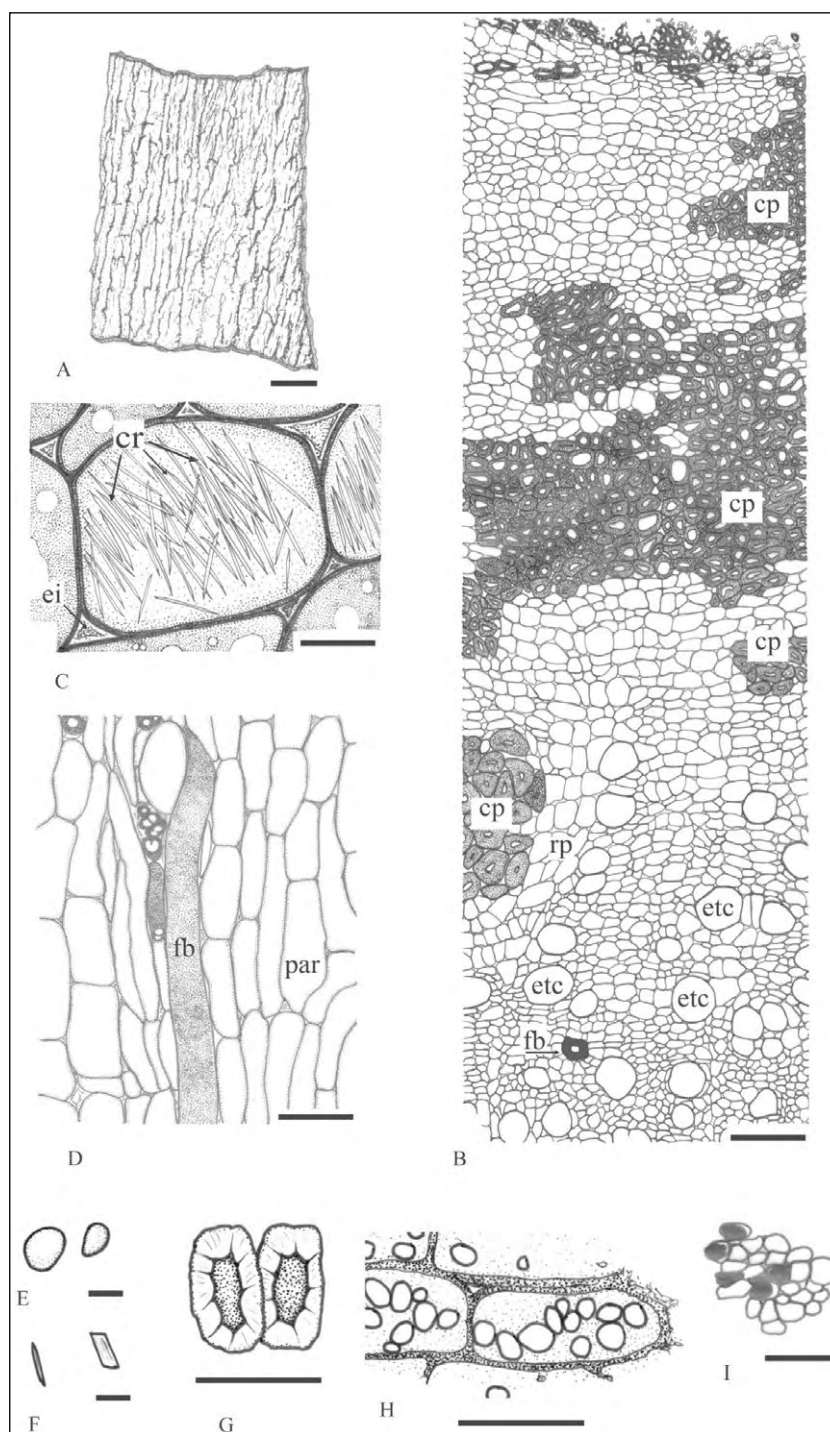


Figure 1 - Macroscopic and microscopic aspects in *Cinnamomum cassia* (L.) J. Presl

Additional information of **Figure 1**. Scales correspond to 5 mm, in **A**; 40 μ m, in **B**; 10 μ m, in **C**; 20 μ m, in **D**; 17.5 μ m, in **E**; 3.8 μ m, in **F**; 25.5 μ m, in **G**; and 37.5 μ m in **H** and **I**.

A - general appearance of the bark portion. **B** - histological appearance of the outer portion of the bark through cross section: stone cells (cp); parenchyma beam (rp); fiber (fb); sieve tube element (etc). **C** - detail of an idioblast containing acicular crystals of calcium oxalate: crystal (cr); intercellular space (ei). **D** - partial detail of phloem in longitudinal section: fiber (fb); parenchyma (par). **E**, **F**, **G**, and **H** - details of the powder. **E** - starch grains. **F** - truncated and acicular crystals. **G** - stone cells. **H** - parenchyma cells with starch grains. **I** - parenchyma cells with lipid inclusion.

CINNAMON, CEYLON

Cinnamomi cortex

Cinnamomum verum J. Presl - LAURACEAE

The drug consists of dry bark, free of periderm and external cortical parenchyma, from the main stem and its branches, containing at least 1.2% of volatile oil, and containing at least 60.0% of *trans*-cinnamaldehyde.

SCIENTIFIC SYNONYM

Cinnamomum zeylanicum Blume

CHARACTERISTICS

Organoleptic characteristics. The drug has a typical aroma of cinnamic aldehyde and spicy and sweet taste.

There are simple starch grains in all tissues of the bark, except in the conducting cells of the phloem, however, they prevail in the nonfunctional phloem and periderm. The secondary phloem is not stratified.

MICROSCOPIC DESCRIPTION OF POWDER

The powder meets all the requirements for the species, unless the macroscopic characters. Characteristics: brown color; abundant starch grains, isolated and/or grouped; isodiametric parenchyma cells containing abundant starch grains, as well as lipid droplets; few fragments of bark; large amount of prismatic and/or acicular crystals of calcium oxalate, truncated apexes, several fibers of 600 µm long, on average, and 35 µm wide, on average, with thick walls, narrow lumen, isolated or associated with fragments of parenchyma; columnar sclereids and abundant stone cells, isolated and/or grouped, separated or within parenchymal tissue fragments.

MACROSCOPIC DESCRIPTION

The dehydrated material has tissue covering itself, forming tubes with up to about 30.0 cm long and 0.2 mm to 0.4 mm thick. The exposed surface, related to the periderm, is smooth or slightly darker longitudinal ribs that may or not be parallel with corrugations that may be regular. The surface color is inhomogeneous brown. The color of the secondary phloem is dark brown to almost red wine color.

IDENTIFICATION

A. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using G silica gel chromatoplate with 250 µm thick as stationary phase and methylene chloride as mobile phase. To separately apply to the chromatoplate, as band, 10 µl of each of the solutions freshly prepared, as described below.

MICROSCOPIC DESCRIPTION

The region has peridermic stone cells in groups of large number of cells without forming a continuous sclerenchymatic band; the stone cells in this region have thick walls. Libriform fibers are observed, which are sparse and usually are isolated. Parenchyma cells are also observed in the peridermic region, which can simultaneously accumulate prismatic crystals of calcium oxalate, with phenolic compounds and lipid idioblasts. The beam mischaracterizes itself in the nonfunctional secondary phloem, where radial anticlinal divisions occur and their derivatives show slight tangential growth, forming dilations, whose cells resemble meristematic regions; not all the beams form dilations. In the functional secondary phloem, the beam has 1-2 cells in width and 6-14 cells in height, homocellular or heterocellular, where procumbent cells prevail. Libriform fibers occur sparse, and can be considered to be rare in the tissue. Sieve tube elements, companion cells, and parenchyma prevail in the functional phloem. Similarly to what occurs in other tissues, parenchyma cells can accumulate simultaneously or not crystals of calcium oxalate, prismatic, rhombohedral, small acicular crystals with acute or truncated apex, but not druses or raphides, and phenolic compounds, besides lipid idioblasts.

Solution (1): to use about 3 g of the powder and stir for 15 minutes with 15 mL of methylene chloride. To filter and evaporate almost to dryness in a water bath. To dissolve the residue in 1 mL of toluene.

Solution (2): to dissolved 10 µL of eugenol in 1 mL of toluene.

To develop the chromatogram. To remove the chromoplate and allow it to dry in a current of air for 5 minutes. Then, aerosolize the plate with vanillin sulfuric SR and put it in an oven at 100 °C - 105 °C for 5 minutes. The chromatogram of *Solution (1)* has grayish spot under visible light, located just below the height of the spot caused by the *Solution (2)*, whose color is brownish, corresponding to eugenol (*R_f* about 0.70).

B. To proceed the identification of eugenol by using an aliquot of 0.05 mL of volatile oil, obtained as described in Dosage, item A. To add 5 mL of ethanol and 0.05 mL of a 5% ferric chloride solution (w/v). The development of blue color characterizes the presence of phenolic compounds.

PURITY TESTS

Foreign matter (5.4.2.2). At most 2.0%.

Total ashes (5.4.2.4). At most 5.0%.

DOSAGE

Volatile oils

To proceed as described in *Determination of volatile oils in vegetable drugs (5.4.2.7)*. To use 1000 mL flask containing

500 mL of water as distillation liquid. To reduce the sample to coarse powder and immediately proceed to the determination of volatile oil from 50 g of drug in powder form. To distill for 4 hours.

***trans*-cinnamaldehyde**

To proceed as described in *Gas chromatography* (5.2.17.5). To use gas chromatograph equipped with flame ionization detector, 30 m long capillary chromatography column and 0.25 mm inner diameter, filled with poly (dimethyl/diphenyl siloxane), with a film thickness of 0.25 µm; column temperature from 60 °C to 300 °C at 3 °C per minute (total 80 minutes); injector temperature at 220 °C, and the detector temperature at 250 °C; use helium at 80 kPa as carrier gas and flow of 1 mL/minute. To use mixture of nitrogen, synthetic air, and hydrogen (1:1:10) as auxiliary gases.

Sample solution: to dilute the volatile oil in diethyl ether (2:100).

Procedure: to inject 1 µL of *Sample solution* into the gas chromatograph, by using 1:50 flow division. The cinnamic aldehyde has relative linear retention time of 1266 (Z) and 1214 (E). The content of cinnamic aldehyde is at least 60.0%. The relative concentrations are obtained by integrating manually or electronically. To calculate the Relative Retention Index (RRI), according to the equation:

$$\text{IRR} = 100 \times n + \frac{100 \times (\text{tr}_x - \text{tr}_z)}{(\text{tr}_{z+1} - \text{tr}_z)}$$

where

n = number of carbon atoms in the alkane with lower molecular weight,

tr_x = retention time of compound “x” (intermediate to tr_z and tr_{z+1});

tr_z = retention time of the alkane with ‘n’ carbons;

tr_{z+1} = retention time of the alkane with ‘n+1’ carbons;

PACKAGING AND STORAGE

In tightly closed container, protected from light and heat.

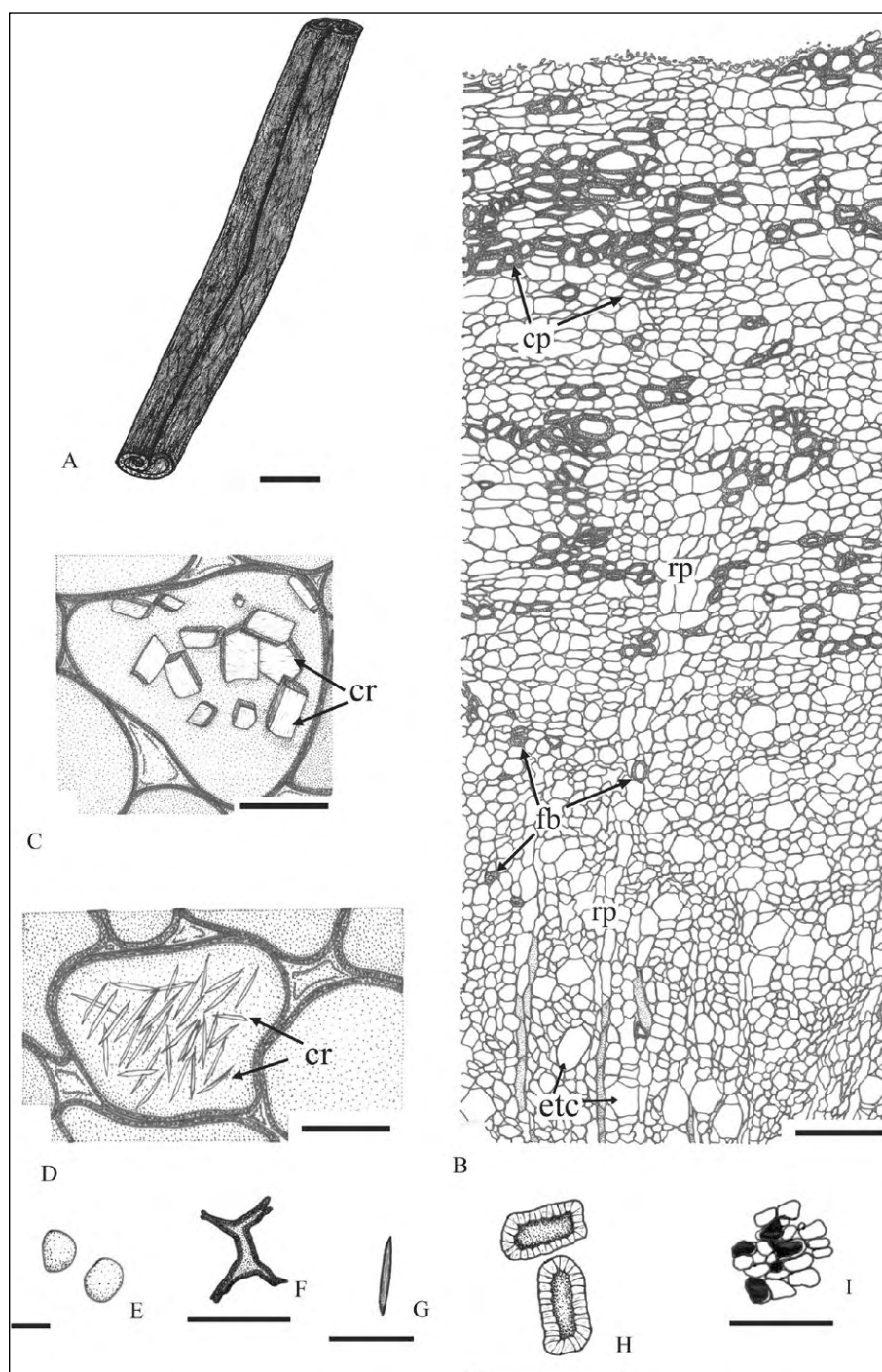


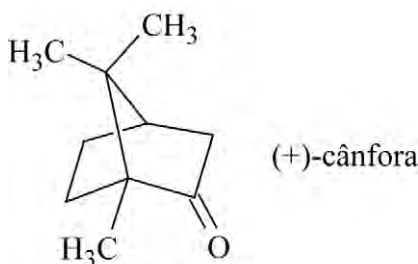
Figure 1 - Macroscopic and microscopic aspects in *Cinnamomum verum* J. Presl

Additional information of **Figure 1**. Scales correspond to 15 mm in **A**; 80 μm , in **B**; 10 μm , in **C** and **D**; 12.5 μm , in **E** and **H**; 37.5 μm , in **F** and **I**; 17.5 μm , in **G**; and 125.0 μm , in **H**.

A - general appearance of the portion of the bark; **B** - histological appearance of the bark through cross section: stone cells; sieve tube element (etc); fiber (fb); parenchyma beam (rp), **C** - idioblast containing prismatic crystals of calcium oxalate: crystal (cr), **D** - idioblast containing raphid crystals of calcium oxalate: crystal (cr), **E** - **H** - details of the powder, **E** - starch grains; **F** - branched columnar sclerids; **G** - acicular crystal, **H** - stone cells, **I** - parenchyma cells with lipid inclusion.

CAMPBOR

Camphora



$C_{10}H_{16}O$; 152.23
camphor; 01677
11,7,7-trimethylbicyclo [2.2.1] heptan-2-one
[76-22-2]

DESCRIPTION

Physical characteristics. Crystals, white or colorless, crystalline masses or granules. Typical penetrating odor, pungent aromatic flavor. It slowly becomes volatile at room temperature.

Solubility. Sparingly soluble in water; very soluble in ethanol, chloroform, and diethyl ether; easily soluble in carbon disulfide, hexane, and fixed and volatile oils.

Physical and chemical constants.

Melting range (5.2.2): 174 °C to 179 °C.

Specific rotation (5.2.8): +41° to +43° for natural camphor. Synthetic camphor is the racemic form optically inactive. To determine at 10% solution (w/v) in ethanol.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the standard camphor spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14) ranging from 200 nm to 400 nm, of 0.1% sample solution (w/v) prepared with ethanol exhibits maxima at 289 ± 1 nm.

C. To add a drop of 1.0% vanillin (w/v) and a drop of sulfuric acid to pulverized camphor (which is obtained by treating it with the same small amount of ethanol); a yellow color that gradually becomes purple, violet, and blue, respectively, will appear. This test is positive only for natural camphor.

PURITY TESTS

Appearance of solution. The 10% solution (w/v) in hexane is clear (5.2.25).

Evaporation residue. To heat in water bath 2.0 g of the sample in tared capsule to obtain complete sublimation. To dry the residue at 120 °C for 3 hours, cool down, and weigh it. The weight of the residue should not exceed 0,05%.

Halogens. To mix 0.1 g of finely powdered camphor with 0.2 g of sodium peroxide in a dry porcelain crucible. To gently heat to obtain total incineration. To dissolve the residue in 25 mL of warm water, acidify with nitric acid, and filter the solution to a comparison tube. To wash the tube and filter with 10 mL of hot water (twice) and filter by adding the washings to the filtrate solution. To add 0.5 mL of 0.1 M silver nitrate to the filtrate, dilute with water to 50 mL, and mix it. The turbidity should not exceed that produced in blank test, with the same amounts of the same reagent and 0.05 mL of 0.02 M hydrochloric acid (0.035%).

PACKAGING AND STORAGE

In airtight containers. To avoid excessive heat.

LABELLING

To comply with the legislation in force. The label should indicate the origin, whether natural or synthetic.

THERAPEUTIC CLASS

Antipruritic, topic.

LEMON GRASS

Cymbopogonis foliae

Cymbopogon citratus (DC.) Stapf - POACEAE

The vegetable drug consists of dried leaves containing at least 0.5% of volatile oil. The volatile oil consists of at least 60% of citral.

POPULAR NAMES

Capim cidró, capim santo.

CHARACTERISTICS

Organoleptic characteristics. The dried leaves have a typical odor of citral and citrus flavor.

D. To heat the powdered camphor and cover the container with a watch glass, thus obtaining a sublimate consisting of piriformis isotropic crystals assembled in radical groups.

MACROSCOPIC DESCRIPTION

Leaves consisting of convoluted sheath and blade. Sheath extended towards the base, 4 cm to 26 cm long, with 0.6 cm to 6.5 cm wide, at the basal region, 1.0 cm to 3.5 cm in the midline, and 0.9 cm to 2.1 cm in the apical region. Ligule with 0.2 cm height, short and truncated, membrane. Simple trichomes located at the base of the adaxial face of

the leaf blade, lower than the ligule and distributed behind this. Blade with 60 cm to 85 cm long, 0.8 cm to 1.1 cm wide, at the basal region, and 1.4 cm to 1.8 cm in the midline, bright green when fresh and grayish-green when dry, linear-lanceolate, flat on the expanded portion, and canaliculate and narrowed in the basal portion, acuminate at the apex, rough due to short and siliceous trichomes, whole margin, with hard and sharp trichomes in amount larger than the rest of the blade; parallel ribs, the median more developed and pronounced on the lower face.

MICROSCOPIC DESCRIPTION

Amphi-hypostomatic leaf. In the leaf sheath, the epidermis in front view, in the adaxial face, displays straight-walled cells, with siliceous trichomes and rare stomata; in the lower face, cells with sinuous walls, arranged in rows and interspersed with sclerified cells, located in the region corresponding to groupings of subepidermal fibers, as well as sparse unicellular siliceous trichomes and stomata, arranged in rows, in the region between the ribs. In cross section, the epidermal cells are rectangular, with the external periclinal thick wall; cells facing the lower face are smaller. The fundamental parenchyma fills almost the entire blade and is formed by voluminous cells; in its innermost portion there are different secretory cells and next to lower face there is a chlorenchyma formed by smaller cells. The vascular bundles are collateral; those further developed are distributed throughout the parenchyma, while others are facing the lower face, next to the chlorenchyma. Subepidermal groupings of fibers occur in larger amounts next to lower face. In the leaf blade, the epidermis in front view, on the adaxial face, shows fundamental cells and specialized cells arranged in rows: guard cells, bulliform, subsidiaries, suberous, and siliceous trichomes. Bulliform cells are voluminous and more or less isodiametric; fundamental cells have lipid droplets, they are rectangular, with sinuous anticlinal walls and interspersed by siliceous trichomes and by 1-3 suberous cells much smaller than the others and straight walls; trichomes are unicellular, short, thick-walled, and have broad base and acute apex towards the leaf apex. The stomata are tetracytic and have guard cells in the form of dumbbells, occurring in greater number on the lower face. In cross section, the epidermis is uniseriate and stomata, on the adaxial face, spread out laterally to the group of fundamental cells, whereas on the lower surface they are distributed along the chlorenchyma.

The vascular bundles are collateral with different sizes; they have specialized sheath, kranz-type, as well as mestomatic sheath in more developed bundles. The fiber strands occur on both sides, always opposite to the vascular bundles, and, on the adaxial face, they are attached only to the most developed beams; chlorenchyma cells are radially distributed around the beams. The fundamental parenchyma occurs both in the mesophyll and in the midrib region. Secretory cells occur in the border region between the chlorenchyma and fundamental parenchyma, with dense content and distinctive shape. The secretory cells of the sheath and blade are shown in reaction with Lugol, showing dense cellular content, brown or red color in fresh or dry material. In the

reaction with sulfuric vanillin, the contents of the secretory cells are brown and dense. Sometimes, this content appears collapsed and concentrated along the cell wall. For the reaction with vanillin sulfuric, cuts should be immersed in ethyl alcohol, passed to vanillin and flambéed, and submerged for 2 minutes. The blade, for observation, should be mounted in ethanol, and cuts should be passed in water.

MICROSCOPIC DESCRIPTION OF POWDER

The powder meets all the requirements for the species, unless the macroscopic characters. Characteristics: bright green color; portions of the epidermis, as described; large amount of fragments of ribs, with siliceous trichomes; portions of leaf mesophyll, as described; portions of edge with siliceous trichomes.

IDENTIFICATION

To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel with 250 µm thick as support and a mixture of toluene and ethyl acetate (93:7) as mobile phase. To separately apply to the plate, as band, 10 µl of each of the solutions freshly prepared, as described below.

Solution (1): to stir about 0.5 g of crushed drug with 10 mL of methylene chloride in a closed container for 10 minutes. To filter it. To concentrate the filtrate to dryness in a water bath at temperature up to 60 °C. To resuspend the residue in 10 mL of toluene.

Solution (2): to dilute 2 µL of volatile oil obtained in *Dosage for volatile oils* in 1 mL of toluene.

Solution (3): to dilute 2 µL of citral in 1 mL of toluene.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (365 nm). Spots obtained with *Solution (1)* and *Solution (2)*, with R_f about 0.60, corresponding in position and intensity to that obtained with *Solution (3)*. To nebulize the plate with vanillin sulfuric SR and put it in an oven at 100 °C - 105 °C for 5 minutes. The spot corresponding to citral has dark blue color.

PURITY TESTS

Foreign matter (5.4.2.2). At most 1%.

Water (5.4.2.3). At most 11%.

Total ashes (5.4.2.4). At most 9%.

DOSAGE

Volatile oils

To proceed as described in *Determination of volatile oils in vegetable drugs* (5.4.2.7). To use 1000 mL flask containing 500 mL of water as distillation liquid and 0.5 mL of xylene.

To use dry scratched plant. To immediately proceed to determine the volatile oil from 50 g of the scratched drug. To distill for 4 hours.

Citral A and citral B

To proceed as described in *Gas chromatography* (5.2.17.5). To use chromatograph equipped with flame ionization detector, by using mixture of nitrogen, synthetic air, and hydrogen (1:1:10) as the auxiliary gas to flame detector; 30 m long capillary column and 0.25 mm inner diameter, filled with poly (dimethyl/diphenyl siloxane), with a film thickness of 0.25 µm; column temperature from 60 °C to 300 °C, at 3 °C per minute (total: 80 minutes), injector temperature at 220 °C, and the detector temperature at 250 °C; use helium at 80 kPa as carrier gas and flow of carrier gas 1 mL/minute.

Sample solution: to dilute the volatile oil obtained in *Dosage for volatile oils*, in the ratio of 2:100 in ethyl ether.

Procedure: to inject 1 µL of *Sample solution* into the gas chromatograph, by using 1:50 flow division. The citral A

(*trans*-citral) shows linear retention time (Kovats index) of 1263 and citral B (*cis*-citral) of 1233. The relative concentrations are obtained by manual or electronic integration.

To calculate the Kovats index, according to the equation:

$$IK = 100 \times n + \frac{100 \times (tr_x - tr_z)}{(tr_{z+1} - tr_z)}$$

where

n = number of carbon atoms in the alkane with lower molecular weight,

tr_x = retention time of compound 'x' (intermediate to tr_z and tr_{z+1});

tr_z = retention time of the alkane with 'n' carbons;

tr_{z+1} = retention time of the alkane with 'n+1' carbons;

PACKAGING AND STORAGE

In tightly closed container, protected from light and heat.

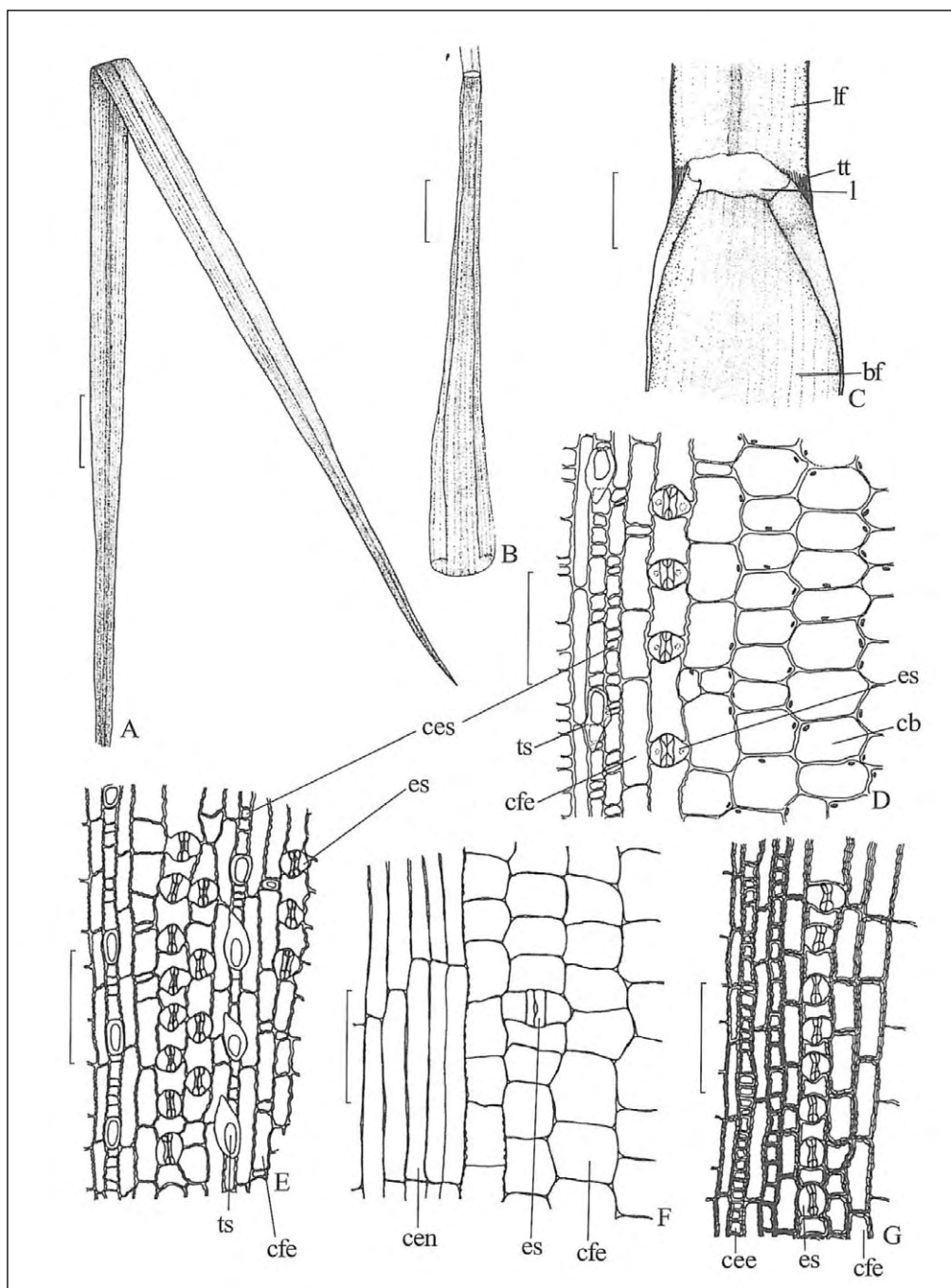


Figure 1 - Macroscopic and microscopic aspects of *Cymbopogon citratus* (DC.) Stapf

Additional information of **Figure 1**. Scales correspond to 3 cm in **A** and **B**; 0.5 cm in **C**; and 100 µm from **D** to **G**.

A - general appearance of the leaf blade. **B** - general appearance of the leaf sheath. **C** - detail of the portion between the sheath and leaf blade, showing the ligule and trichomes: leaf sheath (bf); ligule (l); leaf blade (lf); tector trichomes (tt). **D** - detail of the epidermis of adaxial face of the leaf blade: bulliform cell (cb); fundamental cell of the epidermis (cfe); suberous epidermal cell (ces); stomata (es), siliceous trichomes (ts). **E** - detail of epidermis of lower face in the leaf blade: suberous epidermal cell (ces); fundamental cell of the epidermis (cfe); stomata (es), siliceous trichomes (ts). **F** - detail of the epidermis of adaxial face of the leaf sheath: fundamental cells of the epidermis on a rib (cen); fundamental cells of the epidermis (cfe); stomata (es). **G** - detail of the epidermis of lower face of the leaf sheath: sclerified epidermal cell (cee); fundamental cell of the epidermis (cfe); stomata (es).

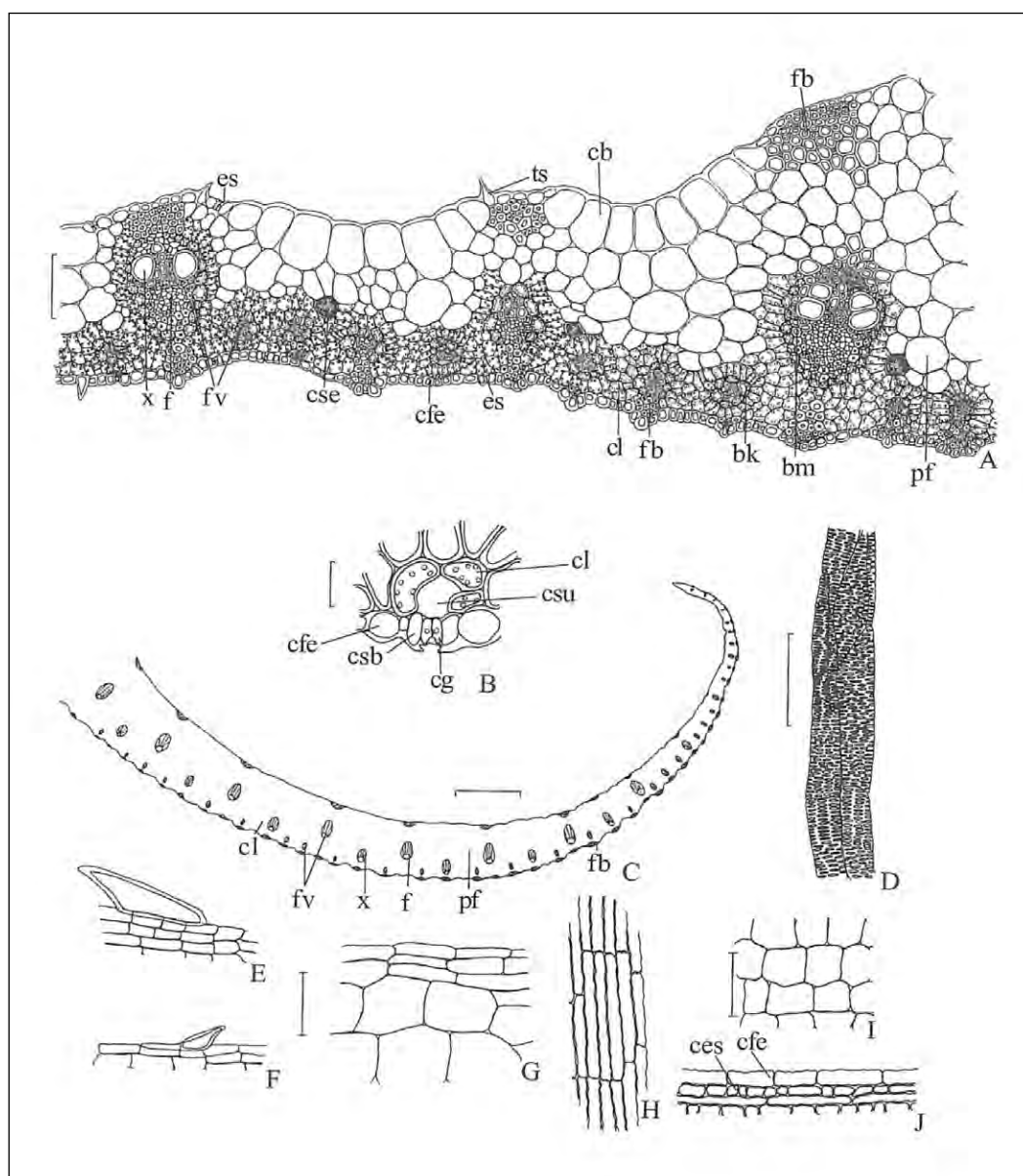


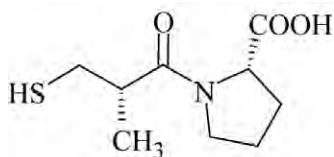
Figure 2 - Microscopic aspects of *Cymbopogon citratus* (DC.) Stapf

Additional information of **Figure 2**. Scales correspond to 100 µm in A; 20 µm, in B; 1 mm, in C; and 100 µm, from D to J.

A — detail of cross section of the leaf blade: Kranz sheath (bk); mesophyll (bm); bulliform cell (cb); fundamental cell of the epidermis (cfe); chlorenchyma (cl); secretory cell (cse); stomata (es), phloem (f); fibers (fb); vascular bundle (fv); fundamental parenchyma (pf); siliceous trichomes (ts); xylem (x). **B** - detail of the leaf blade containing stomata: fundamental cell of the epidermis (cfe); guard-cell (cg); chlorenchyma (cl); subsidiary cell (csb); substomatal chamber (csu). **C** - general appearance of the cross section of portion of the leaf sheath: chlorenchyma (cl); phloem (f); fiber strand (fb); vascular bundle (fv); fundamental parenchyma (pf), xylem (x). **D** - detail of a vessel element with crosslinked thickening. **E** - details of fragments observed in the powder: leaf edge with siliceous trichomes. **F** - details of fragments observed in the powder: epidermis with cells on the rib showing siliceous trichomes. **G** - details of fragments observed in the powder: epidermal cells. **H** - details of fragments observed in the powder: epidermis with cells on the rib. **I** - details of fragments observed in the powder: epidermal cells. **J** - details of fragments observed in the powder: epidermis. suberous cell (ces); fundamental cell of the epidermis (cfe).

CAPTOPRIL

Captoprilum



$C_9H_{15}NO_3S$; 217.29

captopril; 01699

1 - [(2*S*)-3-mercapto-2-methyl-1-oxopropyl]-L-proline
[62571-86-2]

It contains at least 97.5% and at most 102.0% of $C_9H_{15}NO_3S$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white or almost white powder.

Solubility. Soluble in water, freely soluble in methanol and methylene chloride. Soluble in dilute alkalis.

Physical and chemical constants.

Melting range (5.2.2): 105 °C to 108 °C.

Specific rotation (5.2.8): -156° to -161°, in relation to the desiccated substance. To determine in 2% solution (w/v) in carbon dioxide-free water.

IDENTIFICATION

The identification test **A.** may be omitted if the tests **B.** and **C.** are conducted.

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the captopril SQR spectrum similarly prepared.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method **B.** corresponds to that of main peak of *Standard solution*.

C. To dissolve about 20 mg of the sample in 2 mL of water and add 0.5 mL of 0.05 *M* iodine. Color due to the iodine immediately disappears.

PURITY TESTS

pH (5.2.19). 2.0 to 2.6. To determine in 2% solution (w/v) of the sample in carbon dioxide-free water.

Related substances. To proceed as described in *Dosage* method **B.** To prepare *Test solutions* as described below.

Solution (1): to transfer 50 mg of the sample to 100 mL volumetric flask, dissolve with *Mobile phase*, and top up with the same solvent.

Solution (2): to transfer 1 mL of *Solution (1)* to 50 mL volumetric flask and top up with *Mobile phase*.

Solution (3): to dissolve 10 mg of the sample in 20 mL of *Mobile phase*, add 0.25 mL of 0.05 *M* iodine, and top up to 100 mL with the same solvent. To make it homogeneous. To transfer 5 mL of this solution to 50 mL volumetric flask, make it homogeneous, and top up with the same solvent.

Procedure: to separately inject 20 µL of each solution, record the chromatograms at least 3 times the retention time of the captopril, and measure the areas under the peaks. The test is valid if the chromatogram obtained with *Solution (3)* presents 3 peaks and the resolution between the two peaks with long retention time is greater than 2.0. The three peaks respectively correspond to the excess of iodine, to captopril, and to captopril disulfide formed. The area of any secondary peak in the chromatogram obtained with *Solution (1)* is not greater than 0.5 times the area under the main peak in the chromatogram obtained with *Solution (2)* (1.0%). The sum of the areas of all the peaks obtained with *Solution (1)*, except the main peak's one, is not greater than the area under the main peak obtained with *Solution (2)* (2.0%). To disregard the peaks relating to the solvent or area less than 0.1 times the area under the main peak in the chromatogram obtained with *Solution (2)* (0.2%).

Heavy metals (5.3.2.3). To use *Method III*. At most 0.002% (20 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 60°C under reduced pressure for 3 hours. At most 1.0%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.2%.

DOSAGE

To employ one of the methods described below.

A. To exactly transfer about 0.15 g of the sample to 125 mL Erlenmeyer flask and dissolve in 50 mL of water. To titrate with 0.05 *M* SV iodine, to potentiometrically determine the endpoint or by using 1 mL of starch SI. Each mL of 0.05 *M* SV iodine is equal to 21.729 mg of $C_9H_{15}NO_3S$.

B. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 220 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at room temperature; flow of *Mobile phase* of 1 mL/min.

Mobile phase: mixture of 0.11% phosphoric acid (v/v) and methanol (45:55).

Note: to protect the solutions described below from exposure to air and use them within 8 hours.

Sample solution: to accurately dissolve weighed amount of the sample in *Mobile phase* to obtain a 0.5 mg/mL solution.

Standard solution: to accurately dissolve weighed amount of captopril SQR in *Mobile phase* to obtain a 0.5 mg/mL solution.

To inject 20 µL of *Solution (3)* obtained in *Related Substances*. The test is valid if the chromatogram obtained presents 3 peaks and the resolution between the two peaks with long retention time is greater than 2.0. To inject replicates of 20 µL of the *Standard solution*. The relative standard deviation for the replicate areas for the peaks recorded is not greater than 2.0%.

Procedure: to separately inject 20 µL of *Sample and standard solutions*, record the chromatograms, and measure the average area under the peaks. To calculate the content of $C_9H_{15}NO_3S$ from the sample, from the responses obtained for the *Sample and standard solutions*.

PACKAGING AND STORAGE

In airtight containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antihypertensive.

CAPTOPRIL TABLETS

It contains at least 90.0 % and at most 110 % of the amount stated of $C_9H_{15}NO_3S$.

IDENTIFICATION

A. To proceed as described in Thin-layer chromatography (5.2.17.1) by using G silica gel as support and a mixture of toluene, glacial acetic acid, and methanol (75:25:1) as mobile phase. To separately apply to the plate 20 µL of each of the solutions freshly prepared, as described below.

Solution (1): to weigh and pulverize the tablets. To transfer an amount equivalent to 0.1 g of captopril to 25 mL volumetric flask, add 15 mL of methanol, leave it in ultrasound for 30 minutes, and occasionally stir it. To top up with the same solvent, make it homogeneous, and filter it.

Solution (2): to prepare 4 mg/ml solution of captopril SQR in methanol.

To develop the chromatogram. To remove the plate, and let it drying. To nebulize with mercury diphenyl carbazone SR. The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage method B*, corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

Procedure for content uniformity. To transfer each tablet to a suitable volumetric flask containing 5 mL of water. To wait a total disintegration of the tablet. To add mixture of ethanol and water (1:1) corresponding to half the capacity of the flask. To leave it in ultrasound for 15 minutes. To mechanically stir for 15 minutes and top up with the same solvent. To make it homogenous and filter it. To successively dilute with the same solvent to a 0.002% concentration (w/v). To prepare a standard solution at the same concentration by using the same solvent. To measure the absorbance of the resulting solutions at 212 nm (5.2.14), by using ethanol and water (1:1) to adjust zero. To calculate the amount of $C_9H_{15}NO_3S$ in the tablets, from the readings obtained.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 900 mL

Equipment: baskets, 50 rpm

Time: 20 minutes

Procedure: After testing, remove aliquot from the dissolution medium and dilute, if necessary, with 0.1 M hydrochloric acid, to reach suitable concentration. To measure the absorbance at 212 nm (5.2.14), by using the same solvent to adjust zero. To calculate the amount of $C_9H_{15}NO_3S$ dissolved in the medium, by comparing the readings obtained with 0.0025 % captopril SQR solution (w/v) prepared in 0.1 M hydrochloric acid.

Tolerance: not less than 80% (Q) of the stated amount of $C_9H_{15}NO_3S$ dissolves within 20 minutes.

PURITY TESTS

Limit for captopril disulfide. To proceed as described in *Dosage* method B. to separately inject 20 µL of Test solution and Sample solution.

The peak area relative to captopril disulfide in the Sample solution should not be greater than the peak area in relation to the captopril disulfide obtained in the Test solution. At most 3.0%.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To weigh and spray 20 tablets. To use amount of powder equivalent to approximately 0.15 g of captopril, transfer to 125 mL Erlenmeyer flask, and add 50 mL of water. To leave it in ultrasound for 15 minutes and mechanically stir for 15 minutes. To proceed as described in *Dosage Method A.*, in the monograph *Captopril* from “To titrate with 0.05 M SV iodine...” **B.** To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 220 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at room temperature; flow of *Mobile phase* of 1.0 mL/min.

Mobile phase: mixture of 0.11% phosphoric acid (v/v) and methanol (45:55).

Captopril disulfide solution: to prepare 1 mg/ml solution of captopril disulfide SQR in *Mobile phase*.

Test solution: to transfer 3 mL of *Captopril disulfide solution* to 100 mL volumetric flask and top up with *Mobile phase*.

Sample solution: To weigh and spray 20 tablets. To transfer amount of powder equivalent to 50 mg of captopril to a 50 mL volumetric flask and add 30 mL of *Mobile phase*. Then, leave it in ultrasound for 15 minutes and mechanically stir for 15 minutes. To top up the volume with the same solvent. To make it homogenous and filter it.

Standard solution: to transfer 0.1 mL of captopril SQR to 100 mL volumetric flask, add 3 mL of *Captopril disulfide solution*, and top up with *Mobile phase*.

To inject replicates of 20 µL of the *Standard solution*. The relative retention times are about 0.5 to captopril and 1.0 to captopril disulfide. The resolution between captopril and

captopril disulfide should not be less than 2. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2.0%.

Procedure: to separately inject 20 µL of *Sample and standard solutions*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of $C_9H_{15}NO_3S$ in

the tablets, from the responses obtained for the *Sample and standard solutions*.

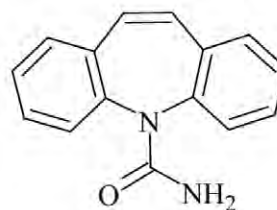
PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

CARBAMAZEPINE Carbamazepinum



$C_{15}H_{12}N_2O$; 236.27
carbamazepine; 01710
5H-dibenz [*b, f*] azepine-5-carboxamide
[298-46-4]

It contains at least 98.0% and at most 102.0% of $C_{15}H_{12}N_2O$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white or yellowish-white powder; odorless. It presents polymorphism.

Solubility. Practically insoluble in water, freely soluble in methylene chloride, soluble in chloroform and methanol, slightly soluble in acetone and ethanol and very slightly soluble in ethyl ether.

Physical and chemical constants

Melting range (5.2.2): 189 °C to 193 °C.

IDENTIFICATION

The identification tests **B.** and **C.** may be omitted if the test **A.** is conducted.

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has

absorption maximum only at the same wavelength, with the same relative intensities to those observed in the carbamazepine SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14) ranging from 200 nm to 400 nm, of sample solution obtained in Dosage, method A., exhibits absorption maxima in 285 nm.

C. To heat about 0.1 g of the sample with 2 mL of nitric acid, in a water bath, for 3 minutes. The red-orange color develops.

PURITY TESTS

Acidity or alkalinity. To weigh 2.5 g of the sample and transfer to a 50 mL volumetric flask. To add 20 mL of water. To stir, top up with water, and make it homogeneous. To filter it. To add a drop of phenolphthalein SI to an aliquot of 20 mL. To titrate with 0.01 M sodium hydroxide. To simultaneously perform sample blank correction. No more than 1 mL is required for every 1 g of sample. To add a drop of methyl red SI to another aliquot of 20 mL. To titrate with 0.01 M hydrochloric acid. To simultaneously perform sample blank correction. No more than 1 mL is required for every 1 g of sample.

Related substances.

To employ one of the methods described below.

A. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel as support and a mixture of toluene and ethanol (70:30) as mobile phase. To separately apply to the plate 2 µL of each of the solutions freshly prepared in mixture of chloroform and ethanol, as described below.

Solution (1): 50 mg/mL solution of the sample.

Solution (2): 0.05 mg/mL solution of the sample.

Solution (3): 50 mg/mL solution of carbamazepine SQR.

Solution (4): 0.05 mg/mL solution of imino dibenzyl.

Solution (5): 0.05 mg/mL solution of carbamazepine, related substance B SQR (iminostilbene).

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm and 365 nm). To nebulize with 0.5% potassium dichromate (w/v) in M sulfuric acid. Any secondary spot with obtained in the chromatogram with *Solution (1)* is not more intense than that obtained with *Solutions (4) and (5)* (0.1 %). To heat at 140 °C for 15 minutes and observe under ultraviolet light (254 nm and 365 nm). Any spot in the chromatogram with *Solution (1)*, with Rf value lower than that of principal spot, is not more intense than that in the *Solution (2)* (0.1%).

B. To proceed as described in *Dosage* method **B.** To prepare the following solutions.

Sample solution: To accurately weigh about 100 mg of the sample. To transfer it to 50 mL volumetric flask, dissolve, and top up with methanol. To transfer 25 mL of this solution to 50 mL volumetric flask and top up with *Mobile phase*.

Standard solution: to dissolve amount accurately weighed of carbamazepine SQR, carbamazepine related substance A SQR (10,11-dihydro carbamazepine), and carbamazepine related substance B SQR

(iminostilbene) in methanol, to obtain 0.02 mg/mL concentration of each component. To transfer 5 mL of this solution to 100 mL volumetric flask and top up with *Mobile phase*, thus obtaining 1 µg/mL solution.

Resolution solution: to dissolve amount accurately weighed of carbamazepine SQR and carbamazepine related substance A SQR (10,11-dihydro carbamazepine) in methanol, to obtain 100 µg/mL and 500 µg/mL solutions, respectively. to transfer to 5 mL of this solution to 50 mL volumetric flask and top up with *Mobile phase*.

System Suitability Test: To inject replicates of 20 µL of the *Resolution solution*. The resolution between carbamazepine related substance A and standard carbamazepine should not be less than 1.70. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2.0 %.

Procedure: to separately inject 20 µL of *Sample and standard solutions*. To record the chromatograms and measure the area under the peaks. To calculate the amount (mg) of any impurity found in the *Sample solution* from the responses.

Chlorides (5.3.2.1). to boil 0.5 g of the sample with 20 mL of water for 10 minutes, cool down, and quantitatively filter it into Nessler tube. At most 0.014% (140 ppm).

Heavy metals (5.3.2.3). To use *Method III*. To heat to boiling 1 g of the sample with 20 mL of water for 10 minutes, cool down, and quantitatively filter it into Nessler tube. At most 0.001% (10 ppm).

Loss on drying (5.2.9). To determine in 2 g of the sample. To desiccate in an oven at 105 °C for 2 hours. At most 0.5 %.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To accurately weigh about 50 mg of the sample. To transfer it to 50 mL volumetric flask,

add 25 mL of methanol, and leave it in ultrasound for 15 minutes. To top up the volume with the same solvent. To successively dilute in methanol to the 0.001% concentration (w/v). To prepare a standard solution at the same concentration by using the same solvent. To measure the absorbance of the resulting solutions at 285 nm, by using methanol to adjust zero. To calculate the $C_{15}H_{12}N_2O$ concentration of the sample from the readings obtained. Alternatively, make the calculations taking into account that $A(1\%, 1\text{ cm}) = 490$ at 285 nm, in methanol.

B. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 230 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μm), kept at room temperature; flow of *Mobile phase* of 1 mL/min.

Mobile phase: methanol and water (70:30).

Sample solution: to dissolve accurately weighed amount of the sample in methanol, to obtain a 2 mg/mL solution. To leave it in ultrasound for 15 minutes. To transfer 5 mL of this solution to 50 mL volumetric flask and top up with *Mobile phase*, thus obtaining 0.2 mg/mL solution.

Standard solution: to dissolve accurately weighed amount of carbamazepine SQR in methanol to obtain a 1 mg/mL solution. To leave it in ultrasound for 15 minutes. To transfer 5 mL of this solution to 25 mL volumetric flask and top up with *Mobile phase*, thus obtaining 0.2 mg/mL solution.

Procedure: to separately inject 20 μL of *Sample and standard solutions*, record the chromatograms, and measure the average area under the peaks. To calculate the content of $C_{15}H_{12}N_2O$ from the sample, from the responses obtained for the *Sample and standard solutions*.

PACKAGING AND STORAGE

In airtight closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Anticonvulsant.

CARBAMAZEPINE TABLETS

It contains at least 92.0 % and at most 108.0 % of the amount stated of $C_{15}H_{12}N_2O$.

IDENTIFICATION

To weigh and pulverize the tablets. To heat in a water bath an amount of powder equivalent to 0.2 g of carbamazepine

with 15 mL of acetone. To filter it. To wash it with two parts of 5 mL of hot acetone. To evaporate the filtrate to about 5 mL and cool down in an ice bath to crystallization. To filter the crystals and wash the filter with 3 mL of cold acetone. To desiccate in a vacuum oven at 70 °C for 30 minutes. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the carbamazepine SQR spectrum similarly prepared.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). At most 5 minutes.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST

Dissolution medium: 1% sodium lauryl sulfate (w/v) in water, 900 mL.

Equipment: blades, 75 rpm

Time: 60 minutes, with sampling rates in 15 and 60 minutes.

Procedure: After testing, remove aliquots from the dissolution medium, according to time set, and filter them. To measure the absorbance at 285 nm (5.2.14), by using the *Dissolution medium* to adjust zero. To calculate the content of $C_{15}H_{12}N_2O$ dissolved in the medium, by comparing the readings obtained with carbamazepine SQR solution at 0.002% concentration (w/v) prepared in 1% sodium lauryl sulfate (w/v) with previous addition of methanol, to ensure solubilization. The methanol concentration in the standard solution must not exceed 1% (v/v).

Tolerance: between 45% and 75% of it dissolves in 15 minutes; not less than 75% (Q) of the stated amount of $C_{15}H_{12}N_2O$ dissolves within 60 minutes.

PURITY TESTS

Water (5.2.20.1). At most 3.0%.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To weigh and spray 20 tablets. To transfer amount of powder equivalent to 50 mg of carbamazepine to a 100 mL volumetric flask, add 70 mL of methanol, and leave it in ultrasound for 30 minutes. To top up with the same solvent, make it homogeneous, and filter it. To successively dilute to 0.001% concentration (w/v) by using methanol as solvent. To prepare a standard solution at the same concentration by using the same solvent.

To measure the absorbance of the resulting solutions at 285 nm, by using methanol to adjust zero. To calculate the amount of $C_{15}H_{12}N_2O$ in the tablets, from the readings obtained. Alternatively, make the calculations taking into account that $A(1\%, 1\text{ cm}) = 490$ at 285 nm, in methanol.

B. To proceed as described in *Dosage*, method **B**, in monograph *Carbamazepine*. To prepare the *Sample solution* as described below.

Sample solution: To weigh and spray 20 tablets. To transfer amount of powder equivalent to 100 mg of carbamazepine to a 50 mL volumetric flask, add 25 mL of methanol, and leave it in ultrasound for 15 minutes. To top up with the same solvent, make it homogeneous, and filter it. To transfer 5 mL of the filtrate to 50 mL volumetric flask and top up with *Mobile phase*, thus obtaining 0.2 mg/mL solution.

Procedure: to separately inject 20 μ L of *Standard solution* and *Sample solution*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of $C_{15}H_{12}N_2O$ in the tablets, from the responses obtained for the *Sample and standard solutions*.

PACKAGING AND STORAGE

In airtight closed containers, protected from light.

LABELLING

To comply with the legislation in force.

CARBONATE, BISMUTH, BASIC Bismuthi subcarbonas

$(BiO)_2CO_3$; 509.97
basic bismuth carbonate; 01747
bismuth carbonate Oxide
[5892-10-4]

It contains at least 97.6% and at most 100.7% of $(BiO)_2CO_3$, in relation to the desiccated substance, equivalent to at least 80.0% and at most 82.5% of bismuth.

DESCRIPTION

Physical characteristics. White, odorless, and tasteless powder.

Solubility. Practically insoluble in water, ethanol, and ethyl ether. It dissolves with effervescence in diluted mineral acids and glacial acetic acid.

IDENTIFICATION

A. It responds to the reactions of bismuth ion (5.3.1.1).

B. It responds to the reactions of carbonate ion (5.3.1.1).

PURITY TESTS

Appearance of solution. To stir 5 g of the sample with 10 mL of water. To add 20 mL of nitric acid and heat to dissolve it. To cool down and dilute to 100 mL with water. The resulting solution is colorless (5.2.12) and less opalescent than a 10% sample suspension (w/v) (5.2.25).

Copper. To add 2 mL of ammonia to 5 mL of the resulting solution in *Appearance of solution*, dilute to 50 mL with water, and filter it. To add 1 mL of 0.1% sodium diethyldithiocarbamate solution (w/v) to 10 mL of filtrate. The color of the solution is not more intense than that of a reference solution simultaneously prepared, under the same conditions, by using mixture of 0.25 mL of *Copper standard solution* (10 ppm Cu) and water sufficient to 10 mL instead of filtrate. At most 0.005% (50 ppm).

Alkali metals and alkaline earth metals. To add 10 mL of water and 10 mL of acetic acid SR to 1 g of sample. To heat to boiling for 2 minutes, cool down, and filter it. To wash the residue with 20 mL of distilled water. To add 2 mL of hydrochloric acid SR and 20 mL of water to the filtrate. To heat to boiling and pass hydrogen sulfide through the solution to precipitate all the bismuth. To filter, wash the residue with water, evaporate it in a water bath to dryness, and add 0.5 mL of sulfuric acid. To carefully incinerate it and let cool down. The residue mass should not be greater than 10 mg (1.0%).

Nitrates. To transfer 0.25 g of the sample to 125 mL Erlenmeyer flask and add 20 mL of distilled water, 0.05 mL of indigo carmine SV, and carefully 30 mL of sulfuric acid. To titrate with indigo carmine SV to change stable blue color. The amount of indigo carmine SV spent is not larger than the volume equivalent to 1 mg of NO_3 (0.4%).

Silver. To add 1 mL of water and 4 mL of nitric acid to 2 g of sample. To carefully heat to dissolve it, cool down, and dilute to 11 mL with water. To add 2 mL of *M* hydrochloric acid, make it homogeneous, and let rest for 5 minutes protected from light. Any opalescence developed is not more intense than that of a standard prepared by mixing 10 mL of silver standard solution (5 ppm Ag), 1 mL of nitric acid, and 2 mL of *M* hydrochloric acid. At most 0.0025% (25 ppm).

Arsenic (5.3.2.5). To transfer 0.6g of the sample to distillation flask. To add 5 mL of water, 7 mL of sulfuric acid, and cool down. To add 5 g of a reducing mixture and 10 mL of hydrochloric acid. To gradually heat to boiling for 15 - 30 minutes, and further heating so that the distillation continues until the volume of the flask is reduced by half, or the capacitor is full of steam for 5 minutes. The distillation should be stopped before the formation of vapor of sulfur trioxide. To collect the distillate in a tube containing 15 mL of water cooled in an ice bath. To wash the capacitor with water and dilute the distilled to 25 mL with the same solvent. Then, proceed as described in *Visual method*. To prepare reference solution by using a mixture of 3 mL of *Arsenic standard solution (1 ppm As)* and 22 mL of water. At most 0.0005% (5 ppm).

Lead. To proceed as described in *Atomic absorption spectrophotometry (5.2.13.1)*, by employing *Method II*. To dissolve 12.5 g in 75 mL of a mixture of equal volumes of water and lead-free nitric acid. To heat to boiling for 1 minute, cool down, and dilute to 100 mL with water. To prepare the lead reference solutions by using appropriate amounts of lead standard solution and 37% lead-free nitric acid (v/v). To measure the absorbance of the solutions at 283.3 nm by using hollow-cathode lamp as source of radiation and air-acetylene flame. At most 0.002% (20 ppm).

Chlorides (5.3.2.1). To determine 0.7 g of the sample and 1 mL of 0.01 M hydrochloric acid. At most 0.05% (500 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample, in an oven, at 105 °C. At most 1.0%.

DOSAGE

To dissolve 0.25 g of the sample in 2 mL of nitric acid and dilute to 100 mL with water. To proceed as described in *Complexometric titrations (5.3.3.4)* for *Bismuth*. Each mL of 0.05 M SV disodium edetate is equal to 12.749 mg of $(\text{BiO})_2\text{CO}_3$, corresponding to 10.449 mg of bismuth.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antacid.

It contains at least 98.5% and at most 100.5% of CaCO_3 , in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Fine, white microcrystalline, odorless, and tasteless powder.

Solubility. Sparingly soluble in water upon the presence of ammonium salts or carbon dioxide, practically insoluble in water and ethanol. It dissolves with effervescence in M acetic acid, 3 M hydrochloric acid, and 2 M nitric acid.

IDENTIFICATION

A. To put about 0.1 g of the sample into the test tube and suspend with 2 mL of water. To add 3 mL of 2 M acetic acid and close the tube with a cork immediately connected to a U shape glass tube. The mixture effervesces. To connecting a second test tube containing 0.1 M barium hydroxide to the other end of the U shape glass tube. To gently heat the tube containing the sample. A precipitate, which dissolves in 6 M hydrochloric acid, is formed in the second tube.

B. It responds to the reactions of the calcium ion (5.3.1.1).

PURITY TESTS

Substances insoluble in acid. To weigh 5 g of the sample and drip hydrochloric acid with stirring until effervescence stops. Then, transfer to 200 mL flask and top up with water. To use suitable filter paper. To wash the residue until the last washing does not present chloride reaction (5.3.1.1). To incinerate and put it in the oven at 100 °C - 105 °C for 1 hour. The weight of the residue is at most 10 mg (0.2%).

Magnesium and alkali metals. To mix 1 g of the sample with 35 mL of distilled water. To carefully add 3 mL of hydrochloric acid and boil the solution for 1 minute. To quickly add 40 mL of oxalic acid SR. To vigorously stir until precipitation occurs. To immediately heat it, add 2 drops of methyl red SI, and add 6 M ammonium hydroxide to the mixture becomes alkaline. To cool down at room temperature and transfer to a 100 mL volumetric flask. To make up to volume with water and let it rest for 4 hours. To use suitable filter paper. To put 50 mL of the filtrate in a porcelain capsule, add 0.5 mL of sulfuric acid, and reduce the volume in a water bath to obtain a small volume. To heat on a electric plate until decomposition and volatilization of ammonium salts. To incinerate the residue at 600 °C to obtain constant weight. The weight of the residue is at most 5 mg (1%).

Arsenic (5.3.2.5). To use *Method I*. To dissolve 5 g of the sample in 80 mL of dilute acetic acid After effervescence stops, boil it for 2 min, cool down, and dilute to 100 mL with dilute acetic acid. To filter, if necessary, by using a sintered glass filter. At most 0.0004% (4 ppm).

Chlorides (5.3.2.1). To weigh 1 g of the sample, add 10 mL of distilled water, and carefully add 10 mL of 2 M nitric acid, by stirring to dissolve it. At most 0.035% (350 ppm).

CARBONATE, CALCIUM

Calcii carbonas

CaCO_3 ; 100.09
calcium carbonate; 01748
Calcium salt, carbonic acid (1:1)
[471-34-1]

Barium. To accurately weigh about 2.5 g of the sample, quantitatively transfer to the beaker, add 3 M hydrochloric acid until effervescence stops, and heat to boiling to remove dissolved carbon dioxide. To quantitatively transfer 25 mL volumetric flask and top up with water. To transfer 5 mL of solution of the sample to the test tube and add 5 mL of calcium sulfate SR.

After 15 minutes, the preparation is not more opalescent than 5 mL of solution of the sample with 5 mL of water.

Iron. To proceed as described in *Atomic absorption spectrophotometry* (5.2.13.1), by employing *Method I*. At most 0.02% (200 ppm).

Sample solution: to accurately weigh about 0.5 g of the sample and transfer to a beaker. To add 5 mL of 3 M hydrochloric acid and heat to boiling to remove carbon dioxide. To quantitatively transfer 25 mL volumetric flask and top up with water.

Sulfate (5.3.2.2). To use 5 mL of solution of the sample from the barium test. At most 0.25% (2500 ppm).

Heavy metals (5.3.2.3). To *Method I*. To use 5 mL of solution of the sample from the barium test. At most 0.002% (20 ppm).

Loss on drying. (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 200 °C for 4 hours. At most 2%.

DOSAGE

To accurately weigh about 0.1 g of the sample previously desiccated and transfer to a 250 mL erlenmeyer flask. To add 50 mL of water and 2 mL of dilute hydrochloric acid, cover with a watch glass, and stir until dissolution of calcium carbonate. To calculate the theoretical equivalence point and titrate with 0.05 M SV edetate disodium to approximately 2 mL prior to this volume. To add 8 mL of sodium hydroxide SR and 150 mg of hydroxy naphthol blue indicator. To proceed the titration with 0.05 M SV edetate disodium to obtain blue color. Each mL of 0.05 M SV disodium edetate is equal to 5.004 mg of CaCO_3 .

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antacid, nutritional supplement, phosphorous binder.

CARBONATE, MAGNESIUM Magnesii carbonas

MgCO_3 ; 84.31

$\text{MgCO}_3 \cdot x\text{H}_2\text{O}$

MgO ; 40.30

magnesium carbonate; 01750

Magnesium salt, carbonic acid (1:1)

[546-93-0]

Magnesium salt, hydrate carbonic acid (1:1)

[23389-33-5]

Magnesium carbonate is a mixture of hydrate magnesium carbonate and basic hydrate magnesium carbonate. It must contain at least 40.0% and at most 43.5% of MgO .

DESCRIPTION

Physical characteristics. It has two varieties: light and heavy.

Light magnesium carbonate: white, light, and brittle mass, or white powder, very fine, light, tasteless and odorless.

Heavy magnesium carbonate: granular, white, tasteless, and odorless powder.

When stirred with water, both of them make it slightly alkaline to litmus paper.

Solubility. Practically insoluble in water and ethanol; in large quantity, cold dissolving in carbon dioxide saturated water.

IDENTIFICATION

A. It produces effervescence when treated with dilute mineral acids.

B. The solution obtained in *Identification* test **A.** responds to the reactions of the magnesium ion (5.3.1.1).

PURITY TESTS

Arsenic (5.3.2.5). To add 10 mL of water and 5 mL of brominated hydrochloric acid SR to 1 g of sample. Then, remove the bromine in excess with a few drops of stannous chloride (II) SR, and proceed as described in *Limit test for arsenic*. To use *Method I*. At most 0.0005% (5 ppm).

Calcium (5.3.2.7). To accurately weigh about 1 g of sample add 22 mL of water and 3 mL of sulfuric acid, carefully. To add 50 mL of ethanol and let the mixture rest at least 12 hours. In case of separation of magnesium sulfate crystals, heat the mixture at approximately 50 °C to dissolve them. To filter with Gooch crucible, coated with asbestos and previously washed with M sulfuric acid, water, and ethanol. It must be calcined and tared.

To wash several times the Gooch crucible with a mixture of 2 volumes of ethanol and 1 volume of M sulfuric acid. To dry it in bright red, cool down, and quickly weigh it. The

weight of the calcium sulfate multiplied by 0.4119 is the weight of CaO in the sample. The sample should contain no more than the equivalent to 0.7% of CaO.

Iron (5.3.2.4). To weigh 2 g of sample and dissolve it in 15 mL of 3 M hydrochloric acid. When effervescence stops, top up to 20 mL with water. To use 5 mL in *Limit test for iron*. At most 0.02% (200 ppm).

Heavy metals (5.3.2.3). To neutralize with concentrated ammonia solution, 4 mL of the solution prepared in the *Limit test for iron*. To add 2 mL of acetic acid SR (Pb) and proceed as described in *Test limit for heavy metals*. At most 0.0025% (25 ppm).

Sulfates (5.3.2.2). To use 10 mL of the solution prepared in the *Limit test for iron*. At most 0.12% (1200 ppm).

Chlorides (5.3.2.1). To accurately weigh about 0.5 g of the sample, add 15 mL of 2 M nitric acid, 25 mL of water, and 1 mL of 0.25 M silver nitrate. To top up to 50 mL with water. In case of opalescence, it should not be more intense than that produced by 0.1 mg of chloride in an equal volume of liquid, by employing the same reagents. At most 0.02% (200 ppm).

Substances insoluble in hydrochloric acid. To mix 5 g of the sample with 75 mL of water and add, by stirring, hydrochloric acid in small portions until complete dissolution. To boil for 5 minutes. To collect the insoluble residue in a filter and wash until the washings no longer produces chloride reaction. To incinerate, cool down, and weigh it. The residue should weigh no more than 0.0025 g (0.05%).

Substances soluble in water. To add 1 g of the sample to 50 mL of freshly boiled water and boil it for 5 minutes. To filter, evaporate the filtrate to dryness, and desiccate the residue at 110 °C for 1 hour. It should weigh no more than 0.015 g (1%).

DOSAGE

To accurately weigh about 1 g of sample, add 50 mL of 0.5 M SV sulfuric acid, 0.5 mL of methyl orange SI, and titrate the acid in excess with M SV sodium hydroxide. To subtract from the amount of 0.5 M SV acid consumed and corresponding to CaO determined in *Purity tests*. The difference is the volume of 0.5 M SV sulfuric acid, which is equivalent to magnesium carbonate. Each mL of 0.5 M SV sulfuric acid is equivalent to 0.02015 g of MgO and 0.02804 g of CaO.

PACKAGING AND STORAGE

In closed containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antacid and laxative.

CARBONATE, POTASSIUM

Kalli carbonas

K_2CO_3 ; 138.21
potassium carbonate; 01751
Potassium salt, carbonic acid (2:1)
[584-08-7]

It contains at least 99.5% and at most 100.5% of K_2CO_3 , in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Granular, white, and hygroscopic powder.

Solubility. Freely soluble in water and practically insoluble in ethanol.

IDENTIFICATION

A. The 0.1% solution (w/v) of the sample responds to the reactions of carbonate ion (5.3.1.1).

B. The 0.1% solution (w/v) of the sample responds to the reactions of potassium ion (5.3.1.1).

PURITY TESTS

Insoluble matter. To dissolve 10 g of the sample in 100 mL water. Put it in a beaker. To cover the beaker and heat it to boiling in a water bath for 1 hour. To filter the solution in tared funnel with porosity from 10 µm to 15 µm. To wash with hot water. To dry at 105 °C, cool down in a desiccator, and weigh it. At most 0.01% (100 ppm).

Calcium and magnesium. To add hydrochloric acid to 20 mL of 10% solution of the sample (w/v) to obtain the acid reaction to litmus paper. Then, add 5 mL of ammonium oxalate SR, 2 mL of dibasic heptahydrate sodium phosphate SR, and 10 mL of ammonium hydroxide. Let it rest in a cool place for 24 hours. In case of precipitate, filter and wash with 2% ammonium hydroxide (v/v). To desiccate and calcinate it to constant weight. The mass of the residue is less than 0.4 mg. At most 0.02%.

Cyanide. To add 0.5 mL of ferrous sulfate SR and 0.5 mL of ferric chloride SR to 15 mL of 10% solution of the sample (w/v). To add hydrochloric acid SR to obtain strongly acidic reaction. No blue color develops.

Chlorides (5.3.2.1). To acidify 10 mL of 10% solution of the sample (w/v) with nitric acid SR to obtain acidic reaction to litmus paper. To add 1 mL of silver nitrate SR and top up to 50 mL with water. In case of opalescence, it should not be more intense than that produced by 0.02 mg of chloride ion (Cl⁻) under the same conditions. At most liquid 0.002% (20 ppm).

Sulfates (5.3.2.2). To acidify 20 mL of 10% solution of the sample (w/v) with hydrochloric acid SR to obtain acidic

reaction to litmus paper. To add 1 mL of barium chloride SR, top up to 50 mL with water, and heat in a water bath for 15 minutes. In case of opalescence, it should not be more intense than that produced by 0.2 mg of sulfate ion (SO_4^{2-}) under the same conditions. At most 0.01% (100 ppm).

Heavy metals (5.3.2.3). To dissolve 4 g of the sample with 10 mL of water, add 15 mL of hydrochloric acid SR, and heat to boiling. To add a drop of phenolphthalein SI and neutralize with *M* sodium hydroxide to obtain light pink color. To cool down and dilute to 25 mL with water. To proceed as described in *Method I*. At most 0.0005% (5 ppm).

Iron (5.3.2.4). To dissolve 10 g of the sample in 25 mL of water, and slowly add 14 mL of hydrochloric acid. When effervescence stops, heat to boiling for a few minutes. To cool down and dilute to 50 mL with water. To proceed as described in *Method I*, by using 5 mL of the solution obtained. To use 1 mL of the *Iron standard solution (10 ppm Fe)*. At most 0.001% (10 ppm).

Arsenic (5.3.2.5). To use 10 mL of 10% solution of the sample (w/v) and add 5 mL of hydrochloric acid SR. To prepare the *Stock standard solution for arsenic (1 ppm As)*. To proceed as described in *Method I*. At most 0.002% (20 ppm). At most 0.001% (10 ppm).

Loss on drying (5.2.9). To determine in 0.3 g of the sample. To desiccate in an oven at 180 °C for 4 hours. At most 0.5%.

DOSAGE

To accurately transfer about 0.3 g of the sample previously desiccated to an erlenmeyer flask. To add 150 mL of water and 4 drops of methyl orange SI. To titrate with *M* SV hydrochloric acid. Each mL of *M* SV hydrochloric acid is equal to 69.105 mg of K_2CO_3 .

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Alkalizing and diuretic.

It contains at least 99.5% and at most 100.5% of Na_2CO_3 , in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Colorless crystals or white powder. Odorless and alkaline and caustic taste. In moist air and cool place, it absorbs water; at 100 °C it becomes anhydrous.

Solubility. Freely soluble in water, boiling water, and glycerol. Insoluble in ethanol.

IDENTIFICATION

A. It responds to the reactions of sodium ion (5.3.1.1).

B. It responds to the reactions of carbonate ion (5.3.1.1).

PURITY TESTS

Appearance of solution. The 10% aqueous solution (w/v) is clear (5.2.25) and colorless (5.2.12).

Alkalinity. The aqueous solution of the sample is strongly alkaline to litmus paper.

Calcium and magnesium. To determine in 20 mL of the solution obtained in *Appearance of solution*. To add hydrochloric acid to obtain acidic reaction to litmus paper. To add 5 mL of 0.25 *M* ammonium oxalate, 2 mL of 0.3 *M* dibasic heptahydrate sodium phosphate SR, and 10 mL of ammonia. Let it rest in a cool place for 24 hours. In case of precipitation, filter, wash with 2% ammonia solution (w/v), desiccate, and calcine to obtain constant weight. The residue should weigh no more than 0.4 g (0.02%).

Cyanide. To determine in 15 mL of the solution obtained in *Appearance of solution*. To add 0.5 mL of 0.5 *M* ferrous sulfate and 0.5 mL of 0.3 *M* ferric chloride. To add 3 *M* hydrochloric acid to obtain strongly acidic reaction. The liquid should not be blue.

Arsenic (5.3.2.5). To use *Method I*. To determine in 10 mL of the solution obtained in *Appearance of solution*. At most 0.001% (10 ppm).

Chlorides (5.3.2.1). To determine in 10 mL of the solution obtained in *Appearance of solution*. To add *M* nitric acid to obtain acid reaction to litmus paper. To add 1 mL of 0.25 *M* silver nitrate and top up to 50 mL with water.

In case of opalescence, it should not be more intense than that produced by 0.1 mg of chloride ion in an equal volume of liquid, by employing the same reagents. At most 0.01% (100 ppm).

Iron (5.3.2.4). To use *Method I*. To determine in 20 mL of the solution obtained in *Appearance of solution*. To add nitric acid to obtain acid reaction to litmus paper. In case of pink or red color, it should not be more intense than that pro-

CARBONATE, SODIUM

Natrii carbonas

Na_2CO_3 ; 105.99

$\text{Na}_2\text{CO}_3 \cdot \text{H}_2\text{O}$; 124.00

sodium carbonate; 01752

Sodium salt, carbonic acid (2:1)

[497-19-8]

Sodium salt, hydrate carbonic acid (2:1:1)

[5968-11-6]

duced by 0.02 mg of ferric ion in an equal volume of liquid, by employing the same reagents. At most 0.001% (10 ppm).

Heavy metals (5.3.2.3). To use *Method I*. To determine in 5 mL of the solution obtained in *Appearance of solution*. To add nitric acid to obtain acid reaction to litmus paper. At most 0.002% (20 ppm).

Sulfates (5.3.2.2). To determine in 20 mL of the solution obtained in *Appearance of solution*. To add 3 M hydrochloric acid to obtain acid reaction to litmus paper. To add 1 mL of 0.5 M barium chloride and top up with water to 50 mL. To heat in water bath for 10 minutes. In case of opalescence, it should not be more intense than that produced by 0.8 mg of sulfate ion in an equal volume of liquid, by employing the same reagents. At most 0.04% (400 ppm).

Loss on drying (5.2.9). To determine in an oven at 105 °C for 4 hours. At most 0.5% for the anhydrous substance, and between 12 and 15% for the hydrated substance.

DOSAGE

To dissolve 1 g of the sample in 25 mL of water. To titrate with M hydrochloric acid, by using 0.2 mL of methyl orange SI. Each mL of M SV hydrochloric acid is equal to 52.99 mg of Na_2CO_3 or 62.0 mg of $\text{Na}_2\text{CO}_3 \cdot \text{H}_2\text{O}$.

PACKAGING AND STORAGE

In airtight containers.

LABELLING

To comply with the legislation in force.

CATEGORY

Pharmacotechnical adjuvant (alkalizing agent).

CARBOPLATIN, INJECTABLE SOLUTION

It contains at least 90.0 % and at most 110 % of the amount stated of $\text{C}_6\text{H}_{12}\text{N}_2\text{O}_4\text{Pt}$.

IDENTIFICATION

A. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using G silica gel as support and a mixture of acetone and water (80:20) as mobile phase. To apply separately to the plate 10 µL of each of the solutions as described below.

Sample solution: injectable solution; if necessary, diluted with water to obtain 10 mg/mL carboplatin solution.

Standard solution: 10 mg/ mL carboplatin solution SQR in water.

Note: to use the *Sample solution* and *Standard solution* within 2 hours.

To develop the chromatogram. To remove the plate, and let it drying for 2 hours. To nebulize with mixture of 5.6 g of tin chloride (II) in 10 mL of hydrochloric acid (dissolution may not be complete; if necessary, filter it) and 90 mL of water containing 1 g of potassium iodide, prepared immediately before using. To heat the plate for 10 minutes at 100 °C. The principal spot in the chromatogram with *Sample solution* corresponds in size, color, and position to that obtained with the *Standard solution*.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

pH (5.2.19). 5.0 to 7.0.

PURITY TESTS

Limit for cyclobutane-1,1-dicarboxylic acid To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultra-violet detector at 220 nm; 300 mm chromatograph column and 3.9 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (10 µm), at room temperature; flow of *Mobile phase* of 2.0 mL/min.

Solution (1): to dissolve 8.5 g of tetrabutylammonium sulfate in 80 mL of water, add 3.4 mL of phosphoric acid, and adjust the pH to 7.55 with sodium hydroxide.

Mobile phase: mixture of water, acetonitrile, and *Solution (1)* (88:10:2). To degas and filter it.

Sample solution: to dilute the injectable solution in water to obtain a 1 mg/mL carboplatin solution. To use this solution within 2 hours.

Standard solution: 0.01 mg/mL cyclobutane-1,1-dicarboxylic acid solution in water.

Resolution solution: mixture of *Sample solution* and *Standard solution* (1:1).

The resolution between carboplatin and cyclobutane-1,1-dicarboxylic acid peaks should be greater than 2.5. The relative standard deviation for replicate areas of the peaks related to cyclobutane-1,1-dicarboxylic acid should not be greater than 2.0%.

Procedure: to separately inject 100 µL of *Standard solution*, *Sample solution*, and *Resolution solution*. To record the chromatograms at least two and a half times the retention time of the peak corresponding to carboplatin. The area under the peak corresponding to cyclobutane-1,1-di-

carboxylic acid obtained in the chromatogram of *Sample solution* is not greater than the area under the peak obtained in the chromatogram of *Standard solution* (1.0%).

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

Bacterial endotoxins (5.5.2.2). At most 0.54 UE/ mg of carboplatin.

DOSAGE

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 230 nm; 300 mm chromatograph column and 3.9 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), at room temperature; flow of *Mobile phase* of 2.0 mL/min.

Mobile phase: to prepare a mixture of acetonitrile and water (87:13). To degas and filter it.

Sample solution: injectable solution dilute in water to obtain a 1 mg/mL carboplatin solution.

Standard solution: 1 mg/ mL carboplatin solution SQR in water.

Note: to use the *Sample solution* and *Standard solution* within 2 hours.

The retention factor is not less than 4.0 for the major peak, the number of theoretical plates is not less than 5000, and tailing factor is not greater than 2.0. The relative standard deviation for replicate areas of the peaks recorded for carboplatin standard is not greater than 2 %.

Procedure: to separately inject 10 µL of *Sample and standard solutions*, record the chromatograms, and measure the areas under the peaks. To calculate the content of C₆H₁₂N₂O₄Pt in the injectable solution, from the responses obtained for the *Sample and standard solutions*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light, and no contact with metals.

LABELLING

To comply with the legislation in force.

CARDAMOM Cardamomi semen

Elettaria cardamomum (L.) Maton - ZINGIBERACEAE

The vegetable drug consists of seeds marketed inside the fruit. The seeds should be used immediately after breaking fruit. It contains at least 5% of volatile oil.

CHARACTERISTICS

Organoleptic characteristics. When crushed, the seeds have a strong odor and slightly bitter and particular taste.

MACROSCOPIC DESCRIPTION OF THE FRUIT

Trilocular dehiscent capsule fruit, 10.0 mm to 25.0 mm long and 5.0 mm to 10.0 mm wide, clear-yellow color, yellow-green to yellow-gray, ovoid or oblong in side view, trigona or rounded in cross section, with apical narrowed-tubulosa portion about 1 mm to 2 mm, with or without visible scar in the floral organs and with scar or remaining in the basal pedicel. Thin, coriaceous, and tasteless pericarp. Epicarp, in front view, with numerous protruding longitudinal strips. In cross section, trilocular capsule, each locule with two to seven seeds of axial placentation adhered to each other, forming three double rows, separate by thin, membranous, and paleocarpel walls. Seeds are marketed inside the fruit, which is immature when collected and artificially sun ripened or in greenhouses.

MACROSCOPIC DESCRIPTION OF THE SEED

Seeds of parietal placentation (**Figure 1**), anatropous, hard, ovoid, triangular or sub-cylindrical, irregularly angular and transversely wrinkled, one side is convex and the other is excavated, 2.0 mm to 4.0 mm long and 2.0 mm to 3.0 mm wide, black, gray or reddish-brown, covered with a thin aril, thin and colorless to whitish. Generally the seeds are clumped into masses, corresponding to the rows limited by carpels. With the aid of the lens, in cross section, we can see in each seed aril, seed coat, perisperm, endosperm, and embryo.

MICROSCOPIC DESCRIPTION OF THE SEED

In front view, the aril has very narrow rectangular cells, longitudinally elongated and irregularly fusiform, thin-walled, arranged in rows, with lipid droplets. The epidermis has cells tangentially elongated, fusiforms, thick and anticline walls with pits and arranged in an oblique angle in relation to aril, with lipid droplets.

For transparency, the reserve parenchyma is visible, formed by massive parenchymal cells of different shapes with thin and corrugated walls that are rich in lipid droplets. In cross-section, aril shows small and flat cells with thin walls. The epidermis consists of small and quadrangular cells with thick walls and has some lipid droplets. The hypodermis have rectangular cells, in general, tangentially flat, with thin walls, divided into fewer layers, usually irregular as for the number of cells. The reserve parenchyma

has some massive cell layers, in different shapes, usually polygonal to rectangular, thin-walled, containing lipid droplets. A regular or not layer, with parenchymal cells of lower volume, can internally occur in the reserve parenchyma. It is followed by one or more layers of columnar sclerenchyma cells, with the inner periclinal and anticlinal walls very thick and orange, with a bowl-shaped lumen with or without silica crystals. The perisperm is whitish and the first layers of parenchyma cells are small, tangentially flattened, thin-walled, filled with starch grains, followed by many layers of parenchyma cells of irregular shape, usually columnar or polygonal, massive, with thin walls and corrugated anticlinal containing large amount of starch grains of very small size, lipid droplets, and crystals of calcium oxalate. The endosperm has elongated parenchyma cells, in various shapes, with thin walls, divided into several parallel layers, completely surrounding the embryo and showing lipid droplets and aleurone grains. The embryo is small, oval, and dark in color, its cells are round to ovoid, thin-walled, with aleurone grains and lipid droplets.

MICROSCOPIC DESCRIPTION OF SEED POWDER

The powder meets all the requirements for the species, unless the macroscopic characters, and it must not contain elements of the pericarp. The following are characteristics by adding chloral hydrate: brownish-gray color, fragments of aril, in front view; fragments of epidermis in front view; fragments of aril from the epidermis and reserve parenchyma, viewed by transparency in front view; fragments of epidermis and reserve parenchyma, viewed by transparency in front view; fragments of epidermis, in cross section; fragments of hypodermis in front view; fragments of reserve parenchyma, in front view; fragments of reserve parenchyma in cross section; fragments of sclerenchyma in front view; fragments of sclerenchymatic layer, in cross section; fragments of sclerenchymatic layer perisperm and in cross section; fragments of perisperm in front view; fragments of perisperm, in cross section; fragments of endosperm, in cross section; isolated parenchyma cells; fibers isolated in longitudinal view; isolated and/or grouped starch grains; crystals of isolated calcium oxalate.

IDENTIFICATION

To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF₂₅₄ with 250 µm thick as support

and a mixture of toluene and ethyl acetate (93:7) as mobile phase. To separately apply to the plate, as band, 20 µl of *Solution (1)* and 10 µL of *Solution (2)* freshly prepared, as described below.

Solution (1): 0.1 g of the grinded drug, add 2 mL of methylene chloride. To stir for 15 minutes, filter, and concentrate it to dryness in a water bath at approximately 60 °C. To resuspend in 2 mL of toluene.

Solution (2): to dissolve 10 µg of terpenila acetate, 10 µg of 1,8-cineole, and 10 µg of linalool in 1 mL of toluene.

To develop the chromatogram. To remove the plate, and let it drying. Then, nebulize the plate with a solution of sulfuric vanillin SR and leave it in an oven at 100 °C - 105 °C for about 5 minutes. The blue spots obtained with *Solution (1)*, at the top of the chromatogram (Rf about 0.7), in the middle area (Rf about 0.5), and the lower area (Rf about 0.4) correspond in position and color to those obtained with *Solution (2)* for the terpenila acetate, 1,8-cineole, and linalool, respectively. Other spots may be observed in the upper half of the chromatogram, one with violet color, near the front, with Rf about 0.9, corresponding to limonene. Among the spots with Rf about 0.8 and 0.9 there is a blue spot. In the bottom half of the chromatogram, we can observe several violet, blue, and green spots.

PURITY TESTS

Total ashes (5.4.2.4). At most 4.0%.

DOSAGE

Volatile oils

To proceed as described in *Determination of volatile oils in vegetable drugs (5.4.2.7)*. To use 500 mL flask containing 200 mL of water as distillation liquid. To add 0.5 mL of xylene through the side opening k. To use the seed just after removal from the fruit without grinding. To immediately proceed to determine the volatile oil from 20 g of the drug. To distill for 5 hours.

PACKAGING AND STORAGE

In tightly closed container, protected from light and heat.

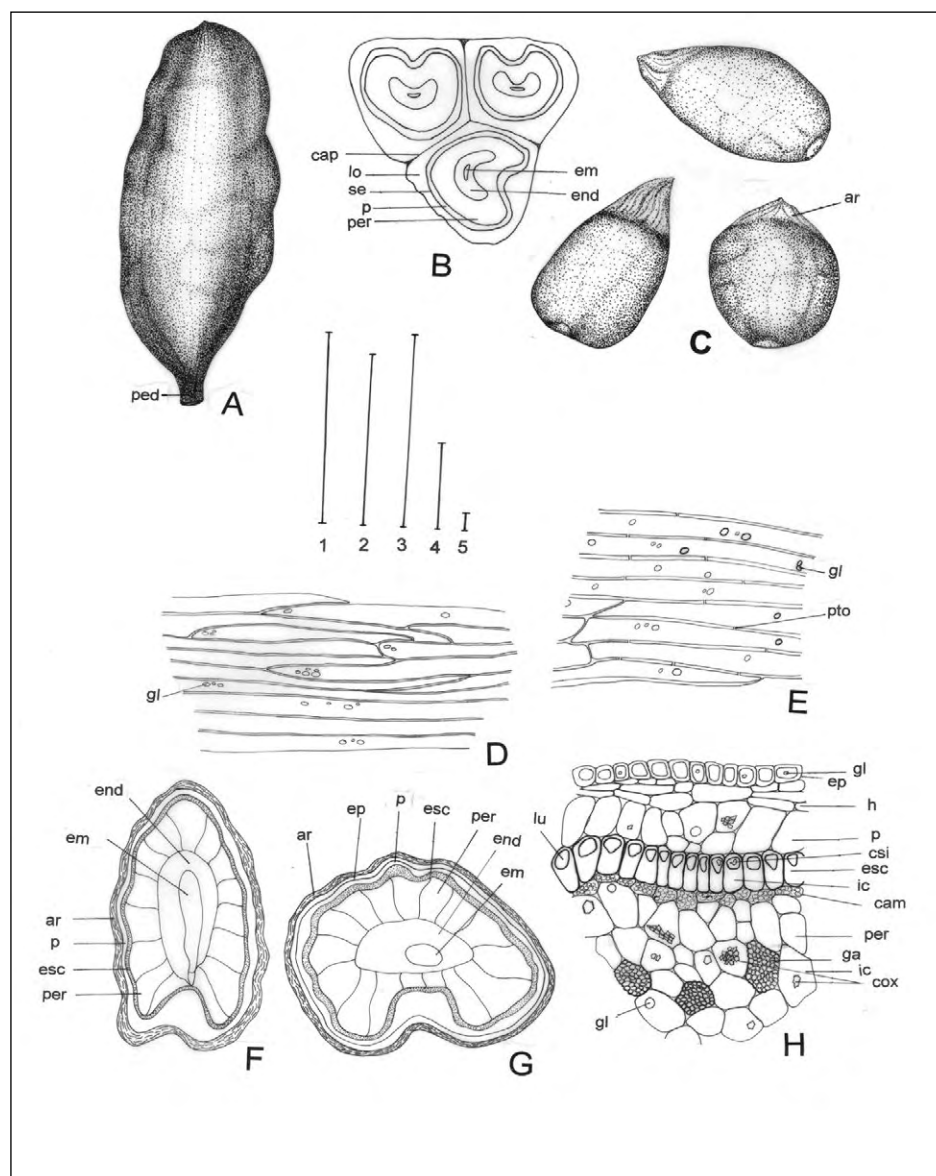


Figure 1 - Macroscopic aspects of fruit and seed and microscopic of seed of *Elettaria cardamomum* (L.) Maton

Additional information of **Figure 1**. Scales correspond to 1cm in **A**(rule 1); in **B**, 0.5 cm (rule 2), in **C**, 0.5 cm (rule 3); in **D**, **E**, and **H**, 100 µm (rule 4); and **F** and **G**, 100 µm (rule 5).

A - general appearance of the fruit, in side view: peduncle (ped). **B** - Schematic representation of the fruit in cross-section: carpel (cap); embryo (em); endosperm (end); locule (lo); parenchyma (p); perisperm (per), seed (se). **C** - general appearance of seeds, in side view: aril (ar). **D** - Detail of portion of the aril in front view: lipid droplet (gl). **E** - detail of portion of the epidermis, in front view: lipid droplet (gl); pit (pto). **F** - schematic representation of the seed in longitudinal section: aril (ar); embryo (em); endosperm (end); sclerenchyma (esc); parenchyma (p); perisperm (per). **G** - Schematic representation of the seed in cross-section: aril (ar); embryo (em); endosperm (end); epidermis (ep); sclerenchyma (esc); parenchyma (p); perisperm (per). **H** - Partial detail of portion of the seed, in cross-section: starch layer (cam); crystal of calcium oxalate (cox), crystal of silica (csi); epidermis (ep), sclerenchyma (esc); crystal idioblast (ic), starch grains (ga); lipid droplet (gl); hypodermis (h), lumen (lu); parenchyma (p); perisperm (per).

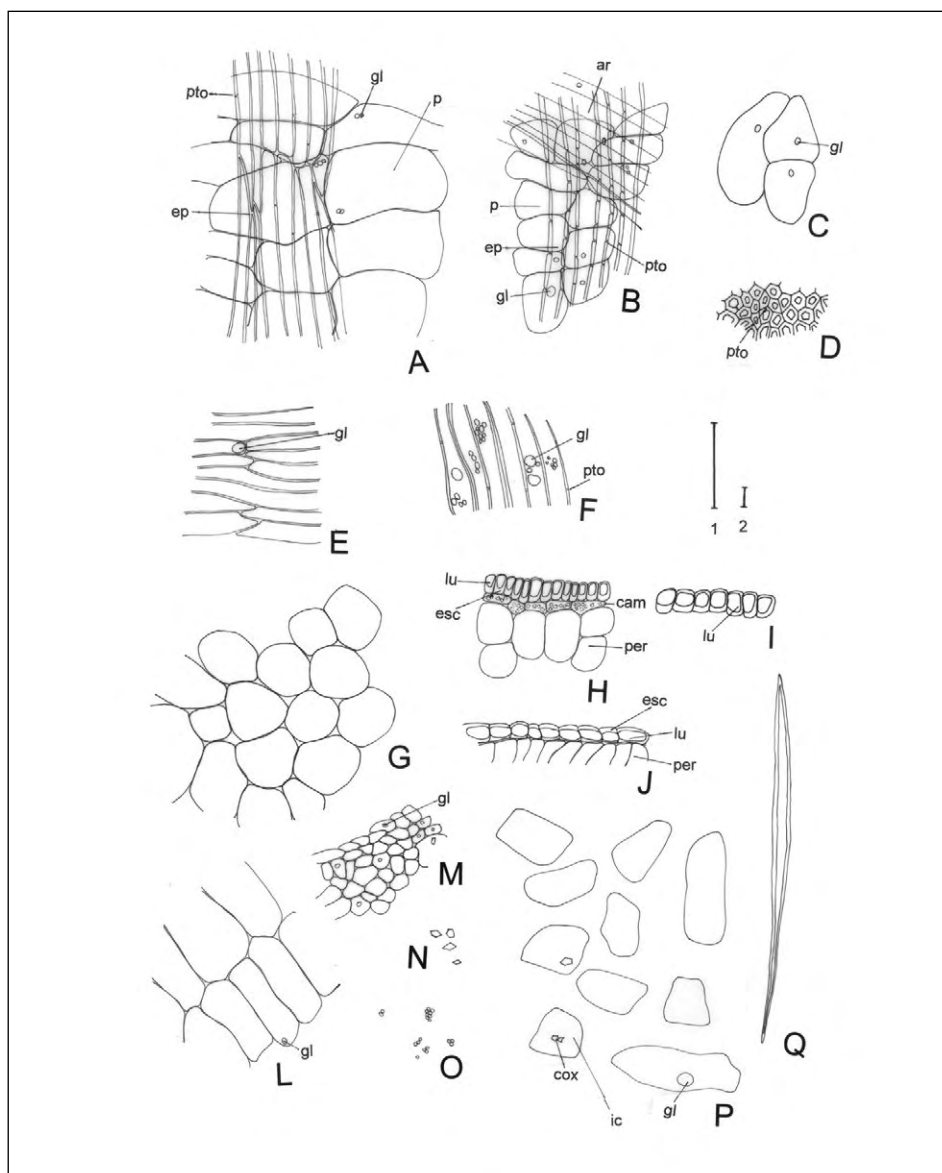


Figure 2 - Microscopic aspects of seed powder of *Elettaria cardamomum* (L.) Maton

Additional information of Figure 2. Scales correspond in A to P μm (rule 1); in Q, 100 μm (rule 2).

A - fragment of epidermis and reserve parenchyma, observed by transparency in front view: epidermis (ep), lipid droplet (gl); parenchyma (p); pit (pto). **B** - fragment of aril, epidermis, and parenchyma, in front view: aril (ar); epidermis (ep), lipid droplet (gl); parenchyma (p); pit (pto). **C** - fragment of endosperm in cross section: lipid droplet (gl). **D** - fragment of sclerenchyma in front view: tip (pto). **E** - fragment of aril, in front view: lipid droplet (gl). **F** - fragment of epidermis, in front view: lipid droplet (gl); pit (pto). **G** - fragment of parenchyma in front view. **H** - fragment of sclerenchymatic layer and perisperm in cross-section: starch layer (cam); sclerenchyma (esc); lumen (lu); perisperm (per). **I** - fragment of sclerenchymatic layer in cross-section: lumen (lu). **J** - fragment of sclerenchymatic layer with perisperm rests in cross-section: sclerenchyma (esc); lumen (lu); perisperm (per). **L** - fragment of parenchyma in cross section: lipid droplet (gl). **M** - fragment of hypodermis in front view: lipid droplet (gl). **N** - crystals of isolated calcium oxalate. **O** - isolated and/or grouped starch grains. **P** - parenchymal cells and isolated crystal idioblasts: crystal of calcium oxalate (cox), lipid droplet (gl); crystal idioblasts (ic). **Q** - isolated fiber in longitudinal view.

CARQUEJA

Baccharis trimerae herbae

Baccharis trimera (Less.) DC. - ASTERACEAE; 09896

The drug consists of winged stems, desiccated, and fragmented containing minimum 1.7% of total caffeic acids calculated as chlorogenic acid.

SCIENTIFIC SYNONYM

Baccharis genistelloides var. *trimera* (Less.) Baker

POPULAR NAMES

Carqueja-bitter.

CHARACTERISTICS

Organoleptic characteristics. The shoots have a bitter taste.

MACROSCOPIC DESCRIPTION

Cylindrical, tri-winged branches up to 1 m in length, with leafless or rare sessile leaves and small nodes. Green wings, glabrous to the naked eye, membrane, 0.5 cm to 1.5 cm wide; wings of flower branches, narrower than the other. Dioecious plants, so when flowering branches are present, these should only be only staminate or pistillate. Inflorescences, when present, cluster-type section, yellowish-white, numerous, sessile, arranged along the upper branches, forming interrupted spikes with flat receptacle, not paleaceous; flowers with pappus, hairy and white.

Clusters staminate with involucre bracts from 0.4 cm to 0.5 cm long, pluriserial, and the external ones are gradually smaller, oval and glabrous; tubulose corolla flower, pentamerous, up to 0.4 cm long and limb divided into long lacinias, volute, five stamens, epipetalous, synantherous; stunted pistil. Clusters pistillate with involucre bracts up to 0.6 cm long, pluriserial, lanceolate, glabrous, filiform corolla flower, pentadentate, with up to 0.4 cm long, bifurcated stylet longer than a corolla, linear-lanceolate, pubescent on the back, with divergent branches, inferior ovary, bicarpelary, gamocarpelary unilocular monospermic; achene-fruit, up to 0.2 cm long, with 10 longitudinal strips.

MICROSCOPIC DESCRIPTION

The stem has three wings or divergent stem expansions, with pronounced strips between each wing. The epidermis is uniseriate, with rectangular cells covered by a striated cuticle. In front view, the epidermal cells are polygonal with winding walls. Few stomata and some trichomes occur, the latter formed by two basal cells and head with

2 sets of 4 cells each. The chlorenchyma cells are elliptical to circular, radially loosely distributed, and arranged in 3 or 4 layers, interrupted in the collenchyma region and secretion schizogenous canals. The chlorenchyma, which

interpolates with chlorenchyma, is modified according to the age of the stem. In young stems, i.e. up to the fifth node, it extends from the epidermis to the secretory canals, partially wrapping them, whereas in the regions between the canals can occur as continuous layer and subepidermal.

In mature stems, that is, from the fifth node, it is distributed to canals in areas opposite to secretory cells, and collenchyma cells may partially or totally become a fiber grouped into three layers: in the remote areas of secretory canals, the collenchyma layer is not modified. The secretory canals, always accompanied by collenchyma, are located externally to the endoderm, thus occurring predominantly opposed to protophloem fibers.

The number of secretory canals ranges from 3 to 10 with epithelial cells from 3 to 14 with thin walls. Internally to the chlorenchyma there is a continuous layer of endoderm with Casparian strips. The vascular system is collateral, thus showing secondary character already in the young branches. The strands of protophloem fibers, from 9 to 20, are formed by up to 7 cell layers with thick and lignified walls. Internally to the xylem, there is a band of fibers almost continuous, with variable thickness, located near the medullary parenchyma.

The pith is relatively large, with spherical or elliptical large cells, with slightly thickened walls, with few intercellular spaces containing prismatic crystals of calcium oxalate, in various shapes, such as acicular, octahedral, and rectangular crystals, arranged predominantly in zones near the xylem. In cross section, the wings exhibit dorsiventral structure with palisade and spongy parenchyma. The epidermis is uniseriate with characteristics similar to those described for the stem.

Anomocytic and anisocytic stomata occur, both spread on both sides of the epidermis. Trichomes predominantly occur in the region of the edges of the wings and the junction with the axis of the stem. There are 4 basic types: a: multicellular, uniseriate, erect, with 3 cells in the body and a conical, erect or inclined apical; b: multicellular, uniseriate, erect, with 5 cells in the body and a conical apical cell with dilated base; c: multicellular, uniseriate, with 1-3 cells in the body and a rounded, globose apical cell that may sometimes be bent; d: multicellular, uniseriate, bent, with 3 cells in the body and a globose apical cell with thickened wall.

The palisade parenchyma consists of elliptical cells, arranged in 3-5 layers on the middle-upper and middle-lower portion of each wing, with their major axes anticlinally oriented. There are up to 18 bundles side in each wing, arranged linearly, by alternating large and small one, accompanied by a few fibers and surrounded by a parenchymatous sheath. Each bundle is accompanied by large 1-2 secretion schizogenous canals, with 4 to 14 epithelial cells, with not thickened walls. The collenchyma is restricted to a subepidermal layer next to the edge of the wing rib; below it, there is a group of fiber with walls strongly thick

involve 3 secretion canals with different sizes.

MICROSCOPIC DESCRIPTION OF POWDER

The powder meets all the requirements for the species, unless the macroscopic characters. Characteristics: fragments of epidermis with striated cuticle and anomocytic and anisocytic stomata besides the trichomes described; portions of the medullary parenchyma with crystals of calcium oxalate; portions of fibers accompanied by secretory canals. Depending on the degree of fragmentation, portions of winged branches with and without clusters may occur.

IDENTIFICATION

To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ with 250 µm thick and a mixture of toluene and ethyl acetate (70:30) as mobile phase. To separately apply to the plate, as band, 10 µl of *Solution (1)* and *Solution (2)* freshly prepared, as described below.

Solution (1): to stir 2 g of the sample with 10 mL of methylene chloride for 10 minutes. To filter and discard the methylene chloride solution. To extract the residue with 10 mL of methanol by magnetic stirring at 40 °C. To filter and concentrate to residue in a rotary evaporator (40 °C). To resuspend the residue in 2 mL of methanol.

Solution (2): to dissolve 1 mg of quercetin SQR and 1 mg of 3-O-methylquercetin SQR in 0.1 mL of methanol.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (365 nm). The principal spot obtained in the chromatogram with *Solution (1)* corresponds in position and intensity to that obtained with *Solution (2)*. Then, nebulize the plate with 1% aminoethanol diphenyl borate (w/v) in methanol. The spots corresponding to quercetin and 3-O-methylquercetin examined in the daylight have orange color.

PURITY TESTS

Foreign matter (5.4.2.2). At most 2.0%.

Water (5.4.2.3). At most 12.0%.

Total ashes (5.4.2.4). At most 8.0%.

DOSAGE

Total caffeic acids

To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 325 nm; pre-column packed with octadecylsilane silica; 75 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (4 µm), kept at room temperature; flow of *Mobile phase* of 0.6 mL/min.

Eluent A: mixture of acetonitrile, water, and trifluoroacetic acid (5:95:0.05).

Eluent B: acetonitrile.

Mobile phase gradient: to adopt the gradient system described in the following table:

Time (minutes)	Eluent A (%)	Eluent B (%)	Elution
0 - 30	100 → 57	0 → 43	linear gradient
30 - 35	57 → 0	43 → 100	linear gradient
35 - 36	0 → 100	100 → 0	linear gradient
36 - 42	100	0	isocratic

Sample solution: To accurately weigh about 0.5 g of dried and grinded drug (250 µm) in a 50 mL beaker. To add 10 mL of a mixture of ethanol and water (50:50) and put it in water bath (40 °C) for 10 minutes. To cool down the extract at room temperature. To use cotton to filter the extract into a 25 mL volumetric flask. To extract again the drug residue collected in the cotton with 10 mL of a mixture of ethanol and water (50:50) and put it in water bath (40 °C) for 10 minutes. To cool down an filter to same 25 mL volumetric flask. To top up the volume with a mixture of ethanol and water (50:50). To dilute 0.12 mL of the resulting solution in 1 mL of acetonitrile, water, and trifluoroacetic acid (5:95:0.05).

Stock solution: To dissolve 5.6 mg of chlorogenic acid in 5 mL of methanol.

Solutions for calibration curve of chlorogenic acid: to dilute a aliquot of 0.2 mL of *Stock solution* with a mixture of acetonitrile and water (5:95) to 0.4 mL, to obtain a 0.56 mg/mL solution. To successively dilute the previous solution in a mixture of acetonitrile and water (5:95), to obtain 0.28 mg/mL, 0.14 mg/mL, 0.07 mg/mL, 0.035 mg/mL, 0.017 mg/mL, and 0.0085 mg/mL concentrations.

Procedure: to separately inject 10 µL of *Solutions for calibration curve of chlorogenic acid* and *Sample solution*. To record the chromatograms and measure the areas under the peaks. The approximate relative retention times compared to chlorogenic acid are: 3,4-dicaffeoylquinic = 1.69; 3,5-dicaffeoylquinic = 1.76; 4,5-dicaffeoylquinic = 1.84. To calculate the content of the sample from the linear equation obtained with *Solutions for calibration curve of chlorogenic acid*. The result is expressed as the mean of the determinations in grams for chlorogenic acid per 100 g of drug (%), by taking into account the water determination. Other peaks may be present in the sample.

PACKAGING AND STORAGE

In tightly closed glass container, protected from light and heat.

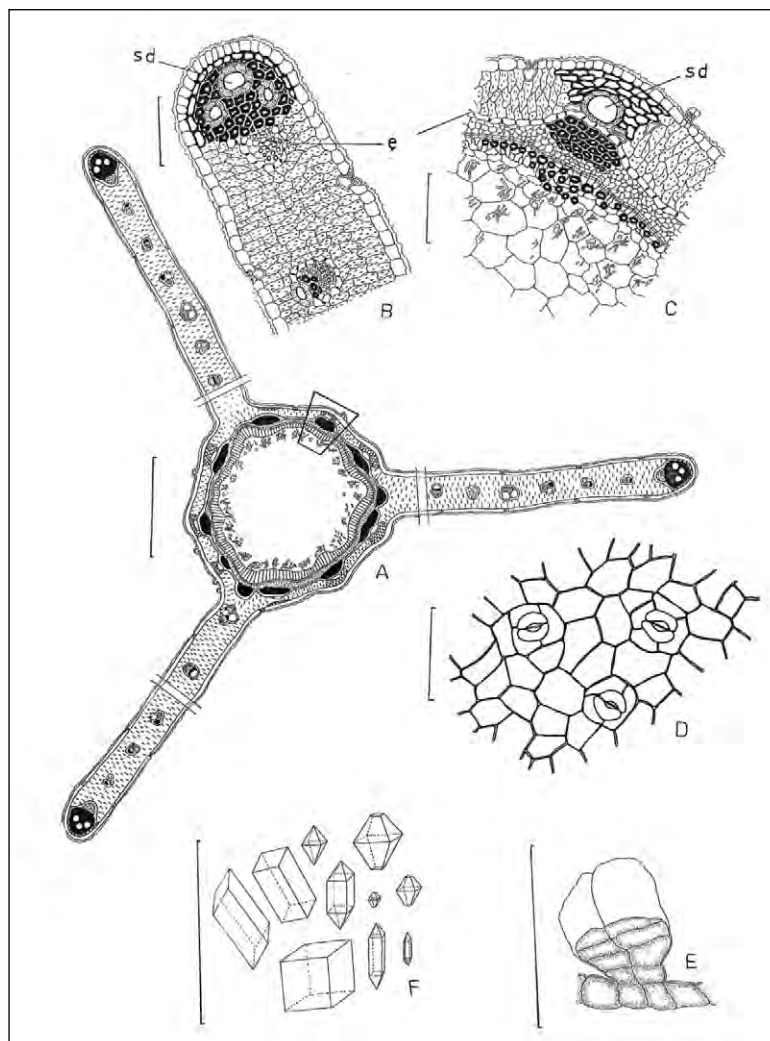


Figure 1 - Macroscopic and microscopic aspects in *Baccharis trimera* (Less.) DC

Additional information of **Figure 1**.

A - representative scheme of the stem with three wings in cross-section. **B** - detail of edge of the wing; endoderm (e); schizogenous canal (sd). **C** - detail of a portion of the stem in cross section, indicated in A; endoderm (e); schizogenous canal (sd). **D** - detail of wing epidermis with striated cuticle and anisocytic stomata. **E** - glandular trichomes. **F** - crystals of calcium oxalate, octahedral prism-shaped and rectangular prisms.

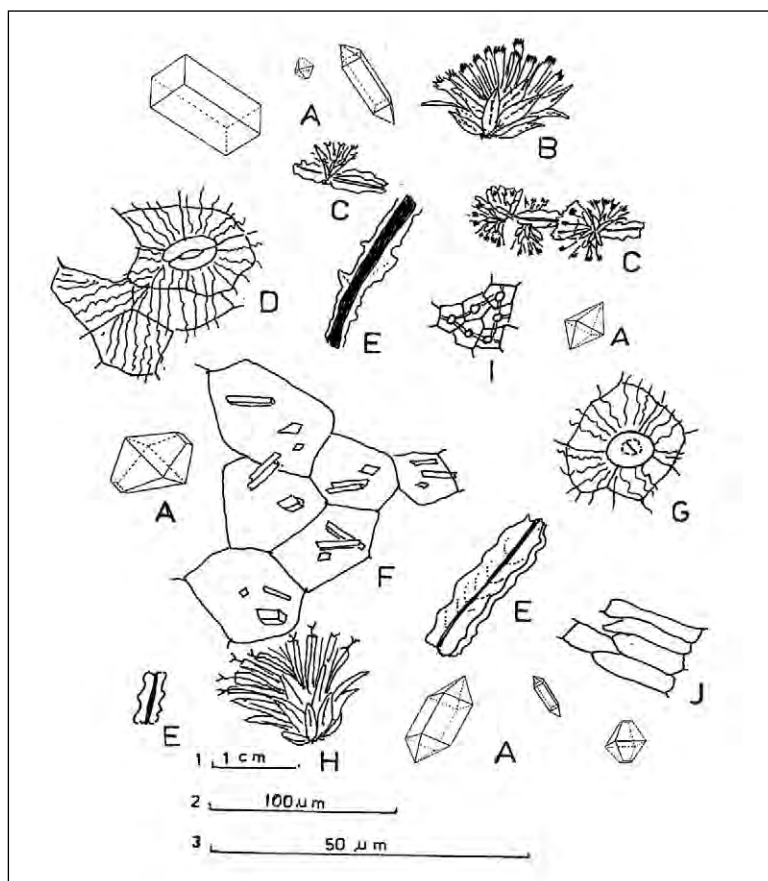


Figure 2 - Microscopic aspects in *Baccharis trimera* (Less.) DC

Additional information of **Figure 2**. Rules correspond to. 1 (B, C, H, E); 2 (D, F, G, I, J); 3 (A).

A - crystals of calcium oxalate. B - clusters of staminate flowers. C - fragment of winged stem with cluster. D - portion of wing epidermis. E - fragment of stem. F - portion of medullary parenchyma with crystals. G - fragment of epidermis with glandular trichomes, in front view. H - cluster of pistillate flowers. I - detail of fibers. J - fragment of palisade parenchyma.

CARRAGEENAN carrageenan; 01798

Carrageenan
[9000-07-1]

Carrageenan is a hydrophilic colloid obtained by extraction with water or an aqueous alkaline solution of some members of *Rhodophyceae* (red seaweeds) class. It is used as a gelling, emulsifier, stabilizer, suspending, and viscosity increase agent. It is a mixture of sulfated polysaccharides, in general consisting of esters potassium sulfate, sodium, calcium, magnesium, and ammonium, and copolymers of galactose and 3,6-anhydrogalactose. Structural families are identified by the position of sulfate group and the presence or absence of anhydrogalactose. These hexoses are alternating in α -1, 3 and β -1,4 in the polymer. The copolymers prevalent in the colloid are designated carrageenan of type *kappa*, *iota*, and *lambda*.

The family *kappa* consists of *kappa* and *iota*. *Kappa*-carrageenan is generally D-galactose-4-sulfate and alternates 3,6-anhydrous-D-galactose and *iota*-carrageenan is similar,

except that the 3,6-anhydrogalactose is sulfated at carbon 2. Between *kappa*-carrageenan and *iota*-carrageenan there are different intermediate compositions, depending on the degree of sulfation at carbon 2. Due to the helical tertiary structure, which allows gelation, the family *kappa* has greater commercial importance. In the *lambda*-carrageenan, the monomer units are generally D-galactose-2-sulfate (1,3 binding) and D-galactose-2,6-disulfate (1,4 binding). This carrageenan is not gelling. The content of the sulfate ester in the carrageenan ranges from 18% to 40%.

DESCRIPTION

Physical characteristics. White or yellowish-white powder; almost odorless

Solubility. Soluble in hot water (80 °C). Dispersed more easily if moistened first in ethanol, glycerol, and syrup.

Physical and chemical constants.

Viscosity (5.2.7): at least 5 cP at 75 °C. To transfer 7.5 g of the sample to a 600 mL beaker previously weighed, add 450 mL of water, and disperse by stirring for 15 minutes.

To add water to 500 g in weight and heat in water bath with continuous stirring until 80 °C is reached. To add water to adjust the loss by evaporation, cooling to a range from 76 °C to 77 °C, and keep it in a water bath at a constant temperature of 75 °C. To use a suitable rotational viscometer and adapt a rotary body (1.88 cm diameter and 6.51 cm height) with immersion depth of 8.10 cm. Rotary body to rotate 6 times in the sample at 30 rpm, then make scale reading. To convert the reading to centipoises by multiplying the constant of rotary body and speed.

IDENTIFICATION

A. To prepare a 2% uniform dispersion (w/v) of the sample in water and heat it in a water bath at 80 °C (*Preparation A*). After cooling down, the dispersion becomes more viscous and may form a gel.

B. To add 4 drops of 10% potassium chloride (w/v) to 10 mL of *Preparation A* obtained in *Identification*, test A. Such *Preparation* must be hot. A fragile texture of the gel indicates the predominance of kappa-type carrageenan; an elastic gel indicates predominance of iota-type carrageenan. If the solution does not form gel, the carrageenan predominant is *lambda*.

C. To dilute a portion of *Preparation A* obtained in *Identification*, test A., in 4 parts of water and add 2-3 drops of 0.05% methylthioninium chloride (w/v) in ethanol. A fibrous blue precipitate is formed.

D. To obtain the infrared absorption spectrum (5.2.14) of the gelling and non-gelling fractions by using the procedure described below. To disperse 2 g of the sample in 200 mL of 2.5% potassium chloride (w/v) and stir for 1 hour. To let it rest for 18 hours, stir again for 1 hour, and transfer to a centrifuge tube. Should the transfer be not performed due to too viscous dispersion, dilute with 200 mL of 2.5% potassium chloride (w/v). To centrifuge at approximately 1000 g for 15 minutes. To remove the clear supernatant, by resuspending the residue in 200 mL of 2.5% potassium chloride (w/v), and centrifuge again. To clot the combined supernatants by adding 2 volumes of 90% ethanol (v/v). To recovery the clot and the sediment. To wash the clot with 250 mL of 90% ethanol (v/v). To remove by pressure the fluid in excess from the clot and dry it at 60 °C for 2 hours. The material thus obtained is a non-gelling fraction, the lambda carrageenan. To disperse the sediment in 250 mL of cold water, heat to 90 °C for 10 minutes, and cool down to 60 °C. To clot the mixture with 2 volumes of 90% ethanol (v/v). To recovery, wash, and dry the clot as described above. The material thus obtained is a gelling fraction, the kappa and iota carrageenan. To prepare for each fraction films of 5 mm thick (when dry) in a non-adherent uniform surface and obtain the infrared absorption spectra for each film. Carrageenan has a broad absorption band typical of the polysaccharides in the region from 1000 cm to 1100 cm. The absorption maxima are 1065 cm and 1020 cm for the gelling and non-gelling fraction, respectively. Further characteristic absorption bands and their relative intensities to absorbance at 1050 cm are shown in the following table.

Wavelength (cm ⁻¹)	Molecular fraction	Absorbance relative to 1 050 cm ⁻¹		
		Kappa	Iota	lambda
1220 a 1260	Ester Sulfate	0,7 a 1,2	1,2 a 1,6	1,4 a 2,0
928 a 933	3,6-Anidrogactose	0,3 a 0,6	0,2 a 0,4	0 a 0,2
840 a 850	Galactose-4-sulfate	0,3 a 0,5	0,2 a 0,4	---
825 a 830	Galactose-2-sulfate	---	---	0,2 a 0,4
810 a 820	Galactose-6-sulfate	---	---	0,1 a 0,3
800 a 805	3,6-anhydrogalactose-2-sulfate	0 a 0,2	0,2 a 0,4	---

PURITY TESTS

Acid insoluble matter. To transfer 2 g of the sample accurately weighed to a 250 mL beaker containing 150 mL of water and 1.5 mL of sulfuric acid. To cover with watch glass and heat in a steam bath for 6 hours. To rub the walls of the beaker with a glass rod with rubber policeman, and replace any water lost by evaporation. To add 500 mg accurately weighed from an auxiliary filtration agent. To filter the preparation into a funnel with filter plate containing a layer of 2,4 cm fibreglass

previously desiccated and weighed. To wash the residue several times with hot water. To dry at 105 °C for 3 hours, cool down in a desiccator, and weigh it. The difference between the final weight and the sum of the weights of the funnel, fiber glass and auxiliary filtration agent is the weight of acid insoluble matter. At most 2%.

Arsenic (5.3.2.5). At most 0.0003% (3 ppm).

Lead (5.3.2.12). At most 0.001% (10 ppm).

Heavy metals (5.3.2.3). To use *Method III*. At most 0.004% (40 ppm).

Loss on drying (5.2.9). To dry under pressure up to 10 mm Hg at 70 °C for 18 hours. At most 12.5%.

Total ashes (5.4.2.4). At most 35%.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). Total bacteria: at most 200 UFC/g.

Survey of pathogenic micro-organisms (5.5.3.1.3).

Absence of *Salmonella* sp and *Escherichia coli*.

PACKAGING AND STORAGE

In airtight containers, preferably in a cool place.

LABELLING

To comply with the legislation in force.

CATEGORY

Excipient.

HORSE CHESTNUT Hippocastani semen

Aesculus hippocastanum L. - HIPPOCASTANACEAE

The vegetable drug consists of desiccated mature seeds containing at least 3.0% of triterpene glycosides, calculated as anhydrous escin.

MICROSCOPIC DESCRIPTION

In front view, the seed coat shows a yellowish-brown epidermis, with uniform cells, mostly polygonal or rounded. In cross section, the epidermal cells are columnar and compact with smooth and thick cuticle and outer periclinal walls much thicker than the internal ones. Below we observe up to 4 distinct zones. The first one, the outermost, is formed by a few layers of yellow-brownish collenchymatic cells. The second one is formed by 10 or more layers of sclerenchyma cells, flat tangentially. The third one consists of 4-10 layers of parenchyma cells colorless, more polyhedral, and walls thinner than the previous ones, with intercellular spaces. In the outer layers of this region we can observe vascular bundles. The fourth region, when present, is formed by a few layers of tangentially flattened cells with thickened walls. The cotyledons are composed of starch parenchyma covered by a single-layered epidermis. In front view, the epidermal cells of the cotyledons are polygonal. The reserve parenchyma has ovate to elliptic cells, with thin walls, smaller in the outer region and gradually larger inside, containing starch grains and lipid droplets. There are delicate vascular bundles in this parenchyma; vessel elements are narrow and have helical wall thickening. The starch grains are simple and can be spherical, oval and piriform, with different sizes, ranging from 2 µm to 80 µm diameter. The smaller grains have hilum usually in form of dot; others, in large amount, show hilum cross-shaped, branched or starlike.

CHARACTERISTICS

Organoleptic characteristics. Odorless seed; faint odor, if broke. Bark with astringent taste and bitter embryo, producing salivation when chewed.

MACROSCOPIC DESCRIPTION

The seeds are hard and exalbuminous from 2.5 cm to 4.0 cm, irregularly subspherical, flattened at both poles or only in the hilum, or irregularly flat by desiccation. The fractured seed coat is brown, brittle, 1.0 mm to 2.0 mm thick, involving the embryo, which has the a small radicle and two large corneal and starch cotyledons, pale brown externally and almost white in the fracture. Absent endosperm. The coat is smooth, coriaceous, brittle, easily detachable from the embryo in some parts, reddish-brown or light brown, usually glossy, unusual opaque, and with large, clear spot corresponding to the hilum. The radicle is curved and occupies a depression on the commissure of the cotyledons or the dorsal side of one of the two cotyledons and is clearly prominent in the outer surface.

MICROSCOPIC DESCRIPTION OF POWDER

The powder meets all the requirements for the species, unless the macroscopic characters. Characteristics: fragments of irregular coats, golden yellow with irregular contours cells, highly interconnected, whose boundaries are not recognizable, with extensions of cell wall like tubes, narrow lumen, similar to the fiber in cross-section; fragments of coat showing cell with thickened walls, fragments of epidermis of the coat, in front view, with periclinal walls uniformly thickened, and when in cross section, with radial and outer periclinal walls strongly thickened, resembling a narrow palisade with reddish-brown cells; fragments of reserve parenchyma, with cells elliptical to flat containing starch grains and lipid droplets; fragments of reserve parenchyma with portions of vascular bundles; plentiful starch grains, isolated or grouped in different sizes and shapes, as described above. When subjected to cold chloral hydrate, starch immediately swells.

In cotyledon tissue fragments, subjected to long cooking, the starch does not lose the usual sticky characteristic. In these tissues, colorless lipid droplets were observed both inside cells and scattered around the fragments.

IDENTIFICATION

To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel with 250 µm thick as support and a mixture of 1-butanol, acetic acid, and water, (50:10:40) as mobile phase. To separately apply to the plate, as band, 20 µl of *Solution (1)* and 10 µl of *Solution (2)* freshly prepared, as described below.

Solution (1): to heat 1 g of the drug sprayed with 10 mL of 70% ethanol (v/v) under reflux, for 15 minutes. To cool down and filter it.

Solution (2): to dissolve 10 mg of escin SQR and 1 mL of 70% ethanol (v/v).

To develop the chromatogram. To remove the plate, and let it drying. To observe under ultraviolet light (254 nm). The principal spot obtained in the chromatogram with *Solution*

(1) corresponds in position, color, and intensity to that obtained with *Solution* (2). Then, nebulize the plate with anisaldehyde SR and put it in an oven at 100 °C - 105 °C for 5-10 minutes. The spot corresponding to escin has bluish-violet color. The chromatogram obtained with *Solution* (1) has smaller spots with faint color, ranging from brown to reddish-brown, a grayish-brown band in the lower third of the chromatogram and just below a brown band.

PURITY TESTS

Foreign matter (5.4.2.2). At most 2%.

Water (5.4.2.3). At most 10%.

Total ashes (5.4.2.4). At most 4%.

DOSAGE

Escin

To proceed as described in *Visible absorption spectrophotometry* (5.2.14). To transfer 1 g of the sprayed drug to a 250 mL flask and add 100 mL of 65% methanol (v/v). To accurately weigh the set and heat it under reflux, in a water bath, for 30 minutes. To cool down, top up to the initial weight with 65% methanol (v/v). To filter it. To evaporate 30 mL of the filtrate to dryness in a 100 mL flask, under reduced pressure. To dissolve the residue in 20 mL of 0.1 M hydrochloric acid, transfer to a 250 mL separatory funnel, and wash the flask with two portions of 5 mL of 0.1 M hydrochloric acid. To gather the acid phases. To extract with a mixture of 20 mL of *n*-propyl alcohol and 50 mL of chloroform. To vigorously stir for 2 minutes. To separate the lower organic phase. To add 30 mL of 0.1 M hydrochloric acid to the phase remaining in

the funnel and extract with a mixture of 20 mL of *n*-propyl alcohol and 50 mL of chloroform. To vigorously stir for 2 minutes. To separate the lower phase and combine it with the lower phase from previous extraction. To evaporate both solutions to dryness and under reduced pressure. To wash the residue with 4 portions of 10 mL of peroxide-free ethyl ether. To filter the ether phase. To wash the filter with 10 mL of peroxide-free ethyl ether. To discard the filtrate. To remove the ethyl ether remaining in the filter and flask. To wash the filter and the flask containing the residue with glacial acetic by transferring to a 50 mL volumetric flask. To top up to volume with glacial acetic acid. To transfer 2 mL of the previous solution to the test tube and add 4 mL of ferric acid chloride SR. To make it homogeneous. To prepare the blank by using 2 mL of glacial acetic acid and 4 mL of ferric acid chloride SR. To heat the test tube in water bath at 60 °C for 25 minutes. To cool down at room temperature and measure the absorbance at 540 nm (5.2.14), by using the same blank to adjust zero. To calculate the content of escin, by taking into account that $A(1\%, 1\text{ cm}) = 60$, according to the equation:

$$\text{Escin } \% = \frac{8,333 \times A}{m}$$

where

Escin% = content of escin;

A = absorbance,

m = mass of drug considering the determination of water (g).

PACKAGING AND STORAGE

In tightly closed containers, protected from light and heat.

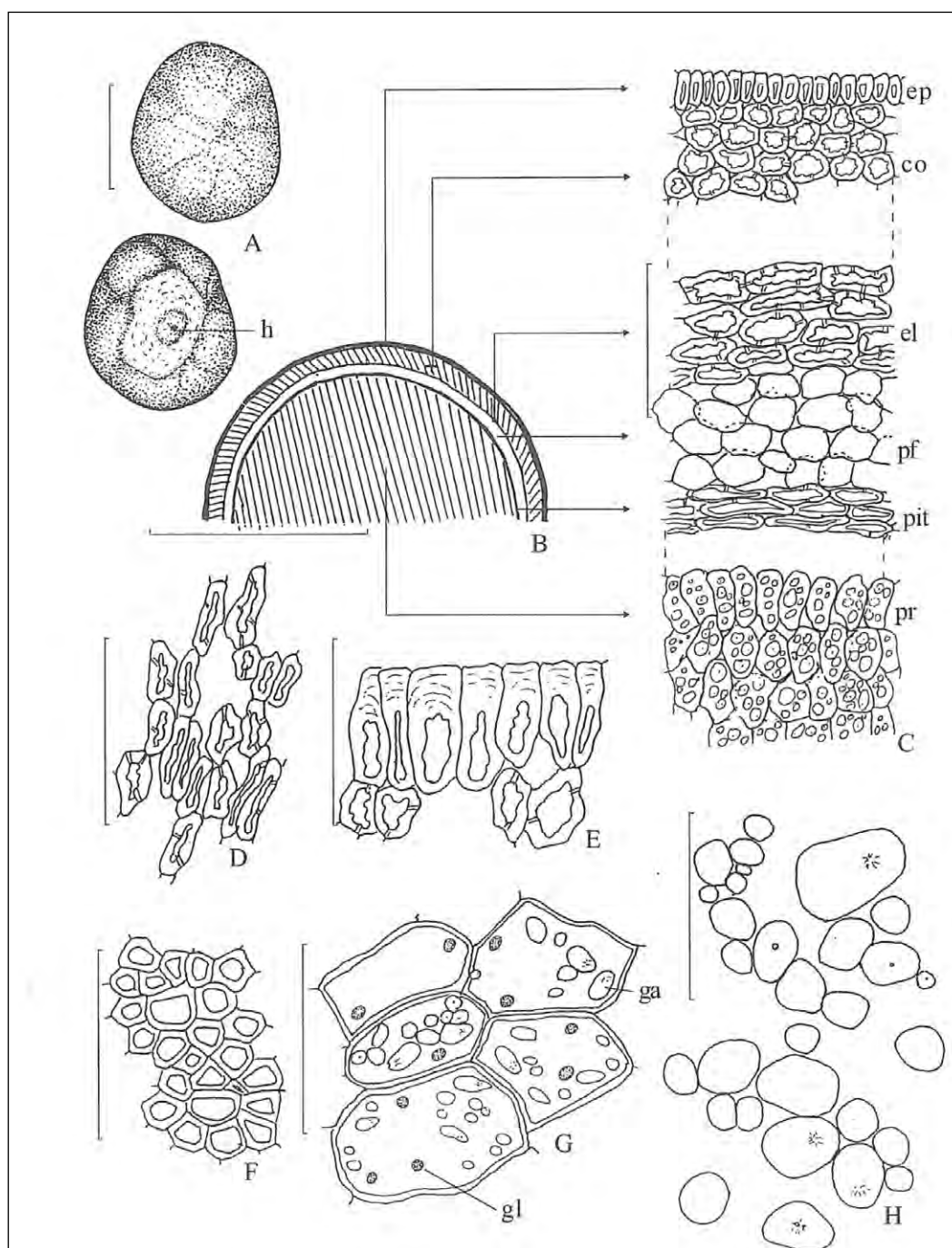


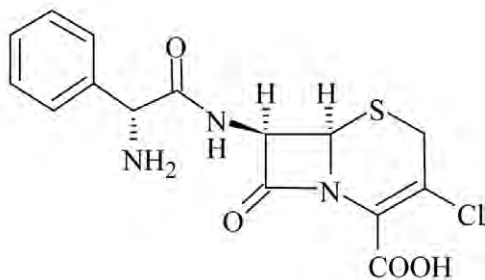
Figure 1 - Macroscopic and microscopic aspects in *Aesculus hippocastanum* L.

Additional information of **Figure 1**. Scales correspond to 0.5 cm in **A** and **B**; in **C**, 300 μ m; and from **D** to **G**, in **H**, 50 μ m.

A - schematic representations of the seed in lower and upper surface view, by showing the hilum region; **B** - schematic representation of the seed, in cross section; **C** - details of the seed, in cross section, as shown in **B**; **D** - detail of the epidermis of the seed tegument in front view; **E** - detail of epidermis of the coat, in cross section; **F** - sclerenchymatic cells, in cross section; **G** - cotyledonal reserve parenchyma cells; **H** - starch grains; collenchyma (co); sclerenchyma (el); epidermis (ep); starch grain (ga); lipid droplet (gl); hilum (h); fundamental parenchyma (pf); internal coat parenchyma (pit), with thick cell walls; cotyledon reserve parenchyma (pr).

CEFACLOR

Cefaclorum



$C_{15}H_{14}ClN_3O_4S$; 367.81

$C_{15}H_{14}ClN_3O_4S \cdot H_2O$; 385.82

cefaclor; 01824

cefaclor monohydrate, 09368

(6R,7R) -6 - [[(2R)-2-amino-2-phenylacetyl] amino] - 3-chloro-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid
[53994-73-3]

(6R, 7R) -7 - [[(2R)-2-amino-2-phenylacetyl] amino] - 3-chloro-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid hydrated (1:1) [70356-03-5]

It contains at least 950 µg and at most 1020 µg of $C_{15}H_{14}ClN_3O_4S$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. Crystalline, white or almost white powder.

Solubility. Sparingly soluble in water; practically insoluble in methanol, chloroform, and benzene.

Physical and chemical constants.

Specific rotation (5.2.8): +101° to +111°, in relation to the anhydrous substance. To determine in 1% solution (w/v) of the sample in 1% hydrochloric acid.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the cefaclor SQR spectrum similarly prepared.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

PURITY TESTS

pH (5.2.19). 3.0 to 4.5. To determine in 2.5% aqueous suspension (w/v).

Related substances. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 220 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm or 10 µm), kept at room temperature; flow of *Mobile phase* of 1.0 mL/min.

Diluent: to dissolve 2.4 g of monobasic sodium phosphate in 1000 mL of water to adjust the pH to 2.5 with phosphoric acid.

Eluent A: to dissolve 6.9 g of monobasic sodium phosphate in 1000 mL of water to adjust the pH to 4.0 with phosphoric acid.

Eluent B: mixture of *Eluent A* and acetonitrile (55:45).

Mobile phase gradient: to adopt the gradient system described in the following table:

Time (minutes)	Eluent A (%)	Eluent B (%)	Elution
0 - 30	95 → 75	5 → 25	linear gradient
30 - 45	75 → 0	25 → 100	linear gradient
45 - 55	0	100	isocratic
55 - 60	0 → 95	100 → 5	linear gradient
60 - 70	95	5	isocratic

Sample solution: to accurately transfer 50 mg of the sample to a 10 mL volumetric flask and perform sonication, if necessary. To top up to volume with *Diluent*, make it homogeneous, and filter it. To use this solution within 3 hours, if stored at room temperature, or within 20 hours, if stored under refrigeration.

Standard solution: to dissolve sufficient amount of cefaclor SQR in *Diluent*, to obtain a 50 µg/mL solution.

Resolution solution: to dissolve amount of delta-3-cefaclor SQR in *Standard solution*, to obtain a 50 µg/mL solution.

To inject replicates of 20 µL of the *Resolution solution*. The retention time for the cefaclor peak is between 23 minutes and 29 minutes. The tailing factor for the cefaclor peak is not greater than 1.2. The resolution between delta-3-cefaclor and cefaclor is greater than 2.0. To inject the *Diluent*. To disregard any peak corresponding to the *Diluent*.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of each related substance according to the equation:

$$0,01 \times C \times P \times (r_i / r_p)$$

where

C = concentration in mg/mL of cefaclor in the *Standard solution*;

P = potency, in µg/mg, of cefaclor SQR;

r = area under the peak of each related substance in the chromatogram obtained with the *Sample solution*;
 r_p = area under the peak corresponding to cefaclor in the chromatogram obtained with the *Standard solution*.

At most 1.0% for any related substance and at most 3.0% for the sum of all related substances. To disregard any peak with a result less than 0.1%.

Water (5.2.20.1). 3.0% to 6.5%.

DOSAGE

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 265 nm; 250 mm column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μ m), flow of *Mobile phase* of 1.5 mL/min.

Mobile phase: to dissolve 1 g of sodium pentane 1-sulfonate in a mixture of 780 mL of water and 10 mL of triethylamine. To adjust the pH to 2.5 ± 0.1 with phosphoric acid. To add 220 mL of methanol and stir.

Sample solution: to accurately transfer about 15 mg of the sample to a 50 mL volumetric flask, top up with *Mobile phase*, and make it homogeneous. To use this solution within 8 hours, if stored at room temperature, or within 20 hours, if stored under refrigeration.

Standard solution: to accurately transfer about 15 mg of cefaclor SQR to a 50 mL volumetric flask, top up with *Mobile phase*, and make it homogeneous. To use this solution within 8 hours, if stored at room temperature, or within 20 hours, if stored under refrigeration.

Resolution solution: to prepare solution in *Mobile phase* containing about 0.3 mg cefaclor SQR and 0.3 mg of delta-3-cefaclor SQR per milliliter.

To inject replicates of 20 μ L of the *Resolution solution*. The tailing factor for cefaclor peak should not be greater than 1.5. The resolution between cefaclor and delta-3-cefaclor is greater than 2.5. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2.0 %.

Procedure: to separately inject 20 μ L of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate, in μ g, the content of cefaclor ($C_{15}H_{14}ClN_3O_4S$) per milligram, from the sample, from the content of standard and responses obtained for the *Sample and Standard solutions*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antibiotic.

CEFACLOR CAPSULES

It contains cefaclor monohydrate equivalent to at least 90.0% and at most 120.0% of the amount stated of $C_{15}H_{14}ClN_3O_4S$.

IDENTIFICATION

The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL

Equipment: baskets, 50 rpm

Time: 30 minutes

Procedure: after testing, remove aliquot from the dissolution medium, filter, and dilute with water to obtain suitable concentration. To measure the absorbances of the solutions at 264 nm (5.2.14), by using the same solvent to adjust zero. To calculate the amount of $C_{15}H_{14}ClN_3O_4S$ dissolved in the medium, by comparing the readings obtained with that of 0.001% cefaclor SQR solution (w/v) prepared in the same solvent.

Tolerance: not less than 80% (Q) of the amount stated of $C_{15}H_{14}ClN_3O_4S$ dissolves within 30 minutes.

PURITY TESTS

Related substances. To proceed as described in *Related substances*, in the monograph *Cefaclor*. To prepare the *Sample solution* as described below.

Sample solution: to weigh 20 capsules, remove the content, and weigh them again. To make the content of the capsules homogeneous. To transfer amount of powder equivalent to 50 mg of cefaclor to a 10 mL volumetric flask. To top up to volume with *Diluent*, make it homogeneous, and filter it.

To use this solution within 3 hours, if stored at room temperature, or within 20 hours, if stored under refrigeration.

To inject replicates of 20 µL of the *Resolution solution*. The retention time for the cefaclor peak is between 23 minutes and 29 minutes. The tailing factor for cefaclor peak is not greater than 1.2.

The resolution between delta-3-cefaclor and cefaclor peaks is not less than 2.0. To inject the *Diluent*. To disregard any peak corresponding to the *Diluent*.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of each related substance according to the equation:

$$0,01 \times CP \times \left(\frac{r_i}{r_p} \right)$$

where

C = concentration in mg/mL of cefaclor in the *Standard solution*;

P = potency, in µg/mg, of cefaclor SQR;

r_i = area under the peak of each related substance in the chromatogram obtained with *Test solution*;

r_p = area under the peak corresponding to cefaclor in the chromatogram obtained with the *Standard solution*.

At most 1.0% to any related substance. The sum of the areas corresponding to all peaks obtained with related substances is at most 3.0%. To disregard peaks with an area less than 0.1%.

Water (5.2.20.1). At most 8.0%.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 265 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at room temperature; flow of *Mobile phase* of 1.5 mL/min.

Mobile phase: to dissolve 1 g of sodium pentane 1-sulfonate in a mixture of 780 mL of water and 10 mL of triethylamine. To adjust the pH to 2.5 ± 0.1 with phosphoric acid. To add 220 mL of methanol and mix.

Sample solution: to weigh 20 capsules, remove the content, and weigh them again. To make the content of the capsules homogeneous. To transfer amount of powder equivalent to 30 mg of cefaclor to a 100 mL volumetric flask, add 70 mL of *Mobile phase*, and put it in ultrasound to dissolve. To top up with the same solvent, make it homogeneous, and filter it.

Standard solution: to dissolve accurately weighed amount of cefaclor SQR in *Mobile phase* to obtain a 0.3 mg/mL concentration.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of C₁₅H₁₄ClN₃O₄S in the capsules from the responses obtained from *Sample and standard solutions*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

CEFACLOR ORAL SUSPENSION

It contains at least 80.0 % and at most 120.0 % of the amount stated of C₁₅H₁₄ClN₃O₄S. The oral suspension may contain coloring, suspension, flavoring, buffer, sweetener, and preservative agents in an aqueous medium.

IDENTIFICATION

A. The ultraviolet absorption spectrum (5.2.14), ranging from 190 nm to 310 nm, of 30 µg/mL solution of the sample, diluted in water and filtered, exhibits maxima at 2264 nm.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

PURITY TESTS

Related substances. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 220 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm); flow of *Mobile phase* of 1.0 mL/min.

Diluent: to dissolve 2.4 g of monobasic sodium phosphate in 1000 mL of water and adjust the pH to 2.5 with phosphoric acid.

Eluent A: to dissolve 6.9 g of monobasic sodium phosphate in 1000 mL of water and adjust the pH to 4.0 with phosphoric acid.

Eluent B: to prepare a mixture of *Eluent A* and acetonitrile (55:45).

Mobile phase gradient: to adopt the gradient system described in the following table:

Time (minutes)	Eluent A (%)	Eluent B (%)	Elution
0-30	95 → 75	5 → 25	linear gradient
30-45	75 → 0	25 → 100	linear gradient
45-55	0	100	isocratic
55-60	0 → 95	100 → 5	linear gradient
60-70	95	5	isocratic

Sample solution: to dilute volume of sample in the *Diluent*, to obtain 5 mg/mL solution. To stir and perform sonication, if necessary. To filter it.

Standard solution: to dissolve accurately weighed amount of cefaclor SQR in *Diluent*, to obtain a 0.05 mg/mL solution.

Resolution solution: to dissolve accurately weighed amount of delta-3-cefaclor SQR in *Standard solution*, to obtain a 0.05 mg/mL solution.

Procedure: To inject 20 µL of *Resolution solution*. The resolution between the delta 3-cefaclor and cefaclor peaks is greater than 2.0. To separately inject 20 µL of *Standard solution* and *Sample solution*, record the chromatograms at least twice the retention time of the main peak, and measure the areas under the peaks. The maximum allowed percentage is 1.0% to each individual impurity and at most 3.0% to the sum of all impurities. To disregard the peaks related to solvent or area less than 0.1%.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_{16}H_{14}ClN_3O_4S$ from the sample, from the responses obtained for the *Sample solution* and *Standard solution*.

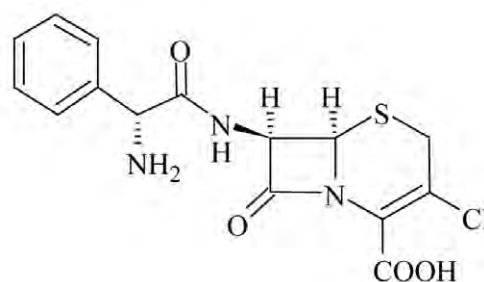
PACKAGING AND STORAGE

In tightly closed containers, protected from light, at room temperature.

LABELLING

To comply with the legislation in force.

CEFADROXIL Cefadroxilum



$C_{16}H_{17}N_3O_5S \cdot H_2O$; 381.40

$C_{16}H_{17}N_3O_5S$; 363.39

cefadroxil; 01825

(6*R*, 7*R*) -7 - [[(2*R*)-2-amino-2-(4-hydroxyphenyl) acetyl] amino]-3-methyl-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid
[50370-12-2]

(6*R*, 7*R*) -7 - [[(2*R*)-2-amino-2-(4-hydroxyphenyl) acetyl] amino]-3-methyl-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid hydrate (1:1) [66592-87-8]

It shows minimum potency of 950 µg and at most 1050 µg of $C_{16}H_{17}N_3O_5S$ per milligram, relative to the anhydrous substance.

DESCRIPTION

Physical characteristics. White or almost white powder.

Solubility. Sparingly soluble in water; very slightly soluble in ethanol; practically insoluble in chloroform and ethyl ether.

Physical and chemical constants.

Specific rotation (5.2.8): +165° to +178°, in relation to the anhydrous substance. To determine at 1% solution (w/v) in water.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the cefadroxil SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14), from 235 nm to 340 nm, of 0.002% solution (w/v) in citro-phosphate buffer pH 6.0, has maximum at 264 nm, similar to that observed in the spectrum of a similar solution of cefadroxil SQR.

C. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using HF_{254} silica gel as support and a mixture of methanol and 15.4 % ammonium acetate (w/v) with

pH 6.2, adjusted with glacial acetic acid (15:85) as mobile phase. To separately apply to the plate 1 μ L of each of the solutions freshly prepared, as described below.

Solution (1): to dissolve 20 mg of sample in 5 mL of a mixture of methanol and citro-phosphate buffer pH 7.0 (1:1).

Solution (2): to dissolve 20 mg of cefadroxil SQR in 5 mL of a mixture of methanol and citro-phosphate buffer pH 7.0 (1:1).

Solution (3): to dissolve 20 mg of cefadroxil SQR and 20 mg of cefalexin SQR in 5 mL of a mixture of methanol and citro-phosphate buffer pH 7.0 (1:1).

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*. The test is valid only if the chromatogram obtained with *Solution (3)* presents two clearly separated spots.

D. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method A. corresponds to that of main peak of *Standard solution*.

PURITY TESTS

pH (5.2.19). 4.0 to 6.0. To determine in 5% aqueous suspension (w/v).

Absorption of light. The absorption of 0.02% solution (w/v) in citro-phosphate buffer pH 6.0, measured at 330 nm, is not greater than 0.05 (5.2.14).

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel as support and a mixture of ethyl acetate, ethanol, water, and formic acid (14:5:5:1) as mobile phase. To separately apply to the plate 20 μ L of *Solutions (1), (2), and (3)* and 4 μ L of *Solution (4)* freshly prepared, as described below.

Diluent: mixture of ethanol, water, and 2.4 M hydrochloric acid (75:22:3).

Solution (1): to accurately dissolve weighed amount of the sample in *Diluent*, to obtain a 25 mg/mL solution.

Solution (2): to transfer 1 mL of *Solution (1)* to 100 mL volumetric flask and top up with *Diluent*.

Solution (3): to dissolve accurately weighed amounts of 7-aminodesacetoxycephalosporanic acid and D- α -4- hydroxyphenylglycine in *Diluent*, to obtain a 0.25 mg/mL solution for each substance.

Solution (4): to mix 1 mL of *Solution (1)* with 1 mL of *Solution (3)*.

To develop the chromatogram. To remove the plate, and let it drying. To nebulize the plate with 3% ninhydrin solu-

tion (w/v) in 4.55% sodium metabisulphite (w/v). To let the plate air drying. Any secondary spot obtained in the chromatogram with *Solution (1)*, corresponding to 7-aminodesacetoxycephalosporanic acid or D- α -4- hydroxyphenylglycine, is not more intense than the spots obtained in the chromatogram with *Solution (3)* (1%). Any spot obtained in the chromatogram with *Solution (1)*, other than the principal one and those corresponding to 7-aminodesacetoxycephalosporanic acid or D- α -4- hydroxyphenylglycine, is not more intense than the spot obtained in the chromatogram with *Solution (2)* (1%). The test is valid only if the chromatogram obtained with *Solution (4)* shows three principal spots clearly separated.

Limit for dimethylaniline. To proceed as described in *Gas Chromatography* (5.2.17.5). To use gas chromatograph equipped with flame ionization detector; chromatographic column packed with silanized diatomaceous support for gas chromatography impregnated with 3% (w/w) of polymethylphenylsiloxane; kept at 120 °C; injector and detector at 150 °C; nitrogen as carrier gas; flow of carrier gas of 30 mL/ min.

Sample solution: to transfer 1 g of the sample to a centrifuge tube and add 5 mL of *M* sodium hydroxide and 1 mL of *Internal standard solution*. To close the tube and stir for 1 minute. To centrifuge, if necessary, and use the clear supernatant.

Internal standard solution: to transfer 50 mg of naphthalene, accurately weighed, to a 50 mL volumetric flask and dissolve in cyclohexane. To top up with the same solvent and make it homogeneous. To transfer 5 mL of this solution to 100 mL volumetric flask and top up with cyclohexane.

Standard solution: to transfer 50 mg of *N,N*-dimethylaniline, accurately weighed, to a 50 mL volumetric flask. To add 2 mL of hydrochloric acid and 20 mL of water. To stir it to dissolve. To top up with water and make it homogeneous. To transfer 5 mL of this solution to 250 mL volumetric flask and top up with water. To transfer 1 mL of this solution to a centrifuge tube and add 5 mL of *M* sodium hydroxide and 1 mL of *Internal standard solution*. To close the tube and stir for 1 minute. To centrifuge, if necessary, and use the clear supernatant.

Procedure: to separately inject 1 μ L of *Standard solution* and *Sample solution*, record the chromatograms, and measure the areas under the dimethylaniline and naphthalene internal standard peaks. The response from relationship dimethylaniline/naphthalene in the *Sample solution* is not greater than that obtained in the *Standard solution* (0.002%).

Water (5.2.20.1). To determine in 0.2 g of the sample. From 4.0% to 6.0%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.5 %.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 230 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at room temperature; flow of *Mobile phase* of 1.5 mL/min.

Buffer pH 5.0: 0.05 M monobasic potassium phosphate, pH 5.0, adjusted with 2 M sodium hydroxide.

Mobile phase: mixture of *Buffer pH 5.0* and acetonitrile (96:4).

Sample solution: to transfer 0.2 g of sample, accurately weighed, to 200 mL volumetric flask by using 120 mL of *Buffer pH 5.0*, and mechanically stir for 15 minutes. To top up with the same solvent, make it homogeneous, and filter it. To use the solution on the same day.

Standard solution: to transfer amount of powder equivalent to 25 mg of cefadroxil SQR to 25 mL volumetric flask, add 15 mL of *Buffer pH 5.0*, and mechanically stir for 15 minutes. To top up with the same solvent and make it homogeneous. To use the solution on the same day.

The column efficiency should be greater than 1800 theoretical plates. The tailing factor should not be greater than 2.0. The capacity factor is 2-3.5. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2.0 %.

Procedure: to separately inject 10 µL of *Sample and standard solutions*, record the chromatograms, and measure the areas under the peaks. To calculate the potency, in mg/mg, of cefadroxil ($C_{16}H_{17}N_3O_5S$), in the sample from the potency of the standard and responses obtained with the *Standard and sample solutions*.

B. To proceed as described in *Microbiological test of antibiotics (5.5.3.3)* by using cylinders with the agar diffusion method.

Micro-organism: *Staphylococcus aureus* ATCC 6538 P.

Culture media: sterile saline solution to the standardization of the inoculum; culture medium number 2, to the base layer culture medium number 1 to inoculum preparation.

Sample solution: To accurately weigh the equivalent to 50 mg of sample and quantitatively transfer it to a 100 mL volumetric flask, by using 60 mL of 1% *Potassium phosphate buffer, sterile, pH 6.0 (Solution 1)*. To put it in ultrasound for 15 minutes. To mechanically stir for 20 minutes and top up with the same solvent. To make it homogenous and filter it. To successively dilute to obtain 10 µg/mL, 20 µg/

mL, and 40 µg/mL concentrations, by using 1% *Potassium phosphate buffer, sterile, pH 6.0* as solvent.

Standard solution: to accurately weigh the equivalent to 25 mg of cefadroxil SQR and quantitatively transfer it to a 50 mL volumetric flask, by using 30 mL of 1% *Potassium phosphate buffer sterile, pH 6.0 (Solution 1)*. To put it in ultrasound for 15 minutes. To mechanically stir for 20 minutes, top up with the same solvent, and make it homogeneous. To successively dilute to obtain 10 µg/mL, 20 µg/mL, and 40 µg/mL concentrations, by using 1% *Potassium phosphate buffer, sterile, pH 6.0* as solvent.

Procedure: to add 20 mL of culture medium number 2 in each plate, wait to solidify, add 5 mL of 0.5% inoculum, and proceed as described in *Microbiological test of antibiotics (5.5.3.3)*.

PACKAGING AND STORAGE

In airtight closed containers, protected from moisture, at temperature below 30 °C.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antibiotic.

CEFADROXIL CAPSULES

It contains at least 90.0 % and at most 120.0 % of the amount stated of $C_{16}H_{17}N_3O_5S$.

IDENTIFICATION

A. To weigh the capsules, remove the content, and weigh them again. To make the content of the capsules homogeneous. To use amount of powder equivalent to 20 mg of cefadroxil and transfer to a 100 mL volumetric flask by using 60 mL of citro-phosphate buffer pH 6.0. To stir for 10 minutes and top up with buffer. To make it homogenous and filter it. To proceed as described in *Identification*, test **B.**, in the monograph *Cefadroxil*.

B. To proceed as described in *Identification*, test **C.**, in the monograph *Cefadroxil*. To prepare *Solution (1)* as described below.

Solution (1): to weigh capsules, remove the content, and weigh them again. To make the content of the capsules homogeneous. To use amount of powder equivalent to 20 mg of cefadroxil, dissolve in 5 mL of a mixture of methanol and citro-phosphate buffer pH 7.0 (1:1), make it homogeneous, and filter it.

C. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in *Dosage* method **A.** corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test. To proceed as described in *Dosage* method **A.**

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL

Equipment: basket, 100 rpm

Time: 30 minutes

Procedure: after testing, remove aliquot from the dissolution medium and dilute with water to obtain suitable concentration. To measure the absorbances at 263 nm (**5.2.14**), by using the same solvent to adjust zero. To calculate the amount of $C_{16}H_{17}N_3O_5S$ dissolved in the medium, by comparing the readings obtained with that of 0.002% cefadroxil SQR solution (w/v) prepared in the same solvent.

Tolerance: not less than 80% (Q) of the amount stated of $C_{16}H_{17}N_3O_5S$ dissolves within 30 minutes.

PURITY TESTS

Water (5.2.20.1). At most 7.0%.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Dosage*, method **A.**, in the monograph *Cefadroxil*. To prepare the *Sample solution* as described below.

Sample solution: to weigh 20 capsules, remove the content, and weigh them again. To make the content of the capsules homogeneous. To transfer amount of powder equivalent to 0.2 g of cefadroxil to 200 mL volumetric flask, add 120 mL of *Buffer pH 5.0*, and mechanically stir for 15 minutes.

To top up with the same solvent, make it homogeneous, and filter it. To use the solution on the same day.

Procedure: to separately inject 10 μ L of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of $C_{16}H_{17}N_3O_5S$ in the capsules from the responses obtained from *Sample* and *standard solutions*.

B. To proceed as described in *Dosage*, method **B.**, in the monograph *Cefadroxil*. To prepare the *Sample solution* as described below.

Sample solution: to weigh 20 capsules, remove the content, and weigh them again. To make the content of the capsules homogeneous. To transfer amount of powder equivalent to 0.125 g of cefadroxil to a 250 mL volumetric flask, by using 150 mL of 1% *Potassium phosphate buffer, sterile, pH 6.0 (Solution 1)*. To put it in ultrasound for 15 minutes. To mechanically stir for 20 minutes and top up with the same solvent. To make it homogenous and filter it. To successively dilute by using the same solvent, to obtain 10 μ g / mL, 20 μ g /mL, and 40 μ g/mL concentrations.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

CEFADROXIL TABLETS

It contains at least 90.0 % and at most 120.0 % of the amount stated of $C_{16}H_{17}N_3O_5S$. Tablets may be coated.

IDENTIFICATION

A. To weigh and pulverize the tablets. To use amount of powder equivalent to 20 mg of cefadroxil and transfer to a 100 mL volumetric flask by using 60 mL of *citro-phosphate buffer pH 6.0*. To stir for 10 minutes and top up with buffer. To make it homogenous and filter it. To proceed as described in *Identification*, test **B.**, in the monograph *Cefadroxil*.

B. To proceed as described in *Identification*, test **C.**, in the monograph *Cefadroxil*. To prepare *Solution (1)* as described below.

Solution (1): to weigh and pulverize the tablets. To use amount of powder equivalent to 20 mg of cefadroxil, dissolve in 5 mL of a mixture of methanol and *citro-phosphate buffer pH 7.0 (1:1)*, and filter it.

C. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

Procedure for content uniformity. To transfer each tablet to 500 mL volumetric flask containing 300 mL of *Phosphate buffer pH 5.0* (described in method **A. Dosage**, in the monograph *Cefadroxil*) and dissolve the tablet in ultrasound for 5 minutes. To mechanically stir for 15 minutes and top up with the same solvent. To make it homogenous and filter it. To transfer 10 mL of this solution to 20 mL volumetric flask and top up with *Phosphate buffer pH 5.0*. To proceed as described in Method **A. Dosage**.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL

Equipment: blades, 50 rpm

Time: 30 minutes

Procedure: after testing, remove aliquot from the dissolution medium and dilute with water to obtain suitable concentration. To measure the absorbances at 263 nm (**5.2.14**), by using the same solvent to adjust zero. To calculate the amount of $C_{16}H_{17}N_3O_5S$ dissolved in the medium, by comparing the readings obtained with that of 0.002% cefadroxil SQR solution (w/v) prepared in the same solvent.

Tolerance: not less than 75% (Q) of the amount stated of $C_{16}H_{17}N_3O_5S$ dissolves within 30 minutes.

PURITY TESTS

Water (5.2.20.1). At most 8.0%.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Dosage*, method **A.**, in the monograph *Cefadroxil*. To prepare the *Sample solution* as described below.

Sample solution: to weigh and pulverize 20 tablets. To transfer amount of powder equivalent to 0.2 g of cefadroxil to 200 mL volumetric flask, add 120 mL of *Buffer pH 5.0*,

and mechanically stir for 15 minutes. To top up with the same solvent and filter it. To use the solution on the same day.

Procedure: to separately inject 10 µL of *Sample and standard solutions*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of $C_{16}H_{17}N_3O_5S$ in the tablets, from the responses obtained for the *Sample and standard solutions*.

B. To proceed as described in *Dosage*, method **B.**, in the monograph *Cefadroxil*. To prepare the *Sample solution* as described below.

Sample solution: to weigh and pulverize 20 tablets. To transfer amount of powder equivalent to 0.125 g of cefadroxil to a 250 mL volumetric flask, by using 150 mL of *1% Potassium phosphate buffer, sterile, pH 6.0 (Solution I)*. To put it in ultrasound for 15 minutes. To mechanically stir for 20 minutes and top up with the same solvent. To make it homogenous and filter it. To successively dilute to obtain 10 µg/mL, 20 µg/mL, and 40 µg/mL concentrations, by using *1% Potassium phosphate buffer, sterile, pH 6.0* as diluent.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

CEFADROXIL POWDER FOR ORAL SUSPENSION

It contains at least 90.0 % and at most 120.0 % of the amount stated of $C_{16}H_{17}N_3O_5S$. The powder for oral suspension is a mixture of cefadroxil monohydrate with one or more coloring, flavoring, buffer, sweetener, and preservative agents.

IDENTIFICATION

A. To reconstitute the contents of three vials as indicated on the label and make them homogeneous. To transfer amount of oral suspension equivalent to 50 mg of cefadroxil to a 250 mL volumetric flask, by using 150 mL of sodium phosphate buffer pH 6.0. To stir for 10 minutes and top up with buffer. To make it homogenous and filter it. To proceed as described in *Identification*, test **B.**, in the monograph *Cefadroxil*.

B. To proceed as described in *Identification*, test **C.**, in the monograph *Cefadroxil*. To prepare *Solution (I)* as described below.

Solution (I): to reconstitute the contents of three vials as indicated on the label and make them homogeneous. To

use amount of oral suspension equivalent to 0.1 g of cefadroxil, dissolve in 25 mL of a mixture of methanol and citro-phosphate buffer pH 7.0 (1:1), and filter it.

C. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in *Dosage* method **A.** corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

pH (5.2.19). 4.5 to 6.0. To determine in the reconstituted suspension as informed on the label.

Determination of weight (5.1.1). It complies with the test. To determine in the powder before reconstituting.

Uniformity of dosage units (5.1.6). It complies with the test. To proceed as described in *Dosage* method **A.**

PURITY TESTS

Water (5.2.20.1). At most 2.0%.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll microorganisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Dosage*, method **A.**, in the monograph Cefadroxil. To prepare the *Sample solution* as described below.

Sample solution: to reconstitute the contents of three vials as indicated on the label and make them homogeneous. To transfer volume of oral suspension equivalent to 0.25 g of cefadroxil to 250 mL volumetric flask, add 150 mL of Buffer pH 5.0, and mechanically stir for 15 minutes. To top up the volume with the same solvent. To filter about 25 mL of this solution through a 0.8 µm filter or less, and use the clear filtrate as a sample solution. To use the solution on the same day.

Procedure: to separately inject 10 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of C₁₆H₁₇N₃O₄S in oral suspension from the responses from the *Standard solution* and *Sample solution*.

B. To proceed as described in *Dosage*, method **B.**, in the monograph Cefadroxil. To prepare the *Sample solution* as described below.

Sample solution: to reconstitute the contents of three vials as indicated on the label and make them homogeneous.

To transfer amount of oral suspension equivalent to 0.1 g of cefadroxil to a 200 mL volumetric flask, by using 120 mL of 1% *Potassium phosphate buffer, sterile, pH 6.0*. To mechanically stir for 20 minutes and top up with the same solvent. To make it homogenous and filter it. To successively dilute by using the same solvent, to obtain 10 µg / mL, 20 µg / mL, and 40 µg / mL concentrations.

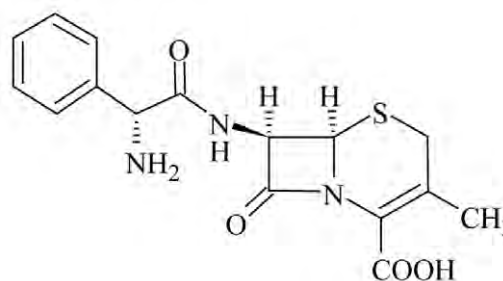
PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

CEPHALEXIN Cefalexinum



C₁₆H₁₇N₃O₄S; 347.39

C₁₆H₁₇N₃O₄S.H₂O; 365.40

cephalexin; 01826

cephalexin monohydrate; 01827

(6R,7R) -7 - [[(2R)-2-amino-2-phenylacetyl] amino] - 3-methyl-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid
[15686-71-2]

(6R, 7R) -7 - [[(2R)-2-amino-2-phenylacetyl] amino] - 3-methyl-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid hydrated (1:1)
[23325-78-2]

It contains at least 95.0% and at most 103.0% of C₁₆H₁₇N₃O₄S, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. Crystalline, white or almost white powder.

Solubility. Sparingly soluble in water, slightly soluble in methanol, and practically insoluble in ethanol and dimethylformamide.

Physical and chemical constants.

Specific rotation (5.2.8): +149° to +158°, in relation to the anhydrous substance. To determine in 0.5 % solution (w/v) in biphtalate buffer pH 4.4.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maxima only at the same wavelength, with the same relative intensities to those observed in the cephalixin SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14), ranging from 200 nm to 400 nm, of solution of the sample in water (1:50), exhibits minimum and maximum at the same wavelength of cephalixin SQR solution, similarly prepared, simultaneously measured, as well as absorptivity, calculated on the anhydrous basis, at the wavelength of maximum absorption around 262 nm is not less than 95% and greater than 104% of cephalixin SQR, by considering the stated potency of cephalixin SQR.

C. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using G silica gel as support and a mixture of ethyl acetate, water, acetonitrile, and glacial acetic acid (42:18:14:14) as mobile phase. To separately apply to the plate 5 µL of each of the solutions freshly prepared, as described below.

Solution (1): 25 mg/mL solution of the sample in 0.01 M hydrochloric acid.

Solution (2): 25 mg/mL cephalixin SQR solution in 0.01 M hydrochloric acid.

To develop the chromatogram until the solvent has moved three-quarters from the plate. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

PURITY TESTS

pH (5.2.19). 3.0 to 5.5. To determine in laqueous suspension containing 50 mg/mL.

Related substances. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 254 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm or 10 µm); flow of *Mobile phase* of 1.0 mL/min.

Eluent A: to dissolve 1 g of sodium pentane 1-sulfonate in a mixture of 1000 mL of water and 15 mL of triethylamine. To adjust the pH to 2.5 + 0.1 with phosphoric acid.

Eluent B: to dissolve 1 g of sodium pentane 1-sulfonate in a mixture of 300 mL of water and 15 mL of triethylamine. To adjust the pH to 2.5 + 0.1 with phosphoric acid. To add 350 mL of acetonitrile and 350 mL of methanol. To make it homogeneous.

Mobile phase: to use various mixtures of *Eluent A* and *Eluent B*. To adopt the gradient system described in the following table.

<i>Time</i> (minutes)	<i>Eluent A</i> (%)	<i>Eluent B</i> (%)	<i>Elution</i>
0	100	0	balance
0 - 1	100	0	isocratic
1 - 33.3	100 → 0	0 → 100	linear gradient
33.3 - 34.3	0	100	isocratic

Diluent: to dissolve 18 g of monobasic potassium phosphate in 1000 mL of water.

Sample solution: to transfer accurately weighed 25 mg of the sample to a 5 mL volumetric flask, and top up with *Diluent*, and mix it.

Standard solution: to dissolve accurately weighed amount of cephalixin SQR in the *Diluent* and properly dilute to obtain 0.08 mg/mL and 0.16 mg/mL cephalixin (C₁₆H₁₇N₃O₄S), by considering the stated potency of cephalixin SQR.

Procedure: to separately inject 20 µL of *Sample and standard solutions*, record the chromatograms, and measure the areas under all the peaks obtained. To construct the analytical curve obtained from the responses to cephalixin with *Standard solutions* versus their concentrations, as calculated on the anhydrous basis, in mg/mL. To determine the concentration *C* in mg/mL for each cephalixin substance obtained with the *Sample solution* beyond the cephalixin peak. To calculate the percentage of each substance related to cephalixin according to the equation below:

$$\frac{500C}{A}$$

where *A* is the calculated amount of an anhydrous basis, in mg, of cephalixin taken to prepare the *Sample solution*. No more than 1.0% of any substance related to cephalixin is found. The sum of the areas of all substances related to cephalixin is not greater than 5.0%.

Water (5.2.20.1). 4.0% to 8.0%.

DOSAGE

To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 254 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm); flow of *Mobile phase* of 1.5 mL/min.

Mobile phase: to prepare 1015 mL of a mixture of water, acetonitrile, methanol, and triethylamine (850:100:50:15). To dissolve 1 g of sodium pentane 1-sulfonate into this mixture, adjust with phosphoric acid to pH 3.0 ± 0.1, and degas it. To make the adjustments as required.

Sample solution: to transfer 0,1 mg of the sample accurately weighed to a 100 mL volumetric flask, dissolve, top up with water, and make it homogeneous. To transfer 10.0 mL of this solution to 25 mL volumetric flask, top up with *Mobile phase*, and make it homogeneous.

Standard solution: to dissolve accurately weighed amount of cephalexin SQR in water and properly dilute to obtain a 1 mg/mL stock solution. To transfer 10 mL to 25 mL volumetric flask, top up with *Mobile phase*, and make it homogeneous.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the main peaks. To calculate the content in µg of C₁₆H₁₇N₃O₄S per mg of the sample by using the following equation:

$$100 \times \left(\frac{C \times P}{M} \right) \times \left(\frac{R_a}{R_p} \right)$$

where *C* is the concentration in mg/mL of cephalexin SQR in the stock solution used to prepare the *Standard solution*. *P* is the stated potency of cephalexin in µg/ mg of cephalexin SQR. *M* is the amount in mg of cephalexin taken to prepare the *Sample solution*; *R_a* and *R_p* are the areas under the *Sample solution* and *Standard solution* peaks, respectively.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antibacteria

CEPHALEXIN TABLETS

The infrared absorption spectrum (5.2.14) of the resulting residue, dispersed in potassium bromide, has absorption maxima only at the same wavelength, with the same relative intensities to those observed in the cephalexin SQR spectrum similarly prepared.

IDENTIFICATION

The identification test **D.** may be omitted if the tests **A.**, **B.**, and **C.** are conducted. The identification test **A.** can be omitted if the tests **B.** and **C.** are carried out.

B. To mix 20 mg of the residue obtained in Test A. *Identification* with 0.25 mL of 1% nitric acid solution (v/v) in 80% sulfuric acid (v/v). The yellow color develops.

C. To mix 20 mg of the residue obtained in Test A. *Identification* with 0.25 mL of 1% glacial acetic acid solution (v/v) and add 0.1 mL of 1% cupric sulfate pentahydrate solution (w/v) and 0.1 mL of 2 M sodium hydroxide. Olive color develops.

D. To proceed as described in *Thin-layer chromatography* (5.2.17.1), by using non-bonded silica gel as support and a mixture of 0.1 M citric acid, 0.1 M dibasic sodium phosphate and, 6.25% ninhydrin (w/v) in acetone (60:40:1.5) as mobile phase. To impregnate the plate with a mixture of n-hexane and tetradecane (95:5). To separately apply to the plate 10 µl of each of the solutions as described below.

Sample solution: To weigh and pulverize the tablets. To dissolve amount of powder in water to obtain about 3 mg/mL of cephalexin.

Standard solution: to prepare an aqueous solution containing 3 mg of cephalexin SQR per milliliter.

To develop the chromatogram. To heat the plate at 100°C for 10 minutes. The principal spot obtained with *Sample solution* corresponds in position, color, and intensity to that obtained with *Standard solution*.

Cephalexin tablets are composed of cephalexin or cephalexin hydrochloride, with one or more suitable diluents and lubricant agents. It contains at least 90.0% and at most 120.0% of the value stated of cephalexin or cephalexin hydrochloride. Cephalexin used in the production meets the specifications described in the monograph *Cephalexin*. Tablets may be coated.

A. To remove any coat from the tablets. To mix amount of finely powdered tablets containing the equivalent to 0.5 g of cephalexin in 1 mL of water and 1.4 mL of M hydrochloric acid. To add 0.1 g of activated charcoal, stir, filter, and wash the filter with 1 mL of water. To slowly add to the filtrate a saturated solution of sodium acetate until precipitation occurs. To add 5 mL of methanol. To dry at most 7 kPa.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). Within 30 minutes.

Uniformity of dosage units (5.1.6). It complies with the test. To proceed as described in *Dosage* method A.

DISSOLUTION TEST (5.1.5)

CEPHALEXIN

Dissolution medium: water, 900 mL

Equipment: baskets, 100 rpm

Time: 30 minutes

Procedure: after testing, remove aliquot from the dissolution medium, filter, and dilute, if necessary, with water, to obtain suitable concentration.

To measure the absorbances at 262 nm (5.2.14), by using the same solvent to adjust zero. To calculate the amount of $C_{16}H_{17}N_3O_4S$ dissolved in the medium, by comparing the readings obtained with that of 0.002% cephalexin SQR solution (w/v) prepared in the same solvent.

Tolerance: not less than 80% (Q) of the amount stated of $C_{16}H_{17}N_3O_4S$ dissolves within 30 minutes.

Cephalexin hydrochloride

Dissolution medium, Equipment, and Procedure: to proceed as described for cephalexin.

Time: 45 minutes

Tolerance: not less than 75% (Q) of the amount stated of $C_{16}H_{17}N_3O_4S$ dissolves within 45 minutes.

PURITY TESTS

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using G silica gel as stationary phase. To impregnate the plate with 5% tetradecane solution (v/v) in hexane. To wait the solvent to evaporate and develop the chromatography in the same direction as the impregnation. To prepare *Solution (1)* as described below.

Mobile phase: mixture of acetone, 7.2% dibasic sodium phosphate dodecahydrate solution (w/v), and 2.1% citric acid solution (w/v) (3:80:120).

Solution (1): to weigh and pulverize the tablets. To dissolve amount of powder equivalent to 0.25 g of cephalexin with 2 M hydrochloric acid and dilute to 10 mL with the same solvent. To make it homogenous and filter it.

Solution (2): to dilute *Solution (1)* to 100 mL with 2 M hydrochloric acid.

Solution (3): solution containing 0.025 % (w/v) of 7-aminodesacetoxycephalosporanic acid in 2 M hydrochloric acid.

Solution (4): solution containing 0.025 % (w/v) of D- α -4-hydroxyphenylglycine in 2 M hydrochloric acid.

Solution (5): solution containing 2.5 % (w/v) of cephalexin and 0.025% (w/v) of each solution of 7-aminodesacetoxycephalosporanic acid and D- α -4-hydroxyphenylglycine in 2 M hydrochloric acid.

To separately apply 5 μ L of each solution to a plate previously impregnated. To develop by 15 cm. To dry the plate at 90 ° C for 3 min. To spray the hot plate with 0.1% ninhydrin solution (w/v) in *Mobile phase*. To heat the plate at 90°C for 15 minutes and cool down.

No 7-aminodesacetoxycephalosporanic acid spot in the chromatogram obtained with the *Solution (1)* is more intense than that spot obtained with the *Solution (3)*.

No D- α -4-hydroxyphenylglycine spot is more intense than the spot obtained with *Solution (4)*; no secondary spot between the principal spot and the 7-aminodesacetoxycephalosporanic acid and D- α -4-parahydroxyphenylglycine spots is more intense than the principal spot in the chromatogram obtained with *Solution (2)*.

The test is not valid unless the chromatogram obtained with *Solution (5)* show three clearly separated spots.

Water (5.2.20.1). At most 9.0% for cephalexin tablets and 8% for cephalexin hydrochloride tablets.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 254 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (3 μ m to 10 μ m), low acidity; flow of *Mobile phase* of 1.5 mL/min.

Mobile phase: to use a mixture of water, acetonitrile, methanol, and triethylamine (850:100:50:15). To dissolve 1 g of sodium pentane 1-sulfonate into this mixture, adjust pH to 3.0 $\pm$ 0.1.

Sample solution: to weigh and pulverize 20 tablets. To transfer amount of powder equivalent to 0.25 mg of cephalexin to 250 mL volumetric flask and add 100 mL of water. To mechanically stir for 30 minutes and top up with the same solvent. To make it homogenous and filter it. To transfer 25 mL of this solution to 50 mL volumetric flask and top up with water.

Standard solution: to accurately dissolve amount of cephalexin SQR with water, to obtain 1 mg/ mL cephalexin ($C_{16}H_{17}N_3O_4S$). To transfer 10 mL of this solution to 50 mL volumetric flask and top up with water.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of $C_{16}H_{17}N_3O_4S$ in the tablets, from the responses obtained for the *Sample solution* and *Standard solution*.

B. To proceed as described in *Microbiological test of antibiotics (5.5.3.3.1)* by using cylinders with the agar diffusion method.

Micro-organism: *Staphylococcus aureus* ATCC 6538P.

Culture media: culture medium number 1, to maintain the micro-organism; sterile saline solution to the standardization of the inoculum; and culture medium number 2, to the base layer; culture medium number 1, to inoculum preparation.

Sample solution: to weigh and pulverize 20 tablets. To transfer an amount of powder equivalent to 250 mg of cephalexin to 250 mL flask, by using 200 mL of 1% *Potassium phosphate buffer, sterile, pH 6.0 (Solution 1)*. To stir for 15 minutes. To top up to volume with 1% *Potassium phosphate buffer, sterile, pH 6.0 (Solution 1)* and filter. To successively dilute in the same solvent to obtain 2.5 µg/mL, 5 µg/mL, and 10 µg/mL concentrations, by using 1% *Potassium phosphate buffer, sterile, pH 6.0 (Solution 1)* as solvent.

Standard solution: to dissolve accurately weighed amount of cephalexin SQR in 1% *Potassium phosphate buffer, sterile, pH 6.0 (Solution 1)* to obtain 1 mg/mL solution. To successively dilute in the same solvent to obtain 2.5 µg/mL, 5 µg/mL, and 10 µg/mL concentrations, by using 1% *Potassium phosphate buffer, sterile, pH 6.0 (Solution 1)* as solvent.

Procedure: to add 20 mL of culture medium number 2 to each plate, wait to solidify, add 5 mL of 1% inoculum, and proceed as described in *Microbiological agar diffusion test (5.5.3.3.1)*. To calculate the potency of the sample in µg per mg of cephalexin, from the potency of the standard and the responses obtained with the *Standard solution* and *Sample solution*.

PACKAGING AND STORAGE

In airtight closed containers, protected from moisture, at temperature below 30 °C.

LABELLING

To comply with the legislation in force.

CEPHALEXIN POWDER FOR ORAL SUSPENSION

Cephalexin powder for oral suspension is a mixture of cephalexin with one or more coloring, flavoring, buffer, sweetener, and preservative agents. It presents potency of

at least 90.0% and at most 120.0% of the value informed in $C_{16}H_{17}N_3O_4S$.

IDENTIFICATION

To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF₂₅₄ silica gel as support and a mixture of 0.1 M citric acid, 0.1 M dibasic sodium phosphate, and ninhydrin solution in acetone (1:15) (60:40:1.5) as mobile phase. To previously place the plate in a chromatographic tank containing a mixture of hexane and tetradecane (95:5), and allow the mixture to run the entire length of the plate.

To remove the plate, and let it drying. To separately apply to the plate 10 µL of each of the solutions freshly prepared, as described below.

Solution (1): to reconstitute the powder as informed on the label. To prepare 3 mg/mL cephalexin solution in water.

Solution (2): to dissolve accurately weighed amount of cephalexin SQR in water, to obtain a 3 mg/mL solution.

To develop the chromatogram. To remove the plate and dry it at 110°C for 10 minutes. The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test. To determine in the powder before reconstituting.

pH (5.2.19). 3.0 to 6.0. To determine in the reconstituted suspension as informed on the label.

PURITY TESTS

Determination of water (5.2.20.1). At most 2%.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Microbiological test of antibiotics (5.5.3.3.1)* by using cylinders with the agar diffusion method.

Micro-organism: *Staphylococcus aureus* ATCC 6538p.

Culture media: culture medium number 1, to maintain the micro-organism; sterile saline solution to the standardiza-

tion of the inoculum; and culture medium number 2, to the base layer and culture medium number 1, to inoculum preparation.

Sample solution: to reconstitute the suspension as informed on the label. To transfer the volume of suspension equivalent to 200 mg of cephalexin to 200 mL volumetric flask, top up with 1% Potassium phosphate buffer, sterile, pH 6.0 (Solution 1). To successively dilute to obtain 4 µg/mL, 8 µg/mL, and 16 µg/mL cephalexin, by using 1% Potassium phosphate buffer, sterile, pH 6.0 as diluent.

Standard solution: to dissolve accurately weighed amount of cephalexin SQR in 1% Potassium phosphate buffer, sterile, pH 6.0 to obtain 1 mg/mL solution.

To successively dilute to obtain 4 µg/mL, 8 µg/mL, and 16 µg/mL cephalexin, by using the 1% Potassium phosphate buffer, sterile, pH 6.0 as diluent.

Procedure: to add 20 mL of culture medium number 2 in a plate, wait to solidify, add 5 mL of 0.05% inoculum in culture medium number 1, and proceed as described in *Microbiological test of antibiotics*, by adding 0.1 mL of *Standard solution* and *Sample solution*, freshly prepared, to the cylinders.

B. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 254 nm; 250 mm chromatograph column and 4 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (3 µm to 10 µm), kept at room temperature; flow of *Mobile phase* of 1.5 mL/min.

Mobile phase: to dissolve 1 g of sodium pentane 1-sulfonate monohydrate in a mixture of water, acetonitrile, methanol, and triethylamine (850:100:50:15). To adjust the pH to 3.0 with phosphoric acid.

Sample solution: to reconstitute the powder as informed on the label. To transfer volume of suspension equivalent to 200 mg of cephalexin to a 200 mL volumetric flask, top up to volume with water, and make it homogeneous. To transfer 10 mL of this solution to 25 mL volumetric flask and top up with *Mobile phase*.

Standard solution: to dissolve accurately weighed amount of cephalexin SQR in water, to obtain a 1 mg/mL solution. To transfer 10 mL of this solution to 25 mL volumetric flask and top up with *Mobile phase*.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_{16}H_{17}N_3O_4S$ from the sample, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light, at room temperature.

LABELLING

To comply with the legislation in force.

CEFALOTIN SODIUM POWDER FOR INJECTABLE SOLUTION

It contains at least 90.0 % and at most 115.0% of the amount stated of cefalotin sodium ($C_{16}H_{15}N_2NaO_6S_2$).

IDENTIFICATION

The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method **B**, corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

pH (5.2.19). 6.0 to 8.5. To determine with diluent after reconstitution of the sample.

Determination of weight (5.1.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

PURITY TESTS

Specific rotation (5.2.8): +124° to +134°, in relation to the anhydrous substance, sodium bicarbonate-free. To determine at 5% cefalotin solution (w/v) in water.

Sodium bicarbonate To dissolve about 1 g of the powder for injectable solution, accurately weighed, in 50 mL of water. To add 0.1% methyl orange (w/v) dissolved in water and titrate with 0.05 M SV sulfuric acid. Each mL of 0.05 M SV sulfuric acid is equivalent to 8.401 mg of $NaHCO_3$. To calculate the percentage of sodium bicarbonate and use the value to calculate *Specific rotation* relative to the sodium bicarbonate-free and anhydrous base.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test. To proceed as described in *Membrane filtration method*.

Bacterial endotoxins (5.5.2.2). At most 0.13 UE/ mg of cefalotin sodium.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To accurately weigh amount of sample equivalent to 0.25 mg of cefalotin sodium. To transfer 100 mL volumetric flask and top up with water. To dilute in the same solvent to obtain 0.0025% concentration (w/v). To prepare a standard solution at the same concentration by using the same solvent. To measure the absorbance of the resulting solutions at 285 nm, by using water to adjust zero. To calculate the content of $C_{16}H_{15}N_2NaO_6S_2$ in the powder for injectable solution, from the readings obtained.

B. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 254 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μ m), kept at 40 °C; flow of *Mobile phase* of 1.5 mL/min.

Mobile phase: to dissolve 17 g of sodium acetate in 790 mL of water, add 0.6 mL of glacial acetic acid and, if necessary, adjust the pH to 5.9 + 0.1 with glacial acetic acid or 0.1 M sodium hydroxide. To add 150 mL of acetonitrile and 70 mL of ethanol and make it homogeneous.

Sample solution: to reconstitute the content of a vial in ultrapure water, stir to obtain total dissolution, transfer the entire volume to 200 mL volumetric flask, wash the flask with ultrapure water, and transfer it to flask. To top up to volume and stir. To transfer 5 mL of this solution to a 50 mL volumetric flask and top up with *Mobile phase*, thus obtaining 0.5 mg/mL cefalotin sodium solution.

Standard solution: to dissolve accurately weighed amount of cefalotin sodium SQR in *Mobile phase* to obtain a 0.5 mg/mL cefalotin sodium concentration.

Procedure: to separately inject 10 μ L of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of $C_{16}H_{15}N_2NaO_6S_2$ in the powder for injectable solution, from the responses from the *Standard solution* and *Sample solution*.

C. To proceed as described in *Microbiological test of antibiotics* (5.5.3.3.1) by using cylinders with the agar diffusion method.

Micro-organism: *Staphylococcus aureus* ATCC 6538p.

Culture media: culture medium number 1, to maintain the micro-organism and inoculum preparation; sterile saline solution, to the standardization of the inoculum and culture medium number 2, to the base layer.

Sample solution: to reconstitute the injectable solution as per label. To transfer volume of injectable solution, accurately measured, to volumetric flask, top up with water, and stir it. To dilute in the same solvent to obtain 10 μ g/mL solution. To transfer aliquots of 6.4 mL, 10 mL, and 15.6 mL of this solution to 100 mL flasks and top up to volume with 1% *Potassium phosphate buffer, sterile, pH*

6.0 (*Solution 1*). The 0.64 μ g/mL, 1.0 μ g/mL, and 1.56 μ g/mL solutions, respectively (A_1 , A_2 , and A_3), are found.

Standard solution: to weigh 20 mg of cephalothin sodium SQR, transfer to a 20 mL flask, and dissolve in water to obtain 1 mg/mL solution. To dilute in the same solvent to obtain 10 μ g/mL solution. To transfer aliquots of 6.4 mL, 10 mL, and 15.6 mL of this solution to 100 mL flasks and top up to volume with 1% Potassium phosphate buffer, sterile, pH 6.0 (*Solution 1*). The 0.64 μ g/mL, 1.0 μ g/mL, and 1.56 μ g/mL solutions, respectively (P_1 , P_2 , and P_3), are found.

Procedure: to pipette 20 mL of culture medium number 2 in a plate, wait to solidify, add 5 mL of 0.1% inoculum in culture medium number 1, and proceed as described in *Microbiological test of antibiotics* (5.5.3.3.1), by adding 0.1 mL of *Standard solution* and *Sample solution*, freshly prepared, to the cylinders. To calculate the potency of the sample, in μ g, of cefalotin sodium per mg, from the potency of the standard and responses obtained with the *Standard and sample solutions*.

PACKAGING AND STORAGE

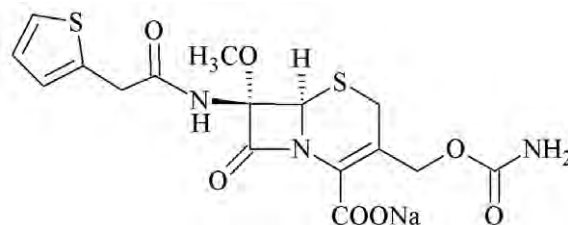
In tightly closed containers, at temperature below 25 °C.

LABELLING

To comply with the legislation in force.

CEFOXITIN SODIUM

Cefoxitinum natricum



$C_{16}H_{16}N_3NaO_7S_2$; 449.43

cefoxitin sodium; 01883

Sodium salt of (6R, 7S) -3 - [[(aminocarbonyl) oxy] methyl]-7-methoxy-8-oxo-7-[(2-thienylacetyl) amino]-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid (1:1) [33564-30-6]

It contains at least 927 μ g and at most 970 μ g of cefoxitin ($C_{16}H_{17}N_3O_7S_2$), per milligram, corresponding to at least 97.5% and at most 102.0% of cefoxitin sodium ($C_{16}H_{16}N_3NaO_7S_2$) in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White or almost white powder, very hygroscopic.

Solubility. Very soluble in water; slightly soluble in ethanol; practically insoluble in ethyl ether.

Physical and chemical constants.

Specific rotation (5.2.8): $+206^{\circ}$ to $+214^{\circ}$, in relation to the anhydrous substance. To determine in 1% solution (w/v) in methanol.

IDENTIFICATION

A. The ultraviolet absorption spectrum (5.2.14), from 200 nm to 400 nm, of 0.002% solution (w/v) in phosphate buffer pH 7.1, has maximum and minima similar to those observed in the spectrum of a similar solution of cefoxitin sodium SQR.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

C. The 5% solution (w/v) of the sample in water responds to the reactions of sodium ion (5.3.1.1).

PURITY TESTS

pH (5.2.19). 4.2 to 7.0. To determine in 1% aqueous solution (w/v).

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using F_{254} silica gel as support and a mixture of glacial acetic acid, water, acetone, and ethyl acetate (10:10:20:50) as mobile phase. To separately apply to the plate 5 μ L of each of the solutions freshly prepared, as described below.

Solution (1): to accurately transfer 0.25 g of the sample to 10 mL volumetric flask, dissolve in water, and top up the volume with the same solvent.

Solution (2): to transfer 0.5 mL of *Solution (1)* to 100 mL volumetric flask and top up with water.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (2)* (0.5%).

Water (5.2.20.1). At most 1%.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

Bacterial endotoxins (5.5.2.2). At most 0.13 UE/ mg of cefoxitin.

DOSAGE

To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped

with an ultraviolet detector at 254 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μ m); flow of *Mobile phase* of 1.0 mL/min.

Mobile phase: mixture of water, acetonitrile, and glacial acetic acid (84:16:1). Alternatively, use a mixture of water, acetonitrile, and trifluoroacetic acid (84:16:1).

Sample solution: to accurately transfer amount of powder equivalent to 0.15 g of cefoxitin to 500 mL volumetric flask, dissolve in phosphate buffer, pH 7.1, and top up the volume with the same solvent. To use this solution within 5 hours.

Standard solution: to accurately weigh and dissolve in phosphate buffer pH 7.1 an amount of cefoxitin SQR sufficient to prepare a 0.3 mg/mL cefoxitin solution. To use this solution within 5 hours.

The column efficiency is greater than 2800 theoretical plates. The tailing factor for cefoxitin peak is not greater than 1.5. The relative standard deviation for replicate areas of the peaks recorded is not greater than 1.0 %.

Procedure: to separately inject 20 μ L of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of cefoxitin ($C_{16}H_{17}N_3O_7S_2$) from the sample of cefoxitin sodium, from the responses obtained for cefoxitin with the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In airtight closed containers, at 2 °C - 8 °C.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antimicrobial.

CEFALOTIN SODIUM POWDER FOR INJECTABLE SOLUTION

It contains cefoxitin sodium equivalent to at least 90.0 % and at most 120.0% of the amount stated for cefoxitin ($C_{16}H_{17}N_3O_7S_2$).

IDENTIFICATION

A. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

B. The 5% solution (w/v) of the sample in water responds to the reactions of sodium ion (5.3.1.1).

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test. To determine in the diluent vial.

pH (5.2.19). 4.2 to 7.0. To determine in 1% aqueous solution (w/v).

Determination of weight (5.1.1). It complies with the test. To determine in the not reconstituted powder.

PURITY TESTS

Water (5.2.20.1). At most 1%.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

Bacterial endotoxins (5.5.2.2). At most 0.13 UE/ mg of cefoxitin.

DOSAGE

To proceed as described in *Dosage*, in the monograph *Cefoxitin sodium*. To prepare the *Sample solution* as described below.

Sample solution: to reconstitute the content of a vial in volume of distilled water, accurately measured, according to the vial of diluent. To quantitatively transfer the reconstituted solution to suitable volumetric flask and dilute with water, to obtain about 0.3 mg/mL cefoxitin ($C_{16}H_{17}N_3O_7S_2$) solution. To use this solution within 5 hours.

Procedure: to separately inject 20 μ L of *Sample and standard solutions*, record the chromatograms, and measure the area under the peaks. To calculate the content of cefoxitin ($C_{16}H_{17}N_3O_7S_2$) in the injectable solution, from the responses obtained for the *Sample and standard solutions*.

PACKAGING AND STORAGE

In airtight closed containers, at 2 °C - 8 °C.

LABELLING

To comply with the legislation in force.

CENTELLA

Centellae folium

Centella asiatica (L.) Urban - APIACEAE; 09898

The vegetable drug consists of dried leaves. It contains at least 2.0% of asiaticoside, in relation to the desiccated material.

MACROSCOPIC DESCRIPTION

The leaf blades are membranous, rarely papyraceas, gray-green on the adaxial face and pale green on the lower face, glabrous to tomentose on both faces, covered with hyaline trichomes up to 2 mm, multicellular, uniseriate, composed of 2-5 cells. The lower cell is derived from a single basal cell. Ovate to orbicular-reniform blade, palm leaf, 5-9 ribs, cordate to truncate base, rounded apex, obtuse to truncate, margin slightly sinuate to toothed to crena, measuring from 1.5 cm - 7 cm long and 1 cm - 6 cm wide. Venation is very dense, actinodromous. The first order ribs are longitudinally straight. The second order ribs have moderate divergence angle. The ramifications of the secondary and tertiary ribs end in the epitema of the hydathodes. The areolas are pentagonal or polygonal, with simple venule, curved or branched only once, and randomly arranged. Petiole up to 15 cm long, broad in basal portion and channeled on the adaxial face, villous-tomentose, greenish-brown to reddish-brown.

MICROSCOPIC DESCRIPTION

In front view, the adaxial face of epidermis shows polygonal cells with straight to curved walls, pronounced stomata, paracytic, rare anisocytic, stomatal index equal to 18, and hydathodes, cuticle is striated. The lower face also has polygonal cells, larger than the adaxial one, pronounced stomata, paracytic and stomatal index equal to 12, cuticle is very striated. Both faces of the epidermis have simple trichomes, uniseriate, twisted, usually tricellular (2-5 cells), rather scarce on the adaxial face. In cross section, faces consisting of rectangular cells, flattened, alternating with quadrangular papillose cells; projection of stomata can be better observed, and the cuticle is thin. The mesophyll shows dorsoventral structure, with 1-3 layers of loose palisade parenchyma and spongy parenchyma occupying more than half of the mesophyll, composed of oblong cells in the horizontal direction; druses of calcium oxalate are found in these parenchymas. Rare secretory canals (ducts) are arranged along the phloem. In the midrib, there are, as a rule, two secretory canals, arranged in the region of the fundamental parenchyma, one is facing the adaxial side, and another, to the lower side, next to the vascular system and rarely in the phloem; the collenchyma is lacunar-type and is found on both faces, and is represented by 1-3 cell layers, especially on the adaxial face. The vascular system is collateral, in open arc, with various fibers in the outer zone to the phloem. The petiole is fistula and, in cross section, shows circular outline, with two opposite edges on the adaxial face, separated by a small slightly concave region, with channeled aspect. The epidermis has quadrangular cells, somewhat papillose, with paracytic stomata and simple trichomes, multicellular, uniseriate, with basal cell much shorter than the other. The cuticle is thin and striated. There is subepidermically an angular collenchyma, continuous, where a predominant cell layer is alternated with narrow regions of 2-3 layers, or even 5 on the edges. Below this, lies a chlorenchyma, containing seven collateral vascular bundles, arranged in a circle, separated by wide bands of fundamental parenchyma, with one smaller beam at each edge. Around the phloem, in the fundamental parenchyma, there may be starch cells. In each vascular bundle, there is a covering of fibers, restricted to the phloem. Se-

cretory canals are also observed in the petiole: one internally to the chlorenchyma, usually and regularly in regions where there is a greater number of such layers, one less frequently arranged throughout the structure, in the region approximately midway between the epidermis and vascular bundles, two opposite each other, in the same vascular bundle, both very close to the xylem, and one by vascular bundle on the edges. The medullary parenchyma is nonexistent, because the petiole is fistulous. Near the fistula there are druses of calcium oxalate, in the fundamental parenchyma cells.

MICROSCOPIC DESCRIPTION OF POWDER

The powder meets all the requirements for the species, unless the macroscopic characters. Characteristics: trichomes or their portions, uniseriate; druses of calcium oxalate, and portions of epidermal cells with paracytic stomata.

IDENTIFICATION

A. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel, with 250 µm thickness as support and a mixture of chloroform, glacial acetic acid, methanol, and water (60:32:12:8) as mobile phase. To separately apply to the plate, as band, 20 µL of *Solution (1)* and 5 µL of *Solution (2)* freshly prepared, as described below.

Solution (1): to boil 3 g of sample in 30 mL of a mixture of ethanol and water (1:1). To filter and concentrate to dryness. To take in 0.5 mL of methanol.

Solution (2): to dissolve 1 mg of asiaticoside in 1 mL of methanol.

To develop the chromatogram. To remove the plate and dry it in the hood for 5 minutes. To nebulize with anisaldehyde SR and heat it in an oven at 100 °C - 105 °C for 10 minutes. To nebulize again with anisaldehyde SR and heat it in an oven at 100 °C - 105 °C for 10 minutes. The brownish principal spot obtained with *Solution (1)*, R_f about 0.50, corresponds in position to that obtained with *Solution (2)*, relating to asiaticoside. A violet secondary spot, R_f about 0.90, is also observed.

B. To transfer 1 g of the powdered or fragmented drug to test tube, add 10 mL of distilled water, and boil it for 2 minutes. To cool down and vigorously stir for 15 seconds. Then, add drops of 10% hydrochloric acid (w/v). A persistent foam is formed, which characterizes the presence of saponins.

PURITY TESTS

Foreign matter (5.4.2.2). At most 2%.

Water (5.4.2.3). At most 6%.

Total ashes (5.4.2.4). At most 11%.

FOAM INDEX

To proceed as described in *Determination of foam index* (5.4.2.10). To weigh 1 g of the pulverized drug, transfer

to a test tube, and boil for 2 minutes. To use 100 mL of distilled water. At most 100.

DOSAGE

Asiaticoside

To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 200 nm; 250 mm column long and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (3 µm to 10 µm), kept at room temperature; flow of *Mobile phase* of 0.5 mL/min.

Eluent A: acetonitrile.

Eluent B: 0.5% phosphoric acid aqueous solution (v/v).

Mobile phase gradient: to adopt the gradient system described in the following table:

<i>Time (minutes)</i>	<i>Eluent A (%)</i>	<i>Eluent B (%)</i>	<i>Elution</i>
0 - 40	25 → 50	75 → 50	linear gradient

Sample solution: to extract 5 g of the dry powdered drug with 150 mL of methanol in a Soxhlet extractor for 4 hours. To evaporate the solvent in a water bath to about 50 mL. To filter by using a sintered glass funnel (G4). To transfer the filtrate to 100 mL volumetric flask and top up with methanol.

Reference solution (1): to dissolve 30 mg of asiaticoside in 5 mL of methanol.

Reference solution (2): to dilute *Reference solution (1)* in methanol, to obtain a 80% solution (v/v).

Reference solution (3): to dilute *Reference solution (1)* in methanol, to obtain a 60% solution (v/v).

Reference solution (4): to dilute *Reference solution (1)* in methanol, to obtain a 40% solution (v/v).

Reference solution (5): to dilute *Reference solution (1)* in methanol, to obtain a 20% solution (v/v).

Procedure: To separately inject 10 µL of *Sample solution* and *Reference solutions (1), (2), (3), (4), and (5)*. To determine the area under the asiaticoside peak (retention time: 30 - 40 minutes). To establish an analytical curve with values from *Reference solutions (1), (2), (3), (4), and (5)*. To determine the area under the asiaticoside peak in the *Sample solution* and, using the analytical curve, determine the content of concentration of asiaticoside in the sample.

PACKAGING AND STORAGE

In tightly closed container, protected from light and heat.

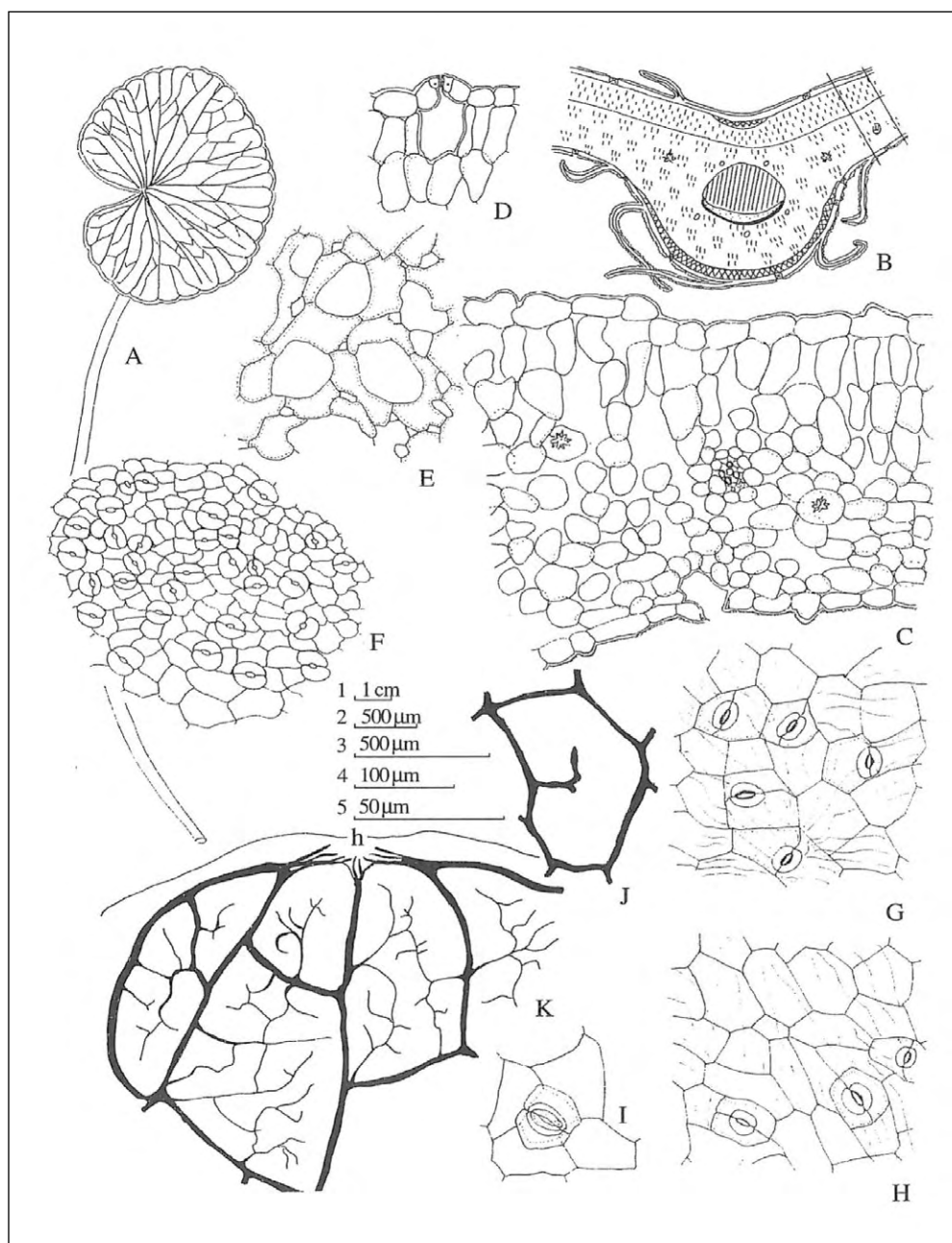


Figure 1 - Macroscopic and microscopic aspects of *Centella asiatica* (L.) Urban

Additional information of **Figure 1**. Scales correspond to 1 cm in **A** (rule 1); in **K**, 500 μm (rule 2), in **B**, **F**, and **J**, 500 μm (rule 3); in **C**, **D**, **E**, **G**, and **H**, 100 μm (rule 4); and **I**, 50 μm (rule 5).

A - appearance of the leaf. **B** - scheme of the cross section of the leaf in midrib. **C** - cross section of the leaf in the region of limb, in **B**. **D** - detail of cross section of leaf with stomata and substomatal chamber. **E** - appearance of parenchyma. **F** - hydathode in the adaxial epidermis. **G** - adaxial epidermis. **H** - lower epidermis. **I** - detail of paracitic stomata. **J** - leaf architecture: areola. **K** - leaf architecture: edge and hydathode.

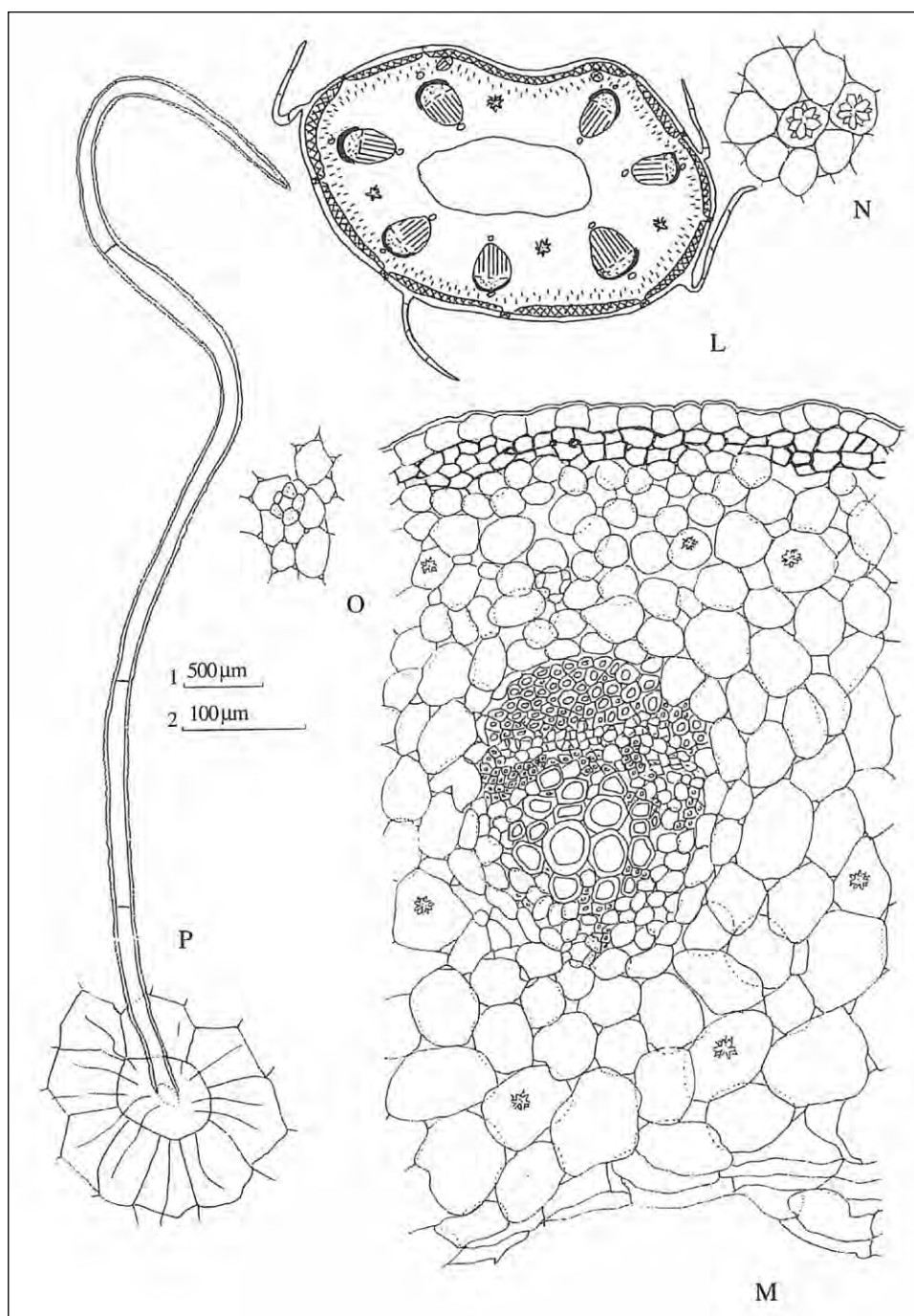


Figure 2 - Microscopic aspects of *Centella asiatica* (L.) Urban

Additional information of **Figure 2**. Scales correspond to 500 μm, in **L** (rule 1); **M** to **P**, 100 μm (rule 2).

L - scheme of petiole in cross-section; **M** - detail of a cross-sectional portion of the petiole. **N** - drusen of calcium oxalate. **O** - secretory canal. **P** - simple multicellular trichomes.

KETOCONAZOLE TABLETS

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_{26}H_{28}Cl_2N_4O_4$.

IDENTIFICATION

To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using G silica gel as support and a mixture of n-hexane, ethyl acetate, methanol, water, and glacial acetic acid (42:40:15:2:1) as mobile phase. To separately apply to the plate 10 μ L of each of the solutions freshly prepared, as described below.

Solution (1): to weigh and pulverize the tablets. To dissolve amount of the sample equivalent to 50 mg of ketoconazole in chloroform, stir for about 2 minutes, top up to 50 mL with the same solvent, and filter it.

Solution (2): to dissolve 10 mg of ketoconazole SQR in chloroform and top up to 10 mL with the same solvent.

To develop the chromatogram in unsaturated tank. To remove the plate, and let it air drying. To examine under ultraviolet light (254 nm). The principal spot obtained with *Solution (1)* corresponds in position and intensity to that obtained with *Solution (2)*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 900 mL

Equipment: blades, 50 rpm

Time: 30 minutes

Procedure: after testing, remove aliquot from the dissolution medium, filter, and dilute in 0.1 M hydrochloric acid, to obtain suitable concentration. To measure the absorbances of the solutions at 270 nm (5.2.14), by using the same solvent to adjust zero. To calculate the amount of $C_{26}H_{28}Cl_2N_4O_4$ dissolved in the medium, by comparing the readings obtained with that of 0.0001% ketoconazole SQR solution (w/v) prepared in the same solvent.

Tolerance: not less than 80% (Q) of the amount stated of $C_{26}H_{28}Cl_2N_4O_4$ dissolves within 30 minutes.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 225 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (3 μ m to 10 μ m), kept at room temperature; flow of *Mobile phase* of 1.0 mL/min.

Mobile phase: mixture of methanol and 0.5 M ammonium acetate (w/v) (95:5).

Sample solution: to weigh and pulverize 20 tablets. To transfer accurately weighed amount of powder equivalent to 0.1 mg of ketoconazole to a 50 mL volumetric flask, add 30 mL of methanol, and put it in ultrasound for 30 minutes. To top up with the same solvent, make it homogeneous, and filter it. To dilute the filtrate to 0.1 mg/mL concentration, by using methanol as solvent.

Standard solution: to dissolve an amount accurately weighed of ketoconazole SQR in methanol and dilute with the same solvent, to obtain a 0.1 mg/mL solution.

Procedure: to separately inject 20 μ L of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of $C_{26}H_{28}Cl_2N_4O_4$ in the tablets, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

KETOCONAZOLE SHAMPOO

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_{26}H_{28}Cl_2N_4O_4$.

IDENTIFICATION

The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 232 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at 40 °C; flow of *Mobile phase* of 1.5 mL/min.

Phosphate buffer pH 7.0: to dissolve 2.8 g of sodium phosphate dibasic anhydrous in 1000 mL of water. To adjust the pH to 7.0 with phosphoric acid.

Mobile phase: to prepare a mixture of acetonitrile, methanol, tetrahydrofuran and *Phosphate buffer pH 7.0* (390:95:15:500), by adding 2.5 mL of 25% tetramethylammonium hydroxide (w/v) in methanol. To filter it and degas it.

Sample solution: to transfer an accurately weighed amount of shampoo equivalent to 20 mg of ketoconazole to a 100 mL volumetric flask. To add 70 mL of methanol to the flask, stir it in ultrasound for 30 minutes to dissolve it. To top up to volume with methanol and filter through filter paper. To transfer 2.5 mL of this preparation to a 10 mL volumetric flask and add 1.5 mL of methanol. To top up with water and mix it.

Standard solution: to accurately weigh about 10 mg of ketoconazole SQR and transfer to a 100 mL volumetric flask. To add 70 mL of methanol to the flask and stir it to dissolve. To top up with methanol and mix it. To transfer an aliquot of 5 mL to a 10 mL volumetric flask. To add 1 mL of methanol and top up with water.

The relative standard deviation for the replicate areas for the peaks recorded is not greater than 2%.

Procedure: to separately inject 20 µL of *Sample and standard solutions*, record the chromatograms, and measure the area under the peaks. To calculate the amount of $C_{26}H_{28}Cl_2N_4O_4$ in the sample from the responses obtained from *Sample and standard solutions*.

PACKAGING AND STORAGE

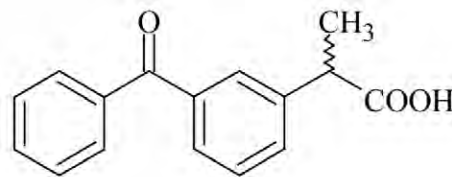
In tightly closed container, protected from excessive heat.

LABELLING

To comply with the legislation in force.

KETOPROFEN

Ketoprofenum



$C_{16}H_{14}O_3$; 254.28

ketoprofen; 01960

3-benzoyl-α-methyl-benzeneacetic acid
[22071-15-4]

It contains at least 98.5% and at most 101.0% of $C_{16}H_{14}O_3$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white or almost white powder.

Solubility. Practically insoluble in water, freely soluble in acetone, ethanol and methylene chloride.

Physical and chemical constants.

Melting range (5.2.2): 94 °C to 97 °C.

Specific rotation (5.2.8): +1° to -1°, in relation to the desiccated substance. To determine at 1% solution (w/v) in ethanol.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the ketoprofen SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14) ranging from 230 nm to 350 nm, of 0.0001% solution (w/v) in ethanol, exhibits maxima absorption at 255 nm. The absorbance at 255 nm is between 0.615 and 0.680.

PURITY TESTS

Chromatographic Purity. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 233 nm; 150 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm); flow of *Mobile phase* of 1 mL/min.

Buffer pH 3.0: to dissolve 68.0 g of monobasic potassium phosphate in 1000 mL of water and adjust the pH to 3.5 ± 0.1 with phosphoric acid.

Mobile phase: mixture of water, acetonitrile, and *Buffer pH* 3.5 (55:43:2).

Sample solution: to dissolve accurately weighed amount of the sample in *Mobile phase* to obtain 200 µg/mL solution. This solution must be protected from light.

Standard solution: to dissolve accurately weighed amount of ketoprofen SQR in *Mobile phase* to obtain 200 µg/mL solution. This solution must be protected from light.

To inject replicates of 20 µL of the *Standard solution*. The column efficiency is greater than 2250 theoretical plates. The tailing factor for ketoprofen peak is not greater than 2.0. The relative standard deviation for replicate areas of the peaks recorded is not greater than 5.0 %.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms at least 3 times the retention time of ketoprofen, and measure the areas corresponding to the impurity peaks. To calculate the percentage of each impurity. At most 0.2% for each individual impurity is found. The sum of all impurities is not greater than 1.0% of ketoprofen area obtained with the *Sample solution*.

Heavy metals (5.3.2.3). To use the *Method I*. At most 0.002% (20 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 60°C under reduced pressure for 4 hours. At most 0.5 %.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1 %.

DOSAGE

To accurately weigh about 0.2 g of the sample, previously desiccated, transfer to a 250 mL erlenmeyer flask, and dissolve in 25 mL of ethanol. To add 25 mL of water and 0.5 mL of phenol red SI. To titrate with 0.1 M SV sodium hydroxide. To potentiometrically determine the endpoint, as an alternative. To perform sample blank correction and adjust it as required. Each mL of 0.1 M SV sodium hydroxide is equal to 25.428 mg of $C_{16}H_{14}O_3$.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Anti-inflammatory

CHAPÉU-DE-COURO *Echinodorus folium*

Echinodorus grandiflorus (Cham. & Schltdl.) Micheli - ALISMATACEAE

The vegetable drug consists of dried leaves containing at least 2.8% of *o*-hydroxycinnamic acid derivatives, expressed as verbascoside ($C_{29}H_{36}O_{15}$, 624.6).

CHARACTERISTICS

Organoleptic characteristics. The dried leaves have a characteristic odor and bitter taste.

MACROSCOPIC DESCRIPTION

Simple leaves, coriaceous, cordate, cordate base, and acute to rounded apex. Leaf blades of various dimensions, depending on the environmental condition, from 10 cm to 35 cm long and from 20 cm to 25 cm wide, at the middle portion; long petiole in circular to oval cross-section, with winged short extensions and longitudinal striations. Venation is the camplodromous-type, with 12-14 ribs of similar calibers, starting from a single point at the base of the limb, prominent on the lower face. Ribs with smaller caliber originate from these, parallel to each other, and tertiary ones, from these as well, culminating in the formation of closed areolas with little branching endings. Blade and petiole are pubescent, relatively rough by the presence of star trichomes. Translucent secretory ducts are abundant throughout the leaf blade, sometimes viewed without the aid of lenses.

MICROSCOPIC DESCRIPTION

Leaf blade with unistratified epidermis covered with a thin cuticle provided with small papillae, which appear as bright spots in microscopy, also present in the petiole. Amphistomatic blade with stomata at the same level as the other epidermal cells, parastomatocytic, with elongated guard-cells, with two pairs of adjacent subsidiary cells, the most proximal is elongated and slender, sometimes viewed with some difficulty due to low contrast obtained in its walls. In front view, on both sides of the blade, there are common epidermal cells with varying sizes and shapes, whose anticlinal walls may be relatively straight to sinuous, although the latter format is more common on the lower face. About the ribs, there are no stomata, and epidermal cells are rectangular, sometimes very elongated in relation to major axis of the leaf. Also on the ribs, there are multicellular, star trichomes. In cross-section, there are voluminous epidermal cells and substomatic chambers are large. The mesophyll is composed of only one layer of palisade parenchyma, with slightly elongated cells, and spongy parenchyma, whose cells have small brachiform expansions.

The ribs of larger caliber are biconvex, with more significant curvature along the lower face, with aerenchyma in abundance, which forms large gaps along the entire

length of the structure. In these gaps, which are arranged transversely, there are trabeculae of brachiform cells with thickened recesses allowing the formation of intercellular triangle spaces. There are secretory ducts throughout the aerenchyma. In the midrib, there are three vascular bundles of larger caliber, collateral, open arc, with raised edges, small gap in protoxylem, partially surrounded by fiber-sclereids calottes, long and lignified walls. There are 8-11 vascular bundles concentrically arranged, with smaller calibers, also in open arc, but with sclerenchyma near phloem. The ribs of smaller caliber, arranged in the mesophyll, are collateral with a callote of few sclerenchymatous elements on both poles of conducting tissues. A single layer of parenchyma cells, voluminous and thin, makes up the vascular bundle. The petiole has polyhedral epidermal cells, longitudinally elongated. In cross-section, this leaf portion is filled with aerenchyma, with the same characteristics of the midrib, including the secretory ducts and trabeculae of brachiform cells. Several cells in this aerenchyma are filled with starch grains. The vascular bundles of greater caliber (1-2) are arranged in the central region of the petiole, with three concentric groups of vascular bundles, smaller caliber towards the periphery, similar to the midrib, but with a gap of large protoxylem. As the midrib, bundles of smaller caliber have sclerenchyma only along the phloematic pole. No positive reactions for polyphenols (10% ferric chloride, (w/v)) or lipidic substances (Sudan IV) have been observed in both tissues of the leaf blade and petiole.

MICROSCOPIC DESCRIPTION OF POWDER

The powder meets all the requirements for the species, unless the macroscopic characters. Characteristics: fragments of the leaf blade epidermis with parallelocytic stomata and epidermal cells of sinuous contours, covered with ornamented cuticle; fiber-sclereids bundles associated with cells of small brachiform expansions, spongy parenchyma; sets of tabular cells, longitudinally elongated, brachiform cells with thickened recesses that make up the trabeculae of midrib and petiole, occur in abundance.

IDENTIFICATION

To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using F₂₅₄ silica gel, with 250 µm thickness as support and a mixture of ethyl acetate, toluene, formic acid, and water (100:10:10:1) as mobile phase. To separately apply to the plate, as band, 10 µL of *Solution* (1) and 5 µL of *Solutions* (2), (3), and (4), freshly prepared, as described below.

Solution (1): to accurately perform turbolysis in about 10 g of the vegetable grinded drug in 100 mL of 70% ethanol (v/v) for 15 minutes, at 5-minute intervals, so that the temperature does not exceed 40 °C. To filter and discard the ethanol in a rotary evaporator under reduced pressure. To extract the resulting aqueous phase with 3 portions (25 mL each) of ethyl acetate in a 125 mL separatory funnel. To let it rest at -18 °C for 15 minutes, to obtain complete separation of the phases. To collect the organic portions

and wash with 50 mL of water. To evaporate the organic portion obtained in a rotary evaporator under reduced pressure, to obtain the residue. To take the residue in 1 mL of methanol.

Solution (2): to weigh about 1 mg of caffeic acid and dissolve in 1 mL of methanol.

Solution (3): to weigh about 1 mg of isoorientin and dissolve in 1 mL of methanol.

To develop the chromatogram. To remove the plate and dry in fume hood. To nebulize the plate with aminoethanol diphenyl borate SR and let it dry in fume hood. The brownish spot obtained with *Solution* (1), Rf about 0.90, corresponds in position to that obtained with *Solution* (2). Immediately below this, there is a greenish spot, Rf about 0.82, in the region of the chromatogram of *Solution* (1). In the lower quadrant of the chromatogram, the intense yellow spot obtained with *Solution* (1), Rf 0.28, corresponds in position to that obtained with *Solution* (3), corresponding to isoorientin. Immediately below, there is other intense yellow spot obtained in the region of the chromatogram of *Solution* (1), Rf 0.22, which corresponds to swertia-japonina.

PURITY TESTS

Foreign matter (5.4.2.2). At most 2.0%.

Water (5.4.2.3). At most 9.0%.

Total ashes (5.4.2.4). At most 11.0%.

Sulfated ashes (5.2.10). At most 13.0 %.

DOSAGE

Derivatives of o-hydroxycinnamic acid

To proceed as described in *Visible absorption spectrophotometry* (5.2.14). To prepare the solutions as described below.

Stock solution: to accurately weigh about 0.5 g of pulverized drug (210 µm) and add 90 mL of 50% ethanol (v/v) to a 250 mL round bottom flask. To take the reflux for 30 minutes. To cool down and filter to 100 mL volumetric flask. To wash the round bottom flask and filter with 10 mL of 50% ethanol (v/v) to the volumetric flask. To top up with 50% ethanol (v/v).

Sample solution: To volumetrically add 1 mL of *Stock solution*, 2 mL of 0.5 M hydrochloric acid, 2 mL of the mixture of 20% sodium nitrite (w/v) and 20% sodium molybdate (w/v) (1:1) to a 10 mL volumetric flask.

To add 2 mL of 8% sodium hydroxide solution (w/v) and top up with 50% ethanol (v/v).

Blank solution: To volumetrically add 1 mL of *Stock solution*, 2 mL of 0.5 M hydrochloric acid, and 2 mL of 8% sodium hydroxide (w/v) (1:1) to a 10 mL volumetric flask, and top up with 50% ethanol (v/v).

To measure the absorbance of the *Sample solution* at 525 nm immediately after preparation, by using *Blank solution* to adjust zero. To calculate the content of o-hydroxycinnamic acid derivatives expressed as a percentage of verbascoside, according to the following equation. To consider the specific absorptivity of verbascoside equal to 185.

$$TA = \frac{A \times 1000}{185 \times m}$$

where

TA = content of o-hydroxycinnamic acid derivatives expressed in verbascoside (%)

A = absorbance of the *Sample solution*

m = mass of the sample used in the test (g) by considering the determination of water

PACKAGING AND STORAGE

In tightly closed container, protected from light and heat.

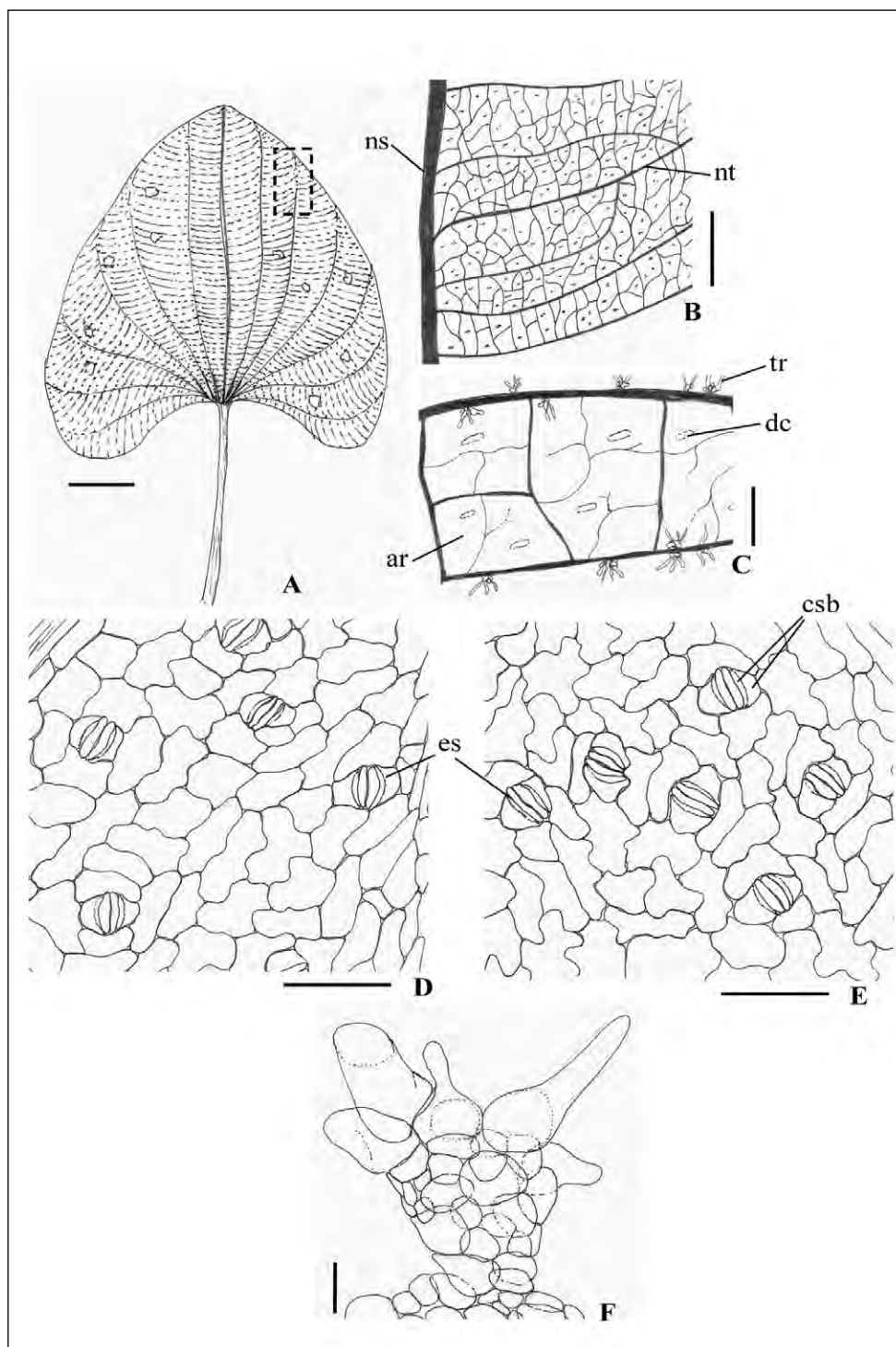


Figure 1 - Macroscopic and microscopic aspects in *Echinodorus grandiflorus* (Cham. & Schltdl.) Micheli

Additional information of **Figure 1**. Scales correspond to 8 cm, in **A**; in **B**, 5 mm; in **C**, 1 mm; in **D** and **E**, 100 μ m; and **F**, 50 μ m.

A - general appearance of the leaf, in front view. **B** - partial detail of secondary rib (ns) and tertiary ribs (nt) in **A**. **C** - detail of some areolas and vascular endings of leaf blade: areola (ar); secretory duct (dc); star trichomes (tr). **D** — detail of the portion of the epidermis in the leaf blade facing the adaxial face, in front view: stomata (es). **E** — detail of the portion of the epidermis in the leaf blade facing the lower face, in front view: subsidiary cells (csb); stomata (es). **F** - detail of a star trichomes.

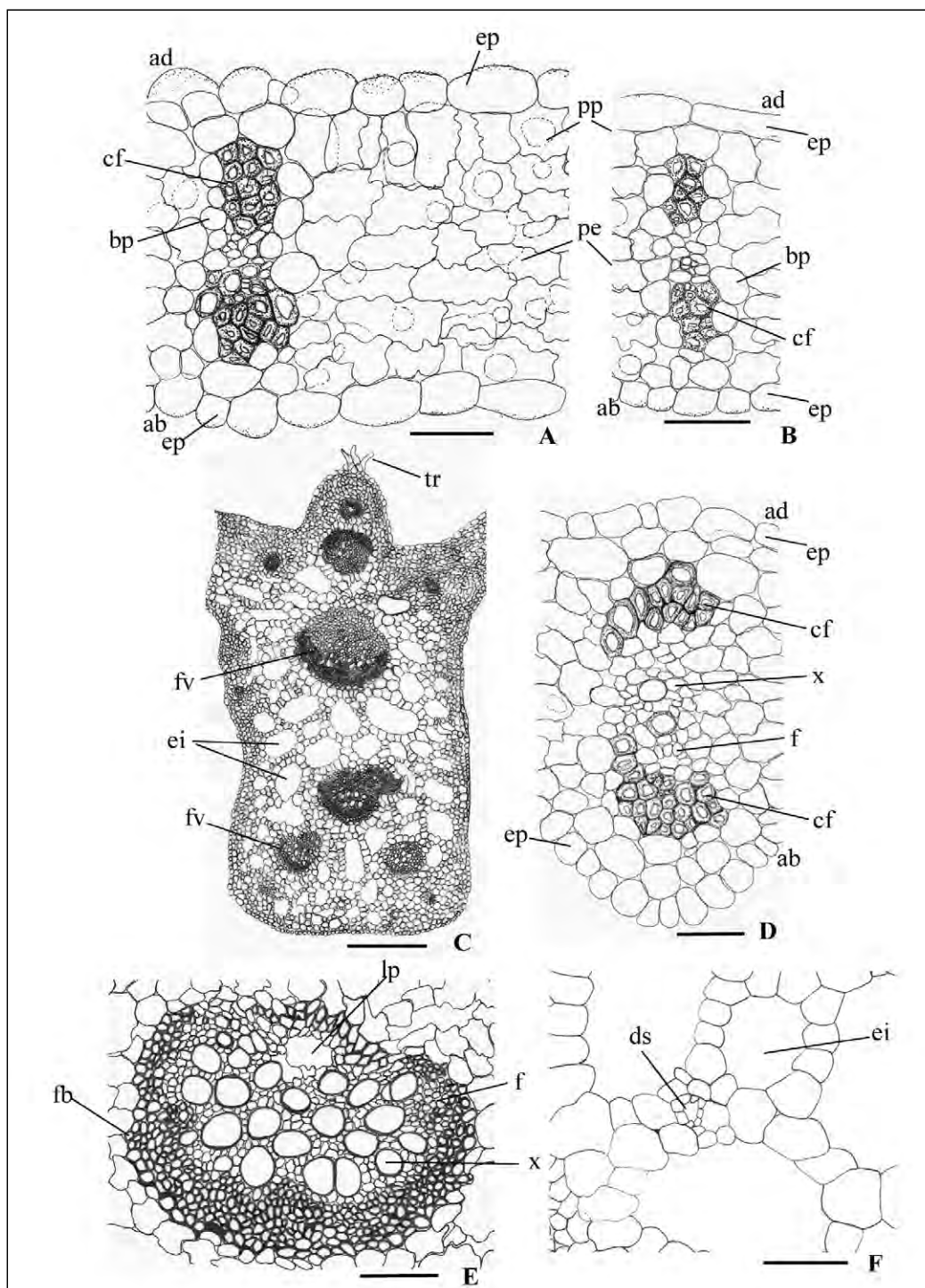


Figure 2 - Microscopic aspects of *Echinodorus grandiflorus* (Cham. & Schltdl.) Micheli

Additional information of **Figure 2**. Scales correspond to 50 μ m in **A**, **B**, and **D**, in **C**, 500 μ m; in **E** and **F**, 100 μ m.

A - detail of the portion of the mesophyll in the middle region of the leaf blade, in cross section: lower face (ab); adaxial face (ad); parenchymatous sheath (bp); fiber calotte (cf); epidermis (ep); spongy parenchyma (pe); palisade parenchyma (pp). **B** - detail of the portion of the mesophyll in the middle region of the leaf blade, showing tertiary bundle, in cross section: lower face (ab); adaxial face (ad); parenchymatous sheath (bp); fiber calotte (cf); epidermis (ep); spongy parenchyma (pe); palisade parenchyma (pp). **C** - detail of midrib, in cross-section: intercellular space (ei); vascular bundle (fv); star trichomes (tr). **D** - detail of portion of the mesophyll showing a vascular bundle, in cross section: lower face (ab); adaxial face (ad); fiber calotte (cf); epidermis (ep); phloem (f), xylem (x). **E** - detail of a vascular bundle of the midrib, in cross-section: phloem (f); fiber-sclereids (fb); gap of protoxylem (lp); xylem (x). **F** - detail of portion of the aerenchyma in the midrib, in cross-section: secretory duct (ds); intercellular space (ei).

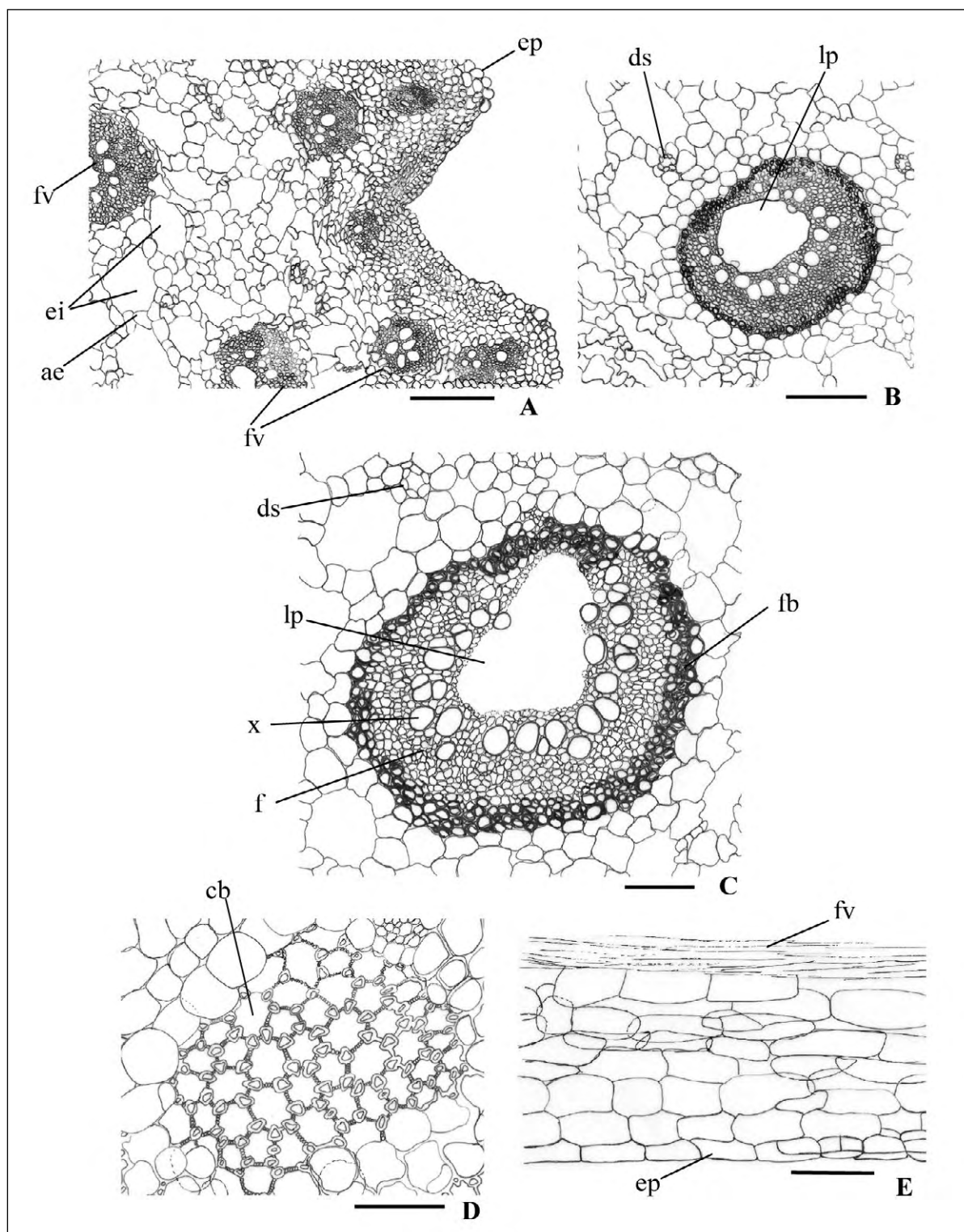


Figure 3 - Microscopic aspects of *Echinodorus grandiflorus* (Cham. & Schltdl.) Micheli

Additional information of **Figure 3**. Scales correspond to 200 µm in **A** and **B**, and **D**, and in **C**, **D**, and **E**, 100 µm.

A to **D** - cross sections of the petiole. **A** - detail of portion of the petiole: aerenchyma (ae); intercellular space (ei); epidermis (ep), vascular bundle (fv). **B** - detail of portion of the petiole, in the region of aerenchyma, showing a vascular bundle: secretory duct (ds); gap of protoxylem (lp). **C** - detail of a vascular bundle in the central region of the petiole: secretory duct (ds); phloem (f); fiber-sclereids (fb); gap of protoxylem (lp); xylem (x). **D** - detail of the trabeculae of petiole: brachiform cell (cb). **E** - partial detail of aerenchyma in longitudinal section: epidermis (ep), vascular bundle (fv).

CYANOCOBALAMIN INJECTABLE SOLUTION

It contains at least 95.0 % and at most 115.0 % of the amount stated of $C_{63}H_{88}CoN_{14}O_{14}P$.

IDENTIFICATION

The ultraviolet and visible absorption spectrum (5.2.14) ranging from 300 nm to 550 nm, of Sample solution obtained in *Dosage*, exhibits absorption maximum similar to those observed in the Standard solution spectrum. The ratio between the absorbance values measured at 361 nm and 550 nm is between 3.15 and 3.40.

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test. **pH (5.2.19).** 4.5 to 7.0.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

Bacterial endotoxins (5.5.2.2). At most 0.4 UE/ mg of cyanocobalamin.

DOSAGE

To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. To transfer volume of injectable solution containing the equivalent to 0.3 mg of cyanocobalamin to volumetric flask, and dilute with water to obtain 0.003% concentration (w/v). To prepare standard solution under the same conditions. To measure the absorbances of the solutions at 361 nm by using water to adjust zero. To calculate the amount of $C_{63}H_{88}CoN_{14}O_{14}P$ in the injectable solution, from the readings obtained.

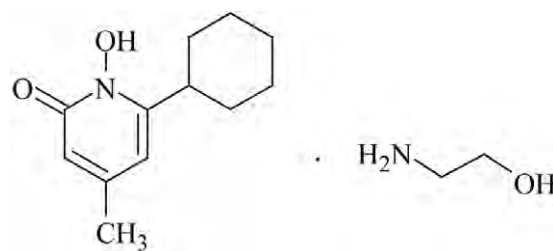
PACKAGING AND STORAGE

Single-dose glass container, type I, at room temperature and protected from light.

LABELLING

To comply with the legislation in force.

CICLOPIROX OLAMINE Ciclopirox olaminum



$C_{12}H_{17}NO_2 \cdot C_2H_7NO$; 268.35

ciclopirox olamine; 09336

6-Cyclohexyl-1-hydroxy-4-methyl-2 (1*H*)-pyridinone with 2-aminoethanol (1:1)

[41621-49-2]

It contains at least 97.5% and at most 101.5% of $C_{12}H_{17}NO_2 \cdot C_2H_7NO$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white to pale yellow powder.

Solubility. Sparingly soluble in water, very soluble in ethanol and methylene chloride, slightly soluble in ethyl acetate, practically insoluble in cyclohexane.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the ciclopirox olamine SQR spectrum similarly prepared.

B. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF_{254} silica gel as support and a mixture of 13.5 *M* ammonia, water, and ethanol (10:15:75) as mobile phase. To prepare two plates. To separately apply to the plate 10 μ L of each of the solutions freshly prepared, as described below.

Solution (1): to dissolve 25 mg of the sample in methanol and dilute in 10 mL volumetric flask, by using the same solvent.

Solution (2): to dissolve 25 mg of ciclopirox olamine SQR in methanol and dilute in 10 mL volumetric flask, by using the same solvent.

Before developing the chromatogram, wash the plates by passing a mixture of 10 volumes of 13.5 *M* ammonia, 15 volumes of water and 75 volumes of ethanol. The mixture should migrate to the top of the plate. To dry the plates at room temperature for 5 minutes. To develop the chromatograms. To remove the plate and allow it to air dry for 10 minutes.

To examine one of the plates under ultraviolet light (254 nm). The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*. To nebulize the plate with 1% ferric chloride (w/v) in methanol. The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*. To nebulize the other plate with ninhydrin SR and heat it up to 110 °C to obtain spots. The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

PURITY TESTS

pH (5.2.19). 8.0 to 9.0. To determine in 1% aqueous solution (w/v).

Heavy metals (5.3.2.3). To use *Method III*. At most 0.001% (10 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in high vacuum oven. At most 1.5 %.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1 %.

DOSAGE

To employ one of the methods described below.

A. To dissolve 0.2 g of the sample, previously desiccated, in 2 mL of methanol. To add 38 mL of water, stir, and titrate with 0.1 M SV sodium hydroxide. To potentiometrically determine the endpoint. To perform sample blank correction and adjust it as required. To determine the correction factor of 0.1 M SV sodium hydroxide by using 0.1 g of benzoic acid, previously weighed, and titrate under the conditions described above. Each mL of 0.1 M SV sodium hydroxide is equal to 26.84 mg of ciclopirox olamine ($C_{12}H_{17}NO_2 \cdot C_2H_7NO$).

To proceed as described in Microbiological test of antibiotics (5.5.3.3.1) by using cylinders with the agar diffusion method.

Micro-organism: Candida albicans ATCC 10231.

Culture media: sterile saline solution to standardize the inoculum and the culture medium number 19 for the inoculated layer.

Sample solution: to accurately weigh 25 mg of the sample and transfer to a 25 mL volumetric flask by using dimethyl sulfoxide. To top up with the same solvent and make it homogeneous. To successively dilute to obtain 56 µg/mL, 84 µg/mL, and 126 µg/mL concentrations, by using phosphate buffer, sterile, pH 7.2, as solvent.

Standard solution: to accurately weigh 25 mg of ciclopirox olamine SQR and transfer to a 25 mL volumetric flask by using dimethyl sulfoxide. To top up with the same solvent

and make it homogeneous. To successively dilute to obtain 56 µg/mL, 84 µg/mL, and 126 µg/mL concentrations, by using phosphate buffer, sterile, pH 7.2, as solvent.

Procedure: to add 8 mL of culture medium number 19, inoculated at 1% with a standardized suspension of microorganism, in each plate, wait to solidify and proceed as described in *Microbiological agar diffusion test (5.5.3.3.1)*. To calculate the potency of the sample in µg per mg of ciclopirox olamine, from the potency of the standard and the responses obtained with the *Standard solution* and *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antifungal.

CICLOPIROX OLAMINE TOPICAL SOLUTION

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_{12}H_{17}NO_2 \cdot C_2H_7NO$.

IDENTIFICATION

The ultraviolet absorption spectra (5.2.14), from 200 nm to 400 nm, of a 0.0008% solution (w/v), in methanol, exhibits maxima and minima similar to those observed in the spectrum of a similar solution of ciclopirox olamine SQR.

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Microbiological test of antibiotics (5.5.3.3.1)* by using cylinders with the agar diffusion method.

Micro-organism: *Candida albicans* ATCC 10231.

Culture media: sterile saline solution to standardize the inoculum and the culture medium number 19 for the inoculated layer.

Phosphate buffer pH 7.2, sterile: to add 250 mL of 0.2 M monobasic potassium phosphate and 175 mL of 0.2 M sodium hydroxide. To top up to 1000 mL. To sterilize the solution at 121 °C, in autoclave, for 20 minutes.

Sample solution: by using a volumetric pipette, transfer 25 mg of cimetiprox olamine to 25 mL volumetric flask, by using dimethyl sulfoxide. To top up with the same solvent and make it homogeneous. To successively dilute to obtain 56 µg/mL, 84 µg/mL, and 126 µg/mL concentrations, by using *Phosphate buffer, sterile, pH 7.2*, as diluent.

Standard solution: to accurately weigh 25 mg of cimetiprox olamine SQR and transfer to a 25 mL volumetric flask by using dimethyl sulfoxide. To top up with the same solvent and make it homogeneous. To successively dilute to obtain 56 µg/mL, 84 µg/mL, and 126 µg/mL concentrations, by using *Phosphate buffer, sterile, pH 7.2*, as diluent.

Procedure: to add 8 mL of culture medium number 19, inoculated at 1% with a standardized suspension of microorganism, in each plate, wait to solidify and proceed as described in *Microbiological agar diffusion test (5.5.3.3.1)*. To calculate the amount, in µg per mg, of cimetiprox olamine in the sample, from the potency of the standard and the responses obtained with the *Standard solution* and *Sample solution*.

B. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 300 nm; 250 mm chromatograph column and 4.0 mm inner diameter, packed with silica chemically bonded to the capped octadecylsilane group (5 µm), kept at 30 °C; flow of *Mobile phase* of 1.0 mL/min.

Mobile phase: mixture of acetonitrile and water (50:50).

Sample solution: to transfer the equivalent to 25 mg of cimetiprox olamine to 25 mL volumetric flask. To dilute with dimethyl sulfoxide and top up with the same solvent.

Standard solution: to transfer the equivalent to 25 mg of cimetiprox olamine to 25 mL volumetric flask. To dilute with dimethyl sulfoxide and top up with the same solvent.

Derivatization procedure: to separately transfer 2 mL of *Standard solution* to a 10 mL test tube. To add 1 mL of 0.1 M sodium hydroxide and 0.2 mL of dimethyl sulfate. To stir in a vortex. To put it in water bath at 37 °C for 15 minutes. To add 0.2 mL of triethylamine. To stir in a vortex. To dilute the resulting solution with *Mobile phase*, to obtain 40 µg/mL concentration. To repeat the same procedure with 2 mL of *Sample solution*.

Procedure: to separately inject 20 µL of *Sample and standard solutions* from derivatization procedure, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_{12}H_{17}NO_2$, C_2H_7NO from the sample, from the responses obtained for the *Sample solution* and *Standard solution*.

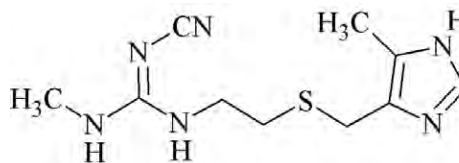
PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

CIMETIDINE Cimetidinum



$C_{10}H_{16}N_6S$; 252.34

cimetidine; 02073

N-Cyano-*N'*-methyl-*N''*-[2 - [[(4-methyl-1*H*-imidazol-5-yl) methyl] thio] ethyl] guanidine
[51481-61-9]

It contains at least 98.5% and at most 101.5% of $C_{10}H_{16}N_6S$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or almost white powder.

Solubility. Slightly soluble in water, soluble in ethanol, and practically insoluble in methylene chloride and ethyl ether. Soluble in dilute mineral acids.

Physical and chemical constants.

Melting range (5.2.2): 139 °C to 144 °C. If necessary, dissolve the substance in isopropyl alcohol, evaporate it to dryness, and determine again the melting range.

IDENTIFICATION

The identification test A. may be omitted if the tests B. and C. are conducted.

A. The infrared absorption spectrum (5.2.14) of the sample, desiccated at 105 °C to obtain constant weight, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the cimetidine SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14), from 200 nm to 400 nm, 0.0005% solution (w/v) in 0.1 M hydrochloric acid, has maximum at 221 nm, similar to that observed in the spectrum of a similar solution of cimetidine SQR.

C. To proceed as described in *Related substances*. The principal spot obtained with *Solution (2)* corresponds in position, color, and dimension to that obtained with *Solution (6)*.

D. To dissolve about 1 mg of the sample in a mixture of 1 mL of absolute ethanol and 5 mL of 2% citric acid solution (w/v) in acetic anhydride freshly prepared. To heat in water bath for 10-15 minutes. Reddish-violet color is developed.

PURITY TESTS

Related substances. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF₂₅₄ silica gel as support and a mixture of 13.5 M ammonia, methanol, and ethyl acetate (15:20:65) as mobile phase. To saturate the tank for 15 minutes with the vapor from the mobile phase. To apply separately to the plate 5 µl of each of the solutions as described below.

Solution (1): to dissolve 0.5 g of the sample in 10 mL of methanol.

Solution (2): to dilute 1 mL of *Solution (1)* to 10 mL with methanol.

Solution (3): to dilute 1 mL of *Solution (1)* to 100 mL with methanol and dilute 20 mL of this solution to 100 mL with methanol.

Solution (4): to dilute 5 mL of *Solution (3)* to 10 mL with methanol.

Solution (5): to dilute 5 mL of *Solution (4)* to 10 mL with methanol.

Solution (6): to dissolve 10 mg of cimetidine SQR in 2 mL of methanol.

To develop the chromatogram. To remove the plate, dry it in a current of air, put it under iodine vapor to obtain maximum contrast of the spots, and examine under ultraviolet light (254 nm). Any secondary spot obtained with *Solution (1)* (5%) is not more intense than the principal spot that obtained with *Solution (3)* (0.001%) and not more than two spots are more intense than the principal spot obtained with *Solution (4)* (0.0005 %). The test is valid only if the chromatogram obtained with *Solution (5)* shows a spot clearly visible.

Heavy metals (5.3.2.3). To use *Method III*. At most 0.002% (20 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 105 °C to obtain constant weight. At most 0.5 %.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.2 %.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. To accurately dissolve about 0.2 g of the sample in 60 mL of acetic anhydride. To titrate with 0.1 M SV perchloric acid, to potentiometrically determine the endpoint. Each mL of 0.1 M SV perchloric acid is equal to 25.234 mg of C₁₀H₁₆N₆S.

B. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 220 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm); flow of *Mobile phase* of 1.2 mL/min.

Mobile phase: to mix 200 mL of methanol and 0.3 mL of phosphoric acid, and top up to 1000 mL with water.

Sample solution: to dissolve accurately weighed amount of the sample in methanol and water (1:4), to obtain a 0.4 mg/mL solution. To put it in ultrasound for 15 minutes. To successively dilute to obtain 8 µg/mL concentration, by using *Mobile phase* as diluent.

Standard solution: to dissolve accurately weighed amount of cimetidine SQR in methanol and water (1:4), to obtain a 0.1 mg/mL solution. To dilute with *Mobile phase*, to obtain 8 µg/mL solution.

The column efficiency is greater than 1000 theoretical plates/meter. The relative standard deviation for the replicate areas for the peaks recorded is not greater than 2.0%.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the area under the peaks. To calculate the amount of C₁₀H₁₆N₆S in the sample, from the responses from the *Standard solution* and *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.=

THERAPEUTIC CLASS

Antihistamine H₂

CIMETIDINE TABLETS

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_{10}H_{16}N_6S$.

IDENTIFICATION

The ultraviolet absorption spectrum (5.2.14), ranging from 200 nm to 400 nm, of sample solution obtained in *Dosage* method A. exhibits maximum at 221 nm, similar to that observed in the cimetidine SQR spectrum.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL

Equipment: basket, 100 rpm

Time: 15 minutes

Procedure: to remove aliquot from the dissolution medium, filter, and dilute, if necessary, with 0.1 M sulfuric acid, to obtain suitable concentration. To measure the absorbances of the resulting solutions at 218 nm (5.2.14), by using 0.1 M sulfuric acid to adjust zero. To calculate the amount of cimetidine ($C_{10}H_{16}N_6S$) dissolved in the medium, by comparing the readings obtained with that of 0.0005% cimetidine SQR solution (w/v) prepared in the same solvent.

Tolerance: not less than 75% (Q) of the amount stated of $C_{10}H_{16}N_6S$ dissolves within 15 minutes.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3).

It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To weigh and pulverize 20 tablets.

To transfer amount of powder equivalent to 0.1 g cimetidine to a 200 mL volumetric flask, add 50 mL of 0.1 M hydrochloric acid, stir for 30 minutes, and top up to volume with the same solvent. To filter it. To successively dilute to obtain 0.0005% concentration (w/v) by using 0.1 M hydrochloric acid as solvent. To prepare standard solution under the same conditions. To measure the absorbances of the solutions at 221 nm, by using 0.1 M hydrochloric acid to adjust zero. To calculate the amount of $C_{10}H_{16}N_6S$ in the tablets, from the readings obtained.

B. To proceed as described in *Dosage*, method B., in monograph *Cimetidine*. To prepare the *Sample solution* as described below.

Sample solution: to weigh and pulverize 20 tablets. To transfer amount of powder equivalent to 40 mg of cimetidine to a 100 mL volumetric flask, add 20 mL of methanol, and put it in ultrasound for 15 minutes. To top up to volume with water, make it homogeneous, and filter it. To successively dilute to obtain 8 µg/mL concentration, by using *Mobile phase* as solvent.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the area under the peaks. To calculate the amount of $C_{10}H_{16}N_6S$ in the tablets, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light, at room temperature.

LABELLING

To comply with the legislation in force.

CIMETIDINE INJECTABLE SOLUTION

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_{10}H_{16}N_6S$. Cimetidine injectable solution is a sterile solution of cimetidine hydrochloride in water for injection.

IDENTIFICATION

A. To successively dilute the injectable solution in 0.1 M hydrochloric acid, to obtain 0.0005% cimetidine (w/v). To use cimetidine hydrochloride SQR and same solvent to prepare solution at the same concentration as cimetidine. The ultraviolet absorption spectrum (5.2.14) ranging from 200 nm to 400 nm exhibits maxima and minima only in the same wavelength of a similar cimetidine hydrochloride SQR solution.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method B. corresponds to that of main peak of *Standard solution*.

C. The injectable solution responds to the reactions of chloride ion (5.3.1.1).

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

pH (5.2.19). 3.8 to 6.0.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test. To employ the *Membrane filtration method* or *Direct inoculation in culture medium method*.

Bacterial endotoxins (5.5.2.2). At most 0.5 UE/mg of cimetidine hydrochloride.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. To transfer a volume of injectable solution containing the equivalent to 0.1 g of cimetidine to a 200 mL volumetric flask, top up with 0.1 M hydrochloric acid, and make it homogeneous. To successively dilute in the same solvent to obtain 0.0005% concentration (w/v). To prepare a standard solution at similar concentration and solvent, by using cimetidine hydrochloride SQR. To measure the absorbances of the solutions at 221 nm, by using 0.1 M hydrochloric acid to adjust zero. To calculate the amount of $C_{10}H_{16}N_6S$ in the injectable solution, from the readings obtained.

B. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 220 nm; 300 mm column and 3.9 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (3 µm to 10 µm), flow of *Mobile phase* of 2.0 mL/min.

Mobile phase: To transfer 200 mL of methanol and 0.3 mL of phosphoric acid to 100 mL volumetric flask, top up with water, make it homogenous, and filter it.

Sample solution: to transfer volume of injectable solution equivalent to 0.25 g of cimetidine hydrochloride to a 100 mL volumetric flask, top up to volume with *Mobile phase*, and make it homogeneous. To transfer 1 mL of this solution to 200 mL volumetric flask, top up with *Mobile phase*, and make it homogeneous.

Standard solution: to dissolve accurately weighed amount of cimetidine hydrochloride SQR in mixture of water and methanol (80:20) to obtain 0.5 mg/mL solution. To transfer 2,5 mL of this solution to 100 mL volumetric flask, top up with *Mobile phase*, and make it homogeneous.

The column efficiency for each analytical peak is greater than 1000 theoretical plates. The tailing factor is not greater than 0.6. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2.0 %.

Procedure: to separately inject 50 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_{10}H_{16}N_6S$ from the sample, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

Single-dose glass container, type I, at room temperature and protected from light.

LABELLING

To comply with the legislation in force.

CIPROFLOXACIN INJECTABLE SOLUTION

It contains at least 90.0% and at most 110.0% of the amount stated of $C_{17}H_{18}FN_3O_3$. Ciprofloxacin injectable solution is a ciprofloxacin solution in water for injection, in 5% glucose (w/v) or 0.9% sodium chloride (w/v) prepared with lactic acid.

IDENTIFICATION

To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF_{254} silica gel as support and a mixture of methylene chloride, methanol, ammonium hydroxide, and acetonitrile (4:4:2:1) as mobile phase. To separately apply to the plate, previously saturated for 15 minutes in ammonia atmosphere, 10 µL of each of the solutions freshly prepared, as described below.

Solution (1): to dilute volume of injectable solution in water, to obtain about 0.05% ciprofloxacin (w/v).

Solution (2): ciprofloxacin hydrochloride SQR solution in water at 0.05% ciprofloxacin (w/v).

To develop the chromatogram. To remove the plate, allow to air dry, and examine it under ultraviolet light (254 nm and 336 nm). The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

pH (5.2.19). 3.5 to 4.6.

PURITY TESTS

Limit of impurity for ciprofloxacin ethylenediamine.

To proceed as described in *Dosage* method B.

To calculate the percentage of impurity from the chromatogram obtained with *Sample solution*, according to the equation:

$$100 \times [0,7R_i / (0,7R_i + R_c)]$$

where

0.7 = response factor between impurity and ciprofloxacin;

R_i = response of impurity peak,

R_c = response of ciprofloxacin peak.

At most 0.5 %.

Limit for lactic acid. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 208 nm; 300 mm chromatograph column and 7.8 mm inner diameter, packed with ion-exchange resin consisting of sulfonated styrene divinylbenzene copolymer, in hydrogenated form (7 μ m to 11 μ m), kept at 40 °C; flow of *Mobile phase* of 0.6 mL/min.

Mobile phase: mixture of 0.0025 M sulfuric acid and acetonitrile (85:15).

Sample solution: to use the undiluted injectable solution.

Standard solution: 0.8 mg/mL sodium lactate SQR solution in water.

The column efficiency is greater than 5000 theoretical plates/meter. The relative standard deviation for the replicate areas for the peaks recorded should not be greater than 2.0%.

Procedure: to separately inject 20 μ L of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the amount, in milligrams, of lactic acid ($C_3H_6O_3$) per milliliter of injectable solution, according to the equation:

$$\left(\frac{90,08}{112,07} \right) \times (C) \times \left(\frac{R_a}{R_p} \right)$$

where

90.08 and 112.07 = molecular weight of lactic acid and sodium lactate, respectively;

C = concentration in mg/mL of sodium lactate SQR;

R_a and R_p = peak responses obtained with *Sample solution* and *Standard solution*, respectively.

The value obtained is between 0.288 mg and 0.352 mg of $C_3H_6O_3$ per mg of ciprofloxacin labeled.

Limit for dextrose. To transfer 50 mL of the injectable solution to 100 mL volumetric flask. To add 0.2 mL of 6 M ammonium hydroxide, top up with water, and stir it. To determine the rotation angle (α) in 200 mm tube at 25 °C (5.2.8). The amount in grams of dextrose ($C_6H_{12}O_6 \cdot H_2O$) in 100 mL of injectable solution is calculated according to the equation:

$$2 \times (1,0425 \times \alpha)$$

The result is between 4.75 and 5.25 g of $C_6H_{12}O_6 \cdot H_2O$ per 100 mL of injectable solution.

Limit for sodium chloride. To transfer 10 mL of injectable solution to erlenmeyer flask, dilute with water to about 150 mL, add 1.5 mL of potassium chromate SR, and titrate with 0.1 M SV silver nitrate. Each mL of silver nitrate is equivalent to 5.844 mg of sodium chloride. The result is between 85.5 mg and 94.5 mg.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test. To use Membrane filtration method.

Bacterial endotoxins (5.5.2.2). At most 1.76 UE/ mL of ciprofloxacin.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To dilute the injectable solution in water to obtain about 0.0004% ciprofloxacin (w/v). To use ciprofloxacin hydrochloride SQR to prepare a standard solution, by using the same solvent. To protect solutions from light. To measure the absorbance of the resulting solutions at 272 nm, by using water to adjust zero. To calculate the content of $C_{17}H_{18}FN_3O_3$ in the sample from the readings obtained.

B. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 278 nm; 250 mm chromatograph column and 4.0 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (3 μ m to 10 μ m), kept at 30 °C; flow of *Mobile phase* of 1.5 mL/min.

Mobile phase: mixture of 0.025 M phosphoric acid with pH pre-adjusted to 3.0 ± 0.1 with triethylamine and acetonitrile (87:13).

Sample solution: to transfer volume of injectable solution equivalent to 25 mg of ciprofloxacin to 100 mL volumetric flask and top up with *Mobile phase*.

Standard solution: to accurately weigh about 30 mg of ciprofloxacin hydrochloride SQR and transfer to a 100 mL

volumetric flask, by using Mobile phase as solvent, to obtain 0.25 mg/mL ciprofloxacin.

Resolution solution: to dissolve an amount of impurity ciprofloxacin ethylene diamine SQR (hydrochloride of 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-2 [2 - (amino)-3-quinoline carboxylic acid) in the *Standard solution*, to obtain 0.25 mg/mL solution.

The column efficiency should be greater than 10000 theoretical plates/meter.

The relative retention times are about 0.7 to impurity of *Resolution solution* and 1.0 to ciprofloxacin. The resolution between impurity and ciprofloxacin peaks should be greater than 6. The relative standard deviation for replicate areas of the peaks recorded is not greater than 1.5 %.

Procedure: to separately inject 10 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of $C_{17}H_{18}FN_3O_3$ in the sample, from the responses from the *Standard solution* and *Sample solution*.

C. To proceed as described in *Microbiological test of antibiotics (5.5.3.3.1)* by using cylinders with the agar diffusion method.

Micro-organism: *Staphylococcus epidermidis* ATCC 12228.

Culture media: culture medium number 1, to maintain the micro-organism; sterile saline solution to the standardization of the inoculum; and culture medium number 11, to the base layer and inoculum preparation.

Sample solution: to transfer volume of injectable solution equivalent to 20 mg of ciprofloxacin to 50 mL volumetric flask, top up with water, and stir it. To successively dilute to obtain 2 µg/mL, 4 µg/mL, and 8 µg/mL concentrations, by using the 0.1 M *Potassium phosphate buffer, sterile, pH 8.0 (solution 2)* as diluent.

Standard solution: to weigh an amount equal to 20 mg of ciprofloxacin SQR, transfer to a 50 mL volumetric flask, and top up with water. To successively dilute to obtain 2 µg/mL, 4 µg/mL, and 8 µg/mL concentrations, by using the 0.1 M *Potassium phosphate buffer, sterile, pH 8.0 (solution 2)* as diluent.

Procedure: to add 20 mL of culture medium number 11 in each plate, wait to solidify, add 5 mL of 1% inoculum, and proceed as described in *Microbiological test of antibiotics (5.5.3.3.1)*, by adding 0.1 mL of freshly prepared solutions to the cylinders. To calculate the potency of the sample, in µg, of ciprofloxacin per mg, from the potency of the standard and responses obtained with the *Standard and sample solutions*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light, at room temperature.

LABELLING

To comply with the legislation in force.

CISPLATIN INJECTABLE SOLUTION

It contains at least 90.0 % and at most 110.0 % of the amount stated of $Cl_2H_6N_2Pt$.

IDENTIFICATION

A. The ultraviolet absorption spectrum (**5.2.14**), from 230 nm to 350 nm, of 0.1% solution (w/v) of the sample, in water, exhibits maxima at 300 nm, similar to that observed in the spectrum of cisplatin SQR solution at the same concentration.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

pH (5.2.19). 3.5 to 6.5.

PURITY TESTS

Limit for amino (trichloro) platinate. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 209 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to quaternary ammonium groups for anion exchange, strong bases, (10 µm), kept at room temperature; flow of *Mobile phase* of 2 mL/min.

Mobile phase: to prepare 0.04% ammonium sulphate solution (w/v) in water and, if necessary, adjust pH between 5.8 and 6.0. To degas and filter it.

Solution (1): dilute, if necessary, the injectable solution in 0.9% sodium chloride solution (w/v), to obtain 0.05% cisplatin solution (w/v).

Solution (2): to dilute accurately weighed amount of potassium amino (trichloro) platinate SQR solution in 0.9% sodium chloride solution (w/v), to obtain 0.0015% amino (trichloro) platinate solution (w/v).

To inject replicates of 20 µL of *Solution (2)*. The resolution between sodium chloride and amino (trichloro) platinate peaks is greater than 2.0. The relative standard deviation for replicate areas for the peaks is not greater than 2.0%.

Procedure: to separately inject 20 µL of *Solution (1)* and *Solution (2)* and record the chromatograms. The area under the peak corresponding to amino (trichloro) platinate obtained in the chromatogram of *Solution (1)* is not greater than the area under the peak obtained in the chromatogram of *Solution (2)* (3%).

Limit for transplatin. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 254 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to cation-exchange groups, strong acids (10 µm), kept at 45 °C; flow of *Mobile phase* of 2 mL/minute for 30 minutes at 0.5 mL/min for further 30 minutes and again at 2 mL/minute for 30 minutes.

Mobile phase: to prepare 2.5 % monobasic potassium phosphate solution in water. To adjust pH to 3.2 with phosphoric acid. To degas and filter it.

Solution (1): 0.005 % transplatin SQR solution (w/v) in 0.9% sodium chloride solution (w/v).

Solution (2): 0.5% thiourea solution (w/v) in water.

Solution (3): 0.005 % cisplatin SQR solution (w/v) in 0.9% sodium chloride solution (w/v).

Solution (4): to dilute, if necessary, the injectable solution in 0.9% sodium chloride solution (w/v), to obtain 0.05% cisplatin solution (w/v).

Solution (5): to transfer 10 mL of *Solution (1)* to a 50 mL volumetric flask. To add 25 mg of cisplatin SQR, accurately weighed. To add 25 mL of 0.9% sodium chloride solution (w/v), stir for 30 minutes, and top up to volume with the same solvent.

Solution (6): to mix 5 mL of *Solution (2)* with 5 mL of *M* hydrochloric acid and 10 mL of *Solution (4)*. To heat it at 60 °C for 1 hour and cool down.

Solution (7): to mix 5 mL of *Solution (2)* with 5 mL of *M* hydrochloric acid and 10 mL of *Solution (5)*. To heat it at 60 °C for 1 hour and cool down.

Solution (8): to mix 10 mL of *Solution (1)* with 10 mL of *Solution (3)*. To heat it at 60 °C for 1 hour and cool down.

To inject 10 µL of replicates of *Solution (7)* and *Solution (8)*. The column efficiency, as determined for transplatin peak in the chromatogram of *Solution (7)* is greater than 2500 theoretical plates. The retention times for transplatin and cisplatin obtained in the chromatogram of *Solution (8)* are approximately 5 minutes and 9 minutes, respectively. If necessary, make adjustments in the composition of *Mobile phase* and recondition the column. The resolution between cisplatin and transplatin, in the chromatogram of *Solution (8)* is not less than 1.7. The relative standard deviation for

replicate areas of peaks obtained for the transplatin standard, in the chromatogram of *Solution (7)* is not greater than 2.0%.

Procedure: to separately inject 10 µL of *Solution (6)* and *Solution (7)* and record the chromatograms. The area under the peak corresponding to transplatin obtained in the chromatogram of *Solution (6)* is not greater than the area under the peak obtained in the chromatogram of *Solution (7)* (2%).

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

Bacterial endotoxins (5.5.2.2). At most 2.0 UE/ mg of cisplatin.

DOSAGE

To proceed as described in *High performance liquid chromatography (5.2.17.4)*.

To use chromatograph equipped with an ultraviolet detector at 220 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the amine group (10 µm), kept at room temperature; flow of *Mobile phase* of 1.5 mL/min.

Mobile phase: mixture of acetonitrile and water (90:10). To degas and filter it.

Solution (1): to dilute, if necessary, the injectable solution in 0.9% sodium chloride solution (w/v), to obtain 0.1% cisplatin solution (w/v).

Solution (2): 0.1 % cisplatin SQR solution (w/v) in 0.9% sodium chloride solution (w/v).

Solution (3): 0.05 % cisplatin SQR solution (w/v) and 0.005 % transplatin SQR solution (w/v) in 0.9% sodium chloride solution (w/v).

To inject 10 µL of *Solution (3)*. The resolution between cisplatin and transplatin is not less than 3.5. To inject replicates of 10 µL of *Solution (2)*. The relative standard deviation for the areas is not greater than 2.0%.

Procedure: to separately inject 100 µL of *Solutions (1)* and *(2)*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of $\text{Cl}_2\text{H}_6\text{N}_2\text{Pt}$ in the injectable solution, from the responses from the *Solution (1)* and *Solution (2)*.

PACKAGING AND STORAGE

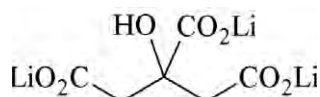
In tightly closed containers, protected from light. It should not be refrigerated.

LABELLING

To comply with the legislation in force.

CITRATE, LITHIUM

Lithii citras



$C_6H_5Li_3O_7$; 209.92

$C_6H_5Li_3O_7 \cdot 4H_2O$; 281.98

lithium citrate; 09575

Lithium salt of 2-hydroxy-1,2,3-propanetricarboxylic acid (3:1)

[919-16-4]

Lithium salt of 2-hydroxy-1,2,3-propanetricarboxylic acid hydrate (3:1:4)

[6080-58-6]

It contains at least 98.0% and at most 102.0% of $C_6H_5Li_3O_7$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. Fine, crystalline, white or almost white powder.

Solubility. Freely soluble in water, sparingly soluble in ethanol.

IDENTIFICATIONA. IT RESPONDS TO THE REACTIONS OF CITRATE ION (5.3.1.1).

B. To dilute 3 mL of solution obtained in *Appearance of solution* in 10 mL of water. To add 3 mL of potassium ferric periodate SR. White or yellowish-white precipitate should be formed.

C. Red color is developed when it is moistened with hydrochloric acid and subjected to non-luminous flame.

PURITY TESTS

Appearance of solution. To dissolve 10 g of the sample and dissolve in carbon dioxide-free water. To dilute to 100 mL with the same solvent. The solution is clear (5.2.25) and colorless (5.2.12).

Acidity or alkalinity. To add 0.1 mL of phenolphthalein SI to 10 mL of solution obtained in *Appearance of solution*. More than 0.2 mL of 0.1 M hydrochloric acid or 0.2 mL of 0.1 M sodium hydroxide is not necessary to change the indicator.

Carbonates. To accurately add about 0.5 g of the sample in 5 mL of 6 M acetic acid. Mild effervescence is developed.

Oxalates. To weigh 0.5 g of the sample and dissolve in 4 mL of water, add 3 mL of hydrochloric acid and 1 g of granulated zinc and heat in water bath for 1 minute. To let it rest for 2 minutes, decant the liquid into a test tube containing 0.25 mL of 1% phenylhydrazine hydrochloride (w/v), and heat

to boiling. To rapidly cool down, transfer to a beaker, and add an equal volume of hydrochloric acid and 0.25 mL of potassium ferricyanide SR. To stir and let it rest for 30 minutes. No pink color developed is more intense than that of standard simultaneously prepared by using 4 mL of 0.005% oxalic acid (w/v). At most 0.03% (300 ppm).

Chlorides (5.3.2.1). To weigh 3.55 g of sample and top up to 40 mL with water. To proceed as described in *Limit test for chlorides*. At most 0.01% (100 ppm).

Sulfates (5.3.2.1). To weigh 2.4 g of sample and top up to 40 mL with water. To proceed as described in *Limit test for sulfates*. At most 0.05% (500 ppm).

Heavy metals (5.3.2.3). To dissolve 2 g of the sample in 2 mL of 0.1 M hydrochloric acid and dilute to 25 mL with water. At most 0.001% (10 ppm).

Water (5.2.20.1). To determine in 0.1 g of the sample, by letting it under stirring for 15 minutes before titration. From 24.0% to 27.0%.

DOSAGE

To proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. To accurately weigh about 80 mg of the sample in 50 mL of glacial acetic acid, heat it at about 50 °C, and cool down. To add 0.25 mL of 1-naphtholbenzein SI and titrate with 0.1 M SV perchloric acid, to change from yellow to green. Each mL of 0.1 M SV perchloric acid is equal to 6.997 mg of $C_6H_5Li_3O_7$.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

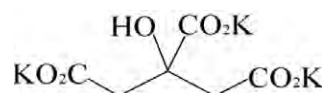
To comply with the legislation in force.

THERAPEUTIC CLASS

Antidepressant.

CITRATE, POTASSIUM

Kalli citras



$C_6H_5K_3O_7$; 306.39

$C_6H_5K_3O_7 \cdot H_2O$; 324.41

potassium citrate; 02181

potassium citrate monohydrate; 09373

Potassium salt of 2-Hydroxy-1-2-3-propanetricarboxylic acid (3:1)

[866-84-2]

Potassium salt of propanetricarboxylic acid hydrate (3:1:1)
[6100-05-6]

It contains at least 99.0% and at most 100.5% of $C_6H_5K_3O_7$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Colorless crystals, or white, granular powder.

Solubility. Very soluble in water; practically insoluble in ethanol.

IDENTIFICATION

A. The solution obtained in *Appearance of solution* responds to the reactions of the potassium ion (5.3.1.1).

B. The solution obtained in *Appearance of solution* responds to the reactions of citrate ion (5.3.1.1).

PURITY TESTS

Appearance of solution. To dissolve 10 g of the sample in carbon dioxide-free water and top up the volume to 100 mL with the same solvent. The solution is clear (5.2.25) and colorless (5.2.12).

Alkalinity. The solution of 1 g of sample in 20 mL of water is alkaline to litmus paper. To add 0.2 mL of 0.05 M sulfuric acid and a drop of phenolphthalein SI to the solution. The solution does not get pink color.

Oxalates. To dissolve 0.5 g of the sample in 4 mL of water, and add 3 mL of hydrochloric acid and 1 g of granulated zinc. To heat it in water bath for 1 minute. To let it rest for 2 minutes, transfer the supernatant to a tube containing 0.25 mL of 1% phenylhydrazine chloride (w/v) and heat to boiling. To rapidly cool down, transfer to a graduated tube, and add an equal volume of hydrochloric acid and 0.25 mL of potassium ferricyanide SR. To make it homogeneous and let it rest for 30 minutes. Any pink color produced is not more intense than that of the standard preparation obtained under the same conditions, by using 4 mL of 0.005% oxalic acid (w/v). At most 0.03% (300 ppm).

Sodium. To proceed as described in *Atomic emission spectrometry* (5.2.13.2). To dissolve 1 g of the sample in water and dilute to 100 mL with the same solvent. To prepare the standard solution by using standard sodium solution (200 ppm Na). To dilute, if necessary. To measure the emission intensity at 589 nm. At most 0.3% (3000 ppm).

Tartrate. To add 1 g of sample, 1.5 mL of water, and 1 mL of 6 M acetic acid into a test tube. To graze the wall of the tube with a glass rod. No crystalline precipitate is produced.

Calcium (5.3.2.7). To dilute 5 mL of the solution obtained in *Appearance of solution* to 15 mL with diluted acetic acid and proceed as described in *Limit test for calcium*. To prepare the standard solution with mixture of 5 mL of *Standard calcium solution* (10 ppm) and 10 mL of water. At most 0.01% (100 ppm).

Chlorides (5.3.2.1). To determine in 7 g of the sample. At most 0.005% (50 ppm).

Iron (5.3.2.4). To use 10 mL of the solution obtained in *Appearance of solution*. At most 0.002% (20 ppm).

Heavy metals (5.3.2.3). To use *Method I*. To dissolve 2 g in 25 mL of water and proceed as described in *Limit test for heavy metals*. There is no need to adjust the pH. At most 0.001% (10 ppm).

Readily carbonisable substances. To add 10 mL of sulfuric acid to 0.2 g of pulverized sample and heat it in water bath at 90 °C for 1 hour.

To quickly cool down. The color of the solution is not more intense than that of the mixture of 75 mL of *Standard solution color SC F* (5.2.12) and 25 mL of 1% hydrochloric acid (w/v) or a mixture of 15 mL of *Standard solution color SC O* and 85 mL of 1% hydrochloric acid (w/v).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 180 °C for 4 hours. From 3% to 6%.

DOSAGE

To dissolve about 0.2 g of the sample, accurately weighed, in 25 mL of glacial acetic acid. To titrate with 0.1 M SV perchloric acid, by using 2 drops of methylrosanilinium chloride SI (crystal violet) as an indicator, to turns into green color. To perform sample blank correction and adjust it as required. Each mL of 0.1 M SV perchloric acid is equal to 10.213 mg of $C_6H_5K_3O_7$.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

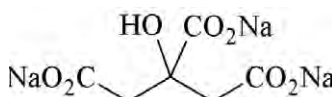
To comply with the legislation in force.

THERAPEUTIC CLASS

Antiurolithic, antacid.

CITRATE, SODIUM

Natrii citras



$C_6H_5Na_3O_7$; 258.07

$C_6H_5Na_3O_7 \cdot 2H_2O$; 294.10

sodium citrate; 02182

sodium citrate dihydrate; 02183

Sodium salt of 2-hydroxy-1,2,3-propanetricarboxylic acid (3:1)

[6804-2]

Sodium salt of 2-hydroxy-1,2,3-propanetricarboxylic acid hydrate (3:1:2)

[6132-04-3]

It contains at least 99.0% and at most 100.5% of $C_6H_5Na_3O_7$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White, crystalline powder, or white, odorless crystals.

Solubility. The hydrate form is freely soluble in water, very soluble in boiling water, and insoluble in ethanol.

IDENTIFICATION

A. The 1:20 solution responds to the test for sodium ion (5.3.1.1).

B. The 1:20 solution responds to the test for citrate ion (5.3.1.1).

C. After incineration, it results in alkaline residue, which effervesces when treated with diluted hydrochloric acid.

PURITY TESTS

Acidity or alkalinity. A solution of 1 g of sample in 20 mL of water is alkaline to litmus paper, but after adding 0.2 mL of 0.05 M sulfuric acid, there is no pink color due to a drop of phenolphthalein SI.

Heavy metals (5.3.2.3). To dissolve 2 g of the sample in 25 mL of water. At most 0.001% (10 ppm).

Tartrate (5.3.1.1). To dissolve 1 g of sample in 2 mL of water, add 1 mL of potassium acetate SR and 1 mL of 6 M acetic acid. To rub the wall of the tube with a glass rod; no crystalline precipitate is produced.

Loss on drying (5.2.9). To desiccate at 180 °C for 18 hours.

At most 1.0% to anhydrous form and at most 10% - 13% to hydrated form.

DOSAGE

To proceed as described in *Titration in non-aqueous medium* (5.3.3.5). To accurately weigh about 350 mg of the sample, previously desiccated, transfer to a 250 mL beaker, and dissolve in 100 mL of glacial acetic acid. To stir to obtain total dissolution. To titrate with 0.1 M SV perchloric acid, to potentiometrically determine the endpoint. To perform sample blank correction and adjust it as required. Each mL of 0.1 M SV perchloric acid is equal to 8.602 mg of $C_6H_5Na_3O_7$.

PACKAGING AND STORAGE

In airtight containers.

LABELLING

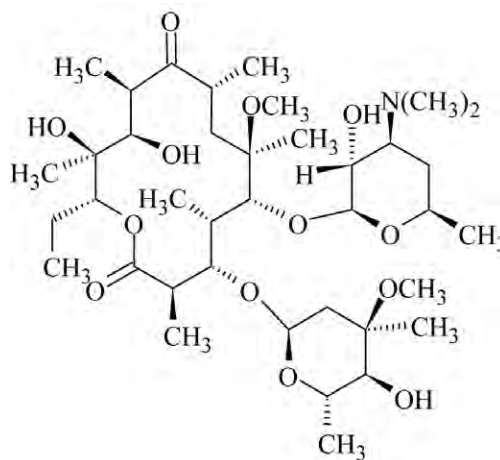
To comply with the legislation in force.

THERAPEUTIC CLASS

Systemic alkalinizing.

CLARITHROMYCIN

Clarithromycinum



$C_{38}H_{69}NO_{13}$; 747.95

clarithromycin; 02200

6-O-Methylerythromycin
[81103-11-9]

It contains at least 96.0% and at most 102.0% of $C_{38}H_{69}NO_{13}$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. Crystalline, white or almost white, and odorless powder.

Solubility. Practically insoluble in water; soluble in acetone; slightly soluble in acetonitrile; sparingly soluble in absolute ethanol and methanol. Sparingly soluble in phosphate buffers pH 2.0 - 5.0.

Physical and chemical constants.

Specific rotation (5.2.8): -87° to -97° , in relation to the anhydrous substance. To determine in 1% solution (w/v) in chloroform at 20°C .

Melting range (5.2.2): 217°C to 225°C with decomposition.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maxima only at the same wavelength, with the same relative intensities to those observed in the clarithromycin SQR spectrum similarly prepared.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

PURITY TESTS

pH (5.2.19). 7.5 to 10.0. To determine at 0.2% suspension (w/v) in a mixture of water and methanol (19:1).

Heavy metals (5.3.2.3). To use *Method I*. At most 0.002% (20 ppm).

Water (5.2.20). At most 2.0%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. To moisten the sample with 2 mL of nitric acid and five drops of sulfuric acid. At most 0.3 %.

DOSAGE

To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 210 nm; 150 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μm), kept at 50°C ; flow of *Mobile phase* of 1.0 mL/min.

Mobile phase: mixture of methanol and 0.067 M monobasic potassium phosphate (65:35). To adjust pH to 4.0 with phosphoric acid, if necessary.

Sample solution: to transfer 50 mg of the sample to 50 mL volumetric flask, and add 35 mL of methanol. To put it in ultrasound for 30 minutes and top up with the same solvent. To make it homogeneous. To transfer 5 mL of this solution to 25 mL volumetric flask and top up with *Mobile phase*, thus obtaining 0.2 mg/mL solution.

Standard solution: to transfer 50 mg of clarithromycin SQR to 50 mL volumetric flask, and add 35 mL of methanol. To put it in ultrasound for 30 minutes and top up with the same solvent. To make it homogeneous. To transfer 5 mL of this solution to 25 mL volumetric flask and top up with *Mobile phase*, thus obtaining 0.2 mg/mL solution.

Procedure: to separately inject 20 μL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of clarithromycin ($\text{C}_{38}\text{H}_{69}\text{NO}_{13}$) per milligram, from the content of the standard and responses obtained for the *Sample* and *Standard solutions*.

PACKAGING AND STORAGE

In opaque containers, tightly closed, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antimicrobial.

CLARITHROMYCIN TABLETS

It contains at least 90.0 % and at most 110.0 % of the amount stated of $\text{C}_{38}\text{H}_{69}\text{NO}_{13}$. Tablets may be coated.

IDENTIFICATION

The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

Procedure for content uniformity. To proceed as described in *Dosage*, by using the solution described below as *Sample solution*. To transfer each tablet to 250 mL volumetric flask, add 100 mL of 0.067 M monobasic potassium phosphate (if necessary, adjust to pH 4.0 with phosphoric acid), and wait for total disintegration of the tablet. To add 130 mL of methanol and put it in ultrasound for 30 minutes. To mechanically stir for 30 minutes. To top up with the methanol, make it homogeneous, and filter it. To transfer volume equal to 5 mg of the sample to a 25 mL volumetric flask and top up with mobile phase.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M sodium acetate buffer, pH 5.0, 900 mL

Equipment: blades, 50 rpm

Time: 30 minutes

Procedure: after testing, remove aliquot from the dissolution medium, filter, and dilute, if necessary, with *Mobile phase*, to obtain about 0.02% concentration (w/v). To proceed as described in *Dosage*. To calculate the amount of $C_{38}H_{69}NO_{13}$ dissolved in the medium from the potency of clarithromycin SQR and responses obtained from *Sample and standard solutions*.

Tolerance: not less than 80% (Q) of the amount stated of $C_{38}H_{69}NO_{13}$ dissolves within 30 minutes.

PURITY TESTS

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 10 °C for 3 hours. At most 6 %.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To proceed as described in *Dosage*, in the monograph *Clarithromycin*. To prepare the *Sample solution* as described below.

Sample solution: to weigh and pulverize 20 tablets. To transfer amount of powder equivalent to 50 mg of clarithromycin to a 50 mL volumetric flask, add 35 mL of methanol, and put it in ultrasound for 30 minutes. To mechanically stir for 30 minutes. To top up with methanol. To make it homogenous and filter it. To transfer 5 mL of this solution to 25 mL volumetric flask and top up with *Mobile phase*, thus obtaining 200 µg/mL solution.

Procedure: to separately inject 20 µL of *Sample and standard solutions*, record the chromatograms, and measure the area under the peaks. To calculate the amount of $C_{38}H_{69}NO_{13}$ dissolved in the medium from the potency of clarithromycin SQR and responses obtained from *Sample and standard solutions*.

PACKAGING AND STORAGE

In opaque containers, tightly closed, protected from light.

LABELLING

To comply with the legislation in force.

CLARITHROMYCIN POWDER FOR ORAL SUSPENSION

It contains at least 90.0% and at most 115.0% of amount stated of $C_{38}H_{69}NO_{13}$. It may contain dispersing, diluent, preservatives, and flavoring agents.

IDENTIFICATION

The retention time of the main peak in the chromatogram of the *Sample solution* obtained in *Dosage* corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test. To determine in the diluent vial.

pH (5.2.19). 4.0 to 5.4. To determine in the reconstituted suspension as informed on the label.

Determination of weight (5.1.1). It complies with the test. To determine in the not reconstituted powder.

PURITY TESTS

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in a vacuum oven at 60 °C for 3 hours. At most 2 %.

DOSAGE

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 210 nm; 150 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at 50 °C; flow of *Mobile phase* of 1 mL/min.

Mobile phase: mixture of methanol and 0.067 M monobasic potassium phosphate (60:40). To adjust pH to 3.5 with phosphoric acid, if necessary.

Sample solution: To reconstitute the suspension as described on the product label. To transfer volume of suspension equivalent to 0.5 g of clarithromycin to a 250 mL volumetric flask containing 100 mL of 0.067 M monobasic potassium phosphate. To mechanically stir for 30 minutes. To add 130 mL of methanol and put it in ultrasound for 60 minutes, by regularly stirring it. To cool down at room temperature. To top up with the methanol, make it homogeneous, and filter it. To transfer 5 mL of the filtrate to 25 mL volumetric flask and top up with *Mobile phase*.

Stock standard solution: to transfer 50 mg of clarithromycin SQR to 25 mL volumetric flask, and add 20 mL of methanol. To put it in ultrasound for 30 minutes and top up with the same solvent, to obtain 2 mg/mL solution. To make it homogeneous.

Standard solution: to transfer 5 mL of *Stock standard solution* to 25 mL volumetric flask and top up with *Mobile phase*. To make it homogeneous.

The column efficiency, as determined from the responses obtained for clarithromycin, is not less than 750 theoretical plates/column. The tailing factor is between 1.0 and 1.7. The capacity factor is between 2.5 and 6.0. The relative standard deviation for replicate areas of the peaks recorded should not be greater than 2.0 %.

Procedure: to separately inject 50 µL of *Sample and standard solutions*, record the chromatograms, and measure the area under the peaks.

To calculate the amount of $C_{38}H_{69}NO_{13}$ in oral suspension reconstituted from the responses from the *Standard solution* and *Sample solution*.

PACKAGING AND STORAGE

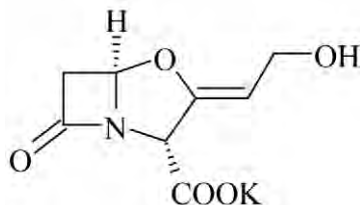
In opaque containers, tightly closed, protected from light.

LABELLING

To comply with the legislation in force.

CLAVULANATE POTASSIUM

Kalli clavulanas



$C_8H_8KNO_5$; 237.25

clavulanate potassium; 00137

Potassium salt of (2R, 3Z, 5R) -3 - (2-hydroxyethylidene) - 7-oxo-4-oxa-1-azabicyclo [3.2.0] heptane-2-carboxylic acid (1:1)

[61177-45-5]

It contains at least 75.5% and at most 92.0% of clavulanic acid ($C_8H_9NO_5$), in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. Crystalline, white or almost white powder; hygroscopic.

Solubility. Very soluble in water, slightly soluble in ethanol, sparingly soluble in acetone.

Physical and chemical constants

Specific rotation (5.2.8): +53° to +63°, in relation to the anhydrous substance. To determine in 2% solution (w/v) in carbon dioxide-free water.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the clavulanate potassium SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14), ranging from 200 nm to 400 nm, of 0.1% solution (w/v) in phosphate buffer pH 7.0 exhibits maxima at 278 nm. The absorbance at 278 nm is approximately 0.40.

C. It responds to the reactions of potassium ion (5.3.1.1).

PURITY TESTS

pH (5.2.19). 5.5 to 8.0. To determine in 1% solution (w/v) in carbon dioxide-free water.

Related substances. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 230 nm; 100 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at 40 °C; flow of *Mobile phase* of 1 mL/min.

Eluent A: to dissolve 7.8 g of monobasic sodium phosphate in 900 mL of water. To adjust pH to 4.0 with phosphoric acid, top up to 1000 mL with water, and make it homogeneous.

Eluent B: mixture of methanol and *Eluent A* (50:50).

Mobile phase gradient: to adopt the gradient system described in the following table:

Time (minutes)	Eluent A (%)	Eluent B (%)	Elution
0 - 4	100	0	isocratic
4 - 15	100 → 50	0 → 50	linear gradient
15 - 18	50	50	isocratic
18 - 24	50 → 100	50 → 0	linear gradient
24 - 39	100	0	isocratic

Solution (1): 10 mg/mL solution of the sample in *Eluent A*.

Solution (2): to dilute 1 mL of *Solution (1)* to 100 mL with *Eluent A*.

Solution (3): to dissolve 10 mg of clavulanate potassium SQR and 10 mg of amoxycillin trihydrate SQR in *Eluent A* and top up to 100 mL with the same solvent.

Procedure: To separately inject 20 µL of each solution. To record the chromatograms and measure the areas under the

peaks. The sum of the areas of all the secondary peaks obtained with *Solution (1)*, except the main peak's one, is not greater than twice the area under the main peak obtained with *Solution (2)* (2.0%), and no peak area is larger than that of the main peak obtained with *Solution (2)* (1.0%). To disregard the peaks with an area less than 0.05 times the area under the main peak obtained with *Solution (2)* (0.05%). The test is valid only if the chromatogram obtained with *Solution (3)* has resolution between the clavulanate and amoxicillin peaks of at least 13.

Limit for aliphatic amines. To proceed as described in *Gas chromatography (5.2.17.5)*. To use gas chromatograph equipped with flame ionization detector, 50 m long capillary chromatography column and 0.53 mm inner diameter, filled with poly (dimethyl/diphenyl siloxane), with a film thickness of 25 μm ; column temperature at 35 °C in the first 7 minutes; 35 °C to 150 °C from 7 minutes to 10.8 minutes, and 150 °C from 10.8 minutes to 25.8 minutes; injector temperature at 200 °C, and the detector temperature at 250 °C; use helium as carrier gas; and flow of carrier gas of 8.0 mL/minute.

Internal standard solution: to dissolve 50 μL of 3-methyl-2-pentanone in water and dilute to 100 mL with the same solvent.

Sample solution: to transfer 1 g of the sample to a centrifuge tube and add 5 mL of *Internal standard solution*, 5 mL of 2 M sodium hydroxide, 10 mL of water, 5 mL of isopropyl alcohol, and 5 g of sodium chloride. To vigorously stir for 1 minute and centrifuge to separate the layers.

Standard solution: to dissolve 80 mg of each of the amines: 1,1-dimethylethylamine, diethylamine, tetramethylethylenediamine, 1,1,3,3 -tetramethylbutylamine, *N, N'*-diisopropylethylenediamine and 2,2'-oxybis (N, N-dimethylethylamine) in 2 M hydrochloric acid and dilute to 200 mL with the same solvent. To transfer 5 mL of resulting solution to a centrifuge tube and add 5 mL of *Internal standard solution*, 10 mL of 2 M sodium hydroxide, 5 mL of isopropyl alcohol, and 5 g of sodium chloride. To vigorously stir for 1 minute and centrifuge to separate the layers.

To separately inject 1 μL of upper layers of *Standard solution* and *Sample solution*. The retention time is approximately 11.4 minutes to 3-methyl-2-pentanone. The relative retention times of amines, in relation to 3-methyl-2-pentanone, are about 0.55 to 1.1-dimethylethylamine, 0.76 to diethylamine, 1.07 to tetramethylethylenediamine, 1.13 to 1,1,3,3-tetramethylbutylamine, 1.33 to *N,N'*-diisopropylethylenediamine, and 1.57 to 2,2'-oxybis (N,N-dimethylethylamine).

Procedure: to separately inject 1 μL of upper layers of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. At most 0.2% of aliphatic amines.

Water (5.2.20.1). To determine in 1 g of the sample. At most 1.5 %.

BIOLOGICAL SAFETY TEST

When the label states that the substance is sterile or indicated to parenteral preparations, the sample complies with the test.

Bacterial endotoxins (5.5.2.2). At most 0.03 IU/mg of clavulanate potassium.

DOSAGE

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 220 nm; 300 mm chromatograph column and 4.0 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μm), kept at room temperature; flow of *Mobile phase* of 1.6 mL/minute.

Buffer pH 4.4: to dissolve 7.8 g of monobasic sodium phosphate in 900 mL of water.

To adjust pH to 4.4 ± 0.1 with phosphoric acid or 10 M sodium hydroxide, top up to 1000 mL with water, and make it homogeneous.

Mobile phase: mixture of *Buffer pH 4.4* and methanol (95:5).

Sample solution: to accurately weigh about 50 g of the sample and transfer to a 200 mL volumetric flask. To dissolve in water and top up with the same solvent.

Standard solution: 0.25 mg/mL clavulanate lithium SQR solution in water.

Resolution solution: solution containing 0.25 mg/mL clavulanate lithium SQR and 0.5 mg/mL amoxycillin trihydrate SQR in water.

To inject replicates of 20 μL of the *Resolution solution*. The column efficiency is greater than 550 theoretical plates. The relative retention times are about 0.5 to clavulanic acid and 1.0 to amoxicillin. The tailing factor is not greater than 1.5. The resolution between clavulanic acid and amoxicillin should not be less than 3.5. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2.0%.

Procedure: to separately inject 20 μL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the area under the peaks. To calculate the content of clavulanic acid ($\text{C}_8\text{H}_9\text{NO}_5$) from the sample, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

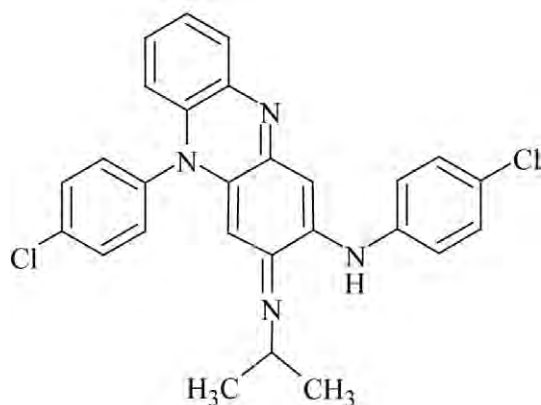
In airtight containers, protected from light, at 2 °C - 8 °C.

THERAPEUTIC CLASS

Beta-lactamase inhibitor.

CLOFAZIMINE

Clofaziminum



$C_{27}H_{22}Cl_2N_4$; 473.40 clofazimine; 02268
N,5-bis(4-chlorophenyl)-3,5-dihydro-3-[(1-methylethyl)imino]-2-fenazinamine
 [2030-63-9]

It contains at least 98.5% and at most 101.5% of $C_{27}H_{22}Cl_2N_4$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Dark red, crystalline, odorless powder.

Solubility. Insoluble in water, soluble in acetone, chloroform, ethyl ether, and sparingly soluble in ethanol.

Physical and chemical constants.

Melting range (5.2.2): 217 °C to 219 °C.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of 5% solution (w/v) of the sample in methylene chloride, dispersed in potassium bromide, has absorption maxima at the same wavelength, with the same relative intensities to those observed in the clofazimine SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14), ranging from 200 nm to 400 nm, of 0.001% solution (w/v) in methanolic 0.1 M hydrochloric acid, exhibits maxima and minima only in the same wavelength of a similar clofazimine SQR solution.

C. The principal spot in the chromatogram of *Solution (1)* in the *Related substances* corresponds in position and color to that obtained with *Solution (2)*.

PURITY TESTS

Related substances. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using HF_{254} silica gel as sup-

port and a mixture of methylene chloride and *n*-propanol (10:1) as mobile phase. To subject the plate to ammonia vapors for 30 minutes immediately before utilization, by suspending the plate in a tank containing surface layer with about 25 mL of 1% ammonia solution (v/v), freshly prepared, to prevent the contact between plate and liquid. To separately apply to the plate 5 µL of each of the solutions freshly prepared, as described below.

Solution (1): to dissolve accurately weighed amount of the sample in methylene chloride, to obtain a 50 mg/mL solution.

Solution (2): to dissolve accurately weighed amount of clofazimine SQR in methylene chloride, to obtain a 0.5 mg/mL solution.

Solution (3): to dilute *Solution (2)* in methylene chloride to obtain a 0.25 mg/mL solution.

Solution (4): to dilute *Solution (2)* in methylene chloride to obtain a 0.1 mg/mL solution.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (2)* (1.0%). The sum of the intensities of secondary spots in the chromatogram with *Solution (1)* does not exceed 2.0%.

Heavy metals (5.3.2.3). To proceed as described in *Method IV*. At most 0.001% (10 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 105 °C for 3 hours. At most 0.5 %.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. To accurately dissolve about 0.3 g of the sample, previously desiccated, in 5 mL of chloroform. To carefully heat, if necessary. To add 20 mL of acetone and 5 mL of glacial acetic acid. To titrate with 0.1 M SV perchloric acid, to potentiometrically determine the endpoint. To perform sample blank correction and adjust it as required. Each mL of 0.1 M SV perchloric acid is equal to 47.340 mg of $C_{27}H_{22}Cl_2N_4$.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Leprostatic.

CLONAZEPAM TABLETS

It contains at least 95.0 % and at most 110.0 % of the amount stated of $C_{15}H_{10}ClN_3O_3$.

IDENTIFICATION

A. To weigh and pulverize the tablets. To transfer amount of powder equivalent to 10 mg of clonazepam to a 125 mL separatory funnel. To add 25 mL of water, stir for 2 minutes, and extract it with 2 portions (40 mL each) of chloroform. To filter the organic extracts by using anhydrous sodium sulfate. To combine them and evaporate at room temperature by using flow of nitrogen.

To wash the residue with 3 portions (10 mL each) of hexane. The infrared absorption spectrum (5.2.14) of the residue, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the clonazepam SQR spectrum similarly prepared.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in *Dosage* corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL

Equipment: blades, 75 rpm

Time: 60 minutes

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 254 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μ m), kept at 25°C; flow of *Mobile phase* of 1.0 mL/min.

Mobile phase: mixture of water, methanol, and acetonitrile (40:30:30).

Sample solution: after testing, use aliquot from the dissolution medium.

Standard solution: to dissolve accurately weighed amount of clonazepam SQR in methanol, to obtain a 0.05 mg/mL solution. To successively dilute with dissolution medium, to obtain a concentration similar to that obtained in the dissolution tanks after the test.

Procedure: to separately inject 100 μ L of *Sample and standard solutions*, record the chromatograms, and measure the area under the peaks. To calculate the amount of $C_{15}H_{10}ClN_3O_3$ dissolved in the medium from the responses obtained from *Sample and standard solutions*.

Tolerance: not less than 75% (Q) of the amount stated of $C_{15}H_{10}ClN_3O_3$ dissolves within 60 minutes.

PURITY TESTS

Related substances. To proceed as described in *Thin-layer chromatography (5.2.17.1)*, by using

GF₂₅₄ silica gel as support and a mixture of ethyl acetate and carbon tetrachloride (1:1) as mobile phase. To separately apply to the plate 25 μ L of each of the solutions freshly prepared, as described below.

Solution (1): to weigh and pulverize the tablets. To stir an amount of powder equivalent to 10 mg of clonazepam in 20 mL of acetone for 1 minute. To filter and evaporate to dryness. To dissolve the residue in 0.5 mL of acetone.

Solution (2): to prepare 0.2 mg/mL 3-amino-4-(2-chlorophenyl) - 6-nitroquinolin-2 (1*H*)-one SQR solution in acetone.

Solution (2): to prepare 0.2 mg/mL (2-amino-5-nitrophenyl) (2-chlorophenyl) methanone SQR solution in acetone.

To develop the chromatogram. To remove the plate, allow it to dry in dry air current, and examine it under ultraviolet light (254 nm). To nebulize the plate with 10% sulfuric acid (v/v) and heat at 105 °C for 15 minutes. To nebulize with 0.1% sodium nitrite (w/v) and allow it to dry in hot air current. To nebulize with 0.5% ammonium sulfamate (w/v) and allow it to dry in hot air current. Any spot obtained in the chromatogram with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (2)* (1.0%). Decreasing in fluorescence intensity is observed on the plate. The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solutions (2)* and (3).

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 254 nm; 250 mm column and 4 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), flow of *Mobile phase* of 0.6 mL/min.

Ammonia phosphate buffer: to transfer 6.6 g of dibasic ammonium phosphate to 1000 mL volumetric flask and add 950 mL of water, adjust pH to 8.0 with 0.33 M phosphoric acid or M sodium hydroxide, and top up with water.

Mobile phase: mixture of Ammonia phosphate buffer, methanol, and tetrahydrofuran (60:52:13). To filter and degas it.

Diluent: mixture of water, methanol, and tetrahydrofuran (60:52:13).

Sample solution: to weigh and pulverize 20 tablets. To transfer amount of powder equivalent to 5 mg of clonazepam to a 50 mL volumetric flask. To initially dissolve in 20 mL of methanol.

To put it in ultrasound for 15 minutes and top up with the *Diluent*. To make it homogenous and filter it.

Standard solution: To transfer amount of powder equivalent to 10 mg of clonazepam SQR to a 100 mL volumetric flask. To initially dissolve in 75 mL of methanol. To put it in ultrasound for about 15 minutes. To top up with *Diluent*, to obtain a 0.1 mg/mL solution. To make it homogenous and filter it.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_{15}H_{10}ClN_3O_3$ in the tablets, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In tightly closed amber glass containers, at temperature below 25 °C.

LABELLING

To comply with the legislation in force.

CLONAZEPAM ORAL SOLUTION

It contains at least 95 % and at most 115 % of the amount stated of $C_{15}H_{10}ClN_3O_3$.

IDENTIFICATION

To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF_{254} silica gel as support and a mixture of toluene, ethyl acetate, and formic acid anhydrous (60:40:5) as

mobile phase. To separately apply to the plate 10 µL of each of the solutions freshly prepared, as described below.

Solution (1): to dilute 2 mL of sample with 10 mL of 0.1 M hydrochloric acid in a centrifuge tube. To add 1 g of sodium chloride and stir to dissolve for about 2 minutes. To add 5 mL of ethyl acetate, stir for 3 minutes, and centrifuge for further 3 minutes. To use the organic phase.

Solution (2): to dissolve 20 mg of clonazepam SQR in 20 mL of ethyl acetate.

To develop the chromatogram. To remove the plate, allow it to dry in dry air current for 10 minutes, and examine it under ultraviolet light (254 nm). Decreasing in fluorescence intensity is observed on the plate. The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

pH (5.2.19). 3.6 to 4.1. To determine in 50% solution (w/v) of the sample in water.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To proceed as described in *Dosage*, in the monograph *Clonazepam tablets*. To prepare the *Sample solution* as described below.

Sample solution: to transfer volume of oral solution containing the equivalent to 5 mg of clonazepam to a 50 mL volumetric flask, to obtain 100 µg/mL solution. To initially solubilize in 20 mL of methanol, put it in ultrasonic bath for 15 minutes, and top up with *Diluent*. To make it homogenous and filter it.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_{15}H_{10}ClN_3O_3$ in the oral solution, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In tightly closed amber glass containers, at temperature below 25 °C.

LABELLING

To comply with the legislation in force.

CLOPIDOGREL TABLETS

Clopidogrel bisulfate tablets contain the equivalent to at least 90.0% and at most 110.0% of the amount stated of $C_{16}H_{16}ClNO_2S$.

IDENTIFICATION

A. The ultraviolet absorption spectrum (5.2.14), ranging from 250 nm to 300 nm, of the sample solution obtained in *Procedure for content uniformity*, exhibits absorption maxima at the same wavelength of the standard solution.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

Procedure for content uniformity. To transfer each tablet to 50 mL volumetric flask To add 30 mL of 0.1 M hydrochloric acid, put it in ultrasound for 5 minutes, top up with the same solvent, and filter it. To transfer 5 mL of this solution to 50 mL volumetric flask and top up with the same solvent. To filter through 0.45 µm filter. To prepare a standard solution at the same concentration by using the same solvent. To measure the absorbance of the resulting solutions at 270 nm (5.2.14), by using 0.1 M hydrochloric acid to adjust zero.

DISSOLUTION TEST (5.1.5)

Dissolution medium: hydrochloric acid buffer pH 2.0, 1000 mL

Equipment: blades, 50 rpm

Time: 30 minutes

Procedure: after testing, remove aliquot from the dissolution medium, immediately filter, and dilute in hydrochloric acid buffer pH 2.0 to obtain suitable concentration. To measure the absorbances of solutions at 240 nm (5.2.14), by using hydrochloric acid buffer pH 2.0 to adjust zero. To calculate the amount of $C_{16}H_{16}ClNO_2S$ dissolved in the medium, by comparing the readings obtained with clopi-

dogrel bisulfate SQR solution containing the equivalent to 0.003% (w/v) of clopidogrel prepared in the same solvent.

Tolerance: not less than 80% (Q) of the amount stated of $C_{16}H_{16}ClNO_2S$ dissolves within 30 minutes.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 220 nm; 150 mm ovomucoid column and 4.6 mm inner diameter, packed with silica, kept at room temperature; flow of *Mobile phase* of 1.0 mL/min.

Mobile phase: mixture of 0.1 M monobasic potassium phosphate and acetonitrile (75:25).

Sample solution: To weigh and pulverize the tablets. To transfer amount of powder equivalent to 50 mg of clopidogrel to a 50 mL volumetric flask.

To add about 30 mL of methanol. To put it in ultrasound for 5 minutes. To mechanically stir for 30 minutes and top up with methanol. To make it homogenous and filter it. To transfer 5 mL of this solution to 50 mL volumetric flask and top up with methanol. To make it homogeneous.

Standard solution: to accurately weigh amount of equivalent to 25 mg of clopidogrel to a 25 mL volumetric flask. To dissolve in 5 mL of methanol. If necessary, put it in ultrasonic bath. To top up to volume with methanol and make it homogeneous. To transfer 5 mL of this solution to 50 mL volumetric flask and top up with methanol. To make it homogeneous.

Procedure: to separately inject 10 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of $C_{16}H_{16}ClNO_2S$ in the tablets, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers, at room temperature.

LABELLING

To comply with the legislation in force.

CHLORIDE, AMMONIUM

Ammonii chloridum

NH₄Cl; 53.49
ammonium chloride; 02362
Ammonium chloride
[12125-02-9]

It contains at least 99.0% and at most 100.5% of NH₄Cl, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White, crystalline powder, or colorless crystals.

Solubility. Freely soluble in water; soluble in glycerol; slightly soluble in ethanol.

IDENTIFICATION

A. The 0.1% solution (w/v) of the sample responds to the reactions of ammonium ion (**5.3.1.1**).

B. The 0.1% solution (w/v) of the sample responds to the reactions of chloride ion (**5.3.1.1**).

PURITY TESTS

Appearance of solution. To dissolve 10 g of the sample in water and top up the volume to 100 mL with the same solvent. The solution is clear (**5.2.25**) and colorless (**5.2.12**).

Acidity or alkalinity. To add 0.05 mL of methyl red SI to 10 mL of the solution obtained in *Appearance of solution*. More than 0.5 mL of 0.01 M hydrochloric acid or 0.5 mL of 0.01 M sodium hydroxide is not necessary to change the indicator.

Bromides and iodides. To add 0.1 mL of hydrochloric acid and 0.05 mL of 2% chloramine-T (w/v) to 10 mL of the solution obtained in *Appearance of the solution*. After 1 minute, add 2 mL of chloroform and vigorously mix it. The chloroform phase remains colorless.

Thiocyanate. To acidify 10 mL of the solution obtained in *Appearance of the solution* with hydrochloric acid and a few drops of 9% ferric chloride. No orange-red color is developed.

Calcium (5.3.2.7). To dilute 5 mL of resulting solution obtained in *Appearance of solution* with 10 mL of water. At most 0.02% (200 ppm).

Iron (5.3.2.4). To use *Method I*. To dilute 5 mL of resulting solution obtained in *Appearance of solution* with 5 mL of water. To use *Iron standard solution (1 ppm Fe)*. At most 0.002% (20 ppm).

Heavy metals (5.3.2.3). To use *Method I*. To determine in 20 mL of the solution obtained in *Appearance of solution*. At most 0.001% (10 ppm).

Sulfates (5.3.2.2). To determine in 8 g of the sample. At most 0.015% (150 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 105 °C for 2 hours. At most 1.0%.

Sulfated ashes (5.2.10). To determine in 2 g of the sample. At most 0.1%.

DOSAGE

To accurately weigh about 1 g of sample, dissolve in 20 mL of water, and add a mixture of 20 mL of water and 5 mL of formaldehyde solution, previously neutralized in the presence of phenolphthalein SI. To titrate with M SV sodium hydroxide, by using phenolphthalein SI as an indicator, after 1-2 minutes. Each mL of M SV sodium hydroxide is equal to 53.490 mg of NH₄Cl.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Systemic acidifier.

CHLORIDE, CALCIUM

Calcii chloridum

CaCl₂; 110.98
CaCl₂·2H₂O; 147.01
calcium chloride; 02369
calcium chloride dihydrate; 02370
Calcium chloride
[10043-52-4]
Calcium chloride dihydrate;
[10035-04-8]

It contains at least 97.0% and at most 103.0% of CaCl₂·2H₂O.

DESCRIPTION

Physical characteristics. White, hygroscopic, crystalline powder.

Solubility. Freely soluble in water; soluble in ethanol.

IDENTIFICATION

A. To dissolve 1 g of the sample in carbon dioxide-free water and top up the volume to 10 mL with the same solvent. Resulting solution responds to the reactions of calcium ion (5.3.1.1).

B. To dissolve 1 g of the sample in carbon dioxide-free water and top up the volume to 10 mL with the same solvent. Resulting solution responds to the reactions of chloride ion (5.3.1.1).

PURITY TESTS

Appearance of solution. To dissolve 10 g of the sample in carbon dioxide-free water and top up the volume to 100 mL with the same solvent. The resulting solution is clear (5.2.25) and it is not more colored than the mixture of 5 mL of *Standard solution color SC F* (5.2.12) and 95 mL of 1% hydrochloric acid (w/v).

Acidity or alkalinity. To add 0.1 mL of phenolphthalein SI to 10 mL of the solution obtained in *Appearance of solution*. If the solution becomes pink, it should become colorless by adding at most 0.2 mL of 0.01 M hydrochloric acid. If no color fades in, it should become pink by adding at most 0.2 mL of 0.01 M sodium hydroxide.

pH (5.2.19). 4.5 to 9.2. To determine in 5% aqueous solution (w/v).

Barium. To add 1 mL of calcium sulfate SR to 10 mL of the solution obtained in *Appearance of solution*. After 15 minutes, any opalescence observed is not more intense than the mixture of 10 mL of the solution obtained in *Appearance of solution* and 1 mL of water.

Iron, aluminum, and phosphate. To dissolve 1 g of the sample in 20 mL of water. To add two drops of 3 M hydrochloric acid and a drop of phenolphthalein SI.

To add dropwise ammonium chloride - ammonium hydroxide SR to obtain light pink color and add further two drops. To heat to boiling. No turbidity or precipitation occurs.

Magnesium and alkali metals. To mix 20 mL of solution obtained in *Appearance of solution* and 80 mL of water. To add 2 g of sample and 2 mL of ammonia SR. To heat to boiling and add 5 g of warm ammonium oxalate solution in 75 mL of water. To let it rest for 4 hours, top up to 200 mL with water, and filter it. To add 0.5 mL of sulfuric acid to 100 mL of filtrate. To evaporate to dryness in a water bath and incinerate at 600 °C to obtain constant weight. The weight of the residue should not exceed 5 mg. At most 0.5%.

Aluminum (5.3.2.10). To add 2 mL of ammonium chloride SR and 1 mL of ammonia SR to 10 mL of the solution obtained in *Appearance of the solution* and boil it. No turbidity or precipitation occurs. If it is used to prepare dialysis solutions, dissolve 4 g of the sample in 100 mL of wa-

ter. To add 10 mL of acetate buffer pH 6.0 and proceed as described in *Limit test for aluminum*. To use as a standard solution a mixture of 2 mL of *Standard aluminum solution* (2 ppm Al), 10 mL of acetate buffer pH 6.0, and 98 mL of water. To prepare the blank by using a mixture of 10 mL of acetate buffer solution pH 6.0 and 100 mL of water. At most 0.0001% (1 ppm).

Arsenic (5.3.2.5). To use *Method I*. To determine in 1 g of the sample. At most 0.0003% (3 ppm).

Iron (5.3.2.4). To use *Method I*. To use 10 mL of the solution obtained in *Appearance of solution*. To use Iron standard solution (1 ppm Fe). At most 0.001% (10 ppm).

Sulfates (5.3.2.2). To determine in 4 g of the sample. At most 0.03% (300 ppm).

Heavy metals (5.3.2.3). To use *Method I*. To use 10 mL of the solution obtained in *Appearance of solution*. To prepare the standard solution by using standard lead solution (2 ppm Pb). At most 0.002% (20 ppm).

DOSAGE

To accurately weigh about 0.28 g of the sample, dissolve in 100 mL of water, and proceed as described in *Complexometric titration for calcium* (5.3.3.4), by using 4 mL of 2 M sodium hydroxide. Each mL of 0.1 M disodium edetate SV is equal to 14.702 mg of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force. The label should indicate whether it can be used to prepare dialysis solutions.

THERAPEUTIC CLASS

Electrolyte replenishment. It can be used as a diuretic, urinary acidifier, and antiallergic.

CHLORIDE, CALCIUM, HEXAHYDRATE
Calcii chloridum hexahydricum

$\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$; 219.08
 calcium chloride hexahydrate; 02371
 Calcium chloride hexahydrate
 [7774-34-7]

It contains at least 97.0% and at most 103.0% of $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$.

DESCRIPTION

Physical characteristics. Colorless crystals or colorless, crystalline mass.

Solubility. Very soluble in water and ethanol.

Physical and chemical constants.

Melting temperature (5.2.2): 30 °C.

IDENTIFICATION

A. To dissolve 15 g of the sample in carbon dioxide-free water and top up the volume to 100 mL with the same solvent. Resulting solution responds to the reactions of calcium ion (5.3.1.1).

B. The solution similarly prepared to that one in *Identification* test **A.** responds to reactions of chloride ion (5.3.1.1).

PURITY TESTS

Appearance of solution. To dissolve 15,0 g of the sample in carbon dioxide-free water and top up the volume to 100 mL with the same solvent. The resulting solution is clear (5.2.25) and intensity color lower than the mixture of 5 mL of *Standard solution color SC F* described in *Color of liquids* (5.2.12).

Acidity or alkalinity. To add 0.1 mL of phenolphthalein SI to 10 mL of the solution obtained in *Appearance of solution*. If the solution becomes pink, it should become colorless by adding at most 0.2 mL of 0.01 M hydrochloric acid. If no color fades in, it should become pink by adding at most 0.2 mL of 0.01 M sodium hydroxide.

Aluminum. To add 2 mL of ammonium chloride SR and 1 mL of ammonia hydroxide SR to 10 mL of the solution obtained in *Appearance of the solution* and boil it. No turbidity or precipitation occurs. If it is used to prepare dialysis solutions, dissolve 6 g of the sample in 100 mL of water.

To add 10 mL of acetate buffer pH 6.0 and proceed as described in *Limit test for aluminum*. To use as a standard solution a mixture of 2 mL of *Standard aluminum solution* (2 ppm), 10 mL of acetate buffer pH 6.0, and 98 mL of water. To prepare the blank by using a mixture of 10 mL of acetate buffer solution pH 6.0 and 100 mL of water. At most 0.0001% (1 ppm).

Barium. To add 1 mL of calcium sulfate SR to 10 mL of the solution obtained in *Appearance of solution*. After 15 minutes, any opalescence observed is not more intense than the mixture of 10 mL of the solution obtained in *Appearance of solution* and 1 mL of water.

Sulfates (5.3.2.2). To determine in 6 g of the sample and proceed as described in *Limit test for sulfates*. At most 0.02% (200 ppm).

Heavy metals (5.3.2.3). To use *Method I*. To use 10 mL of the solution obtained in *Appearance of solution*. To prepare the standard solution by using *Standard lead solution* (2 ppm). At most 0.002% (20 ppm).

DOSAGE

To accurately weigh about 0.2 g of the sample, dissolve in 100 mL of water, and proceed as described in *Complexometric titration for calcium* (5.3.3.4), by using 4 mL of 2 M sodium hydroxide. Each mL of 0.1 M SV disodium edetate is equal to 21.908 mg of $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$.

PACKAGING AND STORAGE

In tightly closed containers.

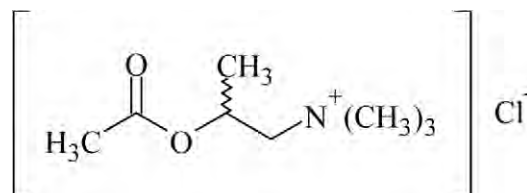
LABELLING

To comply with the legislation in force. The label should indicate whether it can be used to prepare dialysis solutions.

THERAPEUTIC CLASS

Electrolyte replenishment. It can be used as a diuretic, urinary acidifier, and antiallergic.

CHLORIDE, METHACHOLINE
Methacholini chloridum



$\text{C}_8\text{H}_{18}\text{ClNO}_2$; 195.69

methacholine chloride; 02401

Chloride 2 - (acetyloxy)-*N,N,N*-trimethyl-1-propanaminium

(1:1)

[62-51-1]

It contains at least 98.0% and at most 101.0% of $\text{C}_8\text{H}_{18}\text{ClNO}_2$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White, crystalline powder or white or colorless crystals; odorless or slight odor; very hygroscopic.

Solubility. Soluble in water, freely soluble in chloroform and ethanol; insoluble in ethyl ether.

Physical and chemical constants.

Melting range (5.2.2): To transfer 0.1 g of sample to a glass beaker and dissolve it in 3 mL of chloroform. To heat at 110 °C for 1 hour. To determine the melting range of the powder adhered to the walls of the beaker. From 170 °C to 173°C.

IDENTIFICATION

A. To dissolve 0.1 g of the sample in 2 mL of water and add 3 mL of platinum chloride SR. Rhombohedral crystals with melting range between 220 °C and 225 °C are formed.

B. To dissolve 10 mg of the sample in 100 mL of water. To add 1 mL of ethanol and 1 mL of sulfuric acid to 1 mL of this solution. To slowly heat to smell typical odor of ethyl acetate.

C. To prepare a 10% solution (w/v) of the sample. To add 2 g of potassium hydroxide to 5 mL of this solution. To slowly heat to smell typical odor of trimethylamine.

D. To prepare a 2% solution (w/v) of the sample. It responds to the reactions of chloride ion (5.3.1.1).

PURITY TESTS

Acetylcholine chloride. To dissolve 10 mg of the sample in 100 mL of water. To add 3 mL of 20% sodium perchlorate solution (w/v) to 2 mL of this solution, stir, and place it in ice bath for 5 minutes. No precipitate is produced.

Heavy metals (5.3.2.3). To use *Method IV*. At most 0.002% (20 ppm).

Loss on drying (5.2.9). To determine in 2 g of the sample. To desiccate in an oven at 105 °C for 4 hours. At most 1.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To accurately weigh about 0.4 g of the sample, previously desiccated, and dissolve in a mixture of glacial acetic acid and mercury acetate SR (50:10). To titrate with 0.1 M SV perchloric acid, by using methylrosanilinium chloride SI as an indicator, to obtain green color.

To perform sample blank correction and adjust it as required. Each mL of 0.1 M SV perchloric acid is equal to 19.569 mg of $C_8H_{18}ClNO_2$.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Cholinergic.

CHLORIDE, SODIUM Natrii chloridum

NaCl; 58.44
sodium chloride; 02421
Sodium chloride
[7647-14-5]

It contains at least 99.0% and at most 100.5% of NaCl, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White, crystalline, fine powder, or colorless crystals.

Solubility. Very soluble in water; practically insoluble in ethanol.

IDENTIFICATION

A. It responds to the reactions of chloride ion (5.3.1.1).

B. It responds to the reactions of sodium ion (5.3.1.1).

PURITY TESTS

Appearance of solution. The 20% solution (w/v) in carbon dioxide-free water is clear (5.2.25) and colorless (5.2.12).

Acidity or alkalinity. At most 0.5 mL of 0.01 M hydrochloric acid or 0.01 M sodium hydroxide is spent to neutralize 20 mL of the solution described in *Appearance of solution*, by using bromothymol blue SI as indicator.

Barium. To add 5 mL of water and 1 mL of M sulfuric acid to 5 mL of the solution described. After 15 minutes, any opalescence observed is not more intense than the mixture of 5 mL of the solution described in *Appearance of solution* and 7 mL of water.

Bromides. To add 0.5 mL of the solution described in *Appearance of solution*, 4 mL of water, 2 mL of phenol red SR, and 1 mL of 0.01% chloramine-T (w/v) freshly prepared.

To make it homogeneous and let it rest for 2 minutes. To add 0.15 mL of 0.1 M sodium thiosulfate, make it homogeneous, and top up to 10 mL with water. The absorbance of this solution (5.2.14) at 590 nm, by using water to adjust zero, is not greater than that of standard solution similarly prepared by using 5 mL of 0.3% potassium bromide (w/v). At most 0.005% (50 ppm).

Ferrocyanides. To dissolve 2 g of the sample in 6 mL of water. To add 0.5 mL of a mixture of 5 mL of 1% ammo-

nium ferrous sulfate (w/v) in 0.05 M sulfuric acid solution and 95 mL of 1% iron sulfate heptahydrate (w/v). No blue color develops.

Iodides. To moisten 5 g of the sample by adding dropwise a freshly prepared mixture of 0.15 mL of sodium nitrite SR, 2 mL of 0.05 M sulfuric acid, 25 mL of starch SI, and 25 mL of water. No blue color develops after 5 minutes.

Nitrites. To add 10 mL of water to 10 mL of the solution described in *Appearance of solution*. To measure the absorbance (5.2.14) of the solution at 354 nm. The absorbance is not greater than 0.01.

Potassium. It is required for sodium chloride to prepare parenteral or hemodialysis solutions. To proceed as described in *Atomic absorption spectrometry* (5.2.13.1). To prepare the solution as described below.

Sample solution: to dissolve 1 g of the sample in water and dilute to 100 mL with the same solvent.

Standard solution: to dissolve accurately weighed amount of potassium chloride, previously desiccated, between 100 °C and 105 °C, for 3 hours, to obtain 0.1144% solution (w/v) in water. To dilute, if necessary.

To measure the emission intensity at 766.5 nm. At most 0.05% (500 ppm).

Aluminum (5.3.2.10). It is required for potassium hydroxide to prepare hemodialysis solutions. To dissolve 20 g of the sample in 100 mL of water and add 10 mL of acetate buffer pH 6.0. To proceed as described in *Limit test for aluminum*. To use as a standard solution a mixture of 2 mL of *Standard aluminum solution* (2 ppm), 10 mL of acetate buffer pH 6.0, and 98 mL of water. To use as blank a mixture of 10 mL of acetate buffer solution pH 6.0 and 100 mL of water. At most 0.00002% (0.2 ppm).

Arsenic (5.3.2.5). To use 5 mL of the solution described in *Appearance of solution*. At most 0.0001% (1 ppm).

Iron (5.3.2.4). To use 10 mL of the solution described in *Appearance of solution*. At most 0.0002% (2 ppm).

Phosphates (5.3.2.11): To use 2 mL of solution described in *Appearance of solution* and dilute to 100 mL with water. At most 0.0025% (25 ppm).

Magnesium and alkaline earth metals (5.3.2.9). To use 10 g of the sample. The volume of 0.01 M SV disodium edetate used does not exceed 2.5 mL.

At most 0.01% (100 ppm) calculated as calcium.

Heavy metals (5.3.2.3). To use *Method I*. To determine in 12 mL of the solution described in *Appearance of solution*. At most 0.0005% (5 ppm).

Sulfates (5.3.2.2). To use 3 mL of the solution described in *Appearance of solution*. At most 0.025% (250 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 105 °C for 2 hours. At most 0.5%.

DOSAGE

To accurately dissolve about 50 mg of the sample in water and top up the volume to 50 mL with the same solvent. To titrate with 0.1 M SV silver nitrate, to potentiometrically determine the endpoint. Each mL of 0.1 M SV silver nitrate is equivalent to 5.844 mg of NaCl.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Electrolyte replenishment.

CHLORIDE, SODIUM INJECTABLE SOLUTION

It contains at least 95.0 % and at most 105.0 % of the amount stated of NaCl. Solution contains no antimicrobial agents.

IDENTIFICATION

A. It responds to the reactions of sodium ion (5.3.1.1).

B. It responds to the reactions of chloride ion (5.3.1.1).

CHARACTERISTICS

Determination of volume(5.1.2). It complies with the test.

pH (5.2.19). 4.5 to 7.0.

PURITY TESTS

Iron (5.3.2.4). To use *Method III*. To dilute 5 mL of injectable solution to 45 mL with water, and add 2mL of hydrochloric acid. At most 0.0002% (2 ppm).

Heavy metals (5.3.2.3). To transfer volume of injectable solution equivalent to 1 g of sodium chloride to a suitable container. If necessary, evaporate the volume to 20 mL. To add 2 mL of M acetic acid and top up to 25 mL with water. To proceed as described in *Method I*, but using only 1 mL of *Standard lead solution* (10 ppm Pb) in *Standard prepa-*

ration and Preparation of control tube. At most 0.001% (10 ppm).

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

Bacterial endotoxins (5.5.2.2). At most 0.5 EU/mL if the amount stated of sodium chloride is between 0.5% and 0.9% and at most 3.6 EU/mL if the amount stated of sodium chloride is between 3.0 % and 24.3%.

DOSAGE

To transfer volume of injectable solution containing the equivalent to 90 mg of sodium chloride to erlenmeyer flask. To add water, if necessary, to obtain a volume of 10 mL. To add 10 mL of glacial acetic acid and 75 mL of methanol. To titrate with 0.1 M SV silver nitrate. To determine the endpoint by using eosin Y SI as an indicator, to obtain pink color. Each mL of 0.1 M SV silver nitrate is equivalent to 5.844 of NaCl.

PACKAGING AND STORAGE

In plastic or glass container, preferably type I or type II.

LABELLING

To comply with the legislation in force.

CHLORIDE, ZINC Zinci chloridum

ZnCl₂; 136.32
zinc chloride; 02433
Zinc chloride
[7646-85-7]

It contains at least 95.0% and at most 100.5% of ZnCl₂.

DESCRIPTION

Physical and chemical characteristics. Crystalline, white or almost white powder. Melting temperature (5.2.2): around 318 °C.

Solubility. Very soluble in water, freely soluble in ethanol and glycerol.

IDENTIFICATION

A. To dissolve 0.5 g of the sample in nitric acid SR and dilute to 10 mL with the same solvent. It responds to the reactions of chloride ion (5.3.1.1).

B. To add 38 mL of carbon dioxide-free water to 2 G of the sample. To drip hydrochloric acid SR to obtain total solubilization and dilute to 40 mL with carbon dioxide-free water. It responds to the reactions of zinc ion (5.3.1.1).

PURITY TESTS

pH (5.2.19). 4.6 to 5.5. To determine in solution containing 1 g of the sample in 9 mL of carbon dioxide-free water.

Aluminum, calcium, heavy metals, iron, magnesium. To add 2 mL of concentrated ammonia solution to 8 mL of the solution obtained in test B. *Identification* and make it homogeneous. The solution is clear (5.2.25) and colorless (5.2.12). To add 1 mL of dibasic sodium phosphate dodecahydrate SR. The solution remains clear for at least 5 minutes. To add 0.2 mL of sodium sulfide SR. White precipitate is produced. The supernatant remains colorless.

Ammonium salts. To add M sodium hydroxide to 5 mL of 10% solution (w/v) of the sample, to begin the formation of a precipitate. To gently heat. No ammonia odor is released.

Sulfates (5.3.2.2). To dissolve 0.8 g of the sample in 10 mL of water and proceed as described in *Limit test for sulfates*. At most 0.03% (300 ppm).

DOSAGE

To dissolve 0.25 g of the sample in 5 mL of diluted acetic acid. To proceed as described in *Complexometric titrations (5.3.3.4)* for Zinc. Each mL of 0.1 M SV disodium edetate is equal to 13.632 mg of ZnCl₂.

PACKAGING AND STORAGE

In nonmetallic, tightly closed containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Nutritional supplement.

AMILORIDE HYDROCHLORIDE AND HYDROCHLOROTHIAZIDE TABLETS

It contains at least 90.0 % and at most 110.0 % of the amount stated of C₆H₈ClN₇O.HCl and C₇H₈ClN₃O₄S₂.

IDENTIFICATION

A. To weigh and pulverize the tablets. To transfer an amount of powder equivalent to 0.1 g of hydrochlorothiazide to a beaker and dissolve it in 50 mL of acetone. To filter and evaporate the filtrate to dryness. To dry the residue at 105 °C for 1 hour. The infrared absorption spectrum (5.2.14) of the dried residue, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the hydrochlorothiazide SQR spectrum.

B. The retention times of the main peaks in the chromatogram of the *Sample solution* obtained in the *Dosage* correspond to those of main peaks of *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 900 mL

Equipment: blades, 50 rpm

Time: 30 minutes

Procedure: after testing, remove aliquot from the dissolution medium and dilute, if necessary, with *Dissolution medium*, to obtain suitable concentration. To measure the absorbances of the solutions at 363 nm to amiloride hydrochloride and 270 nm to hydrochlorothiazide (5.2.14), by using the same solvent to adjust zero. To calculate the amount of $C_6H_8ClN_7O.HCl$ and $C_7H_8ClN_3O_4S_2$ dissolved in the medium, by comparing the readings obtained with amiloride hydrochloride SQR and hydrochlorothiazide SQR solutions with known concentrations prepared in the same solvent.

Tolerance: not less than 80% (Q) of the stated amount of $C_6H_8ClN_7O.HCl$ and 75% (Q) of the stated amount of $C_7H_8ClN_3O_4S_2$ dissolve within 30 minutes.

PURITY TESTS

Limit for methyl 3,5-diamino-6-chloropyrazine-2-carboxylate. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using F_{254} silica gel as support and a mixture of dioxane and 3 M ammonium hydroxide (90:12) as mobile phase. To separately apply to the plate 10 μ L of each of the solutions freshly prepared, as described below.

Solution (1): to pulverize the tablets and dissolve an amount of powder equivalent to 17.5 mg of anhydrous amiloride hydrochloride in 10 mL of methanol and centrifuge it.

Solution (2): to dissolve 1 mg of methyl 3,5-diamino-6-chloropyrazine-2-carboxylate SQR in methanol and dilute to 100 mL with the same solvent.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (365 nm). Any spot corresponding to methyl 3,5-diamino-6-chloro-

pyrazine-2-carboxylate obtained in the chromatogram with *Solution (1)* is not more intense than that obtained with *Solution (2)*.

Limit for 4-amino-6-chloro-1,3-benzene-disulfonamide.

To proceed as described in *Dosage*. To prepare *Test solution* as described below.

Test solution: to dissolve 1 mg of 4-amino-6-chloro-1,3-benzene-disulfonamide SQR in the mobile phase and dilute to 100 mL with the same solvent.

To separately inject 20 μ L of *Test solution* and 20 μ L of *Sample solution*, record the chromatograms, and measure the areas under the peaks. The area under the 4-amino-6-chloro-1,3-benzene-disulfonamide peak obtained with the *Sample solution* is not greater than the area under the main peak obtained with the *Test solution*. At most 1.0%.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 286 nm; 300 mm chromatograph column and 3.9 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μ m), kept at room temperature; flow of *Mobile phase* of 1.0 mL/min.

Phosphate buffer: to dissolve 136 g of monobasic potassium phosphate in 800 mL of water, adjust the pH to 3.0 with phosphoric acid, and top up to 1000 mL with water.

Mobile phase: mixture of water, methanol, and *Phosphate buffer* (71:25:4).

Sample solution: to weigh and pulverize 20 tablets. To transfer an amount of powder equivalent to 5 mg of amiloride hydrochloride to 50 mL volumetric flask. To add 15 mL of methanol and 2 mL of *M* hydrochloric acid. To put it in ultrasound for 10 minutes, make it homogeneous, and filter it.

Standard solution: to dissolve 20 mg of amiloride hydrochloride SQR in methanol and dilute to 20 mL with the same solvent. To transfer 10 mL of this solution to 100 mL volumetric flask containing about 100 mg of hydrochlorothiazide SQR and 20 mL of methanol. To add 4 mL of *M* hydrochloric acid, top up with water, and make it homogeneous.

To inject replicates of 20 μ L of the *Standard solution*. The relative retention times are about 0.7 to hydrochlorothia-

zide and 1 to amiloride hydrochloride. The resolution between hydrochlorothiazide and amiloride hydrochloride should not be less than 2.0. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2.0%.

Procedure: to separately inject 10 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_6H_8ClN_7O$, HCl and $C_7H_8ClN_3O_4S_2$ from the sample, from the responses obtained for the *Sample and Standard solutions*.

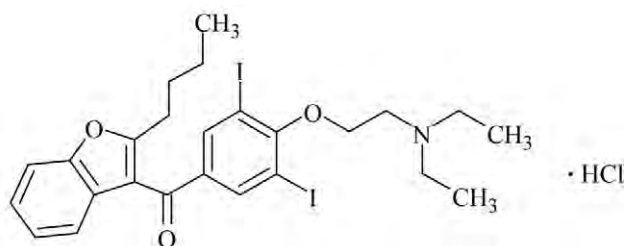
PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

HYDROCHLORIDE, AMIODARONE Amiodaroni hydrochloridum



$C_{25}H_{29}I_2NO_3 \cdot HCl$; 681.77
amiodarone hydrochloride; 00700
(2-butyl-3-benzofuranyl) - [4 - [2 - (diethylamino)ethoxy] -3,5-diiodophenyl] methanone hydrochloride (1:1)
[19774-82-4]

It contains at least 98.5% and at most 101.5% of $C_{25}H_{29}I_2NO_3 \cdot HCl$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Fine, crystalline, white or almost white powder.

Solubility. Practically insoluble in water, freely soluble in methylene chloride, soluble in methanol, slightly soluble in ethanol, and very slightly soluble in n-hexane.

Physical and chemical constants. *Melting range* (5.2.2): 159 °C to 163 °C with decomposition.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative

intensities to those observed in the amiodarone hydrochloride SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14) ranging from 200 nm to 400 nm, of 0.002% solution (w/v) in methanol, exhibits maxima and minima only in the same wavelength of a similar amiodarone hydrochloride SQR solution.

C. The principal spot in the chromatogram of *Solution (2)* in the *Related substances* corresponds in position, color, and intensity to that obtained with *Solution (3)*.

D. It responds to the reactions of chloride ion (5.3.1.1).

PURITY TESTS

Appearance of solution. The 5% solution (w/v) in methanol is clear (5.2.25) and colorless (5.2.12).

pH (5.2.19). 3.2 to 3.8. To determine in solution prepared as described below. To dissolve 1.25 g of the sample in water heated at 80 °C. To cool down and top up with water to 25 mL.

Absorption of light. The absorbance of 0.002% solution (w/v) in methanol, measured at 242 nm, is between 1.03 and 1.05.

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF_{254} silica gel as support and a mixture of formic acid, methanol, and methylene chloride (5:10:85) as mobile phase. To separately apply to the plate 5 µL of each of the solutions freshly prepared and protected from light, as described below.

Solution (1): to dissolve 1 g of the sample in methylene chloride and top up to 10 mL with the same solvent, thus obtaining 100 mg/mL solution.

Solution (2): to dilute 0.5 mL of *Solution (1)* to 10 mL with methylene chloride, thus obtaining 5 mg/mL solution.

Solution (3): to dissolve 25 g of amiodarone hydrochloride SQR in methylene chloride and top up to 5 mL with the same solvent, thus obtaining 5 mg/mL solution.

Solution (4): to dilute 1 mL of *Solution (2)* to 10 mL with methylene chloride, thus obtaining 0.5 mg/mL solution.

Solution (5): to dilute 5 mL of *Solution (4)* to 10 mL with methylene chloride, thus obtaining 0.25 mg/mL solution.

Solution (6): to dissolve 10 mg of (2-chloroethyl) diethylamine hydrochloride in 50 mL of methylene chloride, thus obtaining 0.2 mg/mL solution.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense

than that obtained with *Solution (4)* (0.5%) and not more than a spot is more intense than that obtained in the chromatogram with *Solution (5)* (0.25 %). To nebulize the plate with potassium iodobismuthate SR and 3% hydrogen peroxide SR (w/v) and examine immediately. Any spot corresponding to (2-chloroethyl) diethylamine hydrochloride obtained in the chromatogram with *Solution (1)* is not more intense than that obtained with *Solution (6)* (0.2%).

Volatile organic impurities. To proceed as described in *Gas chromatography (5.2.17.5)*. To use gas chromatograph equipped with flame ionization detector, 30 m long column and 0.25 mm inner diameter, with stationary phase of 35% of diphenyl polysiloxane (0.25 µm thick); column operating as follows. 40 °C per minute; ramp from 40 °C to 200 °C, with a heating rate of 15 °C per minute. To keep the injector and detector temperature at 250 °C, respectively, hydrogen as carrier gas at 85 kPa at the head of the column.

Solution (1): to transfer 0.3 g of sample and 5 mL of dimethylsulfoxide to 10 mL headspace vial containing 1 g of anhydrous sodium sulfate.

Solution (2): to weigh 1 g of methylene chloride and dilute to 0.02% (w/v) (200 ppm) in dimethylsulfoxide. To transfer 5 mL of this solution to *headspace* vial containing 1 g of anhydrous sodium sulfate.

Solution (3): to weigh 1 g of toluene and dilute to 0.02% (w/v) in dimethylsulfoxide. To transfer 5 mL of this solution to *headspace* vial containing 1 g of anhydrous sodium sulfate.

To close the *headspace* vials with polytetrafluoroethylene lid and aluminum seal. To heat the samples at 80°C for 60 minutes.

Procedure: to inject 1 µL of vapor phase of each solution and record the areas of each peak. The retention time of toluene is approximately 2.5 minutes. The resolution between the methylene chloride and toluene peaks is greater than 2. The relative standard deviation for replicate areas of the peaks recorded is not greater than 15% for both solvents. The areas for the methylene chloride and toluene in *Solution (1)* are not greater than the areas for the methylene chloride standards in the *Solution (2)* and toluene standard in *Solution (3)*.

Iodides. To simultaneously prepare the solutions as described below.

Solution (1): to add 1.5 g of sample to 40 mL of water heated at 80 °C and stir to obtain total dissolution. To cool down at room temperature and dilute to 50 mL with water.

Solution (2): To add 1 mL of 0.1 M hydrochloric acid and 1 mL of 0.05 M potassium iodate to 15 mL of *Solution (1)* and dilute to 25 mL with water. Let it rest for 4 hours, protected from light.

Solution (3): To add 1 mL of 0.1 M hydrochloric acid, 1 mL of 0.00882% potassium iodate (w/v), and 0.05 M potassium iodate to 15 mL of *Solution (1)* and dilute to 25 mL with water. Let it rest for 4 hours, protected from light.

To measure the absorbances of *Solution (2)* and *Solution (3)* at 420 nm (V.2.14) by using a mixture of 15 mL of *Solution (1)* and 1 mL of 0.1 M hydrochloric acid to adjust zero, diluted to 25 mL with water. The absorbance of *Solution (2)* is not greater than the half absorbance of the *Solution (3)*. At most 0.015% (150 ppm).

Heavy metals (5.3.2.3). To use *Method IV*. At most 0.002% (20 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate with phosphorus pentoxide in an oven at 50°C under pressure not greater than 0.3 kPa for 4 hours. At most 0.51%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. To accurately dissolve about 0.5 g of the sample in 40 mL of glacial acetic acid. To add 10 mL of 5% mercuric acetate (w/v) in glacial acetic acid. To titrate with 0.1 M SV perchloric acid, to potentiometrically determine the endpoint. To perform sample blank correction and adjust it as required. Each mL of 0.1 M SV perchloric acid is equal to 68.177 mg of $C_{25}H_{29}I_2NO_3 \cdot HCl$.

B. To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. To accurately weigh about 50 mg of the sample and dissolve in methanol. To top up the volume to 100 mL with the same solvent. To successively dilute in methanol to the 0.0008% concentration (w/v). To prepare a amiodarone hydrochloride SQR solution at similar concentration by using the same solvent. To measure the absorbances of the resulting solutions at 242 nm, by using methanol to adjust zero. To calculate the content of $C_{25}H_{29}I_2NO_3 \cdot HCl$ in the sample from the readings obtained.

PACKAGING AND STORAGE

In airtight closed containers, protected from light, at temperature below 30 °C.

LABELLING

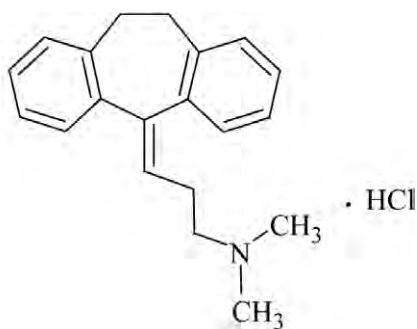
To comply with the legislation in force.

THERAPEUTIC CLASS

Antiarrhythmic and antianginal.

HYDROCHLORIDE, AMITRIPTYLINE

Amitriptylini hydrochloridum



$C_{20}H_{23}N.HCl$; 313.86

amitriptyline hydrochloride; 00712

3 - (10,11-dihydro-5H-dibenzo [a, d] cyclohept-5-ylidene)-N, N-dimethyl-1-propanamine hydrochloride (1:1) hydrochloride
[549-18-8]

It contains at least 99.0% and at most 101.0% of $C_{20}H_{23}N.HCl$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or almost white powder, or colorless crystals.

Solubility. Freely soluble in water, methylene chloride, and ethanol.

Physical and chemical constants

Melting range (5.2.2): 195 °C to 199 °C.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the amitriptyline hydrochloride SQR spectrum similarly prepared.

B. To proceed as described in *Ultraviolet absorption spectrophotometry*. The ultraviolet absorption spectrum (5.2.14), from 200 nm to 400 nm, 0.001% solution (w/v) in methanol has absorption maximum at 239 nm, similar to that observed in the spectrum of a similar solution of amitriptyline hydrochloride SQR.

C. It responds to the reactions of chloride ion (5.3.1.1).

PURITY TESTS

pH (5.2.19). 5.0 to 6.0. To determine in 1% aqueous solution (w/v).

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel as sup-

port and a mixture of chloroform, methanol, and ammonium hydroxide (135:15:1) as mobile phase. To separately apply to the plate 10 µL of each of the solutions freshly prepared, as described below.

Solution (1): 40 mg/mL solution of the sample in methanol.

Solution (2): 0.8 mg/mL amitriptyline hydrochloride SQR solution in methanol.

Solution (3): to dilute *Solution (2)* in methanol to obtain a 0.4 mg/mL solution.

Solution (4): to dilute *Solution (2)* in methanol to obtain a 0.2 mg/mL solution.

Solution (5): to dilute *Solution (2)* in methanol to obtain a 0.16 mg/mL solution.

Solution (6): to dilute *Solution (2)* in methanol to obtain a 80 µg/mL solution.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (4)* (0.5%). The sum of the intensities of all secondary spots obtained with *Solution (1)* corresponds to at most to that obtained with *Solution (3)* (1%). To disregard any spot in the chromatogram with *Solution (1)* that is less intense than that principal spot in the *Solution (7)* (0.1%). To disregard any spot observed at the points of application of solutions.

Volatile organic impurities. To proceed as described in *Gas chromatography* (5.2.17.5). To use gas chromatograph equipped with a flame ionization detector; 30 m long melt-ed silica column, 0.53 mm inner diameter, filled with phenyl (5%) methyl (95%) polysiloxane and 5 m long silica pre-column and 0.53 mm inner diameter, deactivated with phenylmethylsiloxane. To keep the column at 35 °C for 5 minutes, increase to 175 °C at 8 °C per minute, then to 260 °C at 35 °C per minute. To keep it for at least 16 minutes. To keep the injector and detector temperature at 70 °C and 260 °C, respectively; use helium at linear velocity of 35 cm/s as carrier gas.

Sample solution: to dissolve in water free of organic compound an accurately weighed amount of the sample, to obtain a 20 mg/mL solution.

Solution (7): to dilute *Solution (2)* in methanol to obtain a 40 µg/mL solution.

Standard solution: to prepare a solution in water free of organic compounds containing, in each mL, 12 µg of methylene chloride, 1.2 µg of chloroform, 7.6 µg of dioxane, and 1.6 µg of trichlorethylene. To prepare on the day of utilization

To inject replicates of 1 µL of the *Standard solution*. The resolution between any 2 components should not be less than 1.0. The relative standard deviation for replicate areas of the peaks recorded is not greater than 15.0%.

Procedure: to separately inject 1 µL of *Standard solution* and *Sample solution*. To record the chromatograms and measure the areas under the peaks. The identity and response of each peak in the chromatogram of *Sample solution* can be related to the identity and response of volatile organic impurities in the chromatogram of the *Standard solution*. The amount of each volatile organic impurity in the chromatogram of the *Sample solution* does not exceed the limit set in the following table.

<i>Volatile Organic Impurities</i>	<i>Limit (ppm)</i>
chloroform	60
dioxane	380
methylene chloride	600
trichloroethylene	80

Heavy metals (5.3.2.3). To use *Method III*. At most 0.001% (10 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 60 °C, under reduced pressure, to obtain constant weight. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. To dissolve 1 g of the sample in 30 mL of glacial acetic acid, by slightly heating, if necessary, and cool down. To add 10 mL of mercuric acetate SR. To titrate with 0.1 M SV perchloric acid, by using methylrosanilinium chloride SI as an indicator to turns into green color. To perform sample blank correction and adjust it as required. Each mL of 0.1 M SV perchloric acid is equal to 31.391 mg of C₂₀H₂₃N.HCl.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antidepressant.

HYDROCHLORIDE, AMITRIPTYLINE, CAPSULES

It contains at least 90.0 % and at most 110.0 % of the amount stated of C₂₀H₂₃N.HCl.

IDENTIFICATION

A. The ultraviolet absorption spectrum (5.2.14) ranging from 200 nm to 400 nm, of *Sample solution* obtained in *Dosage method A.*, exhibits maximum and minimum similar to those observed in the *Standard solution* spectrum.

B. The principal spot in the chromatogram of *Solution (1)* in the *Related substances* corresponds in position, color, and intensity to that obtained with the *Solution (2)*.

C. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage method B.* corresponds to that of main peak of *Standard solution*.

D. To weigh the capsules, remove the content, and weigh them again. To stir amount of powder equivalent to 10 mg of amitriptyline hydrochloride with 5 mL of water. To filter it. It responds to the reactions of chloride ion (5.3.1.1).

E. To weigh the capsules, remove the content, and weigh them again. To stir amount of powder equivalent to 0.1 mg of amitriptyline hydrochloride with 10 mL of chloroform. To filter and reduce the volume of the filtrate by evaporation. To precipitate by adding ethyl ether to produce turbidity. To let it rest and filter. To dissolve 50 mg of precipitate in 3 mL of water. To add 50 mL of 2.5% quinhydrone to (w/v) in methanol. No red color develops within 15 minutes.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test. Within 15 minutes.

Uniformity of dosage units (5.1.6). It complies with the test.

Procedure for content uniformity. To transfer each capsule to a 50 mL volumetric flask and add 35 mL of 0.1 M hydrochloric acid. To put it in ultrasound for 20 minutes, by occasionally stirring. To make it homogenous and filter it. To transfer 5 mL of the filtrate to a 25 mL volumetric flask and top up with 0.1 M hydrochloric acid. To transfer 5 mL of the resulting solution to 50 mL volumetric flask and top up with the same solvent, thus obtaining 0.001% solution (w/v). To proceed as described in *Method A., Dosage*, from "To prepare standard solution at same concentration..."

Time: 45 minutes

Procedure: after testing, remove aliquot from the dissolution medium and dilute, if necessary, with 0.1 M hydrochloric acid, to obtain suitable concentration. To measure the absorbances at 239 nm (5.2.14), by using the same solvent to adjust zero. To calculate the amount of $C_{20}H_{23}N$.HCl dissolved in the medium, by comparing the readings obtained with that one related to the 0.001% amitriptyline hydrochloride SQR solution (w/v) prepared in the same solvent.

Tolerance: not less than 75% (Q) of the amount stated of $C_{20}H_{23}N$.HCl dissolves within 45 minutes.

PURITY TESTS

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel as support and a mixture of diethylamine, ethyl acetate, and cyclohexane (3:15:85) as mobile phase. To separately apply to the plate 20 µL of each of the solutions freshly prepared, as described below.

Solution (1): to weigh capsules, remove the content, and weigh them again. To transfer an amount of powder equal to 20 mg of amitriptyline hydrochloride to a 5 mL volumetric flask and top up with absolute ethanol. To stir for 10 minutes. To make it homogenous and filter it.

Solution (2): to transfer 20 mg of amitriptyline hydrochloride SQR to 5 mL volumetric flask and top up to volume with absolute ethanol, thus obtaining 4 mg/mL solution.

Solution (3): to transfer 1 mL of *Solution (2)* to 100 mL volumetric flask and top up with absolute ethanol, thus obtaining 40 µg/mL solution.

Solution (4): to transfer 2.5 mL of *Solution (3)* to 10 mL volumetric flask and top up with absolute ethanol, thus obtaining 10 µg/mL solution.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than those obtained with *Solutions (3)* or *(4)* (1% and 0.25%). To nebulize the plate with potassium iodide and bismuth subnitrate SR. Any spot in the chromatogram obtained with *Solution (1)*, other than the principal spot and colored by developer, is not more intense than that obtained with *Solution (3)* (1%).

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3).

It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 900 mL

Equipment: baskets, 100 rpm

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To weigh 20 mg capsules, remove the content, and weigh them again. To transfer an amount of powder equivalent to 10 mg of amitriptyline hydrochloride to 100 mL volumetric flask. To add 75 mL of 0.1 M hydrochloric acid, mechanically stir for 15 minutes, and put it in ultrasound for 15 minutes. To top up the volume with the same solvent. To filter it. To transfer 5 mL of the filtrate to a 50 mL volumetric flask and top up with the same solvent, thus obtaining 0.001% solution (w/v). To prepare a standard solution at the same concentration by using the same solvent. To determine the absorbances of the resulting solutions at 239 nm, by using 0.1 M hydrochloric acid to adjust zero. To calculate the amount of $C_{20}H_{23}N$.HCl in the capsules, from the readings obtained.

B. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 254 nm; 250 mm column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), at room temperature; flow of *Mobile phase* of 2 mL/min.

Triethylamine-phosphate buffer: to transfer 11.04 g of monobasic sodium phosphate and 3 mL of triethylamine to a 1000 mL volumetric flask and dissolve in 900 mL of water. To adjust pH to 2.5 ± 0.5 with phosphoric acid and top up with water.

Mobile phase: mixture of *Triethylamine-phosphate buffer* and acetonitrile (58:42).

Sample solution: to weigh 20 capsules, remove the content, and weigh them again. To transfer an amount of powder equivalent to 50 mg of amitriptyline hydrochloride to a 50 mL volumetric flask, add 35 mL of *Mobile phase*, mechanically stir for 15 minutes, and put it in ultrasound for 15 minutes. To top up with the same solvent, make it homogeneous, and filter it. To transfer 10 mL of the filtrate to 50 mL volumetric flask and top up with *Mobile phase*. To make it homogeneous.

Standard solution: to transfer 50 mg of amitriptyline hydrochloride SQR to 50 mL volumetric flask, dilute in 35 mL of *Mobile phase*, and top up with the same solvent. To transfer 10 mL of the solution to 50 mL volumetric flask and top up with the same solvent, thus obtaining 0.2 mg/mL amitriptyline hydrochloride solution.

The column efficiency should be greater than 800 theoretical plates. The tailing factor should not be greater than 2.5.

The relative standard deviation for replicate areas of the peaks recorded should not be greater than 2.0 %.

Procedure: to separately inject 10 µL of *Sample and standard solutions*, record the chromatograms, and measure the area under the peaks. To calculate the amount of $C_{20}H_{23}N$. HCl in the capsules from the responses obtained from *Sample and standard solutions*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

HYDROCHLORIDE, AMITRIPTYLINE TABLETS

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_{20}H_{23}N$.HCl. Tablets may be coated.

IDENTIFICATION

A. The ultraviolet absorption spectrum (5.2.14) ranging from 200 nm to 400 nm, of *Sample solution* obtained in *Dosage* method **A.**, exhibits maximum and minimum similar to those observed in the *Standard solution* spectrum.

B. The principal spot in the chromatogram of *Solution (1)* in the *Related substances* corresponds in position, color, and intensity to that obtained with the *Solution (2)*.

C. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method **B.** corresponds to that of main peak of *Standard solution*.

D. To weigh and pulverize the tablets. To stir amount of powder equivalent to 10 mg of amitriptyline hydrochloride with 5 mL of water. To filter it. It responds to the reactions of chloride ion (5.3.1.1).

E. To weigh and pulverize the tablets. To stir amount of powder equivalent to 0.1 mg of amitriptyline hydrochloride with 10 mL of chloroform. To filter and reduce the volume of the filtrate by evaporation. To precipitate by adding ethyl ether to produce turbidity. To let it rest and filter. To dissolve 50 mg of precipitate in 3 mL of water. To add 50 mL of 2.5% quinydrone to (w/v) in methanol. No red color develops within 15 minutes.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test. Within 15 minutes.

Uniformity of dosage units (5.1.6). It complies with the test.

Procedure for content uniformity. To transfer each tablet to a 50 mL volumetric flask and add 35 mL of 0.1 M hydrochloric acid. To put it in ultrasound for 20 minutes, by occasionally stirring. To top up with the same solvent, make it homogeneous, and filter it.

To transfer 5 mL of the filtrate to a 25 mL volumetric flask and top up with 0.1 M hydrochloric acid. To transfer 5 mL of the resulting solution to 50 mL volumetric flask and top up with the same solvent, thus obtaining 0.001% solution (w/v). To proceed as described in Method **A.**, *Dosage*, from "To prepare standard solution at same concentration..."

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 900 mL

Equipment: baskets, 100 rpm

Time: 45 minutes

Procedure: after testing, remove aliquot from the dissolution medium and dilute, if necessary, with 0.1 M hydrochloric acid, to obtain suitable concentration. To measure the absorbances at 239 nm (5.2.14), by using the same solvent to adjust zero. To calculate the amount of $C_{20}H_{23}N$. HCl dissolved in the medium, by comparing the readings obtained with that one related to the 0.001% amitriptyline hydrochloride SQR solution (w/v) prepared in the same solvent.

Tolerance: not less than 75% (Q) of the amount stated of $C_{20}H_{23}N$.HCl dissolves within 45 minutes.

PURITY TESTS

Related substances. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF₂₅₄ silica gel as support and a mixture of diethylamine, ethyl acetate, and cyclohexane (3:15:85) as mobile phase. To separately apply to the plate 20 µL of each of the solutions freshly prepared, as described below.

Solution (1): to weigh and pulverize the tablets. To transfer an amount of powder equal to 20 mg of amitriptyline hydrochloride to a 5 mL volumetric flask and top up with absolute ethanol. To stir for 10 minutes. To make it homogeneous and filter it.

Solution (2): to transfer 20 mg of amitriptyline hydrochloride SQR to 5 mL volumetric flask and top up to volume with absolute ethanol, thus obtaining 4 mg/mL solution.

Solution (3): to transfer 1 mL of *Solution (2)* to 100 mL volumetric flask and top up with absolute ethanol, thus obtaining 40 mg/mL solution.

Solution (4): to transfer 2,5 mL of *Solution (3)* to 10 mL volumetric flask and top up with absolute ethanol, thus obtaining 10 mg/mL solution.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than those obtained with *Solutions (3)* or *(4)* (1% and 0.25%). To nebulize the plate with potassium iodide and bismuth subnitrate SR. Any spot in the chromatogram obtained with *Solution (1)*, other than the principal spot and colored by developer, is not more intense than that obtained with *Solution (3)* (1%).

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. To weigh and pulverize 20 tablets. To transfer an amount of powder equivalent to 10 mg of amitriptyline hydrochloride to 100 mL volumetric flask. To add 75 mL of 0.1 M hydrochloric acid, mechanically stir for 15 minutes, and put it in ultrasound for 15 minutes. To top up the volume with the same solvent. To filter it. To transfer 5 mL of the filtrate to a 50 mL volumetric flask and top up with the same solvent, thus obtaining 0.001% solution (w/v). To prepare a standard solution at the same concentration by using the same solvent. To determine the absorbances of the resulting solutions at 239 nm, by using 0.1 M hydrochloric acid to adjust zero. To calculate the amount of $C_{20}H_{23}N.HCl$ in the tablets, from the readings obtained.

B. To proceed as described in *High performance liquid chromatography (5.2.17.4)*. To proceed as described in *Dosage method B.*, in the monograph *Hydrochloride, amitriptyline, capsules*. To prepare the *Sample solution* as described below.

Sample solution: To weigh and pulverize the tablets. To transfer an amount of powder equivalent to 50 mg of amitriptyline hydrochloride to a 50 mL volumetric flask, add 35 mL of *Mobile phase*, mechanically stir for 15 minutes, and put it in ultrasound for 15 minutes. To top up with the same solvent, make it homogeneous, and filter it. To transfer 10 mL of the filtrate to 50 mL volumetric flask and top up with *Mobile phase*. To make it homogeneous.

Procedure: to separately inject 10 µL of *Sample and standard solutions*, record the chromatograms, and measure the area under the peaks. To calculate the amount of $C_{20}H_{23}N.HCl$ in the tablets, from the responses obtained for the *Sample and Standard solutions*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

HYDROCHLORIDE, BIPERIDEN TABLETS

Biperiden hydrochloride tablets contain the equivalent to at least 93.0% and at most 107.0% of the amount stated of $C_{21}H_{29}NO.HCl$.

IDENTIFICATION

A. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF_{254} silica gel as support and a mixture of 25% isopropyl-ammonium hydroxide (v/v) (95:5) as mobile phase. To apply separately to the plate 20 µL of each of the solutions as described below.

Solution (1): to pulverize the tablets. To weigh the equivalent to 10 mg of biperiden hydrochloride, add 5 mL of methylene chloride, and stir. To filter and wash the residue with 5 mL of methylene chloride. To evaporate the filtrate. To dissolve the residue in 1 mL of methanol.

Solution (2): 1% biperiden hydrochloride SQR solution (w/v) in methanol.

To develop the chromatogram. To remove the plate, allow to air dry, and examine under ultraviolet light (254 nm). To expose the plate to iodine vapors, for 10 minutes, in closed tank, previously saturated, with iodine crystals at the bottom. The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

B. To weigh and pulverize the tablets. To use an amount of powder equivalent to 10 mg of biperiden hydrochloride, add 10 mL of water, and heat it for 15 minutes. To filter it. It responds to the reactions of chloride ion (**5.3.1.1**).

C. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.01 M hydrochloric acid, 500 mL

Equipment: blades, 50 rpm

Time: 45 minutes

Standard solution: to dissolve accurately weighed amount of biperiden hydrochloride SQR in methanol and dilute with the same solvent, to obtain about 1.0 mg/mL concentration. To successively dilute in 0.01 M hydrochloric acid, to obtain 4 µg/mL concentration.

Sample solution: after testing, use aliquot from the dissolution medium.

Procedure: after testing, remove aliquot from the dissolution medium and proceed as described in *Dosage* to separately inject 50 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of $C_{21}H_{29}NO.HCl$ dissolved in the medium from the responses obtained from *Sample* and *standard solutions*.

Tolerance: not less than 75% (Q) of the amount stated of $C_{21}H_{29}NO.HCl$ dissolves within 45 minutes.

DOSAGE

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 205 nm; 150 mm column and 4.6 mm inner diameter, packed with silica chemically bonded to the octylsilane group (5 µm), kept at 25°C; flow of *Mobile phase* of 1.2 mL/min.

Sodium phosphate buffer pH 2.5: to dissolve 6.9 g of monobasic sodium phosphate in 500 mL of water. To add 1.011 g of sodium heptane sulphonate and top up to 1000 mL with the same solvent. If necessary, adjust the pH to 2.5 with phosphoric acid.

Mobile phase: mixture of *Sodium phosphate buffer pH 2.5* and methanol (50:50).

Sample solution: to weigh and pulverize 20 tablets. To transfer an amount of powder equivalent to 2 mg of biperiden hydrochloride to 20 mL volumetric flask. To add 10 mL of water and put it in ultrasound for 20 minutes. To mechanically stir for 10 minutes and top up with the same solvent. To make it homogenous and filter it. To transfer 2 mL of previous solution to 10 mL volumetric flask and top up with *Mobile phase*.

Standard solution: to weigh an amount equivalent to 10 mg of biperiden hydrochloride SQR and transfer to 10 mL volumetric flask. To top up to volume with methanol and

make it homogeneous. To transfer 0.2 mL of this solution to 10 mL volumetric flask and top up with *Mobile phase*. To make it homogeneous.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_{21}H_{29}NO.HCl$ in the tablets, from the responses obtained for the *Sample* and *Standard solutions*.

PACKAGING AND STORAGE

In tightly closed containers, at room temperature.

LABELLING

To comply with the legislation in force.

HYDROCHLORIDE, BUPIVACAINE AND GLUCOSE INJECTABLE SOLUTION

It contains at least 93.0 % and at most 107.0 % of the amount stated of $C_{18}H_{28}N_2O.HCl$ and $C_6H_{12}O_6$.

The injectable solution can be prepared in water for injection or another suitable solvent.

IDENTIFICATION

A. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF_{25} silica gel as support and a mixture of 1-butanol, water, ethanol, and glacial acetic acid (6:2:1:1) as mobile phase. To separately apply to the plate 10 µL of *Solution (1)*, *Solution (2)*, and *Solution (3)* freshly prepared, as described below.

Solution (1): injectable solution of bupivacaine hydrochloride and glucose.

Solution (2): bupivacaine hydrochloride SQR solution in water at concentration corresponding to the injectable solution.

Solution (3): glucose SQR solution in water at concentration corresponding to the injectable solution.

Solution (4): to dilute bupivacaine hydrochloride SQR solution in *Solution (3)*, to obtain concentration corresponding to the injectable solution.

To develop the chromatogram. To remove the plate and dry in circulating hot air. To examine the plate under ultraviolet light (254 nm). The position of the principal spot obtained with *Solution (1)* corresponds to that of the spots of *Solution (2)* and *Solution (4)*. To nebulize with naphthalenediol reagent, warm at 90 ° C for 5 minutes, and examine the plate. The position of the brown principal spot obtained with the sample corresponds to that obtained with *Solution (3)*. To cool down the plate, nebulize iodine-platinum

reagent, and examine the plate. Bupivacaine is viewed as blue-violet spot on orange background, and the spot corresponding to glucose gradually fades out. The position of the bupivacaine spot obtained with *Solution (1)* corresponds to those obtained with *Solution (2)* and *Solution (4)*.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method **B.** corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

pH (5.2.19). 4.0 to 6.5.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

Bacterial endotoxins (5.5.2.2). At most 2.5 UE/ mL.

DOSAGE

Bupivacaine hydrochloride To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 263 nm; 250 mm column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at room temperature; flow of *Mobile phase* of 2.0 mL/min.

Phosphate buffer pH 6.8: to dissolve 1.94 g of monobasic potassium phosphate and 2.48 g of dibasic potassium phosphate in 1000 mL of water. If necessary, adjust pH to 6.8 ± 0.05 with *M* potassium hydroxide or *M* phosphoric acid.

Mobile phase: mixture of acetonitrile and *Phosphate buffer pH 6.8* (65:35). If necessary, adjust pH to 7.7 ± 0.02 with *M* phosphoric acid

Sample solution: to transfer a volume of injectable solution equivalent to 25 mg of bupivacaine hydrochloride to a 50 mL volumetric flask. To top up to volume with methanol and make it homogeneous.

Standard solution: to accurately weigh an amount equivalent to 25 mg of bupivacaine hydrochloride SQR and transfer to 50 mL volumetric flask. To dissolve in 5 mL of water, by using ultrasonic bath, if necessary. To top up to volume with methanol and make it homogeneous.

To inject replicates of 20 µL of the *Standard solution*. The relative standard deviation for the replicate areas for the peaks recorded is not greater than 2.0%.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content

of $C_{18}H_{28}N_2O \cdot HCl$ from the sample, from the responses obtained for the *Sample and Standard solutions*.

Glucose. To measure the rotation angle of the sample in a suitable tube (**5.2.8**), by using distilled water to adjust zero. To calculate the content of $C_6H_{12}O_6$ in each mL of the sample, according to the equation:

$$\alpha \times 9.452 \times A$$

where α is the average reading obtained and A is 200/length of the tube in mm.

PACKAGING AND STORAGE

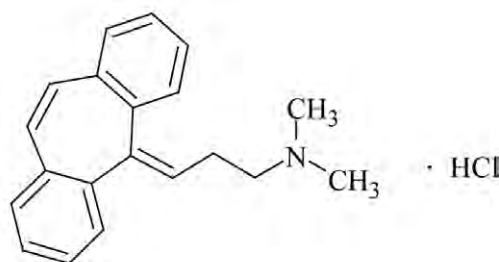
In single-dose containers; preferably type I glass; protected from light.

LABELLING

To comply with the legislation in force.

HYDROCHLORIDE, CYCLOBENZAPRINE

Cyclobenzapriini hydrochloridum



$C_{20}H_{21}N \cdot HCl$; 311.85
cyclobenzaprine hydrochloride; 02013
3 - (5H-dibenzo [a, d] cyclohepten-5-ylidene)-N,N-dimethyl-1-propanamine hydrochloride (1:1)
[6202-23-9]

It contains at least 99.0% and at most 101.0% of $C_{20}H_{21}N \cdot HCl$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White crystalline powder.

Solubility. Freely soluble in water, ethanol, and methanol; slightly soluble in isopropyl alcohol; very slightly soluble in chloroform; insoluble in hydrocarbons.

Physical and chemical constants.

Melting range (5.2.2): 215 °C to 219 °C. The range between the beginning and end of melting range must not exceed 2 °C.

IDENTIFICATION

The identification test **B.** may be omitted if the tests **A.** and **C.** are conducted.

A. The infrared absorption spectrum (**5.2.14**) of the sample, desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the cyclobenzaprine hydrochloride SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (**5.2.14**), from 250 nm to 350 nm, 0.0015% solution (w/v) of the sample in methanol has absorption maximum at 290 nm, similar to that observed in the spectrum of a similar solution of cyclobenzaprine hydrochloride SQR.

C. It responds to the reactions of chloride ion (**5.3.1.1**).

PURITY TESTS

Related substances. To proceed as described in *Thin-layer chromatography* (**5.2.17.1**), by using GF₂₅₄ silica gel as support and a mixture of acetone, toluene, and ammonium hydroxide (75:25:1) as mobile phase. To separately apply to the plate 5 µL of each of the solutions freshly prepared, as described below.

Solution (1): 20 mg/mL solution of the sample in methanol.

Solution (2): 20 mg/mL cyclobenzaprine hydrochloride SQR solution in methanol.

Solution (3): to dilute 0.1 mL of *Solution (1)* in 20 mL with methanol.

To develop the chromatogram. To remove the plate, allow it to dry in air for 15 minutes, and examine it under ultraviolet light (254 nm). The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*. Other spot obtained with *Solution (1)* does not exceed in size or intensity the principal spot obtained from the *Solution (3)* (0.5%).

Heavy metals (**5.3.2.3**). To use *Method III*. To determine in 1 g of the sample. At most 0.001% (10 ppm).

Loss on drying (**5.2.9**). To determine in 1 g of the sample in oven at 105 °C, to obtain constant weight. At most 1.0%.

Sulfated ashes (**5.2.10**). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To proceed as described in *Titrations in non-aqueous medium* (**5.3.3.5**). To accurately weigh about 0.4 g of the desiccated sample. To dissolve in 80 mL of glacial acetic acid and 15 mL of mercuric acetate SR. To titrate with 0.1 M SV perchloric acid, to potentiometrically determine the endpoint. To perform sample blank correction and adjust it as

required. Each mL of 0.1 M SV perchloric acid is equal to 31.185 mg of C₂₀H₂₁N.HCl.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Muscle relaxant.

HYDROCHLORIDE, CYCLOBENZAPRINE TABLETS

It contains at least 90.0 % and at most 110.0 % of the amount stated of C₂₀H₂₁N.HCl.

IDENTIFICATION

A. To weigh and pulverize the tablets. To use an amount of powder equivalent to 50 mg of cyclobenzaprine hydrochloride and add 20 mL of methylene chloride. To filter and evaporate about 5 mL of the filtrate. To transfer to centrifuge tube with 2 mL of ethyl ether. To evaporate by current of air and stir to crystallize it. To wash the crystals with ethyl ether and dry it at room temperature. The infrared absorption spectrum (**5.2.14**) of the residue, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the cyclobenzaprine hydrochloride SQR spectrum.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of weight (**5.1.1**). It complies with the test.

Hardness Test (**5.1.3.1**). It complies with the test.

Friability test (**5.1.3.2**). It complies with the test.

Disintegration test (**5.1.4.1**). It complies with the test.

Uniformity of dosage units (**5.1.6**). It complies with the test.

DISSOLUTION TEST (**5.1.5**)

Dissolution medium: 0.1 M hydrochloric acid, 900 mL

Equipment: basket, 50 rpm

Time: 30 minutes

Procedure: after testing, remove aliquot from the dissolution medium, filter, and dilute, if necessary, with 0.1 M hydrochloric acid, to obtain suitable concentration. To measure the absorbances of the solutions at 290 nm (5.2.14), by using the same solvent to adjust zero. To calculate the amount of $C_{20}H_{21}N.HCl$ dissolved in the medium, by comparing the readings obtained with that of 0.00001 % cyclobenzaprine hydrochloride SQR solution (w/v) prepared in 0.1 M hydrochloric acid.

Tolerance: not less than 75% (Q) of the amount stated of $C_{20}H_{21}N.HCl$ dissolves within 30 minutes.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To proceed as described in *High performance liquid chromatography* (5.2.17.4).

To use chromatograph equipped with an ultraviolet detector at 290 nm; 100 mm column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μ m); flow of *Mobile phase* of 1.5 mL/min.

Mobile phase: mixture of water, acetonitrile, methanol, and methanesulfonic acid (48:28:24:0.2). To adjust pH to 3.6 with diethylamine.

Sample solution: to weigh and pulverize 20 tablets. To transfer an amount of powder equivalent to 10 mg of cyclobenzaprine hydrochloride to 200 mL volumetric flask, add 150 mL of 0.1 M hydrochloric acid, and mechanically stir for 30 minutes. To top up to volume with the same solvent, thus obtaining 50 μ g/mL solution. To make it homogenous and filter it.

Standard solution: to dissolve an accurately weighed amount of cyclobenzaprine hydrochloride SQR in 0.1 M hydrochloric acid, to obtain 50 μ g/mL solution.

To inject replicates of 20 μ L of the *Standard solution*. The column efficiency is greater than 1000 theoretical plates/meter. The relative standard deviation for the replicate areas for the peaks recorded is not greater than 2.0%. The tailing factor for main peak is not greater than 2.

Procedure: to separately inject 20 μ L of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of $C_{20}H_{21}N.HCl$ in the tablets, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

HYDROCHLORIDE, CIPROFLOXACIN TABLETS

Ciprofloxacin hydrochloride tablets contain the equivalent to at least 90.0% and at most 110.0% of the amount stated of $C_{17}H_{18}FN_3O_3$.

IDENTIFICATION

A. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF_{254} silica gel as support and a mixture of methylene chloride, methanol, ammonium hydroxide, and acetonitrile (4:4:2:1) as mobile phase. To separately apply to the plate 5 μ L of each of the solutions freshly prepared, as described below.

Solution (1): to weigh and pulverize the tablets. To transfer an amount of powder equivalent to 0.15 g of ciprofloxacin to a 100 mL volumetric flask.

To add 70 mL of water, stir for about 20 minutes, and top up with the same solvent. To centrifuge and use the clear portion of the supernatant.

Solution (2): aqueous solution of ciprofloxacin hydrochloride SQR containing the equivalent to 0.15% of ciprofloxacin (w/v).

To develop the chromatogram. To remove the plate and allow it to air dry for 15 minutes. To examine under ultraviolet light (254 nm and 336 nm). The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage method B*, corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL *Equipment:* blades, 50 rpm

Time: 30 minutes

Procedure: after testing, remove aliquot from the dissolution medium, filter immediately, and dilute with water, to obtain suitable concentration. To measure the absorbances of the solutions at 272 nm (5.2.14), by using water to adjust zero. To calculate the amount of $C_{17}H_{18}FN_3O_3$ dissolved in the medium, by comparing the readings obtained with that of 0.0004% ciprofloxacin hydrochloride SQR solution (w/v) prepared in the same solvent.

Tolerance: not less than 80% (Q) of the amount stated of $C_{17}H_{18}FN_3O_3$ dissolves within 30 minutes.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To weigh and pulverize 20 tablets. To transfer to a 500 mL volumetric flask an amount of powder equivalent to 1.25 g of ciprofloxacin, by using 400 mL of water. To stir for 30 minutes, top up to volume, and filter it. To successively dilute to obtain 0.0004% concentration (w/v), by using water as solvent. To prepare ciprofloxacin hydrochloride SQR solution in water with concentration similar to that of ciprofloxacin. To measure the absorbance of the resulting solutions at 272 nm, by using water to adjust zero. To calculate the content of $C_{17}H_{18}FN_3O_3$ in the tablets, from the readings obtained.

B. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 278 nm; 250 mm column and 4 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (3 μ m to 10 μ m), kept at 30 °C; flow of *Mobile phase* of 1.5 mL/min.

Mobile phase: mixture of 0.025 M phosphoric acid (pH pre-adjusted to 3.0 ± 0.1 with triethylamine) and acetonitrile (85:15).

Sample solution: to transfer 5 tablets to 500 mL flask. To add about 400 mL of water, put it in ultrasound for about 20 minutes, top up with water, and stir it. To successively dilute to obtain 0.25 mg/mL ciprofloxacin, by using water as solvent, and filter it.

Standard solution: to weigh an amount of ciprofloxacin hydrochloride SQR equal to 25 mg of ciprofloxacin, transfer to a 25 mL volumetric flask, and top up with water. To successively dilute with water, to obtain a 0.25 mg/mL ciprofloxacin solution.

Procedure: to separately inject 20 μ L of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of $C_{17}H_{18}FN_3O_3$ in the tablets, from the responses obtained for the *Sample solution* and *Standard solution*.

C. To proceed as described in *Microbiological test of antibiotics* (5.5.3.3.1) by using cylinders with the agar diffusion method.

Micro-organism: *Staphylococcus epidermidis* ATCC 12228.

Culture media: culture medium number 1, to maintain the micro-organism; sterile saline solution to the standardization of the inoculum; and culture medium number 11, to the base layer and inoculum preparation.

Sample solution: to transfer 5 tablets to 500 mL flask. To add about 400 mL of water, put it in ultrasound for about 20 minutes, top up with water, and stir it. To successively dilute in water to obtain 4 μ g/mL, 8 μ g/mL, and 16 μ g/mL ciprofloxacin concentrations, by using the 0.1 M *Potassium phosphate buffer, sterile, pH 8.0* (Solution 2) as diluent.

Standard solution: to weigh an amount of ciprofloxacin hydrochloride SQR equal to 20 mg of ciprofloxacin, transfer to a 20 mL volumetric flask, and top up with water.

To successively dilute in water to obtain 4 μ g/mL, 8 μ g/mL, and 16 μ g/mL ciprofloxacin concentrations, by using the 0.1 M *Potassium phosphate buffer, sterile, pH 8.0* (Solution 2) as diluent.

Procedure: to add 20 mL of culture medium number 11 to a plate, wait to solidify, add 5 mL of 1% inoculum, and proceed as described in *Microbiological test of antibiotics* (5.5.3.3.1), by adding 0.1 mL of freshly prepared solutions to the cylinders. To calculate the potency of the sample, in μ g, of ciprofloxacin per mg, from the potency of the standard and responses obtained with the *Standard and sample solutions*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light, at room temperature.

LABELLING

To comply with the legislation in force.

HYDROCHLORIDE, CIPROFLOXACIN, OPHTHALMIC SOLUTION

It contains at least 90.0 % and at most 110.0 % of the amount stated of ciprofloxacin ($C_{17}H_{18}FN_3O_3$).

IDENTIFICATION

A. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF_{254} silica gel as support and a mixture of methylene chloride, methanol, ammonium hydroxide, and acetonitrile (4:4:2:1) as mobile phase. To separately apply to the plate 3 μ L of each of the solutions freshly prepared, as described below.

Solution (1): to dilute volume of sample in water, to obtain about 0.3% ciprofloxacin solution (w/v).

Solution (2): 0.3% ciprofloxacin hydrochloride SQR solution (w/v) in water.

To develop the chromatogram. To remove the plate and allow it to air dry for 15 minutes. To examine under ultraviolet light (254 nm and 336 nm). The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage method A* corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

pH (5.2.19). 3.5 to 5.5.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test. To use the *Membrane filtration method*.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 280 nm; 150 mm column and 4 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (3 μ m to 10 μ m), kept at 30 °C; flow of *Mobile phase* of 1.5 mL/min.

Tetrabutylammonium phosphate buffer: to prepare 0.005 M tetrabutylammonium phosphate solution, pH previously adjusted with phosphoric acid to 2.0 ± 0.1 .

Mobile phase: mixture of *Tetrabutylammonium phosphate buffer* and methanol (75:25).

Sample solution: to transfer volume of ophthalmic solution equivalent to 6 mg of ciprofloxacin to 50 mL volumetric flask. To top up the volume with water and stir.

Standard solution: to accurately dissolve weighed amount of ciprofloxacin hydrochloride SQR in water and properly dilute to obtain a 0.12 mg/mL ciprofloxacin solution.

Procedure: to separately inject 20 μ L of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of ciprofloxacin ($C_{17}H_{18}FN_3O_3$) in the ophthalmic solution, from the responses from the *Standard solution* and *Sample solution*.

B. To proceed as described in *Microbiological test of antibiotics (5.5.3.3)* by using cylinders with the agar diffusion method.

Micro-organism: *Staphylococcus epidermidis* ATCC 12228.

Culture media: culture medium number 1, to maintain the micro-organism; sterile saline solution to the standardization of the inoculum; and culture medium number 11, to the base layer and inoculum preparation.

Sample solution: to transfer volume of ophthalmic solution equivalent to 40 mg of ciprofloxacin to 100 mL volumetric flask, top up with water, and stir it. To successively dilute to obtain 2 μ g/mL, 4 μ g/mL, and 8 μ g/mL ciprofloxacin concentrations, by using the 0.1 M *Potassium phosphate buffer, sterile, pH 8.0 (Solution 2)* as diluent.

Standard solution: to weigh an amount of ciprofloxacin hydrochloride equal to 20 mg of ciprofloxacin, transfer to a 50 mL volumetric flask, and top up with water.

To successively dilute to obtain 2 μ g/mL, 4 μ g/mL, and 8 μ g/mL ciprofloxacin concentrations, by using the 0.1 M *Potassium phosphate buffer, sterile, pH 8.0 (Solution 2)* as diluent.

Procedure: to add 20 mL of culture medium number 11 to a plate, wait to solidify, add 5 mL of 1% inoculum, and proceed as described in *Microbiological test of antibiotics (5.5.3.3.1)*, by adding 0.1 mL of freshly prepared solutions to the cylinders. To calculate the potency of the ophthalmic solution, in μ g, of ciprofloxacin per mg, from the potency of the standard and responses obtained with the *Standard and sample solutions*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light, at room temperature.

LABELLING

To comply with the legislation in force.

HYDROCHLORIDE, CLINDAMYCIN, CAPSULES

It contains clindamycin hydrochloride equivalent to at least 90.0% and at most 110.0% of the amount stated of clindamycin ($C_{18}H_{33}ClN_2O_5S$).

IDENTIFICATION

The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

PURITY TESTS

Related substances. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 210 nm; 250 mm long column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μ m); flow of *Mobile phase* of 1.5 mL/min.

Phosphate buffer: to dissolve 6.8 g of monobasic potassium phosphate in 950 mL of water, adjust the pH to 7.5 with 25% potassium hydroxide (w/v), and dilute to 1000 mL with water.

Mobile phase: mixture of *Phosphate buffer* and acetonitrile (55:45). To make the corrections as required.

Note: the reduction in proportion of acetonitrile, in the *Mobile phase*, increases the resolution between 7-epi-clindamycin and clindamycin peaks.

Solution (1): to weigh capsules, remove the content, and weigh them again. To accurately transfer amount of powder equal to 50 mg of clindamycin to a 50 mL volumetric flask and top up with *Mobile phase*. To stir for 15 minutes. To make it homogenous and filter it.

Solution (2): 1 mg /mL clindamycin hydrochloride SQR solution in *Mobile phase*.

Solution (3): to dilute 2 mL of *Solution (1)* to 100 mL with *Mobile phase*.

To inject 20 μ L of *Solution (2)* and record the chromatogram for at least twice the retention time of the main peak. The relative retention times are about 0.4 to lincomycin, 0.65 to clindamycin B, 0.8 to 7-epi-clindamycin, and 1.0 to clindamycin. The resolution between the clindamycin B and 7-epi-clindamycin peaks is not less than 2.4. The res-

olution between 7-epi-clindamycin and clindamycin peaks is not less than 3.0.

Procedure: to separately inject 20 μ L of *Solutions (1)* and (3), record the chromatograms at least twice the retention time of the main peak, and measure the areas under the peaks. The area of any secondary peak corresponding to clindamycin B in the chromatogram obtained with *Solution (1)* is not greater than the area under the main peak in the *Solution (3)* (2.0%). The area of any secondary peak corresponding to 7-epi-clindamycin in the chromatogram obtained with *Solution (1)* is not greater than twice the area under the main peak in the *Solution (3)* (4.0%). The sum of the areas of all the secondary peaks obtained in the chromatogram with *Solution (1)*, except the main peak's one, is not greater than 3 times the area under the main peak obtained with *Solution (3)* (6.0%). To disregard the peaks related to solvent or with an area less than 0.025 times the area under the main peak obtained with *Solution (3)* (0.05%).

Water (5.2.20.1). At most 7.0%.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 210 nm; 250 mm long column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μ m); flow of *Mobile phase* of 1.5 mL/min.

Phosphate buffer: to dissolve 6.8 g of monobasic potassium phosphate in 950 mL of water, adjust the pH to 7.5 with 25% potassium hydroxide (w/v), and dilute to 1000 mL with water.

Mobile phase: mixture of *Phosphate buffer* and acetonitrile (55:45). To make the corrections as required.

Standard solution: to dissolve accurately weighed amount of clindamycin hydrochloride SQR in *Mobile phase*, to obtain a 1.0 mg/mL solution.

Sample solution: to weigh 20 capsules, remove the content, and weigh them again. To transfer an amount of powder equal to 50 mg of clindamycin to a 50 mL volumetric flask and top up with *Mobile phase*. To stir for 15 minutes. To make it homogenous and filter it.

To inject replicates of 20 μ L of *Standard solution* and record the peaks at least twice the retention time of the main peak. The resolution between the clindamycin B and

7-epi-clindamycin peaks is not less than 2.4. The resolution between 7-epi-clindamycin and clindamycin peaks is not less than 3.0. The relative standard deviation for replicate areas of the clindamycin peaks recorded should not be greater than 2.0 %.

Procedure: to separately inject 20 µL of *Standard and sample solutions*, record the chromatograms at least twice the retention time of the main peak, and measure the areas under the peaks. To calculate the content of $C_{18}H_{33}ClN_2O_5S$ from the sample, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

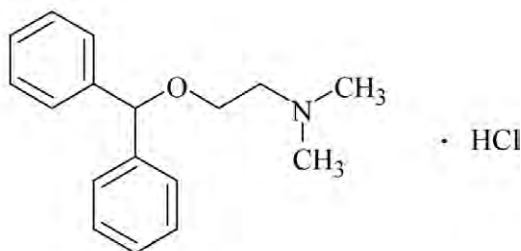
In tightly closed containers.

LABELLING

To comply with the legislation in force.

HYDROCHLORIDE, DIPHENHYDRAMINE

Diphenhydramini hydrochloridum



$C_{17}H_{21}NO \cdot HCl$; 291.82
diphenhydramine hydrochloride; 02979
2-diphenylmethoxy-*N,N*-dimethylethanamine
hydrochloride (1:1)
[147-24-0]

It contains at least 99.0% and at most 101.0% of $C_{17}H_{21}NO \cdot HCl$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white or almost white, and odorless powder.

Solubility. Freely soluble in water, ethanol, and chloroform; practically insoluble in ethyl ether.

Physical and chemical constants. *Melting range* (5.2.2): 167 °C to 172 °C.

IDENTIFICATION

The identification tests B. and C. may be omitted if the tests A. and D. are conducted. The identification tests A. and D. can be omitted if the tests A. and C. are carried out.

A. The infrared absorption spectrum (5.2.14) of the sample, desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the diphenhydramine hydrochloride SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14) ranging from 230 nm to 350 nm, of 0.05% sample (w/v) in ethanol, exhibits maximum at 253 nm.

C. To dissolve 0.05 g of the sample in 100 mL of water. To add 2 mL of sulfuric acid to 0.05 mL of previous solution. Yellow color is developed, which becomes red by adding 0.5 mL of nitric acid. To add 15 mL of water, cool down, add 5 mL of chloroform, and stir it. Violet color develops in chloroform layer.

D. It responds to the reactions of the chloride ion (5.3.1.1).

PURITY TESTS

Appearance of solution. The 0.2% aqueous solution (w/v) and a solution five times diluted are colorless (5.2.12). The 0.2% aqueous solution (w/v) is no colored than the *Standard solution color SC G* (5.2.12).

pH (5.2.19). 4.0 to 6.0. To determine in 5% aqueous solution (w/v).

Acidity or alkalinity. To dissolve 2.5 g of the sample in 50 mL of water. At most 0.25 mL of 0.01 *M* hydrochloric acid is spent to neutralize 10 mL of the sample, by using methyl red SI as an indicator. At most 0.5 mL of 0.01 *M* sodium hydroxide is required to pink becomes yellow.

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using H silica gel as support and a mixture of diethylamine, methanol, and chloroform (1:20:80) as mobile phase. To activate the plate at 105 °C for 1 hour. To apply separately to the plate 5 µl of each of the solutions as described below.

Solution (1): 2% mg/mL solution of the sample in methanol.

Solution (2): 0.02% mg/mL solution of the sample in methanol.

To develop the chromatogram. To remove the plate, dry in a current of air, and nebulize with sulfuric acid. To heat at 120 °C, for 10 minutes, to obtain spots. No spot obtained with *Solution (1)*, other than the principal spot, is more intense than that obtained with *Solution (2)* (1.0%) and is no colored than *Standard solution color SC F* (5.2.12).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 105 °C for 3 hours. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To accurately dissolve about 0.25 g of the sample, previously desiccated, in 20 mL of glacial acetic acid and 10 mL of mercury acetate SR. To add a drop of methylrosanilinium chloride SI and titrate with 0.1 M SV perchloric acid to obtain greenish-blue color. To perform sample blank correction and adjust it as required. To potentiometrically determine the endpoint, as an alternative. Each mL of 0.1 M SV perchloric acid is equal to 29.182 mg of $C_{17}H_{21}NO \cdot HCl$.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antihistamine.

HYDROCHLORIDE, DIPHENHYDRAMINE TABLETS

It contains at least 98.0 % and at most 100.5 % of the amount stated of $C_{17}H_{21}NO \cdot HCl$.

IDENTIFICATION

A. To weigh and pulverize the tablets. To use an amount of powder equivalent to 0.1 g of diphenhydramine hydrochloride. To add 1 mL of 0.1 M sulfuric acid and stir with 3 portions (20 mL each) of ethyl ether. To discard the ether layer. To alkalize the aqueous phase with 5 M sodium hydroxide and extract with 2 portions (50 mL each) of n-heptane. To collect the organic extracts, wash them with 10 mL of water, filter over anhydrous sodium sulfate, and evaporate the filtrate to dryness. To dissolve the residue in 1 mL of carbon disulfide.

The infrared absorption spectrum (5.2.14) of the residue has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the diphenhydramine hydrochloride SQR spectrum similarly prepared.

B. To weigh and pulverize the tablets. To stir an amount of powder equivalent to 0.1 g of diphenhydramine hydrochloride with 2 portions (15 mL each) of chloroform. To

filter each portion. To evaporate the filtrate to dryness in water bath. To desiccate the residue in an oven at 80 °C for 1 hour. The residue melts at around 168 °C.

C. The residue obtained in test B. *Identification* responds to the reactions of chloride ion (5.3.1.1).

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 900 mL

Equipment: baskets, 100 rpm

Time: 45 minutes

Procedure: after testing, remove aliquot from the dissolution medium, filter, and dilute, if necessary, with 0.1 M hydrochloric acid, to obtain suitable concentration. To measure the absorbances at 254 nm (5.2.14), by using the same solvent to adjust zero. To calculate the amount of $C_{17}H_{21}NO \cdot HCl$ dissolved in the medium, by comparing the readings obtained with that one related to diphenhydramine hydrochloride SQR solution, at similar concentration and prepared in the same solvent.

Tolerance: not less than 75% (Q) of the amount stated of $C_{17}H_{21}NO \cdot HCl$ dissolves within 45 minutes.

PURITY TESTS

Related substances. To proceed as described in *Related substances*, in the monograph *Diphenhydramine hydrochloride*. To prepare *Solutions (1) and (2)* as described below.

Solution (1): to weigh and pulverize the tablets. To use an amount of powder equivalent to 50 mg of diphenhydramine hydrochloride and extract with 3 portions (10 mL each) of chloroform. To filter, evaporate the combined extracts to dryness, and dissolve the residue in 5 mL of chloroform.

Solution (2): to dilute 1 mL of *Solution (1)* to 100 mL with chloroform.

Water (5.2.20.1). To determine in 1 g of the sample. At most 1.0%.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To proceed as described in *Titration in non-aqueous medium* (5.3.3.5). To weigh and pulverize 20 tablets. To weigh an amount of powder equivalent to 0.2 g of diphenhydramine hydrochloride, and add 20 mL of anhydrous acetic acid and 10 mL of 6% mercury acetate (w/v) in glacial acetic acid. To titrate with 0.1 M SV perchloric acid, by using methylrosanilinium chloride SI as an indicator. Each mL of 0.1 M SV perchloric acid is equal to 29.180 mg of $C_{17}H_{21}NO.HCl$.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

HYDROCHLORIDE, DIPHENHYDRAMINE ORAL SOLUTION

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_{17}H_{21}NO.HCl$.

IDENTIFICATION

A. To transfer a volume of oral solution containing 50 mg of diphenhydramine hydrochloride to a separatory funnel. To add 0.5 mL of 2 M sulfuric acid and extract with 3 portions (15 mL each) of ethyl ether, by discarding the ether phases. To dissolve 50 mg of diphenhydramine hydrochloride SQR in 25 mL of water into a second separatory funnel. To handle the solutions as follows: to add 2 mL of M sodium hydroxide and extract with 75 mL of n-heptane. To wash the extract of n-heptane with 10 mL of water, evaporate to dryness, and dissolve the residue in 4 mL of chloroform. To filter, if necessary, to clarify the solution. To quickly determine the infrared absorption spectrum of the filtered *Sample and standard solutions*, by using chloroform as blank in a cell of 0.1 mm or 1 mm or 7 μ m - 15 μ m. The infrared absorption spectrum (5.2.14) of the sample has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the diphenhydramine SQR spectrum similarly prepared.

B. To evaporate to dryness 1 mL of *Solution (1)*. To dissolve the residue in 0.15 mL of water and add 2 mL of sulfuric acid. Yellow color is produced. This color becomes red by adding 0.5 mL of nitric acid. To add 15 mL of water,

cool down, add 5 mL of chloroform, and stir it. The chloroform layer turns violet.

Solution (1): to acidify volume of oral solution containing 50 mg of diphenhydramine hydrochloride with 2 M hydrochloric acid, stir with 3 portions (20 mL) of ethyl ether. To discard the ether portion. To extract with 2 portions (20 mL each) of chloroform, dry the combined extracts under anhydrous sodium sulfate, filter, evaporate the chloroform, and dissolve the residue in 5 mL of chloroform.

C. To add an excess of M sodium hydroxide. Ammonium vapors, with typical odor, are released. They can be recognized by developing red color when a paper filter is exposed to such vapors.

CHARACTERISTICS

pH (5.2.19). 4.0 to 6.0.

PURITY TESTS

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using H silica gel as support and a mixture of methanol and chloroform (20:80) as mobile phase. To separately apply to the plate 5 μ L of each of the solutions freshly prepared, as described below.

Solution (1): to use *Solution (1)* as described in Test **B**. Identification

Solution (2): to dilute 1 volume of *Solution (1)* to 100 volumes with chloroform.

To develop the chromatogram. To remove the plate, allow to air dry, and nebulize with diluted potassium iodobismuthate SR. No other secondary spot obtained with *Solution (1)* is more intense than that obtained with *Solution (2)*.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

Diphenhydramine hydrochloride

To acidify volume of oral solution containing about 0.1 g of diphenhydramine hydrochloride with 2 M hydrochloric acid, stir, and extract with 3 portions (20 mL each) of ethyl ether. To discard ether portions.

To alkalinize the aqueous phase with 5 M sodium hydroxide and extract with 4 portions (25 mL each) of ethyl ether. To wash the combined ether extracts with 2 portions (5 mL each) of water. To extract the washings with 15 mL of ethyl ether, collect the ether extracts, and evaporate to dryness.

To dissolve the residue in 15 mL of 0.05 M SV sulfuric acid and titrate the acid in excess with 0.1 M SV sodium hydroxide by using methyl red SI. Each mL of 0.05 M SV perchloric acid is equal to 29.180 mg of $C_{17}H_{21}NO.HCl$.

Ammonium chloride

To perform dosage of ammonium chloride if in the formula. It contains at least 95.0 % and at most 105.0 % of the amount stated of NH_4Cl .

To dissolve a volume of oral solution containing about 1 g of ammonium chloride in 20 mL of water. To add a mixture of 5 mL of formaldehyde previously neutralized in the presence of phenolphthalein SI and 20 mL of water. After 1 - 2 minutes, slowly titrate with M SV sodium hydroxide in the presence of 0.2 mL of the same indicator. Each mL of M SV sodium hydroxide is equal to 53.490 mg of NH_4Cl . Should the sample be colored, previously treat it with activated charcoal to remove the dye.

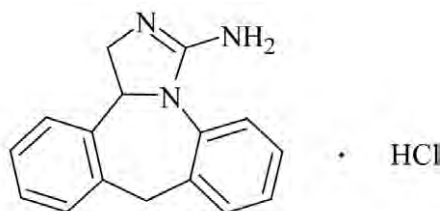
PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

HYDROCHLORIDE, EPINASTINE Epinastini hydrochloridum



$C_{16}H_{15}N_3.HCl$; 285.77
epinastine hydrochloride; 03440
9,13b-dihydro-1H-dibenz [c,f] imidazo [1,5- α] azepine-3-amine hydrochloride (1:1)
[80012-44-8]

It contains at least 98.5% and at most 101.5% of $C_{16}H_{15}N_3.HCl$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white to pale yellow powder.

Solubility. Freely soluble in water.

Physical and chemical constants. *Melting range* (5.2.2): 273 °C to 275 °C.

IDENTIFICATION

The identification tests C. and D. may be omitted if the tests A. and B. are conducted.

A. The infrared absorption spectrum (5.2.14) of the sample, desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the epinastine hydrochloride SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14), from 200 nm to 300 nm, 0.025% solution (w/v) prepared in 0.1 M hydrochloric acid, has absorption maximum at 210 nm, similar to that observed in the spectrum of a similar solution of epinastine hydrochloride SQR.

C. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF_{254} silica gel as support and a mixture of water, 1-butanol, and glacial acetic acid (50:40:10) as mobile phase. To prepare the mobile phase 24 hours in advance. Then, discard the lower layer. To separately apply to the plate 5 μ L of each of the solutions freshly prepared, as described below.

Solution (1): to prepare 1 mg/mL solution of the sample in methanol.

Solution (2): to prepare 1 mg/mL epinastine hydrochloride SQR solution in methanol.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

D. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

PURITY TESTS

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 105 °C to obtain constant weight. At most 0.5%.

DOSAGE

To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 207 nm; 150 mm column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μ m), base-deactivated, kept at room temperature; flow of *Mobile phase* of 1.0 mL/minute.

Mobile phase: mixture of 0.3% triethylamine (v/v), adjust to pH 4.0 with phosphoric acid, and methanol (60:40).

Sample solution: to transfer volume equal to 25 mg of the sample to a 50 mL volumetric flask and top up with *Mobile phase*. To transfer 4 mL of this solution to 100 mL volumetric flask and top up with *Mobile phase*, thus obtaining 20 µg/mL solution.

Standard solution: to transfer an amount equal to 25 mg of epinastine hydrochloride SQR to a 50 mL volumetric flask and top up with *Mobile phase*. To transfer 4 mL of this solution to 100 mL volumetric flask and top up with *Mobile phase*, thus obtaining 20 µg/mL solution.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_{16}H_{15}N_3.HCl$ from the sample, from the responses obtained for the *Sample and Standard solutions*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antihistamine.

HYDROCHLORIDE, EPINASTINE TABLETS

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_{16}H_{15}N_3.HCl$. Tablets may be coated.

IDENTIFICATION

A. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF_{254} silica gel as support and a mixture of water, 1-butanol, and glacial acetic acid (50:40:10) as mobile phase. To prepare the mobile phase 24 hours in advance. Then, discard the lower layer. To separately apply to the plate 10 µL of each of the solutions freshly prepared, as described below.

Solution (1): to weigh and pulverize 20 tablets. To transfer an amount equal to 10 mg of epinastine hydrochloride SQR to a 10 mL volumetric flask by using 5 mL of methanol. To mechanically stir for 10 minutes, top up with the same solvent, and filter it.

Solution (2): to prepare 1 mg/mL epinastine hydrochloride SQR solution in methanol.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm).

The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in *Dosage* corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test. To prepare the final solution containing 20 µg/mL of epinastine hydrochloride.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 900 mL

Equipment: blades, 60 rpm

Time: 45 minutes

To proceed as described in *Dosage*, in the monograph *Epinastine hydrochloride*.

Standard solution: to dissolve accurately weighed amount of epinastine hydrochloride SQR in 0.1 M hydrochloric acid, to obtain a solution whose concentration is (L/900) mg/mL where L is the stated amount in milligrams of epinastine hydrochloride per tablet.

Procedure: after the test, remove aliquot from dissolution medium and filter. To separately inject 20 µL of *Sample and standard solutions*. To record the chromatograms and measure the areas under the peaks. To calculate the content of $C_{16}H_{15}N_3.HCl$ from the sample, from the responses obtained for the *Sample and Standard solutions*.

Tolerance: not less than 75% (Q) of the amount stated of $C_{16}H_{15}N_3.HCl$ dissolves within 45 minutes.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To proceed as described in *Dosage*, in the monograph *Epinastine hydrochloride*. To prepare the *Sample and standard solutions* as described below.

Sample solution: to weigh and pulverize 20 tablets. To transfer an amount of powder equal to 25 mg of epinastine

hydrochloride to a 50 mL volumetric flask and add 30 mL of methanol. To put it in ultrasound for 15 minutes, at room temperature, and mechanically stir for 15 minutes. To top up with the same solvent, stir, and filter it.

To transfer an aliquot equal to 4 mL to a 100 mL volumetric flask and top up with *Mobile phase*.

Standard solution: to transfer an amount equal to 25 mg of epinastine hydrochloride to a 50 mL volumetric flask and add 30 mL of methanol. To mechanically stir for 15 minutes and top up with the same solvent. To transfer an aliquot equal to 4 mL to a 100 mL volumetric flask and top up with *Mobile phase*.

Procedure: to separately inject 20 µL of *Sample and standard solutions*, record the chromatograms, and measure the area under the peaks. To calculate the amount of $C_{16}H_{15}N_3 \cdot HCl$ in the tablets, from the responses obtained for the *Sample solution* and *Standard solution*.

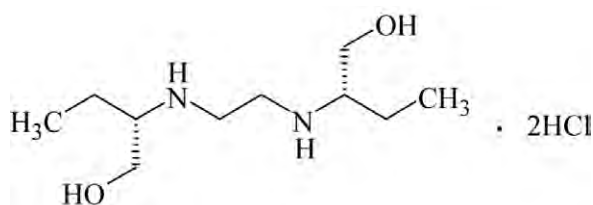
PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

HYDROCHLORIDE, ETHAMBUTOL Ethambutoli hydrochloridum



$C_{10}H_{24}N_2O_2 \cdot 2HCl$; 277.23
ethambutol hydrochloride; 03642
(2S,2'S)-2,2'-(1,2-ethanediy)diimino))bis-1-butanol
hydrochloride (2:1)
[1070-11-7]

It contains at least 97.0% and at most 101.0% of $C_{10}H_{24}N_2O_2 \cdot 2HCl$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white, odorless powder with bitter taste; hygroscopic.

Solubility. Freely soluble in water; soluble in ethanol and methanol; sparingly soluble in chloroform; practically insoluble in ethyl ether.

Physical and chemical constants.

Melting range (5.2.2): 199 °C to 204 °C.

Specific rotation (5.2.8): +5.8° to +6.6°, in relation to the anhydrous substance. To determine in 10% solution (w/v).

IDENTIFICATION

The identification test A. may be omitted if the tests B., C., and D. are conducted. The identification tests B. and C. can be omitted if the tests A. and D. are carried out.

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the ethambutol hydrochloride SQR spectrum similarly prepared.

B. The principal spot in the chromatogram of *Solution (2)*, in the *Limit for amino butanol*, corresponds in position, color, and intensity to that obtained with the *Solution (4)*.

C. To dissolve 0.1 g of the sample in 10 mL of water and add 0.2 mL of cupric sulfate SR. To add 1 mL of *M* sodium hydroxide. Bluer color develops.

D. The 10% aqueous solution (w/v) responds to the reactions of chloride ion (5.3.1.1).

PURITY TESTS

pH (5.2.19). 3.7 to 4.0. To determine in 2% solution (w/v) in carbon dioxide-free water.

Limit for amino butanol. To proceed as described in *Thin-layer chromatography (5.2.17.1)*, by using G silica gel as support and a mixture of ammonium hydroxide, water, and methanol (10:15:75) as mobile phase. To separately apply to the plate 2 µL of each of the solutions freshly prepared, as described below.

Solution (1): 50 mg/mL solution of the sample in methanol.

Solution (2): 5 mg/mL solution of the sample in methanol.

Solution (3): 0.5 mg/mL amino butanol SQR solution in methanol.

Solution (4): 5 mg/mL ethambutol hydrochloride SQR solution in methanol.

To develop the chromatogram. To remove the plate, allow it to dry in air, and heat it at 110 °C for 10 minutes. To cool down and nebulize with ninhydrin SR. To heat the plate at 110 °C for 5 minutes. Any secondary spot related to amino butanol in the chromatogram obtained with *Solution (1)* is not more intense than that obtained with *Solution (3)* (1.0%).

Heavy metals (5.3.2.3).

To use the *Method III*. At most 0.001% (10 ppm).

Loss on drying (5.2.9). To determine in 0.5 g of the sample.

To desiccate in an oven at 105 °C for 3 hours. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. To dissolve 0.2 g of the sample in 100 mL of glacial acetic acid and add 5 mL of mercury acetate SR. To titrate with 0.1 M SV perchloric acid, by using methylrosanilinium chloride SI as an indicator. To perform sample blank correction and adjust it as required. Each mL of 0.1 M SV perchloric acid is equal to 13.862 mg of $C_{10}H_{24}N_2O_2 \cdot 2HCl$.

B. To dissolve 0.1 g of the sample in 20 mL of a solution prepared as follows: mixture of 4 mL of cupric sulfate SR with 70 mL of 2 M ammonium hydroxide, addition of 10 mL of M sodium hydroxide and dilute to 100 mL with water. To top up the volume to 25 mL with the same solvent, after dissolution. To prepare a standard solution at the same concentration by using the same solvent. To measure the rotation angle of the solutions in suitable tube (5.2.8), by using the same solvent to adjust zero. To calculate the content of $C_{10}H_{24}N_2O_2 \cdot 2HCl$ in the sample from the readings of angles.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antibacterial (tuberculostatic).

HYDROCHLORIDE, ETHAMBUTOL TABLETS

It contains at least 95.0 % and at most 105.0% of the amount stated of ethambutol hydrochloride ($C_{10}H_{24}N_2O_2 \cdot 2HCl$). The tablets should be coated.

IDENTIFICATION

A. To weigh and pulverize the tablets. To use an amount of powder equivalent to 0.1 g of ethambutol hydrochloride and mix with 3 mL of methanol in glass mortar. To add 5 mL of methanol, make it homogeneous, and filter.

To collect the filtrate into the beaker containing 100 mL of acetone. To stir the mixture and wait for crystallization within 15 minutes. To remove the supernatant and dry the crystals by using compressed air. To proceed as described in *Identification* test A., in the monograph *Hydrochloride, Ethambutol*.

B. To weigh and pulverize the tablets. To stir amount of powder equivalent to 0.1 g of ethambutol hydrochloride with 10 mL of water.

To add 2 mL of 1% cupric sulfate (w/v) and 1 mL of M sodium hydroxide. Blue color is developed.

C. To weigh and pulverize the tablets. To stir amount of powder equivalent to 1 g of ethambutol hydrochloride with 10 mL of water. Resulting solution responds to the reactions of chloride ion (5.3.1.1).

D. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method A. corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL

Equipment: basket, 100 rpm

Time: 45 minutes

Procedure: after the test, remove aliquot from dissolution medium and filter. To discard the first 10 mL. To determine the amount of ethambutol dissolved as described in method A., *Dosage*. To prepare *Standard solution* as described below.

Standard solution: to accurately weigh 22.2 mg of ethambutol hydrochloride SQR and transfer to 50 mL volumetric flask. To dissolve and top up with dissolution medium. To make it homogenous and filter it.

Tolerance: not less than 75% (Q) of the amount stated of $C_{10}H_{24}N_2O_2 \cdot 2HCl$ dissolves within 45 minutes.

PURITY TESTS

Limit for amino butanol. To proceed as described in *Thin-layer chromatography (5.2.17.1)*, by using G silica gel as support and a mixture of concentrated ammonium hydroxide, water, and methanol (10:15:75) as mobile phase. To separately apply to the plate 2 µL of each of the solutions freshly prepared, as described below.

Solution (1): to weigh and pulverize the tablets. To use an amount of powder equivalent to 0.5 g of ethambutol hydrochloride, add 7 mL of methanol, and stir for 5 minutes. To top up to 50 mL with the same solvent and filter it, to obtain 50 mg/mL solution.

Solution (2): 0.5 mg/mL amino butanol SQR solution in methanol.

To develop the chromatogram. To remove the plate, allow it to dry in air, and heat it at 110 °C for 10 minutes. To cool down and nebulize with ninhydrin SR. To heat at 110 °C for 5 minutes.

Any secondary spot related to amino butanol in the chromatogram obtained with *Solution (1)* is not more intense than that obtained with *Solution (2)* (1%).

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 270 nm; 100 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at 40 °C; flow of *Mobile phase* of 2.0 mL/min.

Ammonium acetate buffer pH 5.0: to quantitatively transfer 50 g of ammonium acetate and 0.2 g of copper acetate to 1000 mL volumetric flask. To top up with water, make it homogeneous, and adjust pH to 5.0 with glacial acetic acid.

Mobile phase: mixture of *Ammonium acetate buffer pH 5.0* and methanol (94:6).

Diluent: water and methanol (94:6).

Sample solution: To weigh and pulverize the tablets. To transfer 20 mg of ethambutol hydrochloride, accurately weighed, to 50 mL volumetric flask. To add *Diluent*, stir, top up with *Diluent*, and filter it. To quantitatively dilute the filtrate with *Mobile phase* to obtain a solution whose concentration is similar to that of *Standard solution*.

Standard solution: to dissolve accurately weighed amount of ethambutol hydrochloride SQR in *Diluent*, to obtain a 0.4 mg/mL solution. To make it homogenous and filter it.

To inject replicates of 20 µL of the *Standard solution*. The column efficiency is greater than or equal to 2000 theoretical plates. The tailing factor is not greater than 2.5. The

relative standard deviation for replicate areas of the peaks recorded should not be greater than 2.0 %.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the area under the peaks. To calculate the content of $C_{10}H_{24}N_2O_2 \cdot 2HCl$ in the tablets, from the responses obtained for the *Sample solution* and *Standard solution*.

B. To weigh and pulverize 20 tablets. To use an amount equivalent to 0.2 g of ethambutol hydrochloride and transfer to a separatory funnel. To add 20 mL of 2 M sodium hydroxide solution and stir for 5 minutes. To extract with 5 portions (25 mL each) of chloroform.

To gather the chloroform extracts into a beaker and evaporate in a water bath to about 25 mL. To filter through paper to an erlenmeyer flask. To wash the beaker and the filter paper with 100 mL of glacial acetic acid anhydrous. To proceed as described in *Titrimetric analysis in non-aqueous medium (5.3.3.5)*, by using 10 drops of 1-naphtholbenzein SI as indicator and 0.1 M SV perchloric acid as agent titrant. To prepare the blank and similarly titrate it. Each mL of 0.1 M SV perchloric acid is equal to 13.862 mg of $C_{10}H_{24}N_2O_2 \cdot 2HCl$ hydrochloride.

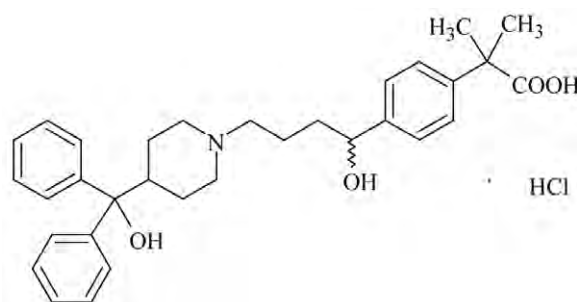
PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

HYDROCHLORIDE, FEXOFENADINE Fexofenadini hydrochloridum



$C_{32}H_{39}NO_4 \cdot HCl$; 538.12

fexofenadine hydrochloride; 04038

4 - [1-hydroxy-4-[4 - (hydroxydiphenylmethyl)-1-piperidinyl] butyl]- α , α -dimethyl-benzeneacetic acid hydrochloride (1:1)
[153439-40-8]

It contains at least 98.0% and at most 102.0% of $C_{32}H_{39}NO_4 \cdot HCl$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white or pale white powder.

Solubility. Slightly soluble in water, very soluble in ethanol and methanol, slightly soluble in chloroform, insoluble in hexane.

Physical and chemical constants.

Melting range (5.2.2): 195 °C to 197 °C.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same intensities to those observed in the fexofenadine hydrochloride SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14), from 200 nm to 370 nm, 0.0014% solution (w/v) in ethanol has 220 nm typical shoulder, similar to that observed in the spectrum of a similar solution of fexofenadine hydrochloride SQR.

C. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel as support and a mixture of 1-butanol, glacial acetic acid, and water (6:3:1) as mobile phase. To separately apply to the plate 10 µL of each of the solutions freshly prepared, as described below.

Solution (1): 1 mg/mL solution of the sample in methanol.

Solution (2): 1 mg/mL fexofenadine hydrochloride SQR solution in methanol.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm) or expose the plate to iodine vapor. The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

D. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

PURITY TESTS

Chlorides. To dissolve 0.3 g of the sample in 50 mL of methanol. To potentiometrically titrate with 0.1 M SV silver nitrate. Each mL of 0.1 M SV silver nitrate is equivalent to 3.545 mg of chloride. It should contain content from 6.45% to 6.75% of chloride, calculated on the anhydrous basis.

Heavy metals (5.3.2.3). To use *Method III*. At most 0.002% (20 ppm).

Water (5.2.20.1). At most 0.5%.

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 105 °C for 2 hours. At most 1.0%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 220 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at 30 °C; flow of *Mobile phase* of 1.0 mL/min.

Mobile phase: mixture of 0.05 M ammonium acetate and acetonitrile (50:50). To adjust pH to 3.2 with *M* hydrochloric acid.

Sample solution: to transfer 20 mg of the sample, accurately weighed, to a 100 mL volumetric flask, top up with *Mobile phase*, and make it homogeneous. To transfer 5 mL of this solution to 25 mL volumetric flask and top up with *Mobile phase*, thus obtaining 40 µg/mL solution.

Standard solution: to dissolve accurately weighed amount of fexofenadine hydrochloride SQR in *Mobile phase*, to obtain a 40 µg/mL solution.

To inject replicates of 20 µL of the *Standard solution*. The column efficiency is equal to or greater than 2500 theoretical plates. The tailing factor is not greater than 2.0. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2.0 %.

Procedure: to separately inject 20 µL of *Sample and standard solutions*, record the chromatograms, and measure the areas under the peaks. To calculate the content of C₃₂H₃₉NO₄.HCl from the sample, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antihistamine.

HYDROCHLORIDE, FEXOFENADINE TABLETS

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_{32}H_{39}NO_4 \cdot HCl$.

IDENTIFICATION

A. To weigh and pulverize sufficient amount of tablets. To accurately transfer an amount of powder equivalent to about 60 mg of fexofenadine hydrochloride to a tube with lid. To add 10 mL of a mixture of acetonitrile and methanol (10:1) and stir in vortex for 1 - 2 minutes, to sample disperses. Let it rest for 10 minutes or centrifuge it for 2-3 minutes. To filter through a 0.45 μm polytetrafluoroethylene filter into a 50 mL bottle. To evaporate the solvent to about 0.5 mL, by using flow of nitrogen with gentle heating (not exceeding 75 °C). To add 5 mL of water and 5 drops of diluted hydrochloric acid, stir, and induce precipitation. To place it in ice bath for 30 minutes. To filter the solution through a 10 μm - 15 μm sintered glass crucible. To dry the precipitate in an oven at 105 °C for 1 hour.

The infrared absorption spectrum (5.2.14) of the resulting residue, dispersed in potassium bromide, has absorption maxima only at the same wavelength, with the same relative intensities to those observed in the fexofenadine SQR spectrum similarly prepared. To transfer about 60 mg of standard to a tube with lid and proceed from "To add 10 mL of acetonitrile and methanol (10:1)...", to prepare a dispersion in potassium bromide with fexofenadine SQR.

B. To weigh and pulverize 20 tablets. To transfer an amount of powder equal to 14 mg of fexofenadine hydrochloride to a 100 mL volumetric flask by using 60 mL of ethanol. To stir for 10 minutes and top up with the same solvent. To make it homogenous and filter it. To proceed as described in *Identification*, test **B.**, in the monograph *Hydrochloride, fexofenadine*.

C. To weigh and pulverize 20 tablets. To stir an amount of powder equivalent to 10 mg of fexofenadine hydrochloride with 10 mL of methanol, make it homogenous, and filter it. To proceed as described in *Identification*, test **C.**, in the monograph *Hydrochloride, fexofenadine*.

D. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.01 M hydrochloric acid, 900 mL

Equipment: baskets, 100 rpm

Time: 45 minutes

Procedure: to proceed as described in *Dosage*, in the monograph *Hydrochloride, fexofenadine*. To prepare the *Sample solution* as described below.

Sample solution: after the test, remove aliquot from dissolution medium and dilute to concentration close to that of *Standard solution*, by using *Mobile phase* as diluent.

Tolerance: not less than 70% (Q) of the amount stated of $C_{32}H_{39}NO_4 \cdot HCl$ dissolves within 45 minutes.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To proceed as described in *Dosage*, in the monograph *Hydrochloride, fexofenadine*. To prepare the *Sample solution* as described below.

Sample solution: To weigh and pulverize 20 tablets. To transfer an amount of powder equivalent to 40 mg of fexofenadine hydrochloride to a 200 mL volumetric flask, add 120 mL of *Mobile phase*, and put it in ultrasound for 15 minutes. To mechanically stir for 30 minutes, top up with the same solvent, make it homogeneous, and filter it. To transfer 5 mL to 25 mL volumetric flask, top up with *Mobile phase*, and make it homogeneous.

Procedure: to separately inject 20 μL of *Sample and standard solutions*, record the chromatograms, and measure the area under the peaks. To calculate the content of $C_{32}H_{39}NO_4 \cdot HCl$ in the tablets, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

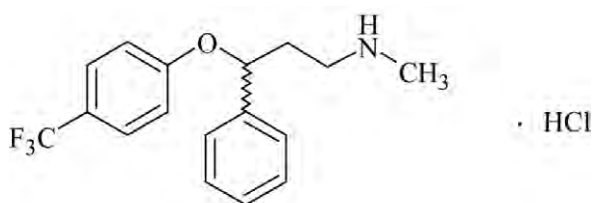
In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

HYDROCHLORIDE, FLUOXETINE

Fluoxetini hydrochloridum



$C_{17}H_{18}F_3NO \cdot HCl$; 345.79

fluoxetine hydrochloride; 04177

N-methyl- γ -[4 - (trifluoromethyl) phenoxy] benzene
propanamine hydrochloride (1:1)
[56296-78-7]

It contains at least 98.5% and at most 101.5% of $C_{17}H_{18}F_3NO \cdot HCl$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. Crystalline, white or almost white powder.

Solubility. Slightly soluble in water; freely soluble in ethanol; practically insoluble in ethyl ether.

Physical and chemical constants.

Melting range (5.2.2): 158.4 °C to 158.9 °C.

Rotation (5.2.8): 0.05° to +0.05°. To determine in 2% solution (w/v) in a mixture of water and methanol (15:85).

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, not desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the fluoxetine hydrochloride SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14) of the sample solution obtained in method A., *Dosage*, ranging from 200 nm to 400 nm, exhibits maxima and minima only in the same wavelength of a similar fluoxetine hydrochloride SQR solution.

C. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method B. corresponds to that of main peak of *Standard solution*.

D. It responds to the reactions of the chloride ion (5.3.1.1).

PURITY TESTS

pH (5.2.19). 4.5 to 6.5. To determine in 1% aqueous solution (w/v) in carbon dioxide-free water.

Related substances. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 215 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μ m), kept at 30 °C; flow of *Mobile phase* of 1.5 mL/min.

Phosphate Buffer pH 3.6: to transfer 3.395 g of tetrabutylammonium sulfate and 1.361 g of monobasic potassium phosphate to 1000 mL volumetric flask, dissolve in 900 mL of water, adjust the pH to 3.6 with ammonium hydroxide, and top up with water.

Mobile phase: mixture of *Phosphate buffer pH 3.6* and acetonitrile (78:22).

Solution (1): to accurately transfer about 30 mg of fluoxetine hydrochloride SQR to a 250 mL volumetric flask, dissolve with *Mobile phase*, and top up to the volume with the same solvent. To transfer 5 mL of the resulting solution to a 50 mL volumetric flask and top up with the same solvent. To transfer 5 mL of this solution to 50 mL volumetric flask and top up with the same solvent, thus obtaining 1.2 μ g/mL solution.

Solution (2): to accurately transfer about 30 mg of the sample to a 25 mL volumetric flask and top up the volume with *Mobile phase*. To make it homogeneous.

To inject replicates of 20 μ L of *Solution (1)*. The tailing factor is not greater than 1.7. The relative standard deviation for replicate areas of the peaks recorded is not greater than 10 %.

Procedure: to separately inject 20 μ L of *Solution (1)* and *Solution (2)*. Then, record the chromatograms at least twice the retention time of the main peak. To calculate the percentage of each impurity for *Solution (2)* by using the equation: $0.1 (r_i/r_p)$, where r_i is the response of the impurity peak in *Solution (2)* and r_p is the response of the fluoxetine peak in *Solution (1)*. At most 0.15% of impurities with relative retention time 0.88 and at most 0.1% of other individual impurity. At most 0.5% of total impurities.

Heavy metals (5.3.2.3). To use *Method III*. To determine in 0.67 g of the sample. To use the Lead standard solution (2 ppm). At most 0.003% (30 ppm).

Water (5.2.20.1). At most 0.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To accurately weigh about 75 mg of the sample, transfer to a 100 mL volumetric flask, dis-

solve in 0.1 M hydrochloric acid, and top up with the same solvent. To successively dilute in 0.1 M hydrochloric acid to obtain 0.0015% concentration (w/v). To prepare a standard solution at the same concentration by using the same solvent. To measure the absorbances of the resulting solutions at 227 nm, by using 0.1 M hydrochloric acid to adjust zero. To calculate the content of $C_{17}H_{18}F_3NO.HCl$ in the sample from the readings obtained.

B. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 227 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), at room temperature; flow of *Mobile phase* of 1.0 mL/min.

Triethylamine buffer pH 6.0: to transfer 10 mL of triethylamine to a 1000 mL volumetric flask, add about 980 mL of water, adjust the pH to 6.0 with phosphoric acid, and top up with water.

Mobile phase: mixture of *Triethylamine buffer pH 6.0*, tetrahydrofuran, and methanol (6:3:1).

Sample solution: to accurately weigh about 27.5 g of the sample and transfer to a 25 mL volumetric flask. To dissolve in *Mobile phase* and top up with the same solvent. To make it homogeneous. To transfer 5 mL of the resulting solution to 50 mL volumetric flask and top up with *Mobile phase*. To make it homogeneous.

Standard solution: to accurately weigh about 27.5 mg of fluoxetine hydrochloride SQR and transfer to 25 mL volumetric flask. To dissolve in *Mobile phase* and top up with the same solvent. To make it homogeneous.

To transfer 50 mL and top up with *Mobile phase*. To make it homogeneous.

To inject replicates of 10 µL of the *Standard solution*. The tailing factor is not greater than 2.5. The relative standard deviation for replicate areas of the peaks recorded should not be greater than 2.0 %.

Procedure: to separately inject 10 µL of *Sample and standard solutions*, record the chromatograms, and measure the area under the peaks. To calculate the content of $C_{17}H_{18}F_3NO.HCl$ from the sample, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antidepressant.

HYDROCHLORIDE, FLUOXETINE TABLETS

It contains fluoxetine hydrochloride equivalent to at least 90.0% and at most 110.0% of the amount stated of fluoxetine ($C_{17}H_{18}F_3NO$).

IDENTIFICATION

The identification tests B. and C. may be omitted if the tests A. and D. are conducted.

A. To weigh and pulverize the tablets. To transfer an amount of powder equivalent to 10 mg of fluoxetine to a beaker, dissolve it in 10 mL of ethanol, and filter. To wash the flask with 5 mL of the same solvent and evaporate the filtrate in water bath to obtain a residue. Such residue, desiccated at 60 °C in a vacuum oven, for 3 hours, responds to test **A. Identification**, in the monograph *Hydrochloride, fluoxetine*.

B. The ultraviolet absorption spectrum (5.2.14) of the sample solution obtained in method **A.**, *Dosage*, ranging from 200 nm to 400 nm, exhibits maxima and minima only in the same wavelength observed in the standard solution spectrum.

C. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method **B.** corresponds to that of main peak of *Standard solution*.

D. To weigh and pulverize the tablets. To transfer an amount of powder equal to 20 mg of fluoxetine to a 5 mL volumetric flask and top up with water. To make it homogeneous and filter it. The resulting solution responds to the reactions of chloride ion (5.3.1.1).

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

Procedure for content uniformity. To transfer each tablet to 100 mL volumetric flask To add 70 mL of 0.1 M hydrochloric acid and put it in ultrasound for 5 minutes. To mechanically stir for 15 minutes, top up with the same solvent, make it homogeneous, and filter it. To successively dilute in the same solvent to obtain a 0.0015% fluoxetine

concentration (w/v). To prepare a standard solution at the same concentration of fluoxetine ($C_{12}H_{18}F_3NO$), by using fluoxetine hydrochloride SQR and the same solvent. To measure the absorbances of the resulting solutions at 227 nm (**5.2.14**), by using 0.1 M hydrochloric acid to adjust zero. To calculate the amount of fluoxetine ($C_{12}H_{18}F_3NO$) in each tablet, from the readings obtained.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 900 mL

Equipment: blades, 50 rpm

Time: 45 minutes

Procedure: after testing, remove aliquot from the dissolution medium and dilute, if necessary, with 0.1 M hydrochloric acid, to obtain suitable concentration. To measure the absorbances at 227 nm (**5.2.14**), by using the same solvent to adjust zero. To calculate the amount of fluoxetine ($C_{12}H_{18}F_3NO$) dissolved in the medium, by comparing the readings obtained with 0.0015% fluoxetine hydrochloride ($C_{12}H_{18}F_3NO$) SQR solution (w/v) prepared with the same solvent.

Tolerance: not less than 70% (Q) of the amount stated of fluoxetine ($C_{12}H_{18}F_3NO$) dissolves within 45 minutes.

PURITY TESTS

Related substances. To proceed as described in *Related substances*, in the monograph *Hydrochloride, fluoxetine*. To prepare *Solution (2)* as described below.

Solution (2): to weigh and pulverize the tablets. to accurately transfer an amount of powder equal to 20 mg of fluoxetine to a 10 mL volumetric flask and add 8 mL of *Mobile phase*. To put it in ultrasound for 15 minutes and top up with the same solvent. To make it homogenous and filter it.

Procedure: to inject replicates of 20 µL of *Solution (2)*, record the chromatograms, and measure the areas under the peaks.

To calculate the percentage of each impurity in the chromatogram for *Solution (2)*, by using the equation: $100 (r_i / r_t)$ where r_i is the response of each peak, excluding the fluoxetine peak, and r_t is the sum of the responses of all the peaks, including the fluoxetine peak. To disregard the peaks related to solvent. At least 0.25% of individual impurity and at most 0.40% of total impurity.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. To weigh and pulverize the tablets. To transfer an amount of powder equivalent to 15 mg of fluoxetine ($C_{17}H_{18}F_3NO$) to a 100 mL volumetric flask. To add 70 mL of 0.1 M hydrochloric acid and put it in ultrasound for 5 minutes. To mechanically stir for 15 minutes, top up with the same solvent, make it homogeneous, and filter it. To successively dilute in the same solvent to obtain a 0.0015% fluoxetine concentration (w/v). To prepare a standard solution at the same concentration of fluoxetine ($C_{17}H_{18}F_3NO$), by using fluoxetine hydrochloride SQR and the same solvent. To measure the absorbances of the resulting solutions at 227 nm, by using 0.1 M hydrochloric acid to adjust zero. To calculate the amount of fluoxetine ($C_{17}H_{18}F_3NO$) in each tablet, from the readings obtained.

B. To proceed as described in *High performance liquid chromatography (5.2.17.4)*. To proceed as described in *Dosage method B.*, in the monograph *Hydrochloride, fluoxetine*. To prepare the *Sample solution* as described below.

Sample solution: To weigh and pulverize the tablets. To transfer an amount of powder equivalent to 10 mg of fluoxetine ($C_{17}H_{18}F_3NO$) to a 100 mL volumetric flask and add 70 mL of *Mobile phase*. To put it in ultrasound for 15 minutes. To mechanically stir for 15 minutes and top up with the same solvent. To make it homogenous and filter it.

Procedure: to separately inject 10 µL of *Sample and standard solutions*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of fluoxetine ($C_{17}H_{18}F_3NO$) in the tablets, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

HYDROCHLORIDE, FLURAZEPAM TABLETS

It contains at least 92.5 % and at most 107.5 % of the amount stated of $C_{21}H_{23}ClFN_3O.HCl$.

IDENTIFICATION

To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF_{254} silica gel as support and a mixture of toluene, acetone, and 25% ammonium hydroxide (v/v) (50:50:2) as mobile phase. To separately apply to the

plate 10 µL of each of the solutions freshly prepared, as described below.

Solution (1): to pulverize the tablets. To transfer an amount of powder equivalent to 20 mg of flurazepam hydrochloride to the beaker, add 10 mL of methanol, stir for 5 minutes, and filter it.

Solution (2): 2 mg/mL flurazepam hydrochloride SQR solution in methanol.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

Procedure for content uniformity. To protect from light. To individually weigh and transfer each tablet to a 100 mL volumetric flask. To add 2 mL of water and leave it in ultrasound for 5 minutes. To add 80 mL of methanol and put it in ultrasound for 5 minutes. To stir for 10 minutes and top up with methanol. To centrifuge and filter it, if necessary. To successively dilute with 0.1 M methanolic sulfuric acid to obtain 0.003% flurazepam hydrochloride (w/v). To prepare a standard solution as described in *Dosage*. To measure the absorbances of the resulting solutions at 284 nm (5.2.14), by using 0.1 M methanolic sulfuric acid to adjust zero. To calculate the amount of $C_{21}H_{23}ClFN_3O.HCl$ in the tablets, from the readings obtained. Alternatively, make the calculations by taking into account that $A(1\%, 1\text{ cm}) = 319$ at 284 nm, in 0.1 M methanolic sulfuric acid.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL

Equipment: baskets, 100 rpm

Time: 20 minutes

Procedure: after testing, remove aliquot from the dissolution medium and filter through 0.45 µm membrane, if necessary. To measure the absorbances of the solutions at 27 nm (5.2.14), by using water to adjust zero. To calculate the amount of $C_{21}H_{23}ClFN_3O.HCl$ dissolved in the medium, by comparing the readings obtained with that of 0.0033% flurazepam hydrochloride SQR solution (w/v).

Tolerance: not less than 80% (Q) of the amount stated of $C_{21}H_{23}ClFN_3O.HCl$ dissolves within 20 minutes.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To perform dosage protected from light. To weigh and pulverize 20 tablets. To transfer an amount of powder equivalent to 0.3 g of flurazepam hydrochloride to 100 mL volumetric flask. To add 2 mL of water and leave it in ultrasound for 5 minutes. To add 80 mL of methanol and put it in ultrasound for 5 minutes. To stir for 10 minutes and top up with methanol. To centrifuge and filter it, if necessary. To successively dilute with 0.1 M methanolic sulfuric acid to obtain 0.003% flurazepam hydrochloride (w/v). To accurately weigh about 30 mg of fluoxetine hydrochloride SQR and transfer to 100 mL volumetric flask. To dissolve it in 80 mL of methanol and put it in ultrasound for 5 minutes. To top up with the same solvent and successively dilute with 0.1 M methanolic sulfuric acid to obtain 0.003% flurazepam hydrochloride (w/v). To measure the absorbances of the resulting solutions at 284 nm (5.2.14), by using 0.1 M methanolic sulfuric acid to adjust zero. To calculate the amount of $C_{21}H_{23}ClFN_3O.HCl$ in the tablets, from the readings obtained. Alternatively, make the calculations by taking into account that $A(1\%, 1\text{ cm}) = 319$ at 284 nm, in 0.1 M methanolic sulfuric acid.

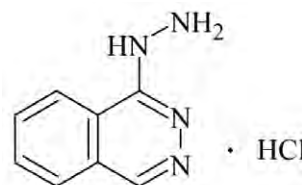
PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

HYDROCHLORIDE, HYDRALAZINE Hydralazini hydrochloridum



$C_8H_8N_4.HCl$; 196.64

hydralazine hydrochloride; 04647

1-hydrazinyl phthalazine hydrochloride (1:1)

[304-20-1]

It contains at least 98.0% and at most 102.0% of $C_8H_8N_4 \cdot HCl$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or almost white, crystalline powder; odorless or almost odorless.

Solubility. Soluble in water, sparingly soluble in ethanol, practically insoluble in ethyl ether and chloroform.

Physical and chemical constants. *Melting range (5.2.2):* 275 °C with decomposition.

IDENTIFICATION

The identification tests B, C, and D, may be omitted if the tests A, and E are conducted.

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium chloride, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the hydralazine hydrochloride SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14) ranging from 230 nm to 350 nm, of 0.002% solution (w/v) in water, exhibits maxima absorption at 240, 260, 305, and 315 nm. The absorbances are about 1.1, 1.1, 0.53, and 0.43, respectively.

C. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method **B**, corresponds to that of main peak of *Standard solution*.

D. To dissolve 0.1 g of the sample in 10 mL of water. To add 5 mL of 2% 2-nitrobenzaldehyde (w/v) in ethanol to 5 mL of the resulting solution. Orange precipitate is produced.

E. The 0.025% aqueous solution (w/v) of the sample responds to the reactions of chloride ion (5.3.1.1).

PURITY TESTS

pH (5.2.19). 3.5 to 4.2. To determine in 2% aqueous solution (w/v).

Related substances. To proceed as described in *Dosage* method **B**. To prepare *Test solution* as described below.

Test solution: To accurately transfer about 25 mg of the sample to 50 mL volumetric flask, dissolve in 30 mL of 0.1 M acetic acid, and top up the volume with the same solvent.

Procedure: to separately inject 20 µL of *Test solution*, record the chromatograms, and measure the areas of all the peaks obtained. The sum of the areas under the secondary peaks, except the main peak's one, is not greater than 1.0% of the total area of the peaks obtained, including the main

peak. Solvent peaks should not be included in the calculations.

Heavy metals (5.3.2.3). To moisten the residue obtained in *Sulfated ashes* with 2 mL of hydrochloric acid, evaporate, dry, and add 20 mL of water. To proceed as described in *Limit test for heavy metals, Method I*. At most 0.002% (20 ppm).

Loss on drying (5.2.9). To determine in 1.0 g of the sample. To desiccate in an oven at 110 °C for 15 hours to obtain constant weight. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 1.0 g of the sample. At most 0.5%.

DOSAGE

To employ one of the methods described below.

A. To accurately weigh about 0.1 g of the sample, transfer to 250 mL erlenmeyer flask, and dissolve in 25 mL of water. To add 35 mL of hydrochloric acid and cool down at room temperature. To add 5 mL of chloroform and titrate with 0.02 M SV potassium iodate. Upon reaching the endpoint, add the titrant dropwise by continuously stirring to purple color fades out from chloroform layer. To potentiometrically determine the endpoint, as an alternative, excluding the chloroform. Each mL of 0.02 M SV potassium iodate is equal to 3.933 mg of $C_8H_8N_4 \cdot HCl$.

B. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 230 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the nitrile group (10 µm), at room temperature; flow of *Mobile phase* of 1 mL/min.

Mobile phase: To dissolve 1.44 g of sodium lauryl sulfate and 0.75 g of tetrabutylammonium bromide in 770 mL of water, add 230 mL of acetonitrile, and make it homogeneous. If necessary, adjust the pH to 3.0 with 0.05 M sulfuric acid.

Sample solution: to dissolve accurately weighed amount of the sample in 0.1 M acetic acid, to obtain 0.4 mg/mL solution.

To transfer 5 mL of this solution to 50 mL volumetric flask and top up with 0.1 M acetic acid, thus obtaining 40 µg/mL solution.

Standard solution: to dissolve accurately weighed amount of hydralazine hydrochloride SQR in 0.1 M acetic acid, to obtain 0.4 mg/mL solution. To transfer 5 mL of this solution to 50 mL volumetric flask and top up with 0.1 M acetic acid, thus obtaining 40 µg/mL solution.

Resolution solution: to prepare a solution in 0.1 M acetic acid containing about 0.25 mg of hydralazine hydrochloride SQR and 50 µg of phthalazine per milliliter. To trans-

fer 5 mL of this solution to 50 mL volumetric flask, top up with 0.1 M acetic acid, and make it homogeneous.

To inject replicates of 20 μL of the *Resolution solution*. The relative retention times are about 0.65 to phthalazine and 1.0 to hydralazine hydrochloride. The resolution between phthalazine and hydralazine hydrochloride peaks should not be less than 4.0. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2.0 %.

Procedure: to separately inject 20 μL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $\text{C}_8\text{H}_8\text{N}_4\cdot\text{HCl}$ from the sample, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Vasodilator.

HYDROCHLORIDE, HYDRALAZINE TABLETS

It contains at least 90.0 % and at most 110.0 % of the amount stated of $\text{C}_8\text{H}_8\text{N}_4\cdot\text{HCl}$.

IDENTIFICATION

A. To weigh and pulverize the tablets. To transfer an amount of powder equivalent to 0.1 g of hydralazine hydrochloride to a separatory funnel and add 2 mL of 6 M ammonium hydroxide and 10 mL of water. To extract with 2 portions (10 mL each) of chloroform. To collect the organic extracts and evaporate to dryness. The residue responds to test **A.**, *Identification*, in the monograph *Hydrochloride, hydralazine*.

B. The ultraviolet absorption spectrum (5.2.14), ranging from 230 nm to 350 nm, of sample solution obtained in method **B**.

Dosage, exhibits maxima at 240 nm, 260 nm, 305 nm, and 315 nm, similar to those observed in the standard solution spectrum.

C. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method **C**. corresponds to that of main peak of *Standard solution*.

D. To weigh and pulverize the tablets. To transfer an amount of powder equivalent to 0.1 g of hydralazine hydrochloride

to a beaker, add 50 mL of a mixture of methanol and water (1:2), mix, and filter it. To concentrate the filtrate in a water bath to 10 mL and wait to cool down. To add 5 mL of 2% 2-nitrobenzaldehyde (w/v) in ethanol to 5 mL of the concentrated solution. Orange precipitate is produced.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

Procedure for content uniformity. To grind each tablet to obtain fine powder, quantitatively transfer it to a 50 mL volumetric flask, and add 25 mL of a mixture of methanol and water (1:2). To proceed as described in method **B.**, *Dosage*, from "To mechanically stir..."

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.01 M hydrochloric acid, 900 mL

Equipment: baskets, 100 rpm

Time: 30 minutes

Procedure: after testing, remove aliquot from the dissolution medium and dilute, if necessary, with 0.01 M hydrochloric acid, to obtain suitable concentration. To measure the absorbances of the solutions at 260 nm (5.2.14), by using the same solvent to adjust zero. To calculate the amount of $\text{C}_8\text{H}_8\text{N}_4\cdot\text{HCl}$ dissolved in the medium, by comparing the readings obtained with that of 0.001 % hydralazine hydrochloride SQR solution (w/v) prepared in 0.01 M hydrochloric acid.

Tolerance: not less than 60% (Q) of the amount stated of $\text{C}_8\text{H}_8\text{N}_4\cdot\text{HCl}$ dissolves within 30 minutes.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To weigh and pulverize 20 tablets. To transfer an amount of powder equivalent to 0.1 g of hydralazine hydrochloride to a beaker and add 25 mL of water. To add 35 mL of

hydrochloric acid and cool down at room temperature. To mechanically stir for 15 minutes. To titrate with 0.02 M SV potassium iodate by continuously stirring. To potentiometrically determine the endpoint. Each mL of 0.02 M SV potassium iodate is equal to 3.933 mg of $C_8H_8N_4.HCl$.

B. To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To weigh and pulverize 20 tablets. To transfer an amount of powder equivalent to 50 mg of hydralazine hydrochloride to a 50 mL volumetric flask and add 25 mL of a mixture of methanol and water (1:2). To mechanically stir for 10 minutes and top up with the same solvent. To filter it. To successively dilute to obtain 0.001% concentration (w/v) by using water as solvent. To prepare standard solution under the same conditions. To measure the absorbance of the resulting solutions at 260 nm, by using water to adjust zero. To calculate the amount of $C_8H_8N_4.HCl$ in the tablets, from the readings obtained.

C. To proceed as described in *Dosage*, method **B.**, in the monograph *Hydrochloride, hydralazine*. To prepare the *Sample solution* as described below.

Sample solution: To weigh and pulverize 20 tablets. To transfer an amount of powder equivalent to 20 mg of hydralazine hydrochloride to a 50 mL volumetric flask, add 40 mL of 0.1 M acetic acid, and put it in ultrasound for 15 minutes. To mechanically stir for 15 minutes. To top up with the same solvent, make it homogeneous, and filter it. To transfer 5 mL of the filtrate to a 50 mL volumetric flask and top up with 0.1 M acetic acid, thus obtaining 40 µg/mL solution.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of $C_8H_8N_4.HCl$ in the tablets, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

HYDROCHLORIDE, HYDRALAZINE INJECTABLE SOLUTION

It contains at least 90.0 % and at most 105.0 % of the amount stated of $C_8H_8N_4.HCl$.

IDENTIFICATION

The identification tests **B.**, **C.**, and **D.** may be omitted if the tests **A.** and **E.** are conducted.

A. To transfer volume of injectable solution equivalent to 0.1 g of hydrazine hydrochloride to a separatory funnel. To add 2 mL of 6 M ammonium hydroxide and 10 mL of water. To extract with 2 portions (10 mL each) of chloroform. To collect the organic extracts and evaporate to dryness. The residue responds to test **A.**, *Identification*, in the monograph *Hydrochloride, hydralazine*.

B. To dilute the injectable solution in water, to obtain a 0.002% concentration (w/v). The ultraviolet absorption spectrum (5.2.14), ranging from 230 nm to 350 nm, of the resulting solution, exhibits absorption maxima at 240 nm, 260 nm, 305 nm, and 315 nm.

C. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method **C.** corresponds to that of main peak of *Standard solution*.

D. To transfer volume of injectable solution equivalent to 0.1 g of hydrazine hydrochloride to a beaker and add water, if necessary, to obtain a final volume of 10 mL. To add 5 mL of 2% 2-nitrobenzaldehyde (w/v) in ethanol to 5 mL of the solution. Orange precipitate is produced.

E. To dilute the injectable solution in water, to obtain a 0.002% concentration (w/v). Resulting solution responds to the reactions of chloride ion (5.3.1.1).

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

pH (5.2.19). 3.4 to 4.4.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To transfer volume of injectable solution equivalent to about 0.1 g of hydrazine hydrochloride to a 250 mL erlenmeyer flask with lid. To add 25 mL of water and proceed as described in method **A.**, *Dosage*, in the monograph *Hydrochloride, hydralazine*, from "To add 35 mL of hydrochloric acid..." Each mL of 0.02 M SV potassium iodate is equal to 3.933 mg of $C_8H_8N_4.HCl$.

B. To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To transfer volume of solution equivalent to about 0.1 g of hydrazine hydrochloride to a 100 mL volumetric flask and top up with a mixture of methanol and water (1:2). To successively dilute to obtain 0.001% concentration (w/v), by using water as solvent.

To prepare standard solution under the same conditions. To measure the absorbances of the solutions at 260 nm by using water to adjust zero. To calculate the amount of

$C_8H_8N_4 \cdot HCl$ in the injectable solution, from the readings obtained.

C. To proceed as described in *Dosage*, method **B.**, in the monograph *Hydrochloride, hydralazine*. To prepare the *Sample solution* as described below.

Sample solution: to transfer volume of injectable solution equivalent to 20 mg of hydralazine hydrochloride to a 50 mL volumetric flask, top up to volume with 0.1 M acetic acid, and make it homogeneous. To transfer 5 mL of the resulting solution to 50 mL volumetric flask and top up with 0.1 M acetic acid, thus obtaining 40 µg/mL solution.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_8H_8N_4 \cdot HCl$ in the injectable solution, from the responses obtained for the *Sample solution* and *Standard solution*.

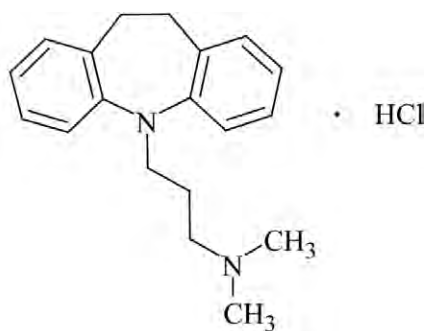
PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

HYDROCHLORIDE, IMIPRAMINE Imipramini hydrochloridum



$C_{19}H_{24}N_2 \cdot HCl$; 316.87
imipramine hydrochloride; 04837
10,11-dihydro-*N*, *N*-dimethyl-5*H*-dibenz [*b*, *f*]
azepina-5-propanamina hydrochloride (1:1)
[113-52-0]

It contains at least 98.0% and at most 102.0% of $C_{19}H_{24}N_2 \cdot HCl$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white to light yellow powder.

Solubility. Freely soluble in water and ethanol.

Physical and chemical constants. *Melting range* (5.2.2): 170 °C to 174 °C.

IDENTIFICATION

The identification tests B. and D. may be omitted if the tests A., C., and E. are conducted. The identification test A. can be omitted if the tests B., C., D., and E. are carried out.

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the imipramine hydrochloride SQR spectrum similarly prepared.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method **B.** corresponds to that of main peak of *Standard solution*.

C. It complies with the requirement for *Melting range*.

D. To dissolve 5 mg of the sample in 2 mL of nitric acid. An intense blue color develops.

E. It responds to the reactions of the chloride ion (5.3.1.1).

PURITY TESTS

pH (5.2.19). 3.6 to 5.0. To determine in 10% solution (w/v) in carbon dioxide-free water.

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel as support and a mixture of hydrochloric acid, water, glacial acetic acid, and ethyl acetate (5:5:35:55) as mobile phase. To separately apply to the plate 10 µL of each of the solutions freshly prepared, as described below.

Solution (1): to dissolve 0.25 g of the sample in methanol and top up to 10 mL with the same solvent.

Solution (2): to dilute 1 mL of *Solution (1)* to 10 mL with methanol. To dilute 1 mL of the resulting solution to 50 mL with the same solvent.

Solution (3): to dissolve 5 mg of imino dibenzyl in methanol and top up to 100 mL with the same solvent.

To develop the chromatogram. To remove the plate, and let it drying. To nebulize with a 0.5% potassium dichromate solution (w/v) in a mixture of water and sulfuric acid (4:1). Any secondary spot related to imino dibenzyl in the chromatogram obtained with *Solution (1)* is not more intense than that obtained with *Solution (3)* (0.2%). Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot and imino dibenzyl spot, is not more intense than that obtained with *Solution (2)* (0.2 %).

Heavy metals (5.3.2.3). To proceed as described in *Reaction methods with tiocetamida, Method III*. At most 0.002% (20 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 105 °C for 2 hours. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To employ one of the methods described below.

A. To accurately weigh about 0.25 g of the sample and dissolve in 50 mL of ethanol. To add 5 mL of 0.01 M hydrochloric acid and make it homogeneous. To titrate with 0.1 M SV sodium hydroxide. To potentiometrically determine the endpoint between the two inflection points. To perform sample blank correction and adjust it as required. Each mL of 0.1 M SV sodium hydroxide is equal to 31.687 mg of $C_{19}H_{24}N_2 \cdot HCl$.

B. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 269 nm; 300 mm chromatograph column and 3.9 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at 40 °C; flow of *Mobile phase* of 1.5 mL/min.

Mobile phase: mixture of 0.06 M sodium perchlorate, acetonitrile, and triethylamine (625:375:1). To adjust pH to 2.0 with perchloric acid.

Sample solution: to dissolve accurately weighed amount of the sample in a mixture of water and acetonitrile (5:3), to obtain a 0.3 mg/mL solution.

Standard solution: to dissolve accurately weighed amount of imipramine hydrochloride SQR in mixture of water and acetonitrile (5:3), to obtain 0.3 mg/mL solution.

Resolution solution: to prepare a solution containing 0.3 mg/mL imipramine hydrochloride SQR and 0.3 mg/mL desipramine hydrochloride SQR in a mixture of water and acetonitrile (5:3).

To inject replicates of 20 µL of the *Resolution solution*. The resolution between imipramine hydrochloride and desipramine hydrochloride peaks should not be less than 1.3. The relative standard deviation for replicate areas of the imipramine hydrochloride peaks recorded is not greater than 2.0 %.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_{19}H_{24}N_2 \cdot HCl$ from the sample, from the responses obtained for the *Sample and Standard solutions*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antidepressant.

HYDROCHLORIDE, IMIPRAMINE TABLETS

It contains imipramine hydrochloride equivalent to at least 92.5% and at most 107.5% of the amount stated of fluoxetine $C_{19}H_{24}N_2 \cdot Ha$.

IDENTIFICATION

A. To weigh and pulverize the tablets. To transfer an amount of powder equivalent to 250 mg of imipramine hydrochloride and add 10 mL of chloroform. To filter and evaporate to reduce the volume. To add ethyl ether to obtain a cloudy solution. Let it rest. After filtration, followed by recrystallization in acetone, precipitate has a melting range around 172 °C.

B. To dissolve 5 mg of the resulting crystals obtained in test **A. Identification** in 2 mL of nitric acid. The blue color develops.

C. The crystals obtained in test **A. Identification** respond to the reactions of chloride ion (**5.3.1.1**).

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.01 M hydrochloric acid, 900 mL

Equipment: baskets, 100 rpm

Time: 45 minutes

Procedure: after testing, remove aliquot from the dissolution medium and dilute, if necessary, with 0.01 M hydrochloric acid, to obtain suitable concentration. To measure the absorbances at 250 nm (**5.2.14**), by using the same solvent to adjust zero. To calculate the amount of $C_{19}H_{24}N_2 \cdot HCl$ dissolved in the medium, by comparing the

readings obtained with that one related to the imipramine hydrochloride SQR solution prepared in the same solvent.

Tolerance: not less than 75% (Q) of the amount stated of $C_{19}H_{24}N_2 \cdot HCl$ dissolves within 45 minutes.

PURITY TESTS

Related substances. To proceed as described in *Related substances*, in the monograph *Hydrochloride, imipramine*. To prepare *Solutions (1), (2), and (3)* as described below.

Solution (1): to weigh and pulverize the tablets. To transfer an exact amount of powder equivalent to 0.2 g of imipramine hydrochloride and add 30 mL of chloroform. To filter it. To evaporate the filtrate to dryness. To dissolve the residue in 10 mL of methanol.

Solution (2): to dilute 3 mL of *Solution (1)* to 100 mL with methanol. To dilute 1 mL of the resulting solution to 10 mL with the same solvent.

Solution (3): to prepare 60 µg/mL imino dibenzyl solution in methanol.

To develop the chromatogram. To remove the plate, and let it drying. To nebulize with 0.5% potassium dichromate solution (w/v) in sulfuric acid (1:4). The principal spot obtained with *Solution (1)* is blue. Any secondary spot corresponding to imino dibenzyl in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than the principal spot obtained with *Solution (3)* (0.2%). Any secondary spot different from the principal spot is not more intense than the principal spot obtained with *Solution (2)* (0.2%).

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To weigh and pulverize 20 tablets. To transfer an amount of powder equivalent to 50 mg of imipramine hydrochloride to a 100 mL volumetric flask, add 70 mL of 0.1 M hydrochloric acid, mechanically stir for 40 minutes, and top up to volume with the same solvent. To filter it. To transfer 5 mL to a 100 mL volumetric flask and top up with 0.1 M hydrochloric acid. To prepare standard solution at the same concentration, by using the same solvent. To measure the absorbances of the solutions at 250 nm, by using 0.1 M hydrochloric acid to adjust zero. To calculate the content of $C_{19}H_{24}N_2 \cdot HCl$ in the tablets, from the readings obtained. Alternatively, make the calculations by taking into account that $A(1\%, 1\text{ cm}) = 264$, at 250 nm.

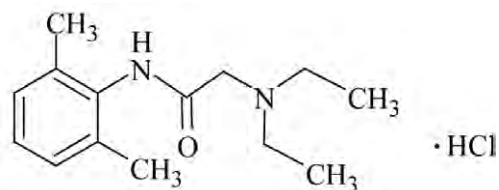
PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

HYDROCHLORIDE, LIDOCAINE Lidocaini hydrochloridum



$C_{14}H_{22}N_2O$; 234.34

$C_{14}H_{22}N_2O \cdot HCl$; 270.80

$C_{14}H_{22}N_2O \cdot HCl \cdot H_2O$; 288.81

lidocaine; 05313

lidocaine hydrochloride; 05314

2 - (Diethylamino)-*N*-(2,6-dimethylphenyl) acetamide
[137-58-6]

2 - (diethylamino)-*N*-(2,6-dimethylphenyl) acetamide
hydrochloride (1:1)

[73-78-9]

2 - (diethylamino)-*N*-(2,6-dimethylphenyl) acetamide
hydrochloride hydrate (1:1)

[6108-05-0]

It contains at least 97.5% and at most 102.5% of $C_{14}H_{22}N_2O \cdot HCl$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White crystalline powder.

Solubility. Very soluble in water, freely soluble in ethanol, soluble in chloroform, insoluble in ethyl ether.

Physical and chemical constants. *Melting range (5.2.2):* 74 °C to 79 °C, determined without previous drying.

IDENTIFICATION

The test B. may be omitted if the tests A. and C. are conducted. The test A. can be omitted if the tests B. and C. are carried out.

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the lidocaine hydrochloride SQR spectrum similarly prepared.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

C. It responds to the reactions of chloride ion (5.3.1.1).

PURITY TESTS

pH (5.2.19). 4.0 to 5.5. To determine in 0.5% aqueous solution (w/v).

Limit for 2,6-dimethylaniline.

Test solution: to transfer 0.25 g of the sample to 10 mL volumetric flask and top up with methanol.

2,6-dimethylaniline solution: to transfer 50 mg of 2,6-dimethylaniline to 100 mL volumetric flask and top up with methanol. To transfer 1 mL of this solution to 100 mL volumetric flask and top up with methanol.

Procedure: to use three Nessler tubes and perform test as follows: first tube: to put 2 mL of *Test solution*; second tube: to put 1 mL of 2,6-dimethylaniline solution and 1 mL of methanol; third tube: 2 mL of methanol (white). To add to each tube 1 mL of 1% *p*-dimethylaminobenzaldehyde solution (w/v) in methanol, freshly prepared, and 2 mL of glacial acetic acid. To let it rest at room temperature for 10 minutes. The intensity of the yellow color obtained in *Test solution* tube should be between white and yellow solution obtained in *2,6-dimethylaniline solution* (100 ppm).

Heavy metals (5.3.2.3). To use 1 g of the sample. To proceed as described in *Limit test for heavy metals, Method III*. At most 0.002% (20 ppm).

Sulfates. To dissolve about 0.2 g of sample in 20 mL of water and add 2 mL of 3 *M* hydrochloric acid. To make it homogeneous and divide it into two parts. To add 1 mL of 12% barium chloride (w/v) to one of the parts. The turbidity obtained by this preparation is not more intense than the turbidity obtained by the preparation with no barium chloride.

Water (5.2.20.1). 5.0% to 7.0%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

Bacterial endotoxins (5.5.2.2). At most 1.1 UE/mg of lidocaine hydrochloride.

DOSAGE

To employ one of the methods described below.

A. To accurately weigh about 0.22 g of the sample, transfer to 125 mL erlenmeyer flask, and dissolve in 50 mL

of ethanol. To add 5.0 mL of 0.01 *M* hydrochloric acid and titrate with 0.1 *M* SV sodium hydroxide, by potentiometrically determining the endpoint between the two inflection points.

Each mL of 0.1 *M* SV sodium hydroxide is equal to 27.08 mg of $C_{14}H_{22}N_2O \cdot HCl$.

B. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 254 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μ m), kept at room temperature; flow of *Mobile phase* of 1.5 mL/min.

Mobile phase: to mix 50 mL of glacial acetic acid with 930 mL of water To adjust pH to 3.4 with 1 *M* sodium hydroxide. To mix 4 volumes of this solution with 1 volume of acetonitrile. The retention time of lidocaine should be 4 - 6 minutes.

Sample solution: to transfer accurately weighed amount of 100 mg of the sample to 50 mL volumetric flask, top up with *Mobile phase*, and mix it.

Standard solution: to transfer 42.5 mg of lidocaine SQR, accurately weighed, to 25 mL volumetric flask and dissolve it by heating, if necessary, in 0.5 mL of 1 *M* hydrochloric acid. To top up to volume with *Mobile phase*, to obtain 1.7 mg/mL lidocaine solution.

Resolution solution: to prepare 220 μ g/mL methylparaben solution by using *Mobile phase* as diluent. To mix 2 mL of this solution with 20 mL of *Standard solution*.

To inject replicates of 20 μ L of the *Resolution solution*. The resolution between lidocaine and methylparaben peaks is not less than 3. To inject replicates of 20 μ L of *Standard solution*. The relative standard deviation for the replicate areas for the peaks recorded should not be greater than 1.5%.

Procedure: to separately inject 20 μ L of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_{14}H_{22}N_2O \cdot HCl$ in the sample, according to the following equation:

$$\left(\frac{270,80}{234,34} \right) (50C) \left(\frac{A_a}{P_p} \right)$$

where

270.80 = Molecular weight of lidocaine hydrochloride,

234.34 = Molecular weight of lidocaine,

C = concentration in mg/mL of lidocaine in the *Standard solution*,

A_a = area under the lidocaine peak in the *Sample solution*,

A_p = area under the lidocaine peak in the *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

When the raw material is used in the manufacturing process of injectable or other sterile pharmaceutical forms,

the label must indicate whether the product is sterile or must be sterilized throughout the process.

When the label states that the substance is sterile, it must comply with Bacterial endotoxins and Sterility tests. When the label indicates that the substance to be sterilized throughout production or substance is intended for non-sterile parenteral preparations, it must comply with the bacterial endotoxins test.

THERAPEUTIC CLASS

Local anesthetic.

HYDROCHLORIDE, LIDOCAINE, GEL

It contains at least 95.0 % and at most 105.0 % of the amount stated of $C_{14}H_{22}N_2O.HCl$ in hydrophilic, viscous, and sterile base.

IDENTIFICATION

A. To transfer an amount of gel equivalent to 80 mg of lidocaine hydrochloride to a test tube, add 4 mL of hydrochloric acid, and heat it in a water bath for 10 minutes. To cool down, transfer to a separatory funnel by using 20 mL of water, add 5 M sodium hydroxide to obtain total precipitation of drug, and extract with 2 portions (20 mL each) of chloroform. To collect the organic phases and filter by using anhydrous sodium sulfate. To evaporate the filtrate in a water bath to dryness under a current of nitrogen. The infrared absorption spectrum (5.2.14) of the resulting residue, dispersed in potassium bromide, has absorption maxima only at the same wavelength, with the same relative intensities to those observed in the lidocaine SQR spectrum similarly prepared.

B. To dissolve 20 mg of the resulting residue obtained in Test A. *Identification*, in 1 mL of ethanol, add 0.5 mL of 10% cobaltous chloride (w/v), 0.5 mL of 5 M sodium hydroxide, and stir for 2 minutes. Bluish-red precipitate is produced.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

PURITY TESTS

pH (5.2.19). 6.0 to 7.0.

Limit for 2,6-dimethylaniline. To mix an amount of gel, accurately weighed, equivalent to 25 mg of lidocaine hydrochloride anhydrous with water enough to make 5 mL and stir in vortex mixer. To add 1 mL of 1% *p*-dimethylanilobenzaldehyde (w/v) in methanol, freshly prepared, to 2 mL of this mixture. To stir in vortex mixer and add 2 mL of glacial acetic acid.

To let it rest at room temperature for 10 minutes. To prepare a standard 2,6-dimethylaniline solution under the same conditions, by using 2 mL of 0.0002% 2,6-dimethylaniline (v/v) in methanol instead of solution of the gel. The intensity of the yellow color obtained in the tube of the test solution is not more intense than that obtained in standard 2,6-dimethylaniline solution.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

DOSAGE

To transfer an amount of gel equivalent to 20 mg of lidocaine hydrochloride to a separatory funnel containing 10 mL of water. To stir to dilute, add 1 mL of 6 M ammonium hydroxide, and extract with 4 portions (20 mL each) of chloroform. To collect the organic phases and evaporate under a hot air current. To add 25 mL of 0.005 M SV sulfuric acid before the last trace of chloroform evaporates. To complete evaporation of chloroform and titrate the acid in excess with 0.01 M SV sodium hydroxide. To potentiometrically determine the endpoint. Each mL of 0.005 M SV sulfuric acid is equivalent to 2.708 mg of $C_{14}H_{22}N_2O.HCl$.

PACKAGING AND STORAGE

In tightly closed containers. Due to sterility, the lid must provide device to open the tube.

LABELLING

To comply with the legislation in force.

**HYDROCHLORIDE,
LIDOCAINE, OINTMENT**

It contains at least 95.0 % and at most 105.0 % of the amount stated of $C_{14}H_{22}N_2O$ in hydrophilic ointment base.

IDENTIFICATION

To transfer an amount of ointment equivalent to 300 mg of lidocaine to a separatory funnel containing 20 mL of water, to extract the drug. To stir to dilute it and extract with 2 portions (30 mL each) of hexane. To wash the com-

bined organic extracts with 10 mL of water and evaporate under hot air current. To dry the residue under vacuum on silica gel for 24 hours. The infrared absorption spectrum (5.2.14) of the crystalline residue obtained upon extracting the drug, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the lidocaine standard SQR spectrum similarly prepared.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

PURITY TESTS

Limit for 2,6-dimethylaniline. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 230 nm; 150 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μ m), kept at room temperature; flow of *Mobile phase* of 0.8 mL/min.

Buffer pH 8.0: to add sufficient amount of 0.408% mono-basic potassium phosphate (w/v) to 0.685% dibasic potassium phosphate solution (w/v) to obtain pH 8.0.

Mobile phase: mixture of *Buffer pH 8.0* and methanol (35:65).

Solution (1): to transfer accurately weighed amount of ointment equivalent to 50 mg of lidocaine to 50 mL volumetric flask, top up with *Mobile phase*, and mix it. To transfer 2 mL of this solution to 20 mL volumetric flask and top up with *Mobile phase*.

Solution (2): to dissolve accurately weighed amount of 2,6-dimethylaniline in methanol, to obtain a 1 mg/mL solution. To successively dilute in *Mobile phase* to obtain 0.04 μ g/mL concentration.

Solution (3): to mix equal volumes of 0.1 mg/mL lidocaine SQR solution with *Mobile phase* and 0.05 g/mL 2,6-dimethylaniline solution with *Mobile phase*.

To inject replicates of 20 μ L of the *Solution (3)*. The relative retention times are about 1 to 2,6-dimethylaniline and 0.5 to lidocaine.

Procedure: to separately inject 20 μ L of *Solution (1)* and 20 μ L of *Solution (2)*, record the chromatograms, and measure the areas under the peaks. The area under the 2,6-dimethylaniline peak obtained in *Solution (1)* is not greater than the area under the main peak obtained in *Solution (2)*. At most 0.04% (400 ppm) in relation to the content of lidocaine.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To proceed as described in *Dosage*, in the monograph *Hydrochloride, Lidocaine, Gel*. Each mL of 0.005 M SV sulfuric acid is equivalent to 2.343 mg of $C_{14}H_{22}N_2O$.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

HYDROCHLORIDE, LIDOCAINE INJECTABLE SOLUTION

It contains at least 95.0 % and at most 105.0 % of the amount stated of $C_{14}H_{22}N_2O.HCl$. Sterile solution of lidocaine hydrochloride in water for injection.

IDENTIFICATION

Test B. can be omitted if tests A. and C. are conducted.

A. To transfer volume of injectable solution equivalent to 0.1 g of lidocaine hydrochloride to a beaker and add volume of 5 M sodium hydroxide, to alkalize the solution. To filter and wash the residue with water. To dissolve the residue in 1 mL of ethanol, add 0.5 mL of 10% cobaltous chloride (w/v), and stir for 2 minutes. Greenish-blue precipitate is produced.

B. To add 10 mL of picric acid SR to obtain volume of injectable solution equivalent to 0.1 g of lidocaine hydrochloride. The melting range of the precipitate obtained after washing with water and drying at 105 °C is around 229 °C.

C. It responds to the reactions of chloride ion (5.3.1.1).

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

pH (5.2.19). 5.0 to 7.0.

PURITY TESTS

Limit for 2,6-dimethylaniline. To add water sufficient to make 10 mL, if necessary, to a volume of injectable solution equivalent to 25 mg of lidocaine hydrochloride. To add sufficient amount of 2 M sodium hydroxide to alkalize the solution and extract with 3 portions (5 mL each) of chloroform. To filter the combined organic extracts over

sodium sulfate anhydrous and wash the filter with 5 mL of chloroform. To evaporate the filtrate to dryness under 2 kPa. To dissolve the residue in 2 mL of methanol, add 1 mL of 1% *p*-dimethylaminobenzaldehyde (w/v) in methanol, freshly prepared, and 2 mL of glacial acetic acid. To prepare a standard 2,6-dimethylaniline solution under the same conditions, by using 10 mL of 0.0001% 2,6-dimethylaniline solution (w/v) in methanol instead of injectable solution. The intensity of the yellow color obtained in the tube of the solution containing sample is not more intense than that obtained in 2,6-dimethylaniline solution.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

Bacterial endotoxins (5.5.2.2). At most 1.1 UE/mg of lidocaine hydrochloride.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. To add 2 M sodium hydroxide to a volume of injectable solution equivalent to 0.1 g of lidocaine hydrochloride to alkalize the solution, and extract with 3 portions (20 mL each) of chloroform. To wash each organic extract with 10 mL of water (always use the same 10 mL of water). To filter the combined organic extractions through chloroform-moistened filter and wash the filter with 10 mL of chloroform. To collect the combined organic filtrates and those related to the washings. To titrate with 0.02 M SV perchloric acid. To potentiometrically determine the endpoint or use methylrosanilinium chloride SI as an indicator. Each mL of 0.02 M SV perchloric acid is equal to 5.416 mg of $C_{14}H_{22}N_2O.HCl$.

B. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 254 nm; 300 mm column and 3.9 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μ m), kept at 20°C - 25°C + 1°C for temperature set; flow of *Mobile phase* of 1.5 mL/minute.

Mobile phase: to mix 50 mL of glacial acetic acid with 930 mL of water To adjust pH to 3.4 with M sodium hydroxide. To mix 4 volumes of this solution with 1 volume of acetonitrile. The composition of *Mobile phase* can be adjusted so that the lidocaine peak has retention time between 4 and 6 minutes.

Sample solution: to transfer volume of injectable solution equivalent to 0.1 mg of lidocaine hydrochloride to a 50 mL volumetric flask, top up to volume with *Mobile phase*, and mix it.

Standard solution: to transfer 42.5 mg of lidocaine SQR, accurately weighed, to 25 mL volumetric flask and dissolve it by heating, if necessary, in 0.5 mL of 1 M hydrochloric

acid. To top up to volume with *Mobile phase*, to obtain 1.7 mg/mL lidocaine solution.

Resolution solution: to prepare 0.22 mg/mL methylparaben solution by using *Mobile phase* as diluent. To mix 2 mL of this solution with 20 mL of *Standard solution*.

To inject replicates of 20 μ L of the *Resolution solution*. The resolution between lidocaine and methylparaben is not less than 3. To inject replicates of 20 μ L of *Standard solution*. The relative standard deviation for the replicate areas for the peaks recorded should not be greater than 1.5%.

Procedure: to separately inject 20 μ L of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of $C_{14}H_{22}N_2O.HCl$ in the sample, from the responses from the *Standard solution* and *Sample solution*.

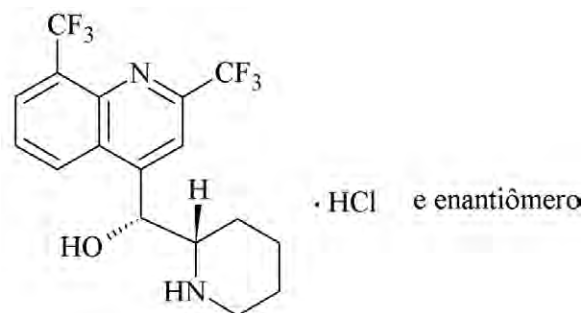
PACKAGING AND STORAGE

In type I glass containers, protected from light.

LABELLING

To comply with the legislation in force.

HYDROCHLORIDE, MEFLOQUINE Mefloquini hydrochloridum



$C_{17}H_{16}F_6N_2O.HCl$; 414.77
mefloquine hydrochloride; 05577
(aS)- α -rel-(2R)-2-piperidinyl-2,8-bis(trifluoromethyl)-4-quinolinemethanol hydrochloride (1:1)
[51773-92-3]

It contains at least 98.0% and at most 102.0% of $C_{17}H_{16}F_6N_2O.HCl$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. Crystalline, white or yellowish powder. It shows polymorphism.

Solubility. Sparingly soluble in water, freely soluble in methanol, soluble in ethanol and ethyl acetate.

Physical and chemical constants.

Melting range (5.2.2): 259 °C to 260 °C.

Rotation (5.2.8): -0.2° to +0.2°. To determine in 5% solution (w/v) in methanol.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the mefloquine hydrochloride SQR spectrum similarly prepared.

To separately dissolve standard and sample in methanol, if the spectra are not identical, and evaporate them to dryness. To obtain new spectra from these residues.

B. To add 0.2 mL of sulfuric acid to 20 mg of the sample. Blue fluorescence under ultraviolet light (365 nm) is developed.

C. It responds to the reactions of chloride ion (5.3.1.1).

PURITY TESTS

Related substances. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 280 nm; 250 mm chromatograph column and 4 mm inner diameter, packed with silica chemically bonded to the capped octadecylsilane group (3 µm to 10 µm), flow of *Mobile phase* of 0.8 mL/min. To balance the column for 30 minutes with a flow of 2 mL/min.

Mobile phase: to dissolve 1 g of tetra heptyl ammonium bromide in a mixture of methanol, 0.15% sodium bisulfite (w/v), and acetonitrile (2:4:4).

Solution (1): 4 mg/mL solution of the sample in *Mobile phase*.

Solution (2): to transfer 1 mL of *Solution (1)* to 100 mL volumetric flask and top up with *Mobile phase*.

Solution (3): to transfer about 8 mg of mefloquine hydrochloride SQR and 8 mg of quinidine sulfate to a 50 mL volumetric flask. To dissolve and top up with *Mobile phase*. To transfer 5 mL to a 100 mL volumetric flask and top up with the same solvent. To make it homogeneous.

To inject 20 µL of *Solution (3)*. The relative retention times are about 0.5 to quinidine and 1.0 to mefloquine. The resolution between the mefloquine and quinidine peaks is greater than 8.5.

Procedure: to inject 20 µL of *Solutions (1)* and *(2)* and record the chromatogram for at least 10 times the retention time of the main peak. The area under any peak, whose relative retention time is about 0.7, obtained with *Solution (1)*, is not greater than twice the area under the main peak obtained with *Solution (2)* (0.2%). The area under any peak obtained with *Solution (1)*, except the main peak and the peak whose relative retention time is about 0.7, is not greater than the area under the main peak obtained with *Solution (2)* (0.1%). The sum of the areas of all the peaks obtained with *Solution (1)*, except the main peak's one, is not greater than 5 times the area under the main peak obtained with *Solution (2)* (0.5%). To disregard the peaks with an area less than 0.2 times the area under the main peak obtained with *Solution (2)*.

Heavy metals (5.3.2.3). To determine in 1 g of the sample. At most 0.002% (20 ppm).

Water (5.2.20.1). To determine in 1 g of the sample. At most 3.0%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To proceed as described in *Titrations in non-aqueous medium* (5.3.3.5). To dissolve 0.3 g of the sample in 15 mL of formic acid anhydrous. To add 40 mL of acetic anhydride. To titrate with 0.1 M SV perchloric acid, to potentiometrically determine the endpoint. Each mL of 0.1 M SV perchloric acid is equal to 41.477 mg of $C_{17}H_{16}F_6N_2O.HCL$.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antimalarial.

HYDROCHLORIDE, MEFLOQUINE TABLETS

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_{17}H_{16}F_6N_2O.HCl$.

IDENTIFICATION

A. The ultraviolet absorption spectrum (5.2.14) ranging from 200 nm to 350 nm, of Sample solution obtained in *Dosage* method A., exhibits maximum and minimum similar to those observed in the Standard solution spectrum.

B. To weigh and pulverize the tablets. To transfer an amount of powder equivalent to 0.25 g of mefloquine hydrochloride to a beaker and add 5 drops of nitric acid. To stir with 50 mL of water and filter through filter paper to slow filtering. To add silver nitrate SR to the filtrate. Casein white precipitate is produced, which is insoluble in nitric acid, but it is soluble in slight excess of 6 M ammonium hydroxide.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 900 mL

Equipment: blades, 100 rpm

Time: 60 minutes

Procedure: after testing, remove aliquot from the dissolution medium, filter, and dilute with *Dissolution medium*, to obtain suitable concentration. To measure the absorbances of solutions at 283 nm (5.2.14), by using the *Dissolution medium* to adjust zero. To calculate the amount of $C_{17}H_{16}F_6N_2O.HCl$ dissolved in the medium, by comparing the readings obtained with that one related to 0.006% mefloquine hydrochloride SQR solution prepared in the same solvent. To use up to 5% (v/v) of methanol at the first dilution to ensure total solubilization of the mefloquine hydrochloride SQR.

Tolerance: not less than 80% (Q) of the amount stated of $C_{17}H_{16}F_6N_2O.HCl$ dissolves within 60 minutes.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. To weigh and pulverize 20 tablets. To transfer an amount of powder equal to 0.1 mg of mefloquine hydrochloride to a 100 mL volumetric flask and add 70 mL of methanol. To put it in ultrasonic bath for 10 minutes and top up with methanol. To filter it. To successively dilute in methanol to obtain 0.004% concentration (w/v). To prepare a standard solution at the same concentration by using the same solvent. To measure the absorbances of the resulting solutions at 283 nm, by using methanol to adjust zero. To calculate the amount of $C_{17}H_{16}F_6N_2O.HCl$ in the tablets, from the readings obtained.

B. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 283 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at 30 °C; flow of *Mobile phase* of 1.0 mL/min.

Buffer pH 3.5: to transfer about 6.80 g of monobasic potassium phosphate to a 1000 mL volumetric flask. To dissolve and top up with water. To adjust the pH to 3.5 with phosphoric acid.

Mobile phase: mixture of *Buffer pH 3.5* and methanol (40:60).

Sample solution: To weigh and pulverize 20 tablets. To transfer an amount of powder equal to 100 mg of mefloquine hydrochloride to a 100 mL volumetric flask, add about 80 mL of methanol, and put it in ultrasound for 10 minutes. To top up with the same solvent and filter it, by discarding the first 10 mL of filtrate. To transfer 5 mL of filtrate to 50 mL volumetric flask, top up with *Mobile phase*, and make it homogeneous.

Standard solution: to accurately weigh about 10 mg of mefloquine hydrochloride SQR and transfer to 100 mL volumetric flask. To add about 80 mL of *Mobile phase* and put it in ultrasound for 10 minutes. To top up with the same solvent and make it homogeneous.

To inject replicates of 20 µL of the *Standard solution*. The tailing factor is not greater than 1.5. The relative standard deviation for the replicate areas for the peaks recorded is not greater than 1.0%.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_{17}H_{16}F_6N_2O \cdot HCl$ in the tablets, from the responses obtained for the *Sample and Standard solutions*.

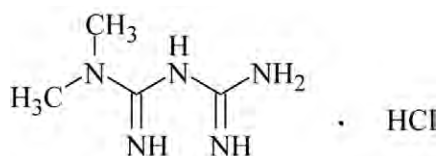
PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

HYDROCHLORIDE, METFORMIN Metformini hydrochloridum



$C_4H_{11}N_5 \cdot HCl$; 165.62

metformin hydrochloride; 05782

N,N-Dimethyl-imido-dicarbonimidic diamide hydrochloride (1:1)

[1115-70-4]

It contains at least 98.5% and at most 101.0% of $C_4H_{11}N_5 \cdot HCl$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white or almost white powder.

Solubility. Freely soluble in water, sparingly soluble in ethanol, practically insoluble in acetone, ethyl ether, chloroform, and methylene chloride.

Physical and chemical constants.

Melting range (5.2.2): 222 °C to 226 °C.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the metformin hydrochloride SQR spectrum similarly prepared.

B. It responds to the reactions of chloride ion (5.3.1.1).

PURITY TESTS

Related substances. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chro-

matograph equipped with an ultraviolet detector at 218 nm; 250 mm column long and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (3 µm to 10 µm), at room temperature; flow of *Mobile phase* of 1.0 mL/min.

Mobile phase: to dissolve 17 g of monobasic ammonium phosphate in 1000 mL of water and adjust the 3.0 ± 0.1 with phosphoric acid.

Solution (1): to accurately dissolve about 20 mg of cyanoguanidine SQR in water and top up to 100 mL. To transfer 1 mL to 200 mL volumetric flask, top up with *Mobile phase*, and make it homogeneous.

Solution (2): to accurately dissolve about 50 mg of the sample in *Mobile phase* and dilute to 100 mL with the same solvent.

Solution (3): to dilute 1 mL of *Solution (2)* to 100 mL with *Mobile phase*. To transfer 1 mL to 100 mL volumetric flask and top up with *Mobile phase*.

Solution (4): to accurately dissolve about 10 mg of melamine in 90 mL of water. To add 5 mL of *Solution (2)* and top up to 100 mL with the same solvent. To dissolve 1 mL to 50 mL with *Mobile phase*.

To inject replicates of 20 µL of *Solution (4)*. The resolution between melamine and metformin hydrochloride should be more than 10. The relative standard deviation for replicate areas of the peaks recorded should not be greater than 2.0 %.

Procedure: to separately inject 20 µL of *Solution (1)*, *Solution (2)*, and *Solution (3)*. Then, record the chromatograms at least twice the retention time of the main peak. The peak obtained in *Solution (2)* corresponding to cyanoguanidine should not be greater than 0.02%, if compared to the peak obtained with *Solution (1)*. No individual impurity obtained with *Solution (2)* can be greater than 0.1%, if compared to the peak obtained with *Solution (3)*.

The sum of the areas of all the peaks obtained with *Solution (2)*, except the solvent peak's one, is not greater than the area under the main peak obtained with *Solution (3)* (0.5%). To disregard the peaks with area less than that in the main peak in the chromatogram obtained with *Solution (4)* (0.2%).

Heavy metals (5.3.2.3). To proceed as described in *Method I*. At most 0.001% (10 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 105 °C for 5 hours. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To proceed as described in *Titration in non-aqueous medium* (5.3.3.5). To dissolve 60 mg of the sample previously desiccated in 4 mL of formic acid anhydrous and add 50 mL of acetic anhydride. To titrate with 0.1 M SV perchloric acid. To potentiometrically determine the endpoint. To perform sample blank correction and adjust it as required. Each mL of 0.1 M SV perchloric acid is equal to 8.281 mg of $C_4H_{11}N_5 \cdot HCl$.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Hypoglycemic.

HYDROCHLORIDE, METFORMIN TABLETS

It contains at least 95.0 % and at most 105.0 % of the amount stated of $C_4H_{11}N_5 \cdot HCl$. Tablets are coated.

IDENTIFICATION

A. To weigh and pulverize the tablets. To dissolve an amount of powder equivalent to 20 mg of metformin hydrochloride in 20 mL of ethanol and stir it. To filter, evaporate the filtrate to dryness in water bath, and desiccate the residue at 105 °C for 1 hour. The residue responds to test **A.**, *Identification*, in the monograph *Hydrochloride, metformin*.

B. To weigh and pulverize the tablets. To transfer an amount of powder equivalent to 50 mg of metformin hydrochloride to a test tube, add 10 mL of water, mix, and filter it. It responds to the reactions of chloride ion (5.3.1.1).

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

Procedure for content uniformity. To transfer each tablet to a 250 mL volumetric flask, add 150 mL of water, me-

chanically stir for 15 minutes, and top up with the same solvent. To filter it. To transfer 5 mL of filtrate to 100 mL volumetric flask and top up with water. To successively dilute to obtain 0.001% concentration (w/v) by using water as solvent. To prepare standard solution under the same conditions. To measure the absorbances of the solutions at 232 nm (5.2.14), by using water to adjust zero. To calculate the amount of $C_4H_{11}N_5 \cdot HCl$ in the tablets, from the reading obtained.

DISSOLUTION TEST (5.1.5)

Dissolution medium: phosphate buffer pH 6.8, 900 mL

Equipment: baskets, 100 rpm

Time: 45 minutes

Procedure: after testing, remove aliquot from the dissolution medium, filter, and dilute, if necessary, with water, to obtain suitable concentration. To measure the absorbances at 233 nm (5.2.14), by using water to adjust zero. To calculate the amount of $C_4H_{11}N_5 \cdot HCl$ dissolved in the medium, by comparing the readings obtained with that one related to the 0.001% metformin hydrochloride SQR solution (w/v) prepared in the same solvent.

Tolerance: not less than 75% (Q) of the amount stated of $C_4H_{11}N_5 \cdot HCl$ dissolves within 45 minutes.

PURITY TESTS

Related substances. To proceed as described in *Related substances*, in the monograph *Hydrochloride, metformin*. To prepare *Solution (2)* as described below.

Solution (2): to weigh and pulverize 20 tablets. To transfer an amount of powder equivalent to 500 mg of sample to a 100 mL volumetric flask, dissolve in 60 mL of *Mobile phase*, stir for 15 minutes, and top up with the same solvent. To filter it.

Procedure: to separately inject 20 µL of *Solution (2)* and *Solution (3)*, record the chromatograms, and measure the areas of all the peaks obtained. No chromatogram may have the retention time twice that of metformin. At most 0.1% of individual impurity and at most 0.6% of total impurity. Solvent peaks should not be included in the calculations.

DOSAGE

To proceed as described in the *Ultraviolet absorption spectrophotometry* (5.2.14). To weigh and pulverize 20 tablets. To transfer an amount of powder equal to 100 mg of metformin hydrochloride to a 100 mL volumetric flask and add 70 mL of water. To mechanically stir for 15 minutes, top up with the same solvent, and filter it. To transfer 10 mL of filtrate to 100 mL volumetric flask and top up with water. To successively dilute to obtain 0.001% concentration (w/v) by using water as solvent. To prepare standard solution un-

der the same conditions. To measure the absorbances of the solutions at 232 nm by using water to adjust zero. To calculate the amount of $C_4H_{11}N_5.HCl$ in the tablets, from the readings obtained.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

HYDROCHLORIDE, METOCLOPRAMIDE TABLETS

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_{14}H_{22}ClN_3O_2.HCl$.

IDENTIFICATION

The identification test A. may be omitted if the tests B., C., D., and E. are conducted. The identification test B. may be omitted if the tests A., C., D., and E. are conducted.

A. The ultraviolet absorption spectrum (5.2.14) ranging from 200 nm to 400 nm, of sample solution obtained in *Dosage* method **B.**, exhibits absorption maximum similar to those observed in the standard solution spectrum.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method **B.** corresponds to that of main peak of *Standard solution*.

C. To weigh and pulverize the tablets. To use an amount of powder equivalent to 10 mg of metoclopramide hydrochloride. To add 1 mL of water, mechanically stir, and filter it. To add 1 mL of 1% *p*-dimethylaminobenzaldehyde (w/v) in *M* hydrochloric acid to the filtrate. Orangish-yellow color is developed.

D. To weigh and pulverize the tablets. To use an amount of powder equivalent to 10 mg of metoclopramide hydrochloride. To add 10 mL of hydrochloric acid SR, cool down at 0 °C, and add 1 mL of 1% sodium nitrite (w/v). Yellow precipitate is formed. To add 1 mL of 2-naphthol SR. Orangish-red precipitate is produced.

E. To weigh and pulverize the tablets. To use an amount of powder equivalent to 10 mg of metoclopramide hydrochloride. To add 10 mL of water, stir, and filter it. It responds to the reactions of chloride ion (5.3.1.1).

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

Procedure for content uniformity. To transfer each table to 100 mL volumetric flask and proceed as described in method **A. Dosage**, from "... add 70 mL of 0.1 *M* hydrochloric acid."

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL

Equipment: baskets, 50 rpm

Time: 30 minutes

Procedure: after testing, remove aliquot from the dissolution medium and dilute with water to obtain suitable concentration. To measure the absorbances at 309 nm (5.2.14), by using water to adjust zero. To calculate the amount of $C_{14}H_{22}ClN_3O_2.HCl$ dissolved in the medium, by comparing the readings obtained with that one related to the 0.001% metoclopramide hydrochloride SQR solution (w/v) prepared in the same solvent.

Tolerance: not less than 75% (Q) of the amount stated of $C_{14}H_{22}ClN_3O_2.HCl$ dissolves within 30 minutes.

PURITY TESTS

Water (5.2.20.1). At most 3.0%.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To weigh and pulverize 20 tablets. To transfer an amount of powder equivalent to 10 mg of metoclopramide hydrochloride to a 100 mL volumetric flask, and add 70 mL of 0.1 *M* hydrochloric acid. To put it in ultrasound for 15 minutes. To mechanically stir for 15 minutes, top up with the same solvent, make it homogeneous, and filter it. To successively dilute in the same solvent to obtain 0.002% concentration (w/v). To prepare a standard solution at the same concentration by using the same solvent. To measure the absorbances of the resulting solutions at 309 nm, by using 0.1 *M* hydrochloric acid to adjust zero.

To calculate the amount of $C_{14}H_{22}ClN_3O_2.HCl$ in the tablets, from the readings obtained.

B. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 215 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed

with silica chemically bonded to the octadecylsilane group (5 μm), kept at room temperature; flow of *Mobile phase* of 1.5 mL/min.

Mobile phase: to dissolve 2.7 g of sodium acetate with 500 mL of water. To add 500 mL of acetonitrile and 2 mL of 20% tetramethylammonium hydroxide (w/v) in methanol. To make it homogeneous. To adjust pH to 6.5 with glacial acetic acid.

Sample solution: to weigh and pulverize 20 tablets. To transfer amount of powder equivalent to 40 mg of metoclopramide hydrochloride to a 50 mL volumetric flask, add 35 mL of 0.01 M phosphoric acid. To put it in ultrasound for 15 minutes. To mechanically stir for 15 minutes, top up with the same solvent, make it homogeneous, and filter it. To transfer 5 mL of the filtrate to a 100 mL volumetric flask and top up with the same solvent.

Stock standard solution: to transfer 40 mg of metoclopramide hydrochloride SQR to 50 mL volumetric flask. To dissolve in 0.01 M phosphoric acid and top up with the same solvent, to obtain 0.8 mg/mL solution.

Standard solution: to transfer 5 mL of *Stock standard solution* to 100 mL volumetric flask and top up with 0.01 M phosphoric acid, thus obtaining 40 $\mu\text{g/mL}$ solution.

Resolution solution: to transfer 12.5 mg of benzenesulfonamide to 25 mL volumetric flask, dissolve in 15 mL of methanol, and top up with 0.01 M phosphoric acid. To transfer 5 mL of the resulting solution to a 100 mL volumetric flask, add 5 mL of *Stock standard solution* and top up with 0.01 M phosphoric acid.

To inject replicates of 20 μL of the *Resolution solution*. The relative retention times are about 0.2 to benzenesulfonamide and 1.0 to metoclopramide hydrochloride. The relative standard deviation for the replicate areas for the peaks recorded is not greater than 2.0%.

Procedure: to separately inject 20 μL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of $\text{C}_{14}\text{H}_{22}\text{ClN}_3\text{O}_2\cdot\text{HCl}$ in the tablets, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

HYDROCHLORIDE, METOCLOPRAMIDE INJECTABLE SOLUTION

It contains at least 90.0 % and at most 110.0 % of the amount stated of $\text{C}_{14}\text{H}_{22}\text{ClN}_3\text{O}_2\cdot\text{HCl}$.

IDENTIFICATION

The identification test A. may be omitted if the tests B., C., D., and E. are conducted. The identification test B. may be omitted if the tests A., C., D., and E. are conducted.

A. The ultraviolet absorption spectrum (5.2.14) ranging from 200 nm to 400 nm, of sample solution obtained in *Dosage* method A., exhibits absorption maximum similar to those observed in the standard solution spectrum.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method B. corresponds to that of main peak of *Standard solution*.

C. To use volume of injectable solution equivalent to 10 mg of metoclopramide hydrochloride. To add 1 mL of water and stir. To add 1 mL of 1% *p*-dimethylaminobenzaldehyde (w/v) in *M* hydrochloric acid. Orangish-yellow color is developed.

D. To use volume of injectable solution equivalent to 10 mg of metoclopramide hydrochloride. To add 10 mL of hydrochloric acid SR, cool down at 0 $^{\circ}\text{C}$, and add 1 mL of 1% sodium nitrite (w/v). Yellow precipitate is formed. To add 1 mL of 2-naphthol SR. Orangish-red precipitate is produced.

E. To use volume of injectable solution equivalent to 10 mg of metoclopramide hydrochloride. To add 10 mL of water and stir. The resulting solution responds to the reactions of chloride ion (5.3.1.1).

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

pH (5.2.19). 2.5 to 6.5.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

Pyrogens (5.5.2.1). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. To transfer a volume of injectable solution containing the equivalent to 10 mg of metoclopr-

amide hydrochloride to a 100 mL volumetric flask, top up with 0.1 M hydrochloric acid, and make it homogeneous.

To successively dilute in the same solvent to a 0.002% concentration (w/v). To prepare a standard solution at the same concentration by using the same solvent. To measure the absorbances of the resulting solutions at 309 nm, by using 0.1 M hydrochloric acid to adjust zero. To calculate the amount of $C_{14}H_{22}ClN_3O_2 \cdot HCl$ in the injectable solution, from the readings obtained.

B. To proceed as described in method **B. Dosage**, in the monograph *Metoclopramide hydrochloride tablets*. To prepare the *Sample solution* as described below.

Sample solution: to transfer volume of injectable solution equivalent to 40 mg of metoclopramide hydrochloride to a 100 mL volumetric flask, top up to volume with 0.01 M phosphoric acid, and make it homogeneous. To transfer 5 mL to a 50 mL volumetric flask and top up with the same solvent.

Procedure: to separately inject 20 μ L of *Standard solution* and *Sample solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_{14}H_{22}ClN_3O_2 \cdot HCl$ in the injectable solution, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

HYDROCHLORIDE, METOCLOPRAMIDE ORAL SOLUTION

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_{14}H_{22}ClN_3O_2 \cdot HCl$.

IDENTIFICATION

The identification test A. may be omitted if the tests B., C., D., and E. are conducted. The identification test B. may be omitted if the tests A., C., D., and E. are conducted.

A. The ultraviolet absorption spectrum (5.2.14) ranging from 400 nm to 800 nm, of sample solution obtained in *Dosage* method A., exhibits absorption maxima similar to those observed in the standard solution spectrum.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method **B.** corresponds to that of main peak of *Standard solution*.

C. To use volume of oral solution equivalent to 10 mg of metoclopramide hydrochloride. To add 1 mL of water and stir. To add 1 mL of 1% *p*-dimethylaminobenzaldehyde (w/v) in *M* hydrochloric acid. Orangish-yellow color is developed.

D. To use volume of oral solution equivalent to 10 mg of metoclopramide hydrochloride. To add 10 mL of hydrochloric acid SR, cool down at 0 °C, and add 1 mL of 1% sodium nitrite (w/v). Yellow precipitate is formed. To add 1 mL of 2-naphthol SR. Orangish-red precipitate is produced.

E. To use volume of oral solution equivalent to 10 mg of metoclopramide hydrochloride. To add 10 mL of water and stir. The resulting solution responds to the reactions of chloride ion (5.3.1.1).

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

pH (5.2.19). 2.0 to 5.5.

BIOLOGICAL SAFETY TEST

Counting of the total viable micro-organisms (5.5.3.1.2). It complies with the test.

Survey and identification of pathogens (5.5.3.1.3).

It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Visible absorption spectrophotometry (5.2.14)*. To protect it from direct light. To transfer volume of oral solution equivalent to 10 mg of metoclopramide hydrochloride to a 100 mL volumetric flask, and top up to volume with water. To make it homogeneous. To transfer 5 mL to 100 mL volumetric flask, add 5 mL of *M* hydrochloric acid, and mix it. To add 5 mL of 1% sodium nitrite (w/v) extemporaneously prepared. To mix it and let it rest for 10 minutes. To add 5 mL of 5% ammonium sulfamate (w/v) extemporaneously prepared. To mix it and let it rest for 25 minutes. To add 5 mL of 0.5% *N*-(1-naphthyl) ethylenediamine dihydrochloride (w/v) in *M* hydrochloric acid, extemporaneously prepared, and mix it. To top up to volume with water and let it rest for 5 minutes, thus obtaining 0.0005% solution (w/v). To prepare standard solution under the same conditions. To measure the absorbances of the resulting solutions at 534 nm, by using water to adjust zero. To calculate the amount of $C_{14}H_{22}ClN_3O_2 \cdot HCl$ in the oral solution, from the readings obtained.

B. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph

equipped with an ultraviolet detector at 215 nm; 250 mm column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μ m), kept at room temperature; flow of *Mobile phase* of 1.5 mL/minute.

Mobile phase: to dissolve 2.7 g of sodium acetate with 600 mL of water. To add 400 mL of acetonitrile and 5 mL of 20% tetramethylammonium hydroxide (w/v) in methanol.

To make it homogeneous. To adjust pH to 6.5 with glacial acetic acid.

Sample solution: to transfer volume of oral solution equivalent to 40 mg of metoclopramide hydrochloride to a 25 mL volumetric flask, top up to volume with 0.01 M phosphoric acid, and make it homogeneous.

Stock standard solution: to transfer 40 mg of metoclopramide hydrochloride SQR to 50 mL volumetric flask. To dissolve in 0.01 M phosphoric acid and top up with the same solvent, to obtain 0.8 mg/mL solution.

Standard solution: to transfer 5 mL of *Stock standard solution* to 25 mL volumetric flask and top up with 0.01 M phosphoric acid, thus obtaining 0.16 mg/mL standard metoclopramide hydrochloride solution.

Resolution solution: to transfer 0.125 g of benzenesulfonamide to 25 mL volumetric flask. To dissolve in 15 mL of methanol. To top up volume with 0.01 M phosphoric acid. To transfer 15 mL of the resulting solution and 5 mL of *Stock standard solution* to a 250 mL volumetric flask, and top up with 0.01 M phosphoric acid.

To inject replicates of 20 μ L of the *Resolution solution*. The relative retention times are about 0.2 to benzenesulfonamide and 1.0 to metoclopramide hydrochloride. The relative standard deviation for the replicate areas for the peaks recorded is not greater than 2.0%.

Procedure: to separately inject 20 μ L of *Sample and standard solutions*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of $C_{14}H_{22}ClN_3O_2$ ·HCl in the oral solution, from the responses obtained from *Sample and standard solutions*.

PACKAGING AND STORAGE

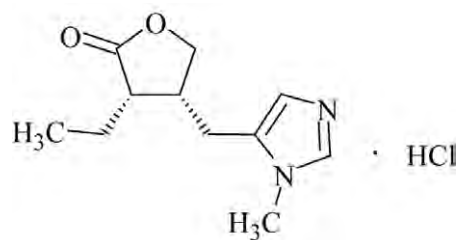
In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

HYDROCHLORIDE, PILOCARPINE

Pilocarpini hydrochloridum



$C_{11}H_{16}N_2O_2$ ·HCl; 244.72

pilocarpine hydrochloride; 07050

(3S, 4S)-3-ethyl dihydro-4-[(1-methyl-1H-imidazol-5-yl) methyl] -2 (3H)-furanone hydrochloride (1:1)
[54-71-7]

It contains at least 98.5% and at most 101.0% of $C_{11}H_{16}N_2O_2$ ·HCl, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White, crystalline powder, or colorless crystals; hygroscopic.

Solubility. Soluble in water and ethanol; sparingly soluble in chloroform, and insoluble in ethyl ether.

Physical and chemical constants.

Melting range (5.2.2): 199 °C to 205 °C. The range between the beginning and end of melting should not exceed 3 °C.

Specific rotation (5.2.8): +89° to +93°, in relation to the desiccated substance. To determine at 2% solution (w/v) in water.

IDENTIFICATION

The identification test A. may be omitted if the tests B., C., and D. are conducted. The identification tests B. and C. can be omitted if the tests A. and D. are carried out.

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in mineral oil, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the pilocarpine hydrochloride SQR spectrum similarly prepared.

B. The principal spot in the chromatogram of *Solution (2)* in the *Related substances* corresponds in position, color, and intensity to that obtained with *Solution (3)*.

C. To dissolve 2.5 g of the sample in carbon dioxide-free water and top up the volume to 50 mL with the same solvent. To dilute 0.2 mL of this solution to 2 mL of water and add 0.05 mL of potassium dichromate SR, 1 mL of 3%

hydrogen peroxide (w/v) and 2 mL of chloroform. To make it homogeneous. Violet color develops in chloroform layer.

D. It responds to the reactions of chloride ion (5.3.1.1).

PURITY TESTS

pH (5.2.19). 3.5 to 4.5. To determine in 5% solution (w/v) in carbon dioxide-free water.

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using G silica gel as support and a mixture of concentrated ammonium hydroxide, methanol, and methylene chloride (1:14:85) as mobile phase. To separately apply to the plate 25 µL of each of the solutions freshly prepared, as described below.

Solution (1): to dissolve 0.25 g of the sample in methanol and top up to 5 mL with the same solvent.

Solution (2): to dilute 1 mL of *Solution (1)* to 10 mL with methanol.

Solution (3): to dissolve 10 mg of pilocarpine hydrochloride SQR in methanol and top up to 2 mL with the same solvent.

Solution (4): to dilute 1 mL of *Solution (2)* to 10 mL with methanol.

To develop the chromatogram in 15 cm. To dry the plate at 100 °C - 105 °C for 10 minutes, let cool down, and nebulize it with potassium iodobismuthate SR and, subsequently, with 3% hydrogen peroxide (w/v). Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (4)* (1%).

Iron (5.3.2.4). To determine in 10 mL of 5% solution (w/v) in carbon dioxide-free water. To prepare the standard by using *Standard iron solution* (1 ppm Fe) and 5 mL of water. At most 0.001% (10 ppm).

Loss on drying (5.2.9). To desiccate in an oven at 100°C - 105 °C for 2 hours. At most 3%.

Sulfated ashes (5.2.10). To determine in 0.5 g of the sample. At most 0.5%.

DOSAGE

To accurately weigh about 2 g of the sample and dissolve in 60 mL of water. To titrate with *M* SV sodium hydroxide, to potentiometrically determine the endpoint. Each mL of *M* SV sodium hydroxide is equal to 244.720 mg of $C_{11}H_{16}N_2O_2 \cdot HCl$.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

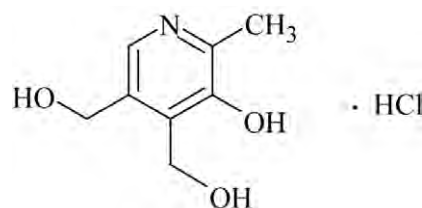
LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antiglaucoma.

HYDROCHLORIDE, PYRIDOXINE Pyridoxini hydrochloridum



$C_8H_{11}NO_3 \cdot HCl$; 205.64
pyridoxine hydrochloride; 07167
5-hydroxy-6-methyl-3,4-pyridine dimethanol
hydrochloride (1:1)
[58-56-0]

It contains at least 98.0% and at most 102.0% of $C_8H_{11}NO_3 \cdot HCl$, in relation to the desiccated substance.

DESCRIPTION

Physical and chemical characteristics. Crystalline, white or almost white powder. Melting range (5.2.2): around 205 °C with decomposition.

Solubility. Freely soluble in water, sparingly soluble in ethanol, practically insoluble in ethyl ether and chloroform.

Physical and chemical constants.

Specific rotation (5.2.8): +28° to +30°. To determine in 2% solution (w/v) in 0.1 M hydrochloric acid.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the pyridoxine hydrochloride SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14) ranging from 250 nm to 350 nm, of 0.001% solution (w/v) in 0.1 M hydrochloric acid, exhibits maximum between 288 nm and 296 nm, with the absorbance at 0.420 to 0.445. In the range from 220 nm to 350 nm, a solution prepared by diluting 1 mL of 0.1% solution (w/v) in 0.1 M hydrochloric acid to 100 mL with 0.025 M equimolar phosphate buffer exhibits maximum between 248 nm and 256 nm and between 320 nm and 327 nm. The absorbances are about 0.175 - 0.195 and 0.345 - 0.365, respectively.

C. The principal spot in the chromatogram of *Solution (2)* in the *Related substances* corresponds in position, color, and intensity to that obtained with *Solution (4)*

D. It responds to the reactions of chloride ion (5.3.1.1).

PURITY TESTS

pH (5.2.19). 2.4 to 3.0. To determine in 5% solution (w/v) of the sample in carbon dioxide-free water.

Related substances. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using G silica gel as support and a mixture of acetone, carbon tetrachloride, tetrahydrofuran, and 13.5 M ammonia (65:13:13:9) as mobile phase. To separately apply to the plate 2 µL of each of the solutions freshly prepared, as described below.

Solution (1): 100 mg/mL aqueous solution of the sample.

Solution (2): 10 mg/mL aqueous solution of the sample.

Solution (3): 0.25 mg/mL aqueous solution of the sample.

Solution (4): 10 mg/mL pyridoxine hydrochloride SQR aqueous solution.

Procedure: To develop the chromatogram. To remove the plate, and let it drying. To nebulize with 5% sodium carbonate solution (w/v) in a mixture of water and ethanol (70:30). To dry in a current of air and nebulize with 0.1% 2,6-dichloroquinone-4-chlorimide (w/v) in ethanol. Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (3)* (0,25%). To disregard remaining spots at the baseline.

Phenolic compounds. In a test tube, add 5 mg of the sample, 0.05 mL of 3 M hydrochloric acid, 1 mL of water, and 1 mL of ferric chloride SR. To make it homogeneous and add 1 mL of potassium ferricyanide SR. No bluish-green color develops after 2 minutes.

Limit for N, N-dimethylaniline. To accurately transfer about 0.5 g of the sample to a 25 mL volumetric flask, add 20 mL of water, and heat it to dissolve. To cool down and add 2 mL of M acetic acid and 1 mL of 1% sodium nitrite (w/v). To top up with water and make it homogeneous. The solution should not exhibit color more intense than the 0.001% N, N-dimethylaniline solution (w/v) (10 ppm) similarly prepared.

Heavy metals (5.3.2.3). To use *Method I*. To determine in 20 mL of 5% solution of the sample (w/v). At most 0.002% (20 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 105 °C. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Titration in non-aqueous medium (5.3.3.5)*. To add 30 mL of glacial acetic acid and 10 mL of mercury acetate SR to 0.4 g of sample, accurately weighed.

To put it in ultrasound, if necessary, to obtain total dissolution. To titrate with 0.1 M SV perchloric acid, by using methylrosanilinium chloride SI as an indicator. To perform sample blank correction and adjust it as required. Each mL of 0.1 M SV perchloric acid is equal to 20.564 mg of $C_8H_{11}NO_3 \cdot HCl$.

B. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 280 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (3 µm to 10 µm), kept at 25 °C; flow of *Mobile phase* of 1.5 mL/min.

Mobile phase: to transfer 20 mL glacial acetic acid, 1.2 g of sodium 1-hexane sulfonate to a 2000 mL volumetric flask and dilute with 1400 mL of water. To adjust pH to 3.0 with glacial acetic acid or M sodium hydroxide. To add 470 mL of methanol, make it homogeneous, and top up with water. To make the adjustments as required.

Internal standard solution: to dissolve an amount of *p*-hydroxybenzoic acid in *Mobile phase*, to obtain a 5 mg/mL concentration.

Sample solution: To transfer 50 mg of the sample, accurately weighed, to a 100 mL volumetric flask, dissolve it, and top up the volume with *Mobile phase*. To make it homogeneous. To transfer 10 mL to 100 mL volumetric flask, add 1 mL of *Internal standard solution*, top up with *Mobile phase*, and make it homogeneous.

Standard solution: to accurately transfer about 50 mg of pyridoxine hydrochloride SQR to a 100 mL volumetric flask, dissolve with *Mobile phase*, top up to the volume with the same solvent, and make it homogeneous. To transfer 10 mL to 100 mL volumetric flask, add 1 mL of *Internal standard solution*, top up with *Mobile phase*, and make it homogeneous.

To inject replicates of 20 µL of the *Standard solution*. The resolution between pyridoxine hydrochloride and *p*-hydroxybenzoic acid peaks is not less than 2.5. The relative standard deviation for the replicate areas for the peaks recorded is not greater than 3.0%.

Procedure: to separately inject 20 µL of *Standard solution* and *Sample solution*, record the chromatograms, and measure the areas under the pyridoxine hydrochloride and *p*-hydroxybenzoic acid peaks. To calculate the content of $C_8H_{11}NO_3 \cdot HCl$ from the sample, from the responses ob-

tained for pyridoxine hydrochloride/*p*-hydroxybenzoic acid with the *Standard solution* and *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Vitamin supplement.

HYDROCHLORIDE, PYRIDOXINE TABLETS

It contains at least 95.0 % and at most 115.0 % of the amount stated of $C_8H_{11}NO_3 \cdot HCl$.

IDENTIFICATION

A. To weigh and pulverize the tablets. To dissolve the amount of powder equivalent to 0.1 g of pyridoxine hydrochloride in 50 mL of methanol by mechanically stirring for 15 minutes. To filter, add sufficient amount of 13.5 *M* ammonia to alkalize the filtrate, and evaporate to dryness. To take up the residue with 15 mL of chloroform and filter. To drip 2 mL of acetyl chloride to the filtrate, add 8 mL of methanol, and evaporate to dryness. The infrared absorption spectrum (**5.2.14**) of the residue, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the pyridoxine hydrochloride SQR spectrum similarly prepared.

B. To weigh and pulverize the tablets. To dissolve the amount of powder equivalent to 20 mg of pyridoxine hydrochloride in 50 mL of 0.025 *M* phosphate buffer and stir for 15 minutes. To transfer to a 100 mL volumetric flask and top up with 0.025 *M* phosphate buffer. The ultraviolet absorption spectrum (**5.2.14**) of the solution, from 230 nm to 350 nm, exhibits absorption maxima at 254 nm and 324 nm.

C. To weigh and pulverize the tablets. To stir amount of powder equivalent to 0.1 g of pyridoxine hydrochloride in 5 mL of water and filter into a test tube. To add 2-3 drops of ferric chloride SR. Reddish-orange color is developed.

D. To weigh and pulverize the tablets. To transfer amount of powder equivalent to 20 mg of pyridoxine hydrochloride to a beaker, add 50 mL of water, and wait for decantation. To add 10 mL of 5% sodium acetate (w/v), 1 mL of water, 1 mL of 0.5% 2,6-dichloroquinone-4-chlorimide (w/v) in ethanol to 1 mL of the supernatant and make it homogeneous. Blue color is developed, but turning rapidly brown. To repeat the operation by adding 1 mL of 0.3% boric acid (w/v) in place of water. No blue color is produced.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness (5.1.3.1). It complies with the test.

Friability (5.1.3.2). It complies with the test.

Disintegration (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

Procedure for content uniformity. To transfer each tablet to 500 mL volumetric flask containing 300 mL of water, wait disintegration, and top up with the same solvent. To filter by discarding the first 25 mL of filtrate. From the filtrate, make appropriate dilutions with 1% hydrochloric acid (v/v), to obtain 0.001% concentration (w/v). To prepare pyridoxine hydrochloride SQR solution at the same concentration of the sample solution, by using the same solvent. To determine the absorbance of the solutions at 290 nm (**5.2.14**), by using 1% hydrochloric acid (v/v) to adjust zero. To calculate the amount of $C_8H_{11}NO_3 \cdot HCl$ in the tablets, from the reading obtained.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 *M* hydrochloric acid, 900 mL

Equipment: blades, 50 rpm *Time:* 45 minutes

Procedure: after testing, remove aliquot from the dissolution medium, filter, and dilute in 0.1 *M* hydrochloric acid, to obtain suitable concentration. To measure the absorbance of the solutions at 290 nm (**5.2.14**), by using the same solvent to adjust zero. To calculate the amount of $C_8H_{11}NO_3 \cdot HCl$ dissolved in the medium, by comparing the both readings from 0.001% pyridoxine hydrochloride SQR (w/v) prepared in the same solvent.

Tolerance: not less than 75% (Q) of the stated amount of $C_8H_{11}NO_3 \cdot HCl$ dissolves within 45 minutes.

PURITY TESTS

Related substances. To proceed as described in *Thin-layer chromatography* (**5.2.17.1**) by using G silica gel as support and a mixture of acetone, carbon tetrachloride, tetrahydrofuran, and 13.5 *M* ammonia (65:13:13:9) as mobile phase. To separately apply to the plate 10 µL of each of the solutions freshly prepared, as described below.

Solution (1): to weigh and pulverize the tablets. To stir amount of powder equivalent to 40 mg of pyridoxine hydrochloride in 10 mL of water for 15 minutes, filter, and use the filtrate.

Solution (2): to dilute 1 mL of *Solution (1)* to 200 mL with water.

To develop the chromatogram. To remove the plate, and let it drying. To nebulize with 5% sodium carbonate decahydrate (w/v) mixture of ethanol and water (30:70). To dry in a current of air and nebulize with 0.1% 2,6-dichloroquinone-4-chlorimide (w/v) in ethanol. Any secondary spot obtained in the chromatogram with *Solution (1)*,

other than the principal spot, is not more intense than that obtained with *Solution (2)* (0.5%).

DOSAGE

To weigh and pulverize 20 tablets. To transfer amount of powder equivalent to 25 mg of pyridoxine hydrochloride and add 50 mL of 0.1 M hydrochloric acid. To heat in water bath for 15 minutes, by occasionally stirring. To cool down, dilute to 100 mL with 0.1 M hydrochloric acid, and filter. To discard the first 20 mL. To dilute 5 mL of the filtrate to 100 mL with 0.1 M hydrochloric acid. To prepare standard solution at the same concentration, by using the same solvent. To measure the absorbance of the solutions at 290 nm (5.2.14), by using 0.1 M hydrochloric acid to adjust zero. To calculate the amount of $C_8H_{11}NO_3 \cdot HCl$ in the tablets, from the reading obtained. Alternatively, make the calculations by taking into account that A (1%, 1 cm) = 430 at 290 nm.

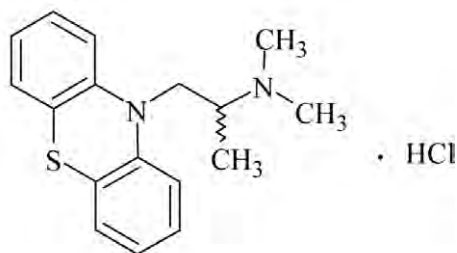
PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

HYDROCHLORIDE, PROMETHAZINE Promethazini hydrochloridum



$C_{17}H_{20}N_2S \cdot HCl$ 320.88

promethazine hydrochloride; 07431

Hydrochloride *N,N*, α -trimethyl-10*H*-phenothiazine-10-ethaneamine (1:1)

[58-33-3]

It contains at least 99.0% and at most 101.0% of $C_{17}H_{20}N_2S \cdot HCl$, in relation to the desiccated substance.

DESCRIPTION

Physical and chemical characteristics. Crystalline powder, white to light yellow, odorless, bitter taste. It melts at around 222 °C with decomposition (5.2.2).

Solubility. Easily soluble in water, ethanol, and chloroform; practically insoluble in ethyl ether.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the promethazine hydrochloride SQR spectrum similarly prepared.

B. It responds to the identifying reactions of phenothiazines (5.3.1.5).

C. To dissolve 0.1 g of the sample in 3 mL of purified water and 1 mL of nitric acid dropwise. Precipitate is formed, which dissolves rapidly and develops red color that turns into orange and then yellow. To heat to boiling. The solution becomes orange and then an orange-red precipitate is formed.

D. It responds to the reactions of chloride ion (5.3.1.1).

PURITY TESTS

pH (5.2.19). 4.0 to 5.0. To determine in 10 % solution (w/v) freshly prepared.

Related substances. To proceed to test for *Substances related to phenothiazines* (5.3.1.6) by using *Mobile Phase B* and separately apply to the plate 10 μ L of each of the three solutions freshly prepared as described below.

Solution (1): 2% solution (w/v) of the sample in mixture of diethylamine and methanol (5:95).

Solution (2): 0.02% isopromethazine hydrochloride SQR solution (w/v) in the same solvent.

Solution (3): 0.01% isopromethazine hydrochloride SQR solution (w/v) in the same solvent.

To develop the chromatogram. To remove the plate, and let it drying. No isopromethazine spot in chromatogram obtained with the *Solution (1)* is more intense than those obtained with the *Solution (2)* (1%). No other secondary spot obtained with *Solution (1)* is more intense than that obtained with *Solution (3)* (0.5%).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 100 °C - 105°C until obtaining constant weight. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To employ one of the methods described below.

A. To dissolve 0.25 g, accurately weighed, in a mixture of 5 mL of 0.01 *M* hydrochloric acid and 50 mL of ethanol. To titrate with 0.1 *M* SV sodium hydroxide, by potentiometrically determining the volume spent between the two inflection points.

Each mL of 0.1 *M* SV sodium hydroxide is equal to 32.088 mg of $C_{17}H_{20}N_2S.HCl$.

B. To accurately dissolve about 700 mg of sample to a mixture of 75 mL of glacial acetic acid and 10 mL of mercuric acetate SR solution. To add a drop of crystal violet SI solution and titrate with 0.1 *M* SV perchloric acid until it turns blue. To perform sample blank correction and adjust it as required. Each mL of 0.1 *M* SV iodine is equal to 32.090 mg of $C_{17}H_{20}N_2S.HCl$.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antiemetic, antihistamine.

HYDROCHLORIDE, PROMETHAZINE TABLETS

It contains at least 95.0 % and at most 110.0% of the amount stated of $C_{17}H_{20}N_2S.HCl$. The tablets should be coated.

IDENTIFICATION

A. To weigh and pulverize the tablets. To weigh amount of powder equivalent to 40 mg of promethazine hydrochloride, add 10 mL of water and 2 mL of *M* sodium hydroxide, stir, and extract with 15 mL of ethyl ether. To wash the ether extract with 5 mL of water, dry with anhydrous sodium sulfate, evaporate to dryness, and dissolve the residue in 0.4 mL of chloroform. The infrared absorption spectrum (5.2.14) has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the promethazine hydrochloride SQR spectrum similarly prepared.

B. To add 5 mL of sulfuric acid to the amount of powdered tablets containing 5 mg of promethazine hydrochloride. Let it rest for 5 minutes. Red color develops.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 *M* hydrochloric acid, 900 mL

Equipment: blades, 100 rpm *Time:* 45 minutes

Procedure: after testing, remove aliquot from the dissolution medium, filter, and dilute with 0.1 *M* hydrochloric acid to obtain suitable concentration. To measure the absorbance of the solutions at 249 nm, by using the same solvent to adjust zero. To calculate the amount of $C_{17}H_{20}N_2S.HCl$. HCL dissolved in the medium, by comparing the readings obtained with promethazine hydrochloride SQR prepared in the same solvent.

Tolerance: not less than 75% (Q) of the stated amount of $C_{17}H_{20}N_2S.HCl$ dissolves within 45 minutes.

PURITY TESTS

Related substances. To proceed as described in *Research of impurities related to phenothiazines in thin-layer chromatography (5.3.1.6)* by using the *Mobile phase B* and separately applying to the plate 20 µL of each of the following solutions, prepared immediately before utilization. Operation in nitrogen atmosphere is not necessary.

Solution (1): to extract amount of powdered tablets, equivalent to 0.1 g of promethazine hydrochloride with 10 mL of mixture of diethylamine and methanol (5:95), and filter it.

Solution (2): 0.01% promethazine hydrochloride SQR solution (w/v) in mixture of diethylamine and methanol (5:95).

Solution (3): to dilute 1 volume of *Solution (1)* to 200 volumes with mixture of diethylamine and methanol (5:95).

To develop the chromatogram. To remove the plate, and let it drying. No isopromethazine spot in the chromatogram obtained with the *Solution (1)* is more intense than those obtained with the *Solution (2)* (1%). No other secondary spot is more intense than that obtained with *Solution (3)* (0.5%).

DOSAGE

To perform dosage protected from light. To pulverize 20 tablets. To weigh amount of powder equivalent to 50 mg of promethazine hydrochloride, add 10 mL of 2 M hydrochloric acid, 200 mL of purified water, stir for 15 minutes, top up to 500 mL with purified water, and centrifuge 50 mL of the mixture. To add 10 mL of 0.1 M hydrochloric acid to 5 mL of the clear and limpid supernatant and top up to 100 mL with purified water. To prepare promethazine hydrochloride SQR solution at the same concentration as 0.01 M hydrochloric acid.

To measure the absorbance (5.2.14) of the solutions at 249 nm, by using 0.01 M hydrochloric acid to adjust zero. To calculate the amount of $C_{17}H_{20}N_2S.HCl$ in the tablets, from the reading obtained with standard and sample solutions.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

PROMETHAZINE, HYDROCHLORIDE INJECTABLE SOLUTION

It contains at least 95.0% and at most 105.0% of the amount stated of $C_{17}H_{20}N_2S.HCl$.

IDENTIFICATION

Test **B**. can be omitted if tests **A**. and **C**. are conducted.

A. To measure the volume of injectable solution equivalent to 25 mg of promethazine hydrochloride and top up to 50 mL with 0.5 M sulfuric acid. To dilute 5 mL to 100 mL by using the same solvent. The ultraviolet absorption spectrum (5.2.14) exhibits maxima and minima only in the same wavelength of a similar promethazine hydrochloride SQR solution.

B. To slowly add 2 mL of sulfuric acid to the solution containing 5 mg of promethazine hydrochloride and let it rest for 5 minutes. Red color is produced.

C. It responds to the reactions of chloride ion (5.3.1.1).

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

pH (5.2.19). 5.0 to 6.0.

PURITY TESTS

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1). To develop the chromatogram, protected from light, under a nitrogen atmosphere, by using GF_{254} silica gel as support and a mixture of diethylamine, hexane, and acetone (15:45:50) as mobile phase. To separately apply to the plate 10 μ L of each of the solutions freshly prepared, as described below.

Solution (1): to dilute injectable solution with mixture of diethylamine and methanol (5:95) to obtain 1.0% promethazine hydrochloride solution (v/v).

Solution (2): to dilute 1 mL of *Solution (1)* to 200 mL with mixture of diethylamine and methanol (5:95).

Solution (3): to prepare a 0.01% isopromethazine hydrochloride solution (w/v) with mixture of diethylamine and methanol (5:95).

Solution (4): to prepare 0.025% promethazine sulfoxide SQR solution (v/v) with a mixture of diethylamine and methanol (5:95).

To develop the chromatogram. To remove the plate, and let it drying. To nebulize with 20% perchloric acid (v/v). To heat the plate at 100°C for 5 minutes. In the chromatogram obtained with *Solution (1)* any spot corresponding to the isopromethazine is no more intense than the corresponding spot in the chromatogram obtained with *Solution (3)* (1.0%). Any spot corresponding to promethazine sulfoxide is not more intense than the spot in the chromatogram obtained with *Solution (4)* (2.5%), and any secondary spot is not more intense than the spot in the chromatogram obtained with *Solution (2)* (0.5%). To disregard any remaining spot in the application line.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

Bacterial endotoxins (5.5.2.2). At most 5.0 UE/mg of promethazine hydrochloride.

DOSAGE

To proceed as described in the *Ultraviolet absorption spectrophotometry* (5.2.14), protected from light. To transfer injectable solution equivalent to about 25 mg of promethazine hydrochloride to a 100 mL volumetric flask and top up with 0.01 M hydrochloric acid. To dilute 10 mL to 100 mL with 0.01 M hydrochloric acid and then dilute 10 mL to 50 mL with the same solvent, thus obtaining 0.0005% concentration (w/v). To prepare standard solution under the same conditions. To measure the absorbance of the solutions at 249 nm, by using 0.01 M hydrochloric acid to adjust zero. To calculate the amount of $C_{17}H_{20}N_2S.HCl$ in the injectable solution, from the readings obtained.

PACKAGING AND STORAGE

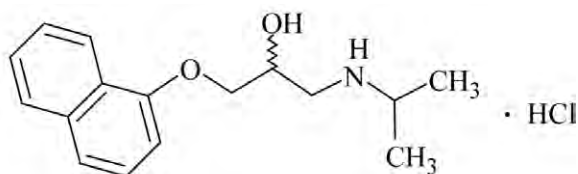
In type I glass container, protected from light.

LABELLING

To comply with the legislation in force.

HYDROCHLORIDE, PROPRANOLOL

Propranololi hydrochloridum



$C_{16}H_{21}NO_2 \cdot HCl$; 295.80
 propranolol hydrochloride; 07482
 Hydrochloride 1 - [(1-methylethyl) amino] -3 -
 (1-naphthalenyloxy) -2 -
 propanol (1:1)
 [318-98-9]

It contains at least 98.0% and at most 101.5% of $C_{16}H_{21}NO_2 \cdot HCl$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or almost white powder, odorless, bitter taste, and crystalline or amorphous appearance.

Solubility. Soluble in water and ethanol, sparingly soluble in chloroform, and insoluble in ethyl ether.

Physical and chemical constants.

Melting range (5.2.2): 163 °C to 166 °C.

Specific rotation (5.2.8): -1.0° to +1.0°. To determine in 4% aqueous solution (w/v) of the desiccated substance.

IDENTIFICATION

The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the propranolol hydrochloride SQR spectrum similarly prepared.

The ultraviolet absorption spectrum (5.2.14) ranging from 200 nm to 400 nm, of 0.004% solution (w/v) in methanol, exhibits maxima absorption at 290 nm, 306 nm, and 319 nm. The absorbances are approximately 0.84, 0.50, and 0.30, respectively.

To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel as support and a mixture

of methanol and concentrated ammonia solution (99:1) as mobile phase. To separately apply to the plate 10 µL of each of the solutions freshly prepared, as described below.

Solution (1): 10 mg/mL solution of the sample in methanol.

Solution (2): 10 mg/mL propranolol hydrochloride SQR solution in methanol.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). To nebulize the plate with a mixture of anisaldehyde, glacial acetic acid, methanol, and sulfuric acid (0.5:10:85:5). To dry at 100 °C - 105 °C. The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

D. It responds to the reactions of chloride ion (5.3.1.1).

PURITY TESTS

pH (5.2.19). 5.0 to 6.0. To determine in 1% aqueous solution (w/v).

Acidity and alkalinity. To dissolve 0.2 g of the sample in carbon dioxide-free water and top up the volume to 20 mL with the same solvent. To add 0.2 mL of methyl red SI and 0.2 mL of 0.01 M hydrochloric acid. Solution is red. To add 0.4 mL of 0.01 M sodium hydroxide. Solution is yellow.

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel as support and a mixture of toluene and methanol (90:10) as mobile phase. To separately apply to the plate 10 µL of each of the solutions freshly prepared, as described below.

Solution (1): 100 mg/mL solution of the sample in methanol.

Solution (2): 0.2 mg/mL solution of the sample in methanol.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). To nebulize the plate with a mixture of anisaldehyde, glacial acetic acid, methanol, and sulfuric acid (0.5:10:85:5). To dry at 100 °C - 105 °C. Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (2)* (0.2%).

Heavy metals (5.3.2.3). At most 0.002% (20 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 105 °C for 4 hours. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Titration in non-aqueous medium (5.3.3.5)*. To dissolve 0.5 g of the sample in a mixture of 50 mL of glacial acetic acid and 10 mL of mercury acetate SR. To titrate with 0.1 M SV perchloric acid, by potentiometrically determining the endpoint or by using methylrosanilinium chloride SI as an indicator. To perform sample blank correction and adjust it as required. Each mL of 0.1 M SV iodine is equal to 29.580 mg of $C_{16}H_{21}NO_2 \cdot HCl$.

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 290 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μ m), at room temperature; flow of *Mobile phase* of 1.5 mL/min.

Mobile phase: to dissolve 0.5 g of sodium lauryl sulfate in 18 mL of 0.15 M phosphoric acid, add 90 mL acetonitrile, 90 mL of methanol, and dilute with water to 250 mL.

Sample solution: to exactly transfer about 50 mg of sample to a 50 mL volumetric flask, add 45 mL of methanol, and put it in ultrasound for 5 minutes. To top up with the same solvent, make it homogeneous, and filter it. To transfer 5 mL to 25 mL volumetric flask, top up with methanol, and make it homogeneous.

Standard solution: to accurately transfer about 50 mg of propranolol hydrochloride SQR to a 50 mL volumetric flask, dissolve with methanol, and top up to the volume with the same solvent, to obtain 1 mg/mL solution. To transfer 5 mL to 25 mL volumetric flask, top up with methanol, and make it homogeneous.

Resolution Solution: to prepare 0.25 mg/mL procainamide hydrochloride solution in methanol. To transfer 5 mL of this solution and 5 mL of the *Standard solution* to 25 mL volumetric flask. To top up to volume with methanol and make it homogeneous.

To inject replicates of 20 μ L of the *Resolution solution*. The resolution between peaks corresponding to procainamide propranolol hydrochloride is not less than 2.0. The tailing factor of the peak corresponding to propranolol hydrochloride is not greater than 3.0. The relative standard deviation for the replicate areas for the peaks recorded is not greater than 2.0%.

Procedure: to separately inject 20 μ L of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_{16}H_{21}NO_2 \cdot HCl$ from the sample, from the responses obtained for the *Sample* and *standard solutions*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antihypertensive, antiarrhythmic, antianginal.

PROPRANOLOL HYDROCHLORIDE TABLETS

It contains at least 90.0% and at most 110.0% of the amount stated of $C_{16}H_{21}NO_2 \cdot HCl$.

IDENTIFICATION

A. To weigh and pulverize the tablets. To suspend in water the amount of powder equivalent to 0.1 g of propranolol hydrochloride and filter. To transfer the filtrate to a separatory funnel, alkalize with M sodium hydroxide, and extract with 3 volumes of 10 mL of ethyl ether. To wash the combined extracts with water to neutralize it. To filter over anhydrous sodium sulfate, evaporate the filtrate, and dry the residue under reduced pressure at 50 °C for 1 hour. The infrared absorption spectrum (5.2.14) of the residue, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the spectrum of the residue obtained after identical treatment carried out with 0.1 g of propranolol hydrochloride SQR.

B. The melting point of the residue obtained in Test A. *Identification* is approximately 94 °C (5.2.2).

C. The 0.004% solution (w/v) obtained in Method A. *Dosage* responds to the test C. *Identification*, in the monograph *Propranolol hydrochloride*.

D. To proceed as described in *Related substances*. The principal spot obtained in the chromatogram with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (3)*.

E. To suspend in water the amount of powder equivalent to 0.1 g of propranolol hydrochloride. To stir and filter through suitable filter paper. The filtrate responds to the reactions of chloride ion (5.3.1.1).

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). Within 30 minutes.

Uniformity of dosage units (5.1.6). It complies with the test.

Procedure for content uniformity. To individually weigh and transfer each tablet to a 100 mL volumetric flask, add 5 mL of 1% hydrochloric acid (v/v), and stir to tablet disintegrates. To add 70 mL of methanol and put it in ultrasound for 1 minute. To top up to volume with methanol, make it homogeneous, and filter through suitable filter paper. To discard the first few milliliters. To dilute the filtrate to the 0.004% concentration (w/v). To prepare a 0.004% methanolic solution (w/v) of propranolol hydrochloride SQR and measure the absorbances of the resulting solutions at 290 nm (5.2.14), by using methanol to adjust zero. To calculate the individual amount of $C_{16}H_{21}NO_2 \cdot HCl$ in the tablets, from the reading obtained.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 1% hydrochloric acid (v/v), 1000 mL
Equipment: basket, 100 rpm *Time:* 30 minutes

Procedure: after testing, remove aliquot from the dissolution medium, and dilute, if necessary, with 1% hydrochloric acid (v/v) to obtain suitable concentration. To measure the absorbances at 289 nm (5.2.14), by using the same solvent to adjust zero. To calculate the amount of $C_{16}H_{21}NO_2 \cdot HCl$ dissolved in the medium, by comparing the readings obtained with 0.004% propranolol hydrochloride SQR solution (w/v) prepared in the same solvent.

Tolerance: not less than 75% (Q) of the amount stated of $C_{16}H_{21}NO_2 \cdot HCl$ dissolves within 30 minutes.

PURITY TESTS

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1), by using GF_{254} silica gel as support and a mixture of toluene, methanol, and concentrated ammonia (80:20:01) as mobile phase. To separately apply to the plate 100 μ L, 20 μ L, and 100 μ L, respectively, of each of the solutions described below.

Solution (1): 1% propranolol hydrochloride SQR solution (w/v) in methanol.

Solution (2): 0.02% propranolol hydrochloride SQR solution (w/v) in methanol.

Solution (3): to weigh and pulverize the tablets. To weigh amount of powder equivalent to 50 mg of propranolol hydrochloride, transfer to 5 mL volumetric flask, top up with methanol, make it homogeneous, and filter it, thus obtaining a 1% solution (w/v).

To develop the chromatogram. To remove the plate, allow to air dry, examine under ultraviolet light (254 nm), and indicate the spots. To nebulize with a mixture of anisaldehyde, glacial acetic acid, methanol, and sulfuric acid

(0.5:10:85:5). To dry at 100 °C - 105 °C. Any secondary spot obtained in the chromatogram with *Solution (3)* is not more intense than that obtained with *Solution (2)*.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in the *Ultraviolet absorption spectrophotometry* (5.2.14). To weigh and pulverize 20 tablets. To transfer amount of powder equivalent to 20 mg of propranolol hydrochloride to 100 mL volumetric flask, add 20 mL of water, and mechanically stir for 10 minutes. To add 50 mL of methanol and stir for 10 minutes, top up with methanol, make it homogeneous, and filter it. To quantitatively transfer 10 mL of the filtrate to a 50 mL volumetric flask and top up with methanol. To prepare a 0.004% propranolol hydrochloride SQR solution (w/v) in methanol and measure the absorbance of the solutions at 290 nm, by using methanol as blank. To calculate the amount of $C_{16}H_{21}NO_2 \cdot HCl$ in the tablets, from the reading obtained.

B. To proceed as described in *High performance liquid chromatography* (5.2.17.4). To proceed as described in method **B. Dosage**, in the monograph *Propranolol hydrochloride*. To prepare the Sample solution as described below.

Sample solution: to weigh and pulverize at least 20 tablets. To accurately transfer amount of powder equivalent to 50 mg of propranolol hydrochloride to 50 mL volumetric flask, add 40 mL of methanol, stir, put it in ultrasonic bath for 5 minutes. To top up with the methanol, make it homogeneous, and filter it. To transfer 5 mL of the above solution to 25 mL volumetric flask, top up with methanol, make it homogeneous, and filter through a 0.45 μ m membrane.

PACKAGING AND STORAGE

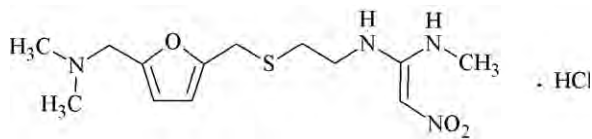
In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

RANITIDINE HYDROCHLORIDE

Ranitidini hydrochloridum



$C_{13}H_{22}N_4O_3S.HCl$; 350.86

ranitidine hydrochloride; 07639

N'-[2 - [[[5 - [(dimethylamino) methyl]-2-furanyl] methyl] thio] ethyl]-*N*-methyl-2-nitro-1,1-ethenediamine hydrochloride (1:1) [66357-59-3]

It contains at least 98.5% and at most 101.0% of $C_{13}H_{22}N_4O_3S.HCl$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline powder, white to pale yellow, odorless, moisture sensitive. It shows polymorphism.

Solubility. Freely soluble in water and methanol, slightly soluble in ethanol, very slightly soluble in methylene chloride, practically insoluble in chloroform. Freely soluble in acetic acid.

Physical and chemical constants. *Melting range* (5.2.2): 133 °C to 134 °C.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously vacuum desiccated at 60°C, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the ranitidine hydrochloride SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14) ranging from 200 nm to 400 nm, of 0.001% solution (w/v) in distilled water, exhibits maxima absorption at 229 nm and 315 nm. The ratio between the absorbance values measured at 229 nm and 315 nm is between 1.01 and 1.07.

C. It responds to the reactions of chloride ion (5.3.1.1).

PURITY TESTS

pH (5.2.19). 4.5 to 6.0. To determine in 1% solution (w/v) in carbon dioxide-free water.

To use the *Method IV*.

Heavy metals (5.3.2.3). At most 0.002% (20 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 60°C under reduced pressure for 3 hours. At most 0.75%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To employ one of the methods described below.

A. To accurately weigh about 0.28 g of the sample and dissolve in 35 mL of water. To titrate with 0.1 *M* SV sodium hydroxide, to potentiometrically determine the endpoint. Each mL of 0.1 *M* SV sodium hydroxide is equal to 38.087 mg of $C_{13}H_{22}N_4O_3S.HCl$.

B. To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To accurately transfer about 50 mg of the sample to a 100 mL volumetric flask. To add 40 mL of water, stir, and top up with the same solvent. To successively dilute in water to the 0.00125% concentration (w/v).

C. To prepare standard solution under the same conditions. To measure the absorbances of the resulting solutions at 314 nm, by using water to adjust zero. To calculate the content of $C_{13}H_{22}N_4O_3S.HCl$ in the sample from the readings obtained.

PACKAGING AND STORAGE

In airtight containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Anti-secretory.

RANITIDINE HYDROCHLORIDE, TABLETS

It contains at least 90.0% and at most 110.0% of the amount stated of $C_{13}H_{22}N_4O_3S$.

IDENTIFICATION

A. The ultraviolet absorption spectrum (5.2.14) ranging from 200 nm to 400 nm, of sample solution obtained in method **A**. **A.**, in *Dosage*, exhibits absorption maximum at 229nm and 315 nm, similar to those observed in the Standard solution spectrum.

B. The retention time of the main peak of the *Sample solution* obtained in the *Dosage* method **B**. corresponds to that of main peak of *Standard solution*.

C. To weigh and pulverize the tablets. To stir amount of powder equivalent to 0.1 g of ranitidine with 2 mL of water and filter it. It responds to the reactions of chloride ion (5.3.1.1).

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness (5.1.3.1). It complies with the test.

Sterility (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL

Equipment: blades, 50 rpm

Time: 45 minutes

Procedure: after testing, remove aliquot from the dissolution medium, filter, and dilute with water to obtain suitable concentration. To measure the absorbance of the solutions at 314 nm (5.2.14), by using the same solvent to adjust zero. To calculate the amount of $C_{13}H_{22}N_4O_3S$ dissolved in the medium, by comparing the readings obtained with 0.00125% ranitidine hydrochloride SQR solution (w/v) prepared with the same solvent.

Tolerance: not less than 80% (Q) of the stated amount of $C_{13}H_{22}N_4O_3S$ dissolves within 45 minutes.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in the *Ultraviolet absorption spectrophotometry* (5.2.14). To weigh and pulverize 20 tablets. To transfer amount of powder equivalent to 0.125 g of ranitidine to a 250 mL volumetric flask, dilute with 150 mL of water, mechanically stir for 30 minutes, and top up to volume with the same solvent. To filter it. To successively dilute to obtain 0.00125% concentration (w/v). To prepare a standard solution at similar concentration by using the same solvent. To measure the absorbance of the resulting solutions at 314 nm, by using water to adjust zero. To calculate the amount of $C_{13}H_{22}N_4O_3S$ in the tablets, from the reading obtained.

B. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 275 nm; 250 mm column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μ m), at room temperature; flow of *Mobile phase* of 1.7 mL/min.

Mobile phase: mixture of methanol and 0.1 M ammonium acetate (85:15).

Sample solution: to weigh and pulverize the tablets. To transfer amount of powder, accurately weighed, to appropriate volumetric flask. To add to *Mobile phase*, stir, top up to volume with the same solvent, and filter. To quantitatively dilute the filtrate with *Mobile phase* to obtain a solution whose concentration is similar to that of *Standard solution*.

Standard solution: to dissolve accurately weighed amount of ranitidine hydrochloride SQR in the *Mobile phase* to obtain a 0.112 mg/mL solution, equivalent to 0.1 mg/mL ranitidine.

Procedure: to separately inject 10 μ L of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_{13}H_{22}N_4O_3S$ in the tablets, from the responses obtained for the *Sample and standard solutions*.

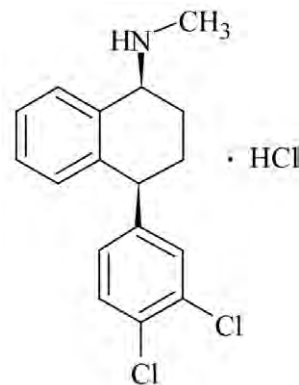
PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

SERTRALINE HYDROCHLORIDE
Sertralini hydrochloridum



$C_{17}H_{17}Cl_2N.HCl$; 342.69
 sertraline hydrochloride; 07964
 (1S, 4S) -4 - (3,4-dichlorophenyl) -1,2,3,4 -
 tetrahydro-N-metil-1-naftalenamina (1:1) hydrochloride
 [79559-97-0]

It contains at least 97.0% and at most 102.0% of $C_{17}H_{17}Cl_2N.HCl$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white or almost white, odorless powder. It shows polymorphism.

Solubility. Soluble in water, acetonitrile, isopropyl alcohol, and methanol; very slightly soluble in ethanol; insoluble in toluene, cyclohexane, and n-hexane.

Physical and chemical constants.

Melting range (5.2.2): 243 °C to 245 °C.

Specific rotation (5.2.8): +37.0° to +42.0°, in relation to the desiccated substance. To determine at 2% solution (w/v) in methanol.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the sertraline hydrochloride SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14), ranging from 200 nm to 400 nm, of sample solution obtained in method **B.** in *Dosage*, exhibits absorption maximum at 266 nm, 274 nm, and 282 nm, similar to those observed in the standard solution spectrum.

C. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method **C.** corresponds to that of main peak of *Standard solution*.

PURITY TESTS

Heavy metals (5.3.2.3). To use the *Method I*. At most 0.002% (20 ppm).

Loss on drying (5.2.9). To determine in 2 g of the sample in oven at 105 °C for 2 hours. At most 1.0%.

Sulfated ashes (5.2.10). At most 0.1 %.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. To transfer about 0.4 g of the sample, accurately weighed, to a 250 mL erlenmeyer flask and dissolved with 80 mL of glacial acetic acid. To add 10 mL of mercury acetate SR. To titrate with 0.1 M SV perchloric acid, by using methylrosanilinium chloride SI as an indicator to turns into blue-green color. Each mL of 0.1 M SV perchloric acid is equal to 34.269 mg of $C_{17}H_{17}Cl_2N.HCl$

B. To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. To weigh approximately 0.5 g of the accurately weighed sample, and transfer to a 200 mL volumetric flask. To add 100 mL of methanol and put it in ultrasound for 15 minutes. To top up with the same solvent, make it homogeneous, and filter it. To successively dilute in methanol to the 0.025% concentration (w/v). To prepare a standard solution at similar concentration by using the same solvent. To measure the absorbances of the resulting solutions at 274 nm, by using methanol to adjust zero. To calculate the $C_{17}H_{17}Cl_2N.HCl$ concentration of the sample from the readings obtained.

C. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 254 nm; 125 mm chromatograph column and 4.0 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at 30 °C; flow of *Mobile phase* of 1.2 mL/min.

Phosphate buffer pH 3.0: to dissolve 2.27 g of monobasic sodium phosphate in 950 mL of water, adjust the pH to 3.0 ± 0.1 with phosphoric acid, and top up to 1000 mL with water.

Mobile phase: mixture of acetonitrile, methanol, and *Phosphate buffer pH 3.0* (30:60:10).

Sample solution: to accurately transfer about 50 mg of the sample to a 100 mL volumetric flask. To add 50 mL of water and put it in ultrasound for 15 minutes. To top up to volume with methanol, to obtain 0.5 mg/mL solution.

Standard solution: to dissolve accurately weighed amount of sertraline hydrochloride SQR in methanol and dilute with the same solvent, to obtain a 0.5 mg/ mL solution.

To inject replicates of 20 µL of the *Standard solution*. The tailing factor should not be greater than 2.0. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2.0 %.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas of the peaks. To calculate the content of $C_{17}H_{17}Cl_2N.HCl$ from the sample, from the responses obtained for the *Sample and Standard solutions*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antidepressant.

**SERTRALINE HYDROCHLORIDE,
TABLETS**

It contains at least 90.0% and at most 110.0% of the amount stated of $C_{17}H_{17}Cl_2N.HCl$.

IDENTIFICATION

A. The ultraviolet absorption spectrum (5.2.14) ranging from 200 nm to 400 nm, of sample solution obtained in *Dosage* method **A.**, exhibits absorption maximum at 266 nm, 274 nm, and 282 nm similar to those observed in the standard solution spectrum.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method **B.** corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

Procedure for content uniformity. To transfer each tablet to a 100 mL volumetric flask and dissolve in 60 mL of methanol. To put it in ultrasound for 20 minutes. After the total disintegration of the tablet, top up to volume with methanol, make it homogeneous, and filter it. To successively dilute to obtain 0.02% sertraline hydrochloride (w/v). To prepare a standard solution at similar concentration by using the same solvent.

To measure the absorbances of the resulting solutions at 274 nm **(5.2.14)**, by using methanol to adjust zero. To calculate the content of $C_{17}H_{17}Cl_2N.HCl$ from each tablet, from the responses obtained for the sample and standard solutions.

DISSOLUTION TEST (5.1.5)

Dissolution medium: sodium acetate buffer, pH 4.5, 900 mL *Equipment:* blades, 75 rpm *Time:* 45 minutes

Procedure: after the test, remove aliquot from dissolution medium and filter. To measure the absorbance of the solutions at 274 nm **(5.2.14)**, by using the same solvent to adjust zero. To calculate the amount of $C_{17}H_{17}Cl_2N.HCl$ dissolved in the medium, by comparing the readings obtained with that one related to the 0.005% sertraline hydrochloride SQR solution (w/v) prepared in the same solvent.

Tolerance: not less than 75% (Q) of the stated amount of $C_{17}H_{17}Cl_2N.HCl$ dissolves within 45 minutes.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in the *Ultraviolet absorption spectrophotometry (5.2.14)*. To weigh and pulverize 20 tablets. To transfer amount of powder equivalent to 0.5 g of sertraline hydrochloride to 200 mL volumetric flask. To proceed as described in method **B.** *Dosage*, in the monograph *Sertraline hydrochloride* from "To add 100 mL of methanol and put it in ultrasound...".

B. To proceed as described in method **C** *Dosage*, in the monograph *Sertraline hydrochloride*. To prepare the *Sample solution* as described below.

Sample solution: To weigh and pulverize 20 tablets. To transfer amount of powder equivalent to 50 mg of sertraline hydrochloride to 100 mL volumetric flask. To add 50 mL of water and put it in ultrasound for 15 minutes. To top up with the same solvent, make it homogeneous, and filter it.

Procedure: to separately inject 20 µL of *Standard solution* and *Sample solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_{17}H_{17}Cl_2N.HCl$ from the tablets, from the responses obtained for the *Sample and standard solutions*.

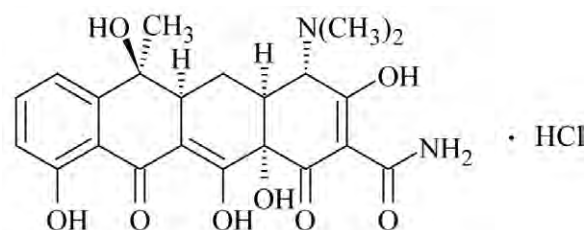
PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

HYDROCHLORIDE, TETRACYCLINE Tetracyclini hydrochloridum



$C_{22}H_{24}N_2O_8.HCl$ 480.90 tetracycline hydrochloride; 08465 (4S, 4aS, 5th, 6S, 12aS) -4 - (dimethylamino) - 1,4,4 a, 5,5 a, 6,11,12 a-octahydro-3, 6,10,12,12 a-pentahydroxy 6-methyl-1,11-dioxo-2-naphthacenecarboxamide (1:1) hydrochloride [64-75-5]

It contains at least 95.0% and at most 102.0% of $C_{22}H_{24}N_2O_8.HCl$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, yellow, odorless, and slightly hygroscopic powder.

Solubility. It is soluble in water, sparingly soluble in ethanol, and practically insoluble in ethyl ether and chloroform.

Physical and chemical constants. *Specific rotation (5.2.8):* -240° to -255°. To determine in a 1% solution (w/v) in 0.01 M hydrochloric acid

IDENTIFICATION

The identification tests **B.**, **C.**, and **D.** may be omitted if the tests **A.** and **E.** are conducted.

The infrared absorption spectrum (5.2.14) of the non desiccated sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the tetracycline hydrochloride SQR spectrum similarly prepared.

The ultraviolet absorption spectrum (5.2.14), ranging from 200 nm to 400 nm, of 0.002% solution (w/v) in 0.25 M sodium hydroxide, exhibits a maximum at 380 nm. The absorbance at 380 nm, in relation to the desiccated substance, is from 96% to 104% of the absorbance obtained with a standard solution similarly prepared.

The determination must be performed 6 minutes after preparing the final solution.

The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

To add 5 mL of sulfuric acid to 2 mg of the sample. Reddish-violet color is developed. To add 2.5 mL of water. The yellow color develops.

It responds to the reactions of chloride ion (5.3.1.1).

PURITY TESTS

pH (5.2.19). 1.8 to 2.8. To determine in 1% solution (w/v) in carbon dioxide-free water.

Limit for 4-epianidrotetracycline hydrochloride. To proceed as described in *Dosage* method **C.** To prepare the solutions as described below.

Solution (1) : to use *Sample solution* described in method **C.**

Solution (2): 10 µg /mL 4-epianidrotetracycline hydrochloride solution SQR in *Mobile phase*.

To separately inject 20 µL of *Solutions (1)* and *(2)*, record the chromatograms, and measure the areas under the peaks. The area of any peak corresponding to 4-epianidrotetracycline in the chromatogram obtained with *Solution (1)* is not greater than the area under the main peak obtained with *Solution (2)*. At most 2.0%.

Heavy metals (5.3.2.3). To use *Method III*. At most 0.005% (50 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 60 °C under reduced pressure up

to 5 mm of Hg for 3 hours, to obtain constant weight. At most 2.0%.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Microbiological test of antibiotics (5.5.3.3.1)* by using cylinders with the agar diffusion method.

Micro-organism: *Staphylococcus epidermidis* ATCC 12228.

Culture media: culture medium number 1, to maintain the micro-organism; sterile saline solution to the standardization of the inoculum; and culture medium number 1, to the base layer and inoculum preparation.

Sample solution: To accurately transfer about 20 mg of the sample to a 100 mL volumetric flask by using 70 mL of 0.01 M hydrochloric acid. To stir and top up to volume with the same solvent. To dilute successively by using sterile water to obtain 2 µg /mL, 4 µg /mL, and 8 µg/mL concentrations.

Standard solution: To accurately transfer about 20 mg of tetracycline hydrochloride SQR to a 100 mL volumetric flask by using 70 mL of 0.01 M hydrochloric acid. To stir and top up to volume with the same solvent. To successively dilute by using sterile water to obtain 2 µg /mL, 4 µg /mL and 8 µg/mL concentrations.

Procedure: to add 20 mL of culture medium number 11 in each plate, wait to solidify, add 5 mL of 2% inoculum, and proceed as described in *Microbiological test of antibiotics*, by adding 0.2 mL of freshly prepared solutions to the cylinders.

B. To proceed as described in the *Ultraviolet absorption spectrophotometry (5.2.14)*. To prepare 0.05% solution (w/v) of the sample in 0.01 M hydrochloric acid. To prepare a standard solution at similar concentration by using the same solvent. To dilute 3 mL of each solution to 100 mL with 0.25 M sodium hydroxide. To make it homogeneous and let it rest for 6 minutes. To simultaneously prepare the blank by diluting 3 mL of 0.01 M hydrochloric acid to 100 mL with 0.25 M sodium hydroxide. To measure the absorbances of the solutions at 380 nm, by using the blank to adjust zero. To calculate the content of $C_{22}H_{24}N_2O_8 \cdot HCl$ in the sample from the readings obtained.

C. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 365 nm; 150 mm chromatograph column and 4.5 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), at room temperature; flow of *Mobile phase* of 0.8 mL/min.

Mobile phase: mixture of 0.01 M oxalic acid, methanol, and acetonitrile (70:20:10).

Sample solution: to transfer 50 mg of the sample to 100 mL volumetric flask and add 70 mL of *Mobile phase*. To put it in ultrasound for 15 minutes and top up with the same solvent. To transfer an aliquot equivalent to 5.0 mL to 25 mL volumetric flask and top up with the same solvent, thus obtaining 0.1 mg/mL solution.

Standard solution: To transfer 25 mg of tetracycline hydrochloride SQR to 50 mL volumetric flask and add 35 mL of *Mobile phase*. To put it in ultrasound for 15 minutes and top up with the same solvent. To transfer an aliquot equivalent to 5.0 mL to 25 mL volumetric flask and top up with the same solvent, thus obtaining 0.1 mg/mL solution.

Resolution Solution: to prepare a solution containing 100 µg/mL tetracycline hydrochloride SQR and 25 µg/mL 4-epianidrotetracycline hydrochloride SQR by using *Mobile phase* as solvent.

To inject 20 µL of *Resolution solution*. The resolution between 4-epianidrotetracycline and tetracycline is greater than 2. To inject replicates of 20 µL of *Standard solution*. The relative standard deviation for the replicate areas under the peaks recorded is not greater than 2.0%.

Tolerance: not less than 80% (Q) of the amount stated of $C_{22}H_{24}N_2O_8 \cdot HCl$ dissolves within 60 minutes (90 minutes to 500 mg capsules).

Procedure: to separately inject 20 µL of *Sample and standard solutions*, record the chromatograms, and measure the area under the peaks. To calculate the content of $C_{22}H_{24}N_2O_8 \cdot HCl$ from the sample, from the responses obtained for the *Sample and standard solutions*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antimicrobial.

TETRACYCLINE HYDROCHLORIDE, CAPSULES

It contains at least 90.0 % and at most 125 % of the amount stated of $C_{22}H_{24}N_2O_8 \cdot HCl$.

IDENTIFICATION

A. To weigh and make the content of the capsules homogeneous. To use amount of powder equivalent to 25 mg of tetracycline hydrochloride, add 25 mL of methanol, and let it rest for 20 minutes. To filter and evaporate the filtrate

in a water bath to obtain a residue. The infrared absorption spectrum (5.2.14) of the resulting residue, previously desiccated in an oven at 60 °C, under reduced pressure to obtain constant weight and dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the tetracycline hydrochloride SQR spectrum similarly prepared.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage method C*, corresponds to that of main peak of *Standard solution*.

C. To add 5 mL of sulfuric acid to 2 mg of the residue obtained in test **A**. *Identification*. Reddish-violet color is produced. Should 2.5 mL of water be added to this solution, it becomes yellow.

D. The residue obtained in test **A**. *Identification* responds to the reactions of chloride ion (5.3.1.1).

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Disintegration test (5.1.4.1). Within 45 minutes.

Uniformity of dosage units (5.1.6). It complies with the test. To proceed as described in *Dosage method A*.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL

Equipment: blades, 75 rpm

Time: 60 minutes (90 minutes to 500 mg capsules).

Procedure: after testing, remove aliquot from the dissolution medium, filter, and dilute with water to obtain suitable concentration. To measure the absorbances of the solutions at 276 nm (5.2.14), by using water to adjust zero. To calculate the amount of $C_{22}H_{24}N_2O_8 \cdot HCl$ dissolved in the medium, by comparing the readings obtained with that one related to 0.0015% tetracycline hydrochloride SQR solution prepared in the same solvent.

PURITY TESTS

Limit for 4-epianidrotetracycline hydrochloride.

To proceed as described in *Dosage method C*. To prepare the solutions as described below.

Solution (1) to use sample solution described in method **C**, *Dosage*.

Solution (2): 10 µg /mL 4-epianidrotetracycline hydrochloride SQR solution in *Mobile phase*.

To separately inject 20 µL of *Solutions (1)* and *(2)*, record the chromatograms, and measure the areas under the peaks. The area of any peak corresponding to 4-epianidrotetracycline in the chromatogram obtained with *Solution (1)* is not greater than the area under the main peak obtained with *Solution (2)*. At most 3.0%.

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 60 °C under reduced pressure up to 5 mm of Hg for 3 hours to obtain constant weight. At most 2.0%.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

To proceed as described in *Microbiological test of antibiotics (5.5.3.3)* by using cylinders with the agar diffusion method.

Micro-organism: Staphylococcus epidermidis ATCC 12228.

Culture media: culture medium number 1, to maintain the micro-organism; sterile saline solution to the standardization of the inoculum; and culture medium number 1, to the base layer and inoculum preparation.

Sample solution: to accurately transfer about 20 mg of sample to a 100 mL volumetric flask by using 70 mL of 0.01 M hydrochloric acid. To stir and top up with the same solvent. To successively dilute by using sterile water to obtain 2 µg/mL, 4 µg/mL, and 8 µg/mL concentrations.

Standard solution: to accurately transfer about 20 mg of tetracycline hydrochloride SQR to a 100 mL volumetric flask by using 70 mL of 0.01 M hydrochloric acid. To stir and top up with the same solvent. To successively dilute by using sterile water to obtain 2 µg/mL, 4 µg/mL, and 8 µg/mL concentrations.

Procedure: to add 20 mL of culture medium number 1 in each plate, wait to solidify, add 5 mL of 2% inoculum, and proceed as described in *Microbiological test of antibiotics (5.5.3.3)*, by adding 0.2 mL of freshly prepared solutions to the cylinders. To calculate the potency of the sample, in µg of tetracycline hydrochloride per milligram, from the potency of the standard and the responses obtained with the *Standard solution* and the *Sample solution*. I have added.

B. To proceed as described in the *Ultraviolet absorption spectrophotometry (5.2.14)*. To prepare 0.05% solution (w/v) of the sample in 0.01 M hydrochloric acid. To prepare a standard solution at similar concentration by using

the same solvent. To dilute 3 mL of each solution to 100 mL with 0.25 M sodium hydroxide. To make it homogeneous and let it rest for 6 minutes. To simultaneously prepare the blank by diluting 3 mL of 0.01 M hydrochloric acid to 100 mL with 0.25 M sodium hydroxide. To measure the absorbances of the solutions at 380 nm, by using the blank to adjust zero. To calculate the amount of $C_{22}H_{24}N_2O_8 \cdot HCl$ in the capsules, in µg per milligram, from the potency of the standard and the readings obtained.

C. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 365 nm; 150 mm chromatograph column and 4.5 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), at room temperature; flow of *Mobile phase* of 0.8 mL/min.

Mobile phase: mixture of 0.01 M oxalic acid, methanol, and acetonitrile (70:20:10).

Sample solution: to transfer 50 mg of the sample to 100 mL volumetric flask, and add 70 mL of *Mobile phase*. To put it in ultrasound for 15 minutes and top up with the same solvent. To transfer an aliquot equivalent to 5 mL to 25 mL volumetric flask and top up with the same solvent, thus obtaining 0.1 mg/mL solution.

Standard solution: To transfer 25 mg of tetracycline hydrochloride SQR to 50 mL volumetric flask, and add 35 mL of *Mobile phase*. To put it in ultrasound for 15 minutes and top up with the same solvent.

To transfer an aliquot equivalent to 5 mL to 25 mL volumetric flask and top up with the same solvent, thus obtaining 0.1 mg/mL solution.

Resolution Solution: to prepare a solution containing 100 µg/mL tetracycline hydrochloride SQR and 25 µg/mL 4-epianidrotetracycline hydrochloride SQR by using the *Mobile phase* as solvent.

To inject 20 µL of *Resolution solution*. The resolution between 4-epianidrotetracycline and tetracycline is not less than 2. To inject replicates of 20 µL of *Standard solution*. The relative standard deviation for the replicate areas for the peaks recorded is not greater than 2.0%.

Procedure: to separately inject 20 µL of *Sample and standard solutions*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_{22}H_{24}N_2O_8 \cdot HCl$ from the capsules, from the responses obtained for the *Sample* and *standard solutions*.

PACKAGING AND STORAGE

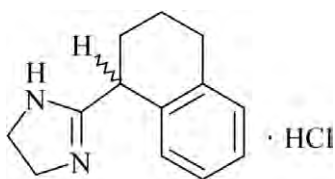
In opaque containers, tightly closed, protected from light.

LABELLING

To comply with the legislation in force.

HYDROCHLORIDE, TETRYZOLINE

Tetracyclini hydrochloridum



$C_{13}H_{16}N_2 \cdot HCl$; 236.74

tetryzoline hydrochloride; 08484

4,5-dihydro-2-(1,2,3,4-tetrahydro-1-naphthalenyl) -
1H-imidazol hydrochloride (1:1)

[522-48-5]

It contains at least 98.0% and at most 100.5% of $C_{13}H_{16}N_2 \cdot HCl$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white or almost white, and odorless powder.

Solubility. It is easily soluble in water and ethanol, very slightly soluble in chloroform; practically insoluble in ethyl ether.

Physical and chemical constants.

Melting range (5.2.2): 253 °C to 259 °C.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the tetryzoline hydrochloride SQR spectrum similarly prepared.

B. The ultraviolet absorption spectra (5.2.14), from 200 nm to 400 nm, 0.025% solution (w/v) in water, exhibits maximum at 264 nm and 271 nm, similar to those observed in the spectrum of a similar solution of tetryzoline hydrochloride SQR.

C. The 0.5% aqueous solution (w/v) responds to the reactions of chloride ion (5.3.1.1).

PURITY TESTS

Heavy Metals (5.3.2.3). To dissolve 0.4 g of the sample in 23 mL of water and add 2 mL of diluted acetic acid. At most 0.005% (50 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 105 °C for 2 hours. At most 1.0%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To accurately weigh about 0.4 g of the sample, transfer to a 250 mL erlenmeyer flask, and dissolve in 60 mL of glacial acetic acid, by heating, if necessary. To add 5 mL of acetic anhydride, 5 mL of mercury acetate SR, and a drop of quinidine red SI. To titrate with 0.1 M SV perchloric acid. To perform sample blank correction and adjust it as required. Each mL of 0.1 M SV perchloric acid is equal to 23.674 mg of $C_{13}H_{16}N_2 \cdot HCl$.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

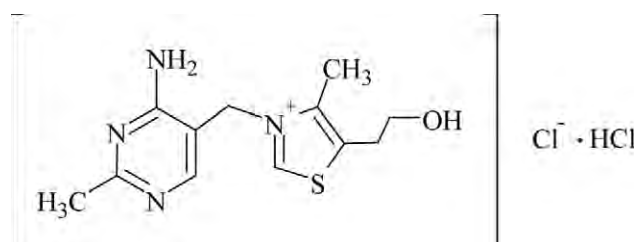
To comply with the legislation in force.

THERAPEUTIC CLASS

Adrenergic (nasal).

HYDROCHLORIDE, THIAMINE

Thiaini hydrochloridum



$C_{12}H_{17}ClN_4OS \cdot HCl$; 337.27

thiamine hydrochloride; 08511 3 - [(4-amino-2-methyl-5-pyrimidinyl methyl) -5 - (2-hydroxyethyl)-4-methylthiazolium chloride hydrochloride (1:1:1) [67-03-8]

It contains at least 98.0% and at most 102.0% of $C_{12}H_{17}ClN_4OS \cdot HCl$, in relation

DESCRIPTION

Physical characteristics. White crystals or crystalline powder with typical odor. When exposed to air, it rapidly absorbs about 4% of water.

Solubility. Freely soluble in water, soluble in glycerol, slightly soluble in ethanol, insoluble in ethyl ether and benzene.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated at 105°C for 2 hours, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to

those observed in the thiamine hydrochloride SQR spectrum similarly prepared.

B. To dissolve 5 mg of the sample in 4 mL of water, add 2 mL of sodium hydroxide SR, 1 mL of potassium ferrocyanide SR, and 5 mL of isobutyl alcohol. To vigorously stir for 2 minutes and let it rest for 30 minutes. The alcoholic layer shows intense blue fluorescence, but the sharper when viewed under ultraviolet light. The fluorescence disappears by acidification, and it reappears by alkalization.

C. To dissolve 5 mg of the sample in 1 mL of water, and add 1 mL of lead acetate SR and 1 mL sodium hydroxide SR. The mixture develops yellow color. To heat in water bath for a few minutes. The color gradually darkens and turns into black precipitate of lead sulfide.

D. To dissolve 0.1 g of the sample in 10 mL of water and divide into 4 fractions. To separately react each fraction with 0.5 mL of the following solutions: a) mercuric chloride SR (white precipitate is produced), b) iodine SR (red-brown precipitate is produced), c), mercury potassium iodide

SR (pale yellow precipitate is produced), d) trinitrophenol SR (yellow precipitate is produced). To filter the precipitate produced with trinitrophenol SR, wash the residue with water, and dry it at 105 °C for 30 minutes. The resulting solid melts at 206 °C - 208 °C with decomposition and darkening after agglomeration at 200 °C.

E. It responds to the reactions of chloride ion (5.3.1.1).

PURITY TESTS

pH (5.2.19). 2.17 to 3.4. To determine in 1% aqueous solution (w/v).

Absorption of light. The absorbance of 10% aqueous solution (w/v) after filtration through fine porosity sintered funnel, measured at 400 nm, does not exceed 0.025.

Chromatographic purity. To proceed as described in method **B**. *Dosage*, except for using flow of *Mobile phase* of 0.75 mL/min. To prepare the *Sample solution* as described below.

Sample solution: to dissolve 10 mg of the sample in *Mobile phase* and top up to 10 mL with the same solvent, to obtain 1.0 mg/mL solution.

Procedure: to inject 10 µL of *Sample solution* and record the chromatogram for at least 3 times the retention time of the main peak. To measure the areas under the peaks. The sum of the area under the secondary peaks is not greater than 1.0% of the total area of all peaks in the chromatogram. To disregard the peaks related to solvent.

Nitrate. To add 2 mL of sulfuric acid to 2 mL of 2% solution (w/v), cool down, and add 2 mL of ferrous sulfate SR. No brown ring is produced at the junction of both layers.

Sulfates (5.3.2.2). To determine in 0.5 g of the sample. At most 0.03% (300 ppm).

Heavy metals (5.3.2.3). At most 0.002% (20 ppm).

Water (5.2.20.1). To determine in 0.4 g of the sample. At most 5.0%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.2%.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Titration in non-aqueous medium (5.3.3.5)*. To dissolve 0.15 g of the sample in 5 mL of anhydrous formic acid. To add 65 mL of anhydrous acetic acid and, by stirring, 10 mL of mercuric acetate SR. To titrate with 0.1 M SV perchloric acid, to potentiometrically determine the endpoint. Each mL of 0.1 M SV perchloric acid is equal to 16.864 mg of $C_{12}H_{17}ClN_4OS.HCl$.

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 254 nm; 150 mm chromatograph column and 4 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (3 µm to 10 µm), at room temperature; flow of *Mobile phase* of 1 mL/min.

Solution A: 0.005 M sodium 1-octane sulfonate solution in 1% acetic acid (v/v).

Solution B: mixture of methanol and acetonitrile (3:2).

Mobile phase: mixture of Solution A and Solution B (60:40).

Internal standard solution: To transfer 2 mL of methyl benzoate to 100 mL volumetric flask, top up with methanol, and make it homogeneous.

Sample solution: To accurately weigh about 0.2 g of the sample, transfer it to a 100 mL volumetric flask, top up with *Mobile phase*, and make it homogeneous. To transfer 10 mL to 50 mL volumetric flask, add 5 mL of *Internal standard solution*, top up with *Mobile phase*, make it homogeneous.

Standard solution: to dissolve accurately weighed amount of thiamine hydrochloride SQR in *Mobile phase*, to obtain a 1 mg/mL solution. To transfer 20 mL to 50 mL volumetric flask, add 5 mL of *Internal standard solution*, top up with *Mobile phase*, and make it homogeneous.

The column efficiency for the peak corresponding to thiamine is not less than 1500 theoretical plates/column. The tailing factor for the peak corresponding to thiamine is not greater than 2.0. The resolution between peaks corresponding to thiamine and methyl benzoate is not less than 4.0.

The relative standard deviation for replicate areas of the peaks recorded is not greater than 2.0 %.

Procedure: to separately inject 10 µL of *Standard solution* and *Sample solution*, record the chromatograms, and measure the areas under the peaks corresponding to thiamine and methyl benzoate. To calculate the content of $C_{12}H_{17}ClN_4OS.HCl$ from the sample, from the responses obtained for relationship thiamine/methyl benzoate with the *Sample* and *Standard solutions*.

PACKAGING AND STORAGE

In nonmetallic containers, tightly closed, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Vitamin B complex.

HYDROCHLORIDE, THIAMIN TABLETS

It contains at least 92.5% and at most 107.5% of the amount stated of $C_{12}H_{17}ClN_4OS.HCl$.

IDENTIFICATION

A. The retention time of the main peak of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

B. To weigh and pulverize the tablets. To dissolve amount of powder equivalent to 10 mg of thiamine hydrochloride in 10 mL of water and filter it. To separate 2 portions of 2 mL of the filtrate. To add 1.27% iodine solution (w/v) to the first portion of the filtrate. A reddish-brown precipitate is produced. To add mercuric chloride SR to the second portion of the filtrate. A white precipitate is produced, which responds to the chloride test (5.3.1.1.).

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL

Equipment: blades, 50 rpm

Time: 45 minutes

Procedure: after testing, remove aliquot from the dissolution medium and dilute, if necessary, with *Dissolution medium* to obtain suitable concentration. To measure the absorbances at 246 nm (5.2.14), by using the same solvent to adjust zero. To calculate the amount of $C_{12}H_{17}ClN_4OS.HCl$ dissolved in the medium, by comparing the readings obtained with 0.002% thiamine hydrochloride SQR solution (w/v) prepared in the same solvent.

Tolerance: not less than 75% (Q) of the stated amount of $C_{12}H_{17}ClN_4OS.HCl$ dissolves within 45 minutes.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Titration in non-aqueous medium (5.3.3.5)*. To weigh and pulverize 20 tablets. To use the amount of powder equivalent to 150 mg of thiamine. By stirring, add 5 mL of anhydrous formic acid, 65 mL of glacial acetic acid, and 10 mL of 6% mercury acetate (w/v) in glacial acetic acid. To titrate with 0.1 M SV perchloric acid. To potentiometrically determine the endpoint. Each mL of 0.1 M SV perchloric acid is equal to 16.860 mg of $C_{12}H_{17}ClN_4OS.HCl$.

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 246 nm; 125 mm chromatograph column and 4.0 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm); flow of *Mobile phase* of 1.0 mL/min.

Mobile phase: to dissolve 1 g of sodium heptane sulphonate in a mixture of 180 mL of methanol and 10 mL of triethylamine. To top up to 1000 mL with water and adjust pH to 3.2 with phosphoric acid.

Standard solution: it contains 0.06 mg/mL thiamine hydrochloride SQR in 0.005 M hydrochloric acid.

Sample solution: for tablets containing less than 10 mg of thiamine hydrochloride. To weigh and pulverize the tablets. To dissolve amount equivalent to 6 mg of thiamine hydrochloride in 5 mL of 0.1 M hydrochloric acid and 50 mL of water. To stir for 20 minutes, dilute to 100 mL with water, and filter it.

Sample solution: for tablets containing amount equal or greater than 10 mg of thiamine hydrochloride. To weigh and pulverize the tablets. To dissolve amount equivalent to 30 mg of thiamine hydrochloride in 25 mL of 0.1 M hydrochloric acid and 250 mL of water. To stir for 20 minutes, dilute to 500 mL with water, and filter it.

Procedure: to separately inject 20 µL of *Sample* and *standard solutions*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of $C_{12}H_{17}ClN_4OS \cdot HCl$ in the tablets, from the responses obtained for the *Sample* and *Standard solutions*.

PACKAGING AND STORAGE

In nonmetallic containers, protected from light.

LABELLING

To comply with the legislation in force.

HYDROCHLORIDE, THIAMINE INJECTABLE SOLUTION

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_{12}H_{17}ClN_4OS \cdot HCl$. Sterile solution of thiamine hydrochloride in water for injection.

IDENTIFICATION

The identification test **B**. may be omitted if the tests **A**. and **C**. are conducted.

A. To dissolve the volume of the injectable solution equivalent to 0.1 g of thiamine hydrochloride in 10 mL of distilled water and divide it into 4 portions. To separately react the solution, in each fraction, with 0.5 mL of mercuric chloride SR, iodine SR, mercury potassium iodide SR, and trinitrophenol SR, thus obtaining respectively white, red-brown, light yellow, and yellow precipitates.

B. To dilute portion of injectable solution with water to obtain 10 mg/mL thiamine hydrochloride. To add 5 mL of 0.5 M sodium hydroxide to 0.5 mL of this solution. Then, add 0.5 mL of potassium ferricyanide and 5 mL of isobutyl alcohol, vigorously stir for 2 minutes, and let it rest for 30 minutes. The alcoholic layer should show intense blue fluorescence, but the sharper when viewed under ultraviolet light. The fluorescence disappears by acidification, and it reappears by alkalization.

C. It responds to the reactions of chloride ion (5.3.1.1).

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

pH (5.2.19). 2.5 to 4.5.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

Bacterial endotoxins (5.5.2.2). At most 3.5 UE/mg of thiamine hydrochloride.

DOSAGE

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 254 nm; 250 mm chromatograph column and 3.9 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm); flow of *Mobile phase* of 1 mL/min.

Mobile phase: to prepare a filtered and degassed mixture of 0.04 M monobasic potassium phosphate and methanol (55:45).

Internal standard solution: to prepare a methylparaben solution in *Mobile phase* at 0.1 mg/mL concentration.

Sample solution: to transfer equal volume of injectable solution to obtain a solution containing 0.5 mg/mL of thiamine hydrochloride. To pipette 10 mL of the resulting solution and 10 mL of *Internal standard solution* to 100 mL volumetric flask, top up with *Mobile phase*, and stir.

Standard solution: to prepare a thiamine hydrochloride solution SQR in *Mobile phase* with a 0.5 mg/mL concentration. To pipette 10 mL of the resulting solution and 10 mL of *Internal standard solution* to 100 mL volumetric flask, top up with *Mobile phase*, and stir, to obtain a 50 µg/mL solution.

To inject replicates of 20 µL of the *Standard solution*. The relative retention times are about 0.3 to thiamine and 1.0 to methylparaben. The resolution between thiamine and methylparaben peaks should not be less than 6.0. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2.0 %.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the amount, in mg, of thiamine hydrochloride in each mL of the injectable solution.

PACKAGING AND STORAGE

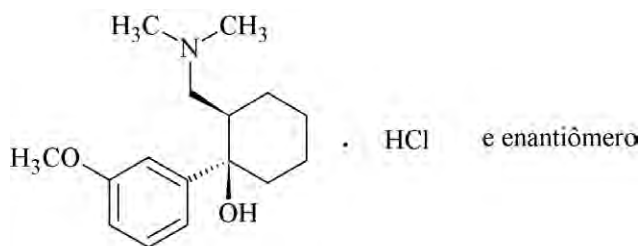
In type I glass containers, protected from light.

LABELLING

To comply with the legislation in force.

HYDROCHLORIDE, TRAMADOL

Ramadoli hydrochloridum



$C_{16}H_{25}NO_2 \cdot HCl$; 299.84

tramadol hydrochloride; 08807

(1 *R*, 2 *R*) -*rel*-2- [(dimethylamino) methyl] -1 - (3 - methoxyphenyl) cyclohexanol hydrochloride (1:1) [36282-47-0]

It contains at least 99.0% and at most 101.0% of $C_{16}H_{25}NO_2 \cdot HCl$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. Crystalline, white, and odorless powder.

Solubility. Soluble in water, ethanol, and methanol; very slightly soluble acetone.

Physical and chemical constants.

Melting range (5.2.2): 180 °C to 184 °C.

Specific rotation (5.2.8): - 0.10 to + 0.10. To determine in 5% aqueous solution (w/v).

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the tramadol hydrochloride SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14), ranging from 250 nm to 300 nm, of 0.1mg/mL solution, exhibits absorption maximum at 270 nm, similar to that observed in the tramadol hydrochloride SQR.

C. It responds to the reactions of chloride ion (5.3.1.1).

PURITY TESTS

Appearance of solution. The 5% solution (w/v) in water is clear (5.2.25) and colorless (5.2.12).

Acidity or alkalinity. To dissolve 1 g of the sample in 20 mL of water and add 0.2 mL of methyl red SI and 0.2 mL of 0.01 *M* hydrochloric acid. Red color is developed. To add 0.4 mL of 0.01 *M* sodium hydroxide. Yellow color is developed.

Related substances. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 270 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (3 µm to 10 µm), flow of *Mobile phase* of 1.0 mL/min.

Mobile phase: to use a mixture of 0.2% trichloroacetic acid (w/v) and acetonitrile (705:295).

Sample solution: to dissolve accurately weighed amount of the sample in *Mobile phase* to obtain a 1.5 mg/mL solution.

Standard solution: to dissolve accurately weighed amount of tramadol hydrochloride SQR in *Mobile phase* to obtain a 0.003 mg/mL solution.

Resolution solution: to accurately transfer about 5 mg of tramadol hydrochloride related substance A SQR ((1*RS*, 2*SR*) -2 - [(dimethylamino) methyl] -1 - (3 - methoxyphenyl) cyclohexanol) and 4 mL of the *Sample solution* to a 100 mL volumetric flask and top up with *Mobile phase*.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution* and record the chromatograms. The relative retention time is 1.0 to tramadol hydrochloride (retention time: approximately 5 minutes) and about 0.85 to tramadol hydrochloride related substance A. The resolution between tramadol hydrochloride and tramadol hydrochloride related substance A peaks is at least 2.0. In the chromatogram obtained with *Sample solution*, the area under the tramadol hydrochloride related substance A peak is not greater than the area obtained with the main peak of *Standard solution* (0.2%). Any other impurity recorded in the chromatogram of the *Sample solution* does not have an area greater than 0.5 times the area of the main peak obtained with the *Standard solution* (0.1%).

Heavy Metals (5.3.2.3). To use the *Method I*. To dissolve 2 g of the sample in 20 mL of water. To determine in 12 mL of the solution obtained and proceed as described in *Limit test for heavy metals*. At most 0.002% (20 ppm).

Water (5.2.20.1). To determine in 1 g of the sample. At most 0.5%.

Sulfated Ashes (5.2.10). To determine in 1g of the sample. At most 0.1%.

DOSAGE

To accurately weigh about 0.25 g of the sample and dissolve in 80 mL of acetic anhydride. To titrate with 0.1 *M* SV perchloric acid, to potentiometrically determine the endpoint. Each mL of 0.1 *M* SV perchloric acid is equal to 29.984 mg of $C_{16}H_{25}NO_2 \cdot HCl$.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Analgesic.

HYDROCHLORIDE, TRAMADOL INJECTABLE SOLUTION

It contains at least 95.0 % and at most 105.0 % of the amount stated of $C_{16}H_{25}NO_2 \cdot HCl$. Sterile solution in water for injection.

IDENTIFICATION

A. The ultraviolet absorption spectrum (5.2.14), ranging from 250 nm to 300 nm, of sample solution obtained in *Dosage* exhibits absorption maximum at 270 nm, similar to those observed in the standard solution spectrum.

B. It responds to the reactions of chloride ion (5.3.1.1).

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test. 5.5 to 6.3.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test. To use direct inoculum method or membrane filtration method.

Bacterial endotoxins (5.5.2.2). At most 7.0 UE/ mL of tramadol hydrochloride.

DOSAGE

To proceed as described in the *Ultraviolet absorption spectrophotometry* (5.2.14). To transfer injectable solution equivalent to 50 mg of tramadol hydrochloride to 100 mL volumetric flask and top up with water. To transfer 10 mL to 50 mL volumetric flask and top up with water, thus obtaining 0.01% concentration (w/v). To prepare standard solution at the same concentration, by using the same solvent. To measure the absorbances of the resulting solutions at 270 nm by using the same solvent to adjust zero. To calculate the amount of $C_{16}H_{25}NO_2 \cdot HCl$ in the injectable solution from the readings obtained.

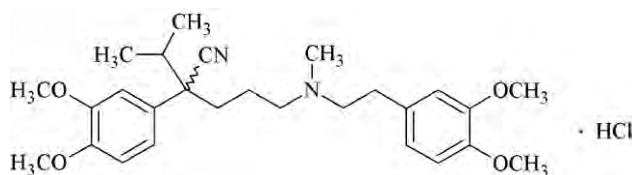
PACKAGING AND STORAGE

In type I glass containers, protected from light, in dry and cool place.

LABELLING

To comply with the legislation in force.

HYDROCHLORIDE, VERAPAMIL Verapamili hydrochloridum



$C_{27}H_{38}N_2O_4 \cdot HCl$; 491.06

verapamil hydrochloride; 09119

α -[3 - [[2 - (3,4-dimethoxyphenyl) ethyl] methylamino] propyl] -3, 4- dimethoxy- α -(1-methylethyl)-benzene

acetonitrile hydrochloride (1:1)

[152-11-4]

It contains at least 99.0% and at most 100.5% of CHN_2O_4HCl , in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or almost white, crystalline powder; odorless or almost odorless.

Solubility. Soluble in water, very soluble in chloroform, sparingly soluble in ethanol.

Physical and chemical constants.

Melting range (5.2.2): 140 °C to 144 °C.

IDENTIFICATION

The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the verapamil hydrochloride SQR spectrum similarly prepared.

The ultraviolet absorption spectrum (5.2.14), ranging from 210 nm to 340 nm, of 0.002% solution (w/v) in 0.01 M hydrochloric acid, exhibits maxima at 229 nm and 278 nm. The ratio between the absorbance values measured at 278 nm and 229 nm is between 0.35 and 0.39.

To add 0.2 mL of 5% mercury chloride (II) (w/v) to 2 mL of 15% aqueous solution (w/v) of the sample. White precipitate is produced.

To add 0.5 mL of 3 M sulfuric acid (w/v) and 0.2 mL of 1.0% potassium permanganate to 2 mL of 1% aqueous solution of the sample (w/v). Violet precipitate is produced, which rapidly dissolves and results in a pale yellow solution.

It responds to the reactions of chloride ion (5.3.1.1).

PURITY TESTS

pH (5.2.19). 4.5 to 6.5. To determine in 5% solution (w/v) in carbon dioxide-free water.

Related substances. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 278 nm; 125 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (3 µm to 5 µm), kept at room temperature; flow of *Mobile phase* of 0.9 mL/min.

Acetate buffer: 0.015 M sodium acetate aqueous solution containing 3.3% glacial acid acetic (v/v).

Mobile phase: mixture of *Acetate buffer*, acetonitrile, and 2-amino heptane (70:30:0.5).

Solution (1): 1.9 mg/mL solution of the sample in *Mobile phase*.

Solution (2): 5.6 µg/mL verapamil hydrochloride SQR solution in *Mobile phase*.

Solution (3): 9.4 µg/mL verapamil hydrochloride SQR solution in *Mobile phase*.

Resolution solution: to dissolve sufficient amount of verapamil hydrochloride SQR and α-[2 - [[2 - (3,4-dimethoxyphenyl) ethyl] methylamino] ethyl] -3, 4- dimethoxy- α-(1-methylethyl)-benzene acetonitrile monohydrochloride (verapamil related compound B) SQR in *Mobile phase*, thus obtaining resolution solution with 1.9 and 1.5 mg/mL concentration already known, respectively.

The retention times are approximately 0.88 to verapamil related compound B and 1.0 to verapamil. The resolution between verapamil related compound B and verapamil peaks is greater than 1.5. The relative standard deviation for injection replicates is not greater than 2.0%.

Procedure: to separately inject 10 µL of each solution, record the chromatograms at least 4 times the retention time of the main peak, and measure the areas under the peaks. The sum of the areas under the peaks obtained with *Solution (1)*, except the main peak's one, is not greater than the area under the main peak obtained with *Solution (3)* (0.5%). The sum of any individual peak obtained with *Solution (1)*, except the main peak's one, is not greater than the area under the main peak obtained with *Solution (2)* (0.3%).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 105 °C for 2 hours. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. To dissolve 0.4 g of the sample in 40 mL of glacial acetic acid, add 5 mL of acetic anhydride and 10 mL of 6% mercury acetate (w/v) in glacial acetic acid. To titrate with 0.1 M SV perchloric acid, to potentiometrically determine the endpoint. To perform sample blank correction and adjust it as required. Each mL of 0.1 M SV perchloric acid is equal to 49.106 mg of C₂₇H₃₈N₂O₄.HCl.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Anti-arrhythmic.

HYDROCHLORIDE, VERAPAMIL TABLETS

It contains at least 90.0 % and at most 110 % of the amount stated of C₂₇H₃₈N₂O₄.HCl.

IDENTIFICATION

The identification tests C. and D. may be omitted if the tests A. and B. are conducted.

A. To weigh and pulverize the tablets. To transfer amount of powder equivalent to 25 mg of verapamil hydrochloride to a separatory funnel. To add 25 mL of water and stir for 30 minutes. To add 1 mL of M sodium hydroxide and extract with 25 mL of chloroform, by stirring for 10 minutes. To filter through filter paper containing anhydrous sodium sulfate and evaporate to dryness. To dry at 105 °C for 2 hours. The infrared absorption spectrum (5.2.14) of the residue, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the verapamil hydrochloride SQR spectrum similarly prepared.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method **B.** corresponds to that of main peak of *Standard solution*.

C. To weigh and pulverize the tablets. To transfer amount of powder equivalent to 0.1 g of verapamil hydrochloride to a beaker. To add 10 mL of methylene chloride and make it homogeneous. To filter, evaporate the filtrate to dryness, and dissolve the residue in 10 mL of water. To add 0.2 mL of 5% mercury (II) solution (w/v) to 2 mL of the resulting solution. White precipitate is produced.

D. To add 0.5 mL of 3 *M* sulfuric acid and 0.2 mL of 1.0% potassium permanganate (w/v) to 2 mL of the solution obtained in test **C**. *Identification*. Violet precipitate is produced, which dissolves rapidly and produces a pale yellow solution.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). To use 900 mL of 0.1 *M* hydrochloric acid as disintegration medium. Within 30 minutes.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 *M* hydrochloric acid, 900 mL

Equipment: blades, 50 rpm

Time: 30 minutes

Procedure: after testing, remove aliquot from the dissolution medium, filter, and dilute, if necessary, with 0.1 *M* hydrochloric acid to obtain suitable concentration. To measure the absorbance at 278 nm and 300 nm (**5.2.14**), by using the same solvent to adjust zero. To calculate the amount of $C_{27}H_{38}N_2O_4 \cdot HCl$ dissolved in the medium, by the difference between measurements at 278 nm and 300 nm, by comparing the readings obtained with the verapamil hydrochloride SQR solution at the same concentration prepared in the same solvent.

Tolerance: not less than 75% (Q) of the stated amount of $C_{27}H_{38}N_2O_4 \cdot HCl$ dissolves within 30 minutes.

PURITY TESTS

Chromatographic Purity. To proceed as described in *Dosage* method **B**. To prepare the solutions as described below.

Solution (1): to weigh and pulverize the tablets. To transfer a sufficient amount of powder to 25 mL volumetric flask, to prepare a 1.9 mg/mL solution. To add 15 mL of *Mobile phase* and mechanically stir for 15 minutes. To top up to volume with the *Mobile phase*, make it homogeneous, and filter it.

Solution (2): to dissolve accurately weighed amount of verapamil hydrochloride SQR in *Mobile phase* to obtain a 5.6 µg/mL solution.

Solution (3): to dissolve accurately weighed amount of verapamil hydrochloride SQR in *Mobile phase* to obtain a 9.4 µg/mL solution.

Procedure: To separately inject 10 µL of each solution. To record the chromatograms and measure the areas under the peaks. The sum of the areas under the peaks obtained with *Solution (1)*, except that of verapamil hydrochloride, is not greater than the area under the peak obtained with *Solution (3)* (0.5%). No peak has an area greater than that of the peak obtained with *Solution (2)* (0.3%).

Water (5.2.20.1). At most 4.0%.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. To weigh and pulverize 20 tablets. To transfer amount of powder equivalent to 100 mg of verapamil hydrochloride to 250 mL volumetric flask

and dissolve in 0.01 *M* hydrochloric acid by stirring. To top up the volume with the same solvent. To filter it. To transfer 10 mL of the filtrate to a 100 mL volumetric flask and top up with 0.01 *M* hydrochloric acid. To prepare standard solution under the same conditions. To measure the absorbances of the solutions at 278 nm, by using 0.1 *M* hydrochloric acid to adjust zero. To calculate the amount of $C_{27}H_{38}N_2O_4 \cdot HCl$ in the tablets, from the readings obtained.

B. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 278 nm; 150 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at room temperature; flow of *Mobile phase* of 0.8 mL/min.

Acetate buffer: aqueous solution of 0.01 *M* sodium acetate containing 3.3% glacial acetic acid (v/v).

Mobile phase: mixture of *Acetate buffer*, acetonitrile, and diethylamine (65:35:1). To filter and degas.

Sample solution: To weigh and pulverize 20 tablets. To transfer amount of powder equivalent to 35 mg of verapamil hydrochloride to 50 mL volumetric flask and add 30 mL of *Mobile phase*. To mechanically stir for 15 minutes. To top up to volume with *Mobile phase*, make it homogeneous, and filter it, to obtain a 70 µg/mL solution.

Standard solution: to dissolve accurately weighed amount of verapamil hydrochloride SQR in *Mobile phase*, to obtain a 70 µg/mL solution.

Resolution solution: to dissolve amount of verapamil hydrochloride SQR and α -[2 - [[2 - (3,4-dimethoxyphenyl) ethyl] methylamino] ethyl] -3, 4- dimethoxy- α -(1-methylethyl)-benzene acetonitrile monohydrochloride (verapamil related compound B) SQR in *Mobile phase*, thus obtaining 70 µg/mL solution for each compound.

To inject replicates of 10 µL of the *Resolution solution*. The relative retention times are about 0.88 to verapamil related compound B and 1.0 to verapamil hydrochloride. The resolution between verapamil related compound B and verapamil hydrochloride peaks is not less than 1.5. The standard deviation between injection replicates is not greater than 2.0%.

Procedure: to separately inject 10 µL of *Standard solution* and *Sample solution*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of $C_{27}H_{38}N_2O_4 \cdot HCl$ in the tablets, from the responses obtained for the *Sample solution* and *Standard solutions*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

HYDROCHLORIDE, VERAPAMIL INJECTABLE SOLUTION

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_{27}H_{38}N_2O_4 \cdot HCl$.

IDENTIFICATION

The identification tests C. and D. may be omitted if the tests A. and B. are conducted.

A. To dilute a volume of the sample containing the equivalent of 10 mg of verapamil hydrochloride in 5 mL of 0.1 M hydrochloric acid, extract with 5 mL of ethyl ether, discard the extract, and alkalize the aqueous phase by using potassium carbonate sesquihydrate. To extract with 5 mL of ethyl ether, filter the ether phase through anhydrous sodium sulfate, and evaporate to dryness. The infrared absorption spectrum (5.2.14) of the sample has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the verapamil hydrochloride SQR spectrum similarly prepared.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage method B.* corresponds to that of main peak of *Standard solution*.

C. To add 0.2 mL of 5% mercury chloride (II) (w/v) to a injectable solution containing 5 mg of verapamil hydrochloride. White precipitate is produced.

D. To add 0.5 mL of 3 M sulfuric acid and 0.2 mL of 1.0% potassium permanganate (w/v) to a injectable solution containing 5 mg of verapamil hydrochloride. Violet precipitate is produced, which dissolves rapidly and produces a pale yellow solution.

CHARACTERISTICS

Determination of volume (5.1.2).

pH (5.2.19). 4.0 to 6.5.

It complies with the test for injectables with small volumes.

PURITY TESTS

Related substances. To proceed as described in *Dosage method B.* To prepare the solutions as described below.

Solution (1): solution of the sample containing 2.5 mg/mL of verapamil hydrochloride.

Solution (2): to dissolve accurately weighed amount of verapamil hydrochloride SQR in *Mobile phase* to obtain a 5.6 µg/mL solution already known.

Solution (3): to dissolve accurately weighed amount of verapamil hydrochloride SQR in *Mobile phase* to obtain a 9.4 µg/mL solution already known.

Procedure: to separately inject 10 µL of *Solution (1)* 10 µL of *Solution (2)* and 10 µL of *Solution (3)*. To record the chromatograms and measure the responses of the peaks. The sum of the responses of the peaks, except that of verapamil hydrochloride obtained in *Solution (1)* is not greater than the peak obtained with *Solution (3)* (0.5%) and no peak is greater than the response of the peak obtained with *Solution (2)* (0.3%).

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

Bacterial endotoxins (5.5.2.2). At most 16.7 UE/mg of verapamil hydrochloride.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in the *Ultraviolet absorption spectrophotometry (5.2.14)*. To transfer a volume of a solution containing 5 mg of verapamil hydrochloride to 100 mL volumetric flask and dissolve with 0.01 M hydrochloric acid. To prepare standard solution under the same conditions. To measure the absorbances of the solutions at 278 nm, by using 0.1 M hydrochloric acid to adjust zero.

To calculate the amount of $C_{27}H_{38}N_2O_4 \cdot HCl$ in the tablets, from the readings obtained. Alternatively, make the calculations by taking into account that $A (1\%, 1\text{ cm}) = 118$ at 278, in 0.01 *M* hydrochloric acid.

B. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 278 nm; 150 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μm), at room temperature; flow of *Mobile phase* of 0.8 mL/min.

Acetate buffer: aqueous solution of 0.015 *M* sodium acetate containing 3.3% glacial acetic acid (v/v).

Mobile phase: to prepare a mixture of *Acetate buffer*, acetonitrile, and diethylamine (65:35:1.0). To filter and degas it.

Sample solution: to quantitatively dilute the injectable solution, if necessary, with *Mobile phase* so as to obtain a 70 $\mu\text{g/mL}$ solution.

Standard solution: to dissolve accurately weighed amount of verapamil hydrochloride SQR in *Mobile phase* to obtain a 70 $\mu\text{g/mL}$ solution.

Resolution solution: to dissolve amount of verapamil hydrochloride SQR and α -[2 - [[2 - (3,4 - dimethoxyphenyl) ethyl] methylamino] ethyl] -3,4-dimethoxy- α -(1-methylethyl) benzene acetonitrile monohydrochloride SQR (verapamil related compound B) in *Mobile phase* thus obtaining 70 $\mu\text{g/mL}$ solution for each compound.

To inject 10 μL of *Solution resolution* and record the responses of the peaks. The relative retention times

are about 0.88 to verapamil related compound B and 1.0 to verapamil hydrochloride SQR. The resolution between verapamil related compound B and verapamil hydrochloride SQR is greater than 1.5. The standard deviation between injection replicates is not greater than 2.0%.

Procedure: to separately inject 10 μL of internal *Standard solution* and *Sample solution*. To calculate the content of $C_{27}H_{38}N_2O_4 \cdot HCl$ in the injectable solution, from the responses obtained for the *Sample solution* and *Standard solutions*.

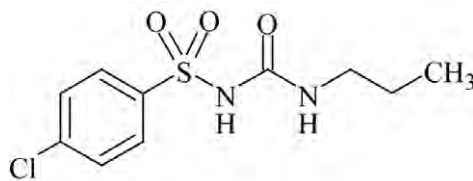
PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

CHLORPROPAMIDE Chlorpropamidum



$C_{10}H_{13}ClN_2O_3S$; 276.74
chlorpropamide; 02505
4-Chloro-*N*-[(propylamino) carbonyl]
benzenesulfonamide [94-20-2]

It contains at least 99.0% and at most 100.5% of $C_{10}H_{13}ClN_2O_3S$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White crystalline powder.

Solubility. Practically insoluble in water, freely soluble in methylene chloride and acetone, soluble in ethanol. Soluble in alkalis.

Physical and chemical constants.

Melting range (5.2.2): 126 °C to 130 °C.

IDENTIFICATION

The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the chlorpropamide SQR spectrum similarly prepared. Should the obtained spectra be different, dissolve the standard and the sample in methylene chloride, evaporate to dryness and carry out a new spectrum by using the residue.

B. To prepare a 0.01% sample of the solution (w/v) in methanol and dilute 10 mL of this solution in 100 mL of 0.01 *M* hydrochloric acid. The ultraviolet absorption spectrum (5.2.14) from 220 nm to 350 nm of the sample solution exhibits maximum absorption at 232 nm and the absorbance at 232 nm is 0.57 to 0.63.

C. To mix 0.1 g of the sample with 2 g of anhydrous sodium carbonate and warm to obtain a strong red color to remain for 10 minutes. To cool down and extract the residue with approximately 5 mL of water. To dilute to 10 mL with water and filter it. To acidify 2 mL of the resulting solution with nitric acid and add 0.4 mL of silver nitrate SR. To make it homogeneous and let it rest. A cheesy white precipitate should be formed. Let the precipitate decant and wash it 3 times with 1 mL of water. To protect from direct light, by discarding the supernatant solution because it is not completely clear. To suspend the precipitate in 2 mL of water and add 1.5 mL of ammonia. The precipitate readily

dissolves with the possible presence of some large sized particles that slowly dissolve.

PURITY TESTS

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel as support and a mixture of ammonia, cyclohexane, methanol, and methylene chloride (11.5:30:50:100) as mobile phase. To separately apply to the plate 5 µl of each of the solutions freshly prepared, as described below.

Solution (1): to dissolve 0.5 g of the sample in acetone and dilute to 10 mL with the same solvent.

Solution (2): to dissolve 15 mg of chloro benzene sulfonamide (chlorpropamide impurity A) SQR in acetone and dilute to 10 mL with the same solvent.

Solution (3): to dissolve 15 mg of chlorpropamide impurity B SQR in acetone and dilute to 100 mL with the same solvent.

Solution (4): to dilute 0.3 mL of *Solution (1)* to 100 mL with acetone.

Solution (5): to dilute 5 mL of *Solution (3)* to 15 mL with acetone.

Solution (6): to dissolve 0.1 g of the sample, 5mg of 4-chloro benzene sulfonamide (chlorpropamide impurity A) SQR and 5 mg of chlorpropamide impurity B SQR in acetone and dilute to 10 mL with the same solvent.

To develop the chromatogram over 15 cm of the plate. To dry the plate in an oven at 110 °C for 10 minutes. To put a mixture of 1 volume of hydrochloric acid, 1 volume of water, and 2 volumes of a 5% potassium permanganate solution (w/v) at the bottom of the chromatographic tank. To close the tank and wait for 15 minutes. To put the plate in contact with chlorine vapor for 2 minutes. To remove the plate and put it in a current of cold air until the chlorine in excess is removed and the cover area below the point of application does not show blue color with a drop of iodine starch SR1. To nebulize with iodine starch SR1. In the chromatogram obtained with *Solution (1)*, any spot corresponding to impurity A is not more intense than the spot obtained with *Solution (2)* (0.3%); any spot corresponding to impurity B is not more intense than that obtained in the chromatogram of the *Solution (3)* (0.3%); any spot, except the principal spot, and any spot corresponding to impurities A and B is not more intense than the spot in the chromatogram obtained with *Solution (4)* (0.3%); no more than 2 spots are more intense than the spot obtained in the chromatogram of *Solution (5)* (0.1%). The test is not valid unless the chromatogram obtained with *Solution (6)* shows 3 clearly separated spots with R_f values about 0.4 to chlorpropamide, 0.6 to impurity A, and 0.9 to impurity B.

Heavy metals (5.3.2.3). To prepare a 10% solution (w/v) of the sample in acetone or dioxane containing at least

15% (v/v) of water. To proceed as described in *Method III*. To use the *Lead standard solution* (2 ppm Pb). At most 0.002% (20 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 100 °C - 105 °C to obtain constant weight. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.4%.

DOSAGE

To employ one of the methods described below.

A. To dissolve 0.25 g in 50 mL of ethanol, previously neutralized in the presence of phenolphthalein SI solution and add 25 mL of water. Titrate with 0.1 M SV sodium hydroxide to obtain pink color. Each mL of 0.1 M SV sodium hydroxide is equal to 27.674 of C₁₀H₁₃ClN₂O₃S.

B. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 240 nm; 150 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at room temperature; flow of *Mobile phase* of 1.5 mL/min.

Mobile phase: to prepare a mixture of equal volumes of diluted glacial acetic acid (1:100) and acetonitrile. To filter and degas the mixture.

Note: do not exceed 50% of acetonitrile. To adjust, if necessary.

Sample solution: to transfer accurately weighed amount of 50 mg of the sample to a 100 mL volumetric flask and top up with *Mobile phase* and mix it. To transfer 10 mL of this solution to another 100 mL volumetric flask, top up with *Mobile phase*, and mix it.

Standard solution: to accurately weigh and dissolve an amount of chlorpropamide SQR in the *Mobile phase*, and appropriately dilute with *Mobile phase*, so as to obtain a 0.05 mg/mL solution.

To inject replicates of 20 µL of the *Standard solution*. The retention time is about 2.2 minutes to chlorpropamide. The tailing factor is not greater than 1.5, and the relative standard deviation for injection replicate is not greater than 2.0%.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of C₁₀H₁₃ClN₂O₃S from the sample, from the responses obtained for the *Sample solution* and *Standard solutions*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Hypoglycemic.

CHLORPROPAMIDE TABLETS

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_{10}H_{13}ClN_2O_3S$.

IDENTIFICATION

A. To weigh and pulverize the tablets. To transfer amount of powder equivalent to 1 g chlorpropamide, add 4 mL of acetone, filter, and evaporate to dryness. The infrared absorption spectrum (5.2.14) of the residue, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the chlorpropamide SQR spectrum similarly prepared.

B. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF_{254} silica gel as support and a mixture of methylene chloride, methanol, cyclohexane, and ammonium hydroxide (100:50:30:10) as mobile phase. To separately apply to the plate 10 μ L of each of the solutions freshly prepared, as described below.

Solution (1): to mix a amount of powder from the tablet equivalent to 100 mg of chlorpropamide, 20 mL of *M* hydrochloric acid and extract with 50 mL of chloroform. To filter the solution and wash the cotton with chloroform in a beaker. To evaporate to dryness the chloroform in a water bath. To dry the residue at 105 °C for 1 hour. From the resulting residue, prepare a 1 mg/mL solution in acetone.

Solution (2): to prepare a 1 mg/mL chlorpropamide SQR solution in acetone.

To develop the chromatogram. To remove the plate from the chromatographic tank and allow to air dry. To examine under ultraviolet light (254 nm). The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL

Equipment: blades, 50 rpm

Time: 60 minutes

Procedure: after testing, remove aliquot from the dissolution medium, filter, and dilute, if necessary, with 0.1 *M* hydrochloric acid, to obtain suitable concentration. To measure the absorbances at 230 nm, by using the same solvent to adjust zero. To calculate the amount of $C_{10}H_{13}ClN_2O_3S$ dissolved in the medium, by comparing the readings obtained with chlorpropamide SQR solution (w/v) prepared in 0.1 *M* hydrochloric acid.

Note: a volume of ethanol that does not exceed 10% (v/v) can be added in the preparation of the standard solution to dissolve the chlorpropamide SQR.

Tolerance: not less than 75% (Q) of the stated amount of $C_{10}H_{13}ClN_2O_3S$ dissolves within 60 minutes.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

A. To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To weigh and pulverize 20 tablets. To transfer amount of powder equivalent to 0.25 g of chlorpropamide by stirring with 40 mL of methanol for 20 minutes, and top up to 50 mL volumetric flask with the same solvent. To filter and dilute the sample up to a 0.001% concentration (w/v) with 0.1 *M* hydrochloric acid. To prepare a chlorpropamide SQR at the same concentration. To measure the absorbances of the solutions at 232 nm.

To calculate the amount of $C_{10}H_{13}ClN_2O_3S$ in the tablets, from the readings obtained. Alternatively, to consider *A* (1%, 1 cm) = 598 at 232 nm.

B. To proceed as described in *Dosage*, method **B.** in monograph *Chlorpropamide*. To prepare the *Sample solution* as described below.

Sample solution: to weigh and triturate 20 tablets. To transfer amount of powder equivalent to 50 mg of chlorpropamide to 100 mL volumetric flask. To add 75 mL of *Mobile phase*, mix for 10 minutes, and top up with *Mobile phase*.

To filter it by discarding the first 10 mL of the filtrate. To pipette 10 mL of the filtrate to another 100 mL volumetric flask, top up with *Mobile phase*, and mix it.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_{10}H_{13}ClN_2O_3S$ from the sample, from the responses obtained for the *Sample solution* and *Standard solutions*.

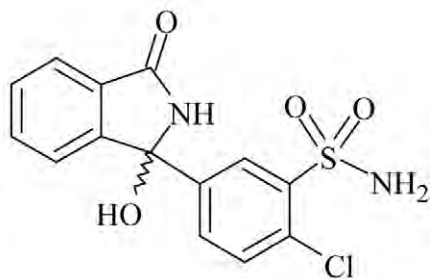
PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

CHLORTHALIDONE Chlorthalidonum



$C_{14}H_{11}ClN_2O_4S$; 338.77
chlorthalidone; 02510

2-Chloro-5-(2,3-dihydro-1-hydroxy-3-oxo-1*H*-isoindol-1-yl)-benzenesulfonamide
[77-36-1]

It contains at least 98.0% and at most 102.0% of $C_{14}H_{11}ClN_2O_4S$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or yellowish-white powder.

Solubility. Practically insoluble in water, soluble in acetone and methanol, sparingly soluble in ethanol, and practically insoluble in methylene chloride. Soluble in dilute alkalis.

Physical and chemical constants. *Specific rotation* (5.2.8): -0.15° to $+0.15^\circ$. To determine in 1% solution (w/v) in methanol.

IDENTIFICATION

The identification tests **B.**, **C.**, **D.**, and **E.** may be omitted if the test **A.** is conducted.

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the chlorthalidone SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14) ranging from 230 nm to 340 nm, of 0.01% solution (w/v) in ethanol, exhibits maxima and minima only in the same wavelength of a similar chlorthalidone SQR solution. The ratio between the absorbance values measured at 284 nm and 275 nm is between 0.73 and 0.88.

C. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF_{254} silica gel as support and a mixture of water and ethyl acetate (1.5:98.5) as mobile phase. To separately apply to the plate 5 µl of each of the solutions freshly prepared, as described below.

Solution (1): 1 mg/mL solution of the sample in mixture of water and ethyl acetate (1.5:98.5).

Solution (2): to dissolve 10 mg of chlorthalidone SQR in acetone and dilute to 10 mL in a mixture of water and ethyl acetate (1.5:98.5).

Solution (3): to dissolve 10 mg of chlorthalidone SQR and 10 mg of hydrochlorothiazide SQR in acetone and dilute to 10 mL in mixture of water and ethyl acetate (1.5:98.5).

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*. The test is valid only if the chromatogram obtained with *Solution (3)* presents two clearly separated spots.

D. To dissolve about 10 mg of the sample in 1 mL of sulfuric acid. An intense yellow color is developed.

E. To proceed as described in *Specific rotation*.

PURITY TESTS

Acidity. To stir 1 g of the sample in 50 mL of acetone and carbon dioxide-free water (1:1) by heating. To cool down. At most 0.75 mL of 0.1 *M* sodium hydroxide is spent to neutralize by potentiometrically determining the endpoint.

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF_{254} silica gel as support and a mixture of toluene, xylene, ammonium hydroxide, dioxane, and isopropyl alcohol (5:10:20:30:30) as mobile phase. To separately apply to the plate 5 µl of each of the solutions freshly prepared, as described below.

Solution (1): to dissolve 200 mg of the sample in a mixture of water and acetone (1:4) and dilute to 5 mL with mobile phase.

Solution (2): to dissolve 20 mg of 2 - (4-chloro-3-sulfamoyl benzoyl) benzoic SQR acid and 20 mg of chlorthalidone SQR in a mixture of water and acetone (1:4) and dilute to 50 mL with mobile phase

Solution (3): to transfer 1 mL of the *Solution (1)* to a 200 mL volumetric flask and top up with a mixture of water and acetone (1:4).

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). Any secondary spot related to 2 - (4-chloro-3-sulfamoyl benzoyl) benzoic SQR acid in the chromatogram obtained with *Solution (1)* is not more intense than that obtained with *Solution (2)* (1%). Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot and the spot in the 2 - (4-chloro-3-sulfamoyl benzoyl) benzoic, is not more intense than that obtained with *Solution (3)* (0.5 %). The test is valid only if the chromatogram obtained with *Solution (2)* shows two principal spots clearly separated.

Chlorides (5.3.2.1). To pulverize 0.3g of the sample. To add 30 mL of water, stir for 5 minutes, and filter it. To use 15 mL of the filtrate to perform *Limit test for chlorides*. To prepare the standard by using 10 mL of solution to 5 ppm of chlorides. At most 350 ppm (0.035%).

To use the *Method IV*.

Heavy metals (5.3.2.3). At most 0.001% (10 ppm).

Loss on drying (5.2.9). To determine in 2 g of the sample. To desiccate in an oven at 105 °C for 4 hours. At most 0.4%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To employ one of the methods described below.

A. To dissolve about 0.2 g, precisely weighed, of the sample in 50 mL of acetone. To titrate with 0.1 M SV tetrabutylammonium hydroxide in nitrogen atmosphere. To potentiometrically determine the endpoint. To perform sample blank correction and adjust it as required. Each mL of 0.1 M SV tetrabutylammonium hydroxide is equal to 33.877 mg of $C_{14}H_{11}ClN_2O_4S$.

B. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 254 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at room temperature; flow of *Mobile phase* of 1 mL/min.

Mobile phase: mixture of 0.01 M dibasic ammonia phosphate and methanol (3:2). To adjust the pH to 5.5 ± 0.1 with phosphoric acid.

Internal standard solution: to dissolve accurately weighed amount of 2,7-naphthalenediol in methanol and quantitatively dilute to obtain a 1 mg/mL solution.

2 - (4-chloro-3-sulfamoyl benzoyl) benzoic acid solution: to dissolve accurately weighed amount of (4-chloro-3-sulfamoyl benzoyl)-benzoic acid SQR in methanol and quantitatively dilute to obtain a 5 µg/mL solution.

Sample solution: to dissolve 50 mg of the sample in methanol and dilute to 50 mL with the same solvent. To transfer 5 mL of this solution to a 50 mL volumetric flask. To add 5 mL of *Internal standard solution* and 10 mL of methanol. To top up with water and make it homogeneous.

Standard solution: to dissolve accurately weighed amount of chlorthalidone SQR in methanol and quantitatively dilute to obtain a 1 mg/mL solution. To transfer 5 mL of this solution to 50 mL volumetric flask. To add 5 mL of *Internal standard solution* and 10 mL of 2-(4-chloro-3-sulfamoyl benzoyl) benzoic acid solution. To top up with water and make it homogeneous.

To inject replicates of 25 µL of the *Standard solution*. The relative retention times are about 0.5 to 2-(4-chloro-3-sulfamoyl benzoyl) benzoic acid, 0.8 to chlorthalidone, and 1.0 to 2,7-naphthalenediol. The resolution between chlorthalidone and 2 - (4-chloro-3-sulfamoyl benzoyl) benzoic acid peaks and between chlorthalidone and 2,7-naphthalenediol peaks is not less than 1.5. The tailing factor for the chlorthalidone and 2 - (4-chloro-3-sulfamoyl benzoyl) benzoic acid peaks is not greater than 2.0.

The relative standard deviation for the replicate areas for the peaks recorded is not greater than 2.0%

Procedure: to separately inject 20 µL of *Standard solution* and *Sample solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_{14}H_{11}ClN_2O_4S$ from the sample, from the responses obtained for the *Sample* and *Standard solutions*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Diuretic.

CHLORTHALIDONE, TABLETS

It contains at least 92.0 % and at most 108.0 % of the amount stated of $C_{14}H_{11}ClN_2O_4S$.

IDENTIFICATION

To weigh and pulverize the tablets. To transfer the amount of powder equivalent to 0.1 g of chlorthalidone to a beaker and dissolve it in 10 mL of acetone. To heat in water bath for 5 minutes. To cool down and filter it. To add 20 mL of water to the filtrate and heat it in water bath for 5 minutes. To gently apply stream of air over the solution to remove the acetone. To cool in an ice bath, filter, and dry the crystals at 105 °C for 4 hours. The dry residue responds to test **A.**, in *Identification*, in the monograph *Chlorthalidone*.

The retention time of the main peak in the chromatogram of the *Sample solution* obtained in method **B.**, *Dosage*, corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness (5.1.3.1). It complies with the test.

Friability (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL *Equipment:* blades, 75 rpm

Time: 60 minutes

Procedure: after testing, remove aliquot from the dissolution medium and dilute, if necessary, with water, to obtain suitable concentration. To measure the absorbances of the solutions at 275 nm (**5.2.14**), by using the same solvent to adjust zero. To calculate the amount of $C_{14}H_{11}ClN_2O_4S$ dissolved in the medium, by comparing the readings obtained with that of 0.5 % chlorthalidone SQR solution (w/v) in methanol. To successively dilute this solution in water to obtain suitable concentration.

Tolerance: not less than 70% (Q) of the stated amount of $C_{14}H_{11}ClN_2O_4S$ dissolves within 60 minutes.

PURITY TESTS

Related substances. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using F_{254} 60 silica gel as support and a mixture of toluene, xylene, ammonium hydroxide, dioxane, and isopropyl alcohol (v/v) (5:10:20:30:30) as mobile phase.

To separately apply to the plate 5 µl of each of the solutions freshly prepared, as described below.

Solution (1): to weigh and pulverize the tablets. To dissolve amount of powder equivalent to 100 mg of chlorthalidone in 5 mL of ethanol. To put it in ultrasound for 15 minutes and centrifuge it. To use the clear supernatant.

Solution (2): to transfer 1 mL of *Solution (1)* to 200 mL volumetric flask and top up with ethanol.

Solution (3): to dissolve amount accurately weighed of 2 - (4-chloro-3-sulfamoyl benzoyl) benzoic SQR acid in ethanol and dilute to obtain 0.02% solution (w/v) in the same solvent.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). Any spot related to 2 - (4-chloro-3-sulfamoyl benzoyl) benzoic acid in the chromatogram obtained with *Solution (1)* is not more intense than that obtained with *Solution (3)* (1%). Any secondary spot in the chromatogram obtained with *Solution (1)* is not more intense than that obtained with *Solution (2)* (0.5%).

DOSAGE

A. To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. To weigh and pulverize 20 tablets. To boil under reflux amount of powder equivalent to 100 mg of chlorthalidone in 30 mL of methanol for 5 minutes. To mechanically stir for 15 minutes. To cool down and filter it. To wash the residue with methanol, add the washings to the filtrate, and dilute to 100 mL with the same solvent. To transfer 5 mL of this solution to a 50 mL volumetric flask, add 2 mL of *M* hydrochloric acid, and top up with methanol. To measure the absorbances of the resulting solutions at 275 nm, by using methanol to adjust zero. To calculate the content of $C_{14}H_{11}ClN_2O_4S$ in the tablets, from the readings obtained. Alternatively, make the calculations by taking into account that $A(1\%, 1\text{ cm}) = 57.4$ at 275 nm.

B. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, according to *Dosage*, method **B.**, in the monograph *Chlorthalidone*. To prepare the *Sample* and *standard solutions* as described below.

Sample solution: To weigh and pulverize 20 tablets. To transfer amount of powder equivalent to 100 mg of chlorthalidone to 100 mL volumetric flask. To add 50 mL of methanol and mechanically stir for 30 minutes. To top up with the same solvent, make it homogeneous, and filter it. To transfer 5 mL of the filtrate to a 50 mL volumetric flask containing 5 mL of the *Internal standard solution* and 10 mL of methanol. To top up with water and make it homogeneous.

Standard solution: to prepare as described in the monograph *Chlorthalidone*. To substitute 10 mL of methanol for 2 - (4-chloro-3-sulfamoyl benzoyl) -benzoic acid solution.

To inject replicates of 25 µL of the *Standard solution*. The resolution between the chlorthalidone and 2,7-naphthalenediol peaks should not

be less than 1.5. The tailing factor to chlorthalidone and 2,7-naphthalenediol peaks should not be greater than 2.0. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2.0 %.

Procedure: to separately inject 25 µL of *Sample* and *standard solutions*, record the chromatograms, and measure the areas under the peaks. To calculate the content of C₁₈H₁₉ClN₄S in the tablets, from the responses obtained for the *Sample solution* and *Standard solutions*.

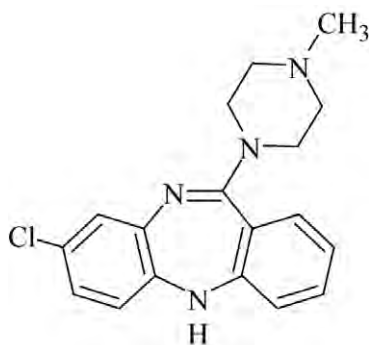
PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

CLOZAPINE Clozapine



C₁₈H₁₉ClN₄; 326.82

clozapine; 02540

8-Chloro-11-(4-methyl-1-piperazinyl)-5H-dibenzo [b, e] [1,4]

diazepine

[5786-21-0]

It contains at least 99.0% and at most 101.0% of C₁₈H₁₉ClN₄ in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Yellow, crystalline powder.

Solubility. Practically insoluble in water, freely soluble in methylene chloride, soluble in ethanol. Soluble in diluted acetic acid.

Physical and chemical constants.

Melting range (5.2.2): 182 °C to 186 °C.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the clozapine SQR spectrum similarly prepared.

B. The principal spot in the chromatogram of *Solution (2)* in the *Related substances* corresponds in position, color, and intensity to that obtained with the *Solution (1)*.

PURITY TESTS

Related substances. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using 0.25 mm silica gel as support and a mixture of chloroform and methanol (3:1) as mobile phase. To separately apply to the plate 20 µL of each of the solutions freshly prepared, as described below.

Solution (1): to dissolve 0.1 g of the sample in ethanol and top up with chloroform.

Solution (2): to transfer 2.5 mg of clozapine SQR to a 25 mL volumetric flask. To dissolve in chloroform and top up with the same solvent.

Solution (3): to transfer 3 mL of *Solution (2)* to a 10 mL volumetric flask and top up with chloroform. Percent for comparing with the 0.3% sample.

Solution (4): to transfer 1 mL of *Solution (2)* to 5 mL volumetric flask and top up with chloroform. Percent for comparing with the 0.2% sample.

Solution (5): to transfer 1 mL of *Solution (2)* to 10 mL volumetric flask and top up with chloroform. Percent for comparing with the 0.1% sample.

Solution (6): to transfer 1 mL of *Solution (2)* to 20 mL volumetric flask and top up with chloroform. Percent for comparing with the 0.05% sample.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm) and compare the intensity of any secondary spot observed in the chromatogram of *Solution (1)* with that of the principal spot in the chromatogram of *Solution (2)*. Any spot in the chromatogram of *Solution (1)* with R_f = 0.82 does not correspond to non 11,11 - (piperazine-1,4-diyl) bis (8-chloro-5H-dibenzo [b, e] [1, 4] diazepine), R_f = 0.67 does not correspond to 8-chloro-5,10-dihydro-11H-dibenzo [b, e] [1,4] diazepine-11-one, and R_f = 0.10 does not correspond to 8-chloro-11-(1-piperazin-1-yl)-5H-dibenzo [b, e] [1,4] diazepine) or more intense than *Solution (3)*, *Solution (4)*, and *Solution (5)*, respectively. Any other secondary spot in the chromatogram of *Solution (1)* is not wider or more intense than the principal spot obtained in the *Solution (5)*. The sum of intensity of all secondary spots obtained in *Solution (1)* is up to 0.6%

Heavy metals (5.3.2.3). To use *Method III*. To prepare a standard with 2 mL of 10 ppm lead (Pb) solution. At most 0.002% (20 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 100 °C - 105°C for 4 hours to obtain constant weight. At most 0.5 %.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To proceed as described in *Titration in non-aqueous medium (5.3.3.5)*. To accurately weigh about 0.1 g of the sample, transfer to a 250 mL erlenmeyer flask, and dissolve in 50 mL of glacial acetic acid. To titrate with 0.1 M SV perchloric acid. To potentiometrically determine the endpoint. Each mL of 0.1 M SV perchloric acid is equal to 16.341 mg of $C_{18}H_{19}ClN_4$.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antipsychotic

CLOZAPINE, TABLETS

It contains at least 90 % and at most 110 % of the amount stated of $C_{18}H_{19}ClN_4$.

IDENTIFICATION

A. The principal spot in the chromatogram of *Solution (2)* in the *Related substances* corresponds in position, color, and intensity to that obtained with the *Solution (1)*.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

Procedure for content uniformity. To transfer each tablet to 1000 mL volumetric flask To add 640 mL of methanol, put it in ultrasound for 10 minutes, and top up with water. To proceed as described in *Dosage* method from "To make it homogeneous..."

DISSOLUTION TEST (5.1.5)

Dissolution medium: acetate buffer pH 4.0, 900 mL *Equipment:* baskets, 100 rpm *Time:* 45 minutes

Procedure: after testing, remove aliquot from the dissolution medium, filter, and dilute, if necessary, with *Dissolution medium*, to obtain suitable concentration. To measure the absorbances of the solutions at 290 nm (5.2.14), by using the same solvent to adjust zero. To calculate the amount of $C_{18}H_{19}ClN_4$ dissolved in the medium, by comparing the readings obtained with 1.11 % clozapine SQR solution (w/v) prepared in the same solvent.

Tolerance: not less than 85% (Q) of the stated amount of $C_{18}H_{19}ClN_4$ dissolves within 45 minutes.

PURITY TESTS

Related substances. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using 0.25mm silica gel as support and a mixture of *n*-heptane, chloroform, absolute ethanol, and ammonium hydroxide (30:30:30:1) as mobile phase. To separately apply to the plate 20 µL of each of the solutions freshly prepared, as described below.

Diluent solution: to prepare a mixture of chloroform and methanol (4:1).

Solution (1): to weigh and pulverize 20 tablets. To transfer equivalent to 125 mg of clozapine to a 25 mL volumetric flask and dissolve it by using 20 mL of *Diluent solution*. To mechanically stir for 15 minutes and top up with *Diluent solution*. To filter and make it homogeneous.

Solution (2): 5 mg/mL clozapine SQR solution of the sample in *Diluent solution*.

Solution (3): to transfer 1 mL of *Solution (2)* to a 200 mL volumetric flask and top up with chloroform. Percent for comparing with the 0.5% sample.

Solution (4): to transfer 1 mL of *Solution (2)* to a 250 mL volumetric flask and top up with chloroform. Percent for comparing with the 0.4% sample.

Solution (5): to transfer 3 mL of *Solution (2)* to a 1000 mL volumetric flask and top up with chloroform. Percent for comparing with the 0.3% sample.

Solution (6): to transfer 1 mL of *Solution (2)* to a 500 mL volumetric flask and top up with chloroform. Percent for comparing with the 0.2% sample.

Solution (7): to transfer 1 mL of *Solution (2)* to 1000 mL volumetric flask and top up with chloroform. Percent for comparing with the 0.1% sample.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light at short wavelength, by comparing the intensity of any secondary spot observed in the chromatogram of *Solution (1)* with that of the principal spot in the chromatogram of *Solutions (2), (3), (4), (5), (6), and (7)*. No other secondary spot in the chromatogram of *Solution (1)* is wider or more intense than the principal spot obtained in the chromatogram of *Solution (3)* (0.5%). The sum of the intensities of all the secondary spots obtained in *Solution (1)* corresponds up to 2.0%.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 257 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at room temperature; flow of *Mobile phase* of 1.0 mL/min.

Mobile phase: mixture of methanol, water, and triethylamine (800:200:0.75).

Sample solution: To weigh and pulverize at least 20 tablets. To transfer amount of powder, exactly weighed, equivalent to 125 mg of clozapine to a 1000 mL volumetric flask, dissolve in 640 mL of methanol, put it in an ultrasound for 10 minutes, top up with water, and make it homogeneous, thus obtaining a 0.125 mg/mL solution.

Standard solution: to dissolve accurately weighed amount of clozapine SQR in methanol to obtain a 0.125 mg/mL solution. The final composition of solvent methanol: water should be about 8:2.

Resolution solution: to accurately transfer about 10 mg of clozapine to a suitable container. To add 5 mL of 0.1 M hydrochloric acid and heat for 2 hours at 90 °C. To transfer this solution to a 100 mL volumetric flask, add 15 mL of water, and top up with methanol. To make it homogeneous. To transfer 10 mL of this preparation to a suitable container, add 10 mL of the *Standard solution*, and mix it.

To inject replicates of 10 µL of the *Standard solution*. The column efficiency should be not less than 1500 theoretical plates, the relative standard deviation for replicate is not greater than 2.0%. To inject 10 µL of *Resolution solu-*

tion. The resolution between clozapine and any other peak should be not less than 1.5.

Procedure: to separately inject 10 µL of *Sample* and *standard solutions*, record the chromatograms, and measure the areas under the peaks. To calculate the content of clozapine (C₁₈H₁₉ClN₄) in the tablets, from the responses obtained for the *Sample solution* and *Standard solution*.

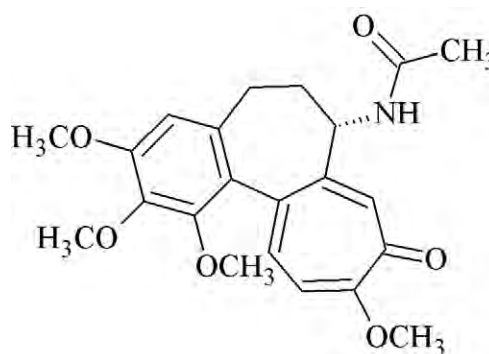
PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

COLCHICINE Colchicinum



C₂₂H₂₅NO₆; 399.44
colchicine; 02567

N - [(7*S*) -5,6,7,9-Tetrahydro-1,2,3,10-tetramethoxy-9-oxobenzene [*a*] heptalen-7-yl] acetamide
[64-86-8]

It contains at least 94.0% and at most 101.0% of C₂₂H₂₅NO₆, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. Amorphous or crystalline pale yellow powder; odorless.

Solubility. Very soluble in water, freely soluble in ethanol and chloroform, slightly soluble in petroleum ether.

Physical and chemical constants.

Melting range (5.2.2): 142 °C to 150 °C (powder), 157 °C (crystal).

Specific rotation (5.2.8): -240° to -250° determined in 1% solution (w/v) in ethanol, in relation to the anhydrous substance.

IDENTIFICATION

A. To dissolve the sample in 0.5 mL of chloroform, disperse in potassium bromide, mix, and firstly evaporate the solvent in a stream of air and then by heating at 80 °C for 60 minutes. The infrared absorption spectrum (5.2.14) of the sample has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the colchicine SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14), from 200 nm to 400 nm, 0.001% solution (w/v) in ethanol has absorption maximum at 243 nm and 350 nm, similar to those observed in the spectrum of a similar solution of colchicine SQR.

C. To dissolve 30 mg of the sample in 1 mL of ethanol and add 0.15 mL of ferric chloride SR. Reddish brown color is produced.

D. To mix 1 mg of colchicine with approximately 0.2 mL of sulfuric acid SR. Yellow color is produced. To add 0.1 mL of nitric acid SR. The solution turns blue-green and turns rapidly to red and finally yellow (almost colorless) color. To add a few drops of sodium hydroxide SR. The solution becomes red.

PURITY TESTS

Appearance of solution. The 0.5% solution (w/v) in water is clear (5.2.25), with no color more intense (5.2.12) than that of a solution prepared by diluting 8.5 mL of the *Color reference solution SC O* in 91.5 mL of 1% hydrochloric acid (w/v).

Acidity or alkalinity. To add 10 mL of the 0.5% sample solution (w/v) in water, 0.1 mL of bromothymol blue SI. There is neither color change nor green color is produced. No more than 0.1 mL of 0.01 M SV sodium hydroxide is required to indicator changes to blue color.

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using HF₂₅₄ silica gel as support and a mixture of concentrated ammonia, chloroform, and acetone (1:25:50) as mobile phase. To separately apply to the plate 10 µL of each of the solutions freshly prepared, as described below.

Solution (1): 10 mg/mL solution of the sample in chloroform.

Solution (2): to dilute 2 mL of *Solution (1)* to 100 mL with chloroform.

Solution (3): to dilute 5 mL of *Solution (2)* to 10 mL with chloroform.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). Any secondary spot obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained in the chromatogram with *Solution (2)* (2%) and not more than a spot is more intense than that obtained in the chromatogram with *Solution (3)* (1 %).

Water (5.2.20.1). To determine in 0.5 g of the sample. At most 2.0%.

Sulfated ashes (5.2.10). To determine in 0.5 g of the sample. At most 0.1%.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Titration in non-aqueous* (5.3.3.5). To accurately weigh about 0.25 g of the sample, dissolve in a mixture of 10 mL of acetic anhydride and 20 mL of toluene, and heat it, if necessary. To titrate with 0.1 M SV perchloric acid, to potentiometrically determine the endpoint. Each mL of 0.1 M SV perchloric acid is equal to 39.940 mg of C₂₂H₂₅NO₆.

B. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 254 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (3 µm to 10 µm), kept at room temperature; flow of *Mobile phase* of 1.0 mL/min.

Mobile phase: mixture of 0.5 M monobasic potassium phosphate, water, and methanol (4.5:45:53). To adjust pH to (5.50 ± 0.05) with phosphoric acid.

Sample solution: 6 µg/mL solution of the sample in a mixture of methanol and water (1:1). To immediately prepare before using it.

Standard solution: 6 µg/mL colchicine SQR solution in a mixture of methanol and water (1:1). To immediately prepare before using it.

The column efficiency is greater than 4500 theoretical plates/meter. The retention time for colchicine is between 5.5 and 9.5. The relative standard deviation for replicate areas of the peaks recorded should not be greater than 2.0%.

Procedure: to separately inject 20 µL of *Sample* and *standard solutions*, record the chromatograms, and measure the areas under the peaks. To calculate the content of C₂₂H₂₅NO₆ from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antigout agent.

COLCHICINE, TABLETS

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_{22}H_{25}NO_6$.

IDENTIFICATION

To weigh and pulverize the tablets. To triturate amount of powder equivalent to 20 mg of colchicine with 20 mL of water. To filter it. To transfer the filtrate to a separatory funnel. To extract with 30 mL of chloroform. To evaporate the chloroform under mild heat to obtain a residue. To proceed as described in *Identification test A.*, in the monograph *Colchicine* by using the resulting residue.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 500 mL

Equipment: basket, 100 rpm

Time: 30 minutes

Procedure: to perform the test without delay under low light, by using low actinism glassware. After the test, proceed as described in method **B. Dosage**, in the monograph *Colchicine*.

Tolerance: not less than 75% (Q) of the stated amount of $C_{22}H_{25}NO_6$ dissolves within 30 minutes.

DOSAGE

To proceed as described in method **B. Dosage**, in the monograph *Colchicine*. To prepare the *Sample solution* as described below.

Sample solution: To weigh and pulverize 20 tablets. To transfer amount of powder equivalent to 0.6 mg of colchicine to a 100 mL volumetric flask, by using 50 mL of a mixture of methanol and water (1:1). To mechanically stir for 15 minutes and top up with the same solvent. To immediately prepare before using it.

Procedure: to separately inject 20 μ L of *Sample* and *standard solutions*, record the chromatograms, and measure the areas under the peaks. To calculate the amount

of $C_{22}H_{25}NO_6$ in the tablets, from the responses obtained for the *Sample solution* and *Standard solutions*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

CRATEGO *Crataegi folium cum flore*

Crataegus spp. - ROSACEAE

The vegetable drug consists of dried, whole or scratched flowering branches of *Crataegus monogyna* Jacq., *Crataegus oxyacantha* L., *Crataegus laevigata* (Poir.) DC., *Crataegus pentagyna* Waldst. et Kit., *Crataegus nigra* Waldst. et Kit., *Crataegus azarolus* L., including leaves and flowers containing at least 1.5% of total flavonoids expressed as hyperoside ($C_{21}H_{20}O_{12}$; 464.4) in relation to the dried drug.

CHARACTERISTICS

Organoleptic characteristics. The dried leaves have a characteristic odor and are tasteless.

MACROSCOPIC DESCRIPTION

Simple leaves with three or more lobes, fractured to lobed, alternate, pilose, and long petiole. Blade with acute base and apex, irregularly serrated edge, "peninervea-type", with secondary ribs starting at an acute angle relative to the midrib and ending at the limb edge; higher ribs form closed areolas with few terminal branches. Pentamerous flowers, small, long-stalked. Calice with lacinia of acute apex, forming a structure with pilose hypanthium hairy and greenish brown color in dried samples. Corolla with white petals, lightly brown in the dehydrated samples, petals free from rounded contour and short nails. Numerous stamens with visible anthers.

MICROSCOPIC DESCRIPTION

Hypostomatic leaves with dorsiventral mesophyll. In front view, the epidermis is covered with cuticle more thickened in adaxial face and has fundamental cells with variable dimensions and sinuous anticlinal walls, with sinuosity more pronounced in lower face. In cross section, the epidermis is uniseriate with voluminous cells in the adaxial face; cyclocytic stomata with typical reniform guard-cells and pronounced thickening in the inner anticlinal; cuticle is concentrically striated to guard-cells over subsidiary cells.

On both sides, there are unicellular, pointed, long trichomes with a thickened wall; at its base occur 7-8 epidermal cells arranged in a rosette, covered with pronounced

accumulation of cuticle. The mesophyll has two or, more rarely, three layers of palisade parenchyma; the spongy parenchyma has elongated cells with short arms, but which allow the formation of wide intercellular spaces. Druzen with eroded and/or misshapen edges of calcium oxalate are common throughout the chlorenchyma, while prismatic crystals (cubic and rhombic) of varying sizes are located near the vascular bundles. The midrib of plano-convex to concave-convex cross-sectional is prominent in the lower face, with 3-4 layers of annular collenchyma strata in this region. The vascular bundle of the midrib is collateral in open arc with lignified fibers at both poles of conducting tissues, the element is surrounded by a sheath of parenchyma cells. According to the leaf analyzed, there may be 1, 2, or 3 bundles. The vascular bundles of smaller caliber are also collateral with fiber calotte at both poles of conducting tissues, surrounded by a parenchymatous sheath. Petiole, in cross section, has plano-convex to concave-convex contour, with uniseriate epidermis covered with thickened cuticle followed by 5-6 strata of annular collenchyma, and conducting tissues organized into a single vascular bundle in open arc with edges slightly high. In some samples there is a small bending on the edges of the arc consisting of phloem only. The epidermis has papillose petals, covered with ornamented cuticle with small projections, also present in the sepals and anthers. The mesophyll of the petals is homogeneous, composed of 10-12 strata in the central-median region and 2-3 strata on the edges and upper third. In the anthers, the endothecium has anticlinal thickening parallel with each other, sometimes woven diagonally. The pollen grains are three colpi and ornamented with small spherical papillae. On the inner face at the base of the sepals there is the floral nectary.

MICROSCOPIC DESCRIPTION OF POWDER

The powder meets all the requirements for the species, unless the macroscopic characters. Characteristics: unicellular trichomes with thickened walls, fragmented or intact; fragments of leaf epidermal with cells arranged in rosette at the base of trichomes; ciclocytic stomata with subsidiary cells with striated cuticle; fundamental cells of the epidermis with sinuous walls, with sinuosity more or less pronounced, depending on the sample and leaf face; fragments of dorsiventral mesophyll with misshapen druzen and prismatic crystals following the vascular bundles.

IDENTIFICATION

A. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using F_{254} silica gel, with 250 μm thickness as support and a mixture of anhydrous formic acid, water, methyl ethyl ketone, and ethyl acetate (10:10:30:50) as mobile phase. To separately apply to the plate, as band, 20 μl of *Solution (1)*, *Solution (2)*, and *Solution (3)* freshly prepared, as described below.

Solution (1): to accurately weigh about 1 g of milled drug (355 μm), add 10 mL of methanol, and heat under reflux for 5 minutes at 65 °C. After cooling down at room tem-

perature, filter under reduced pressure the solution obtained through filter paper.

Solution (2): to dissolve 1 mg of chlorogenic acid in 10 mL of methanol.

Solution (3): to dissolve 1 mg of hyperoside in 5 mL of methanol.

To develop the chromatogram. To remove the plate, dry it in an oven at 100 °C - 105 °C, and nebulized it if still warm with aminoethanol diphenyl borate SR, followed by a 5% macrogol 400 solution (w/v) in methanol. To remove the plate, allow to air dry for 30 minutes, and examine under ultraviolet light (365 nm). Four fluorescent spots in *Solution (1)* are observed, and the top one has a yellowish green color, below there is one with yellow orange color with R_f 0.65 corresponding to hyperoside; a blue color spot corresponding to the chlorogenic acid with R_f 0.53, and the lower spot is yellowish green.

B. To heat under reflux about 3 g of pulverized drug with 60 mL of water for 15 minutes To cool down and filter it. To add 2 drops of hydrochloric acid SR to 2 mL of extract and drip gelatin SR. The appearance of clear precipitate indicates a positive reaction for total tannins.

C. To add 10 mL of distilled water and 2-4 drops of 1% ferric chloride solution (w/v), in ethanol, to 2 mL of the extract obtained in *Identification*, test **B**. The development of dark-gray color indicates a positive reaction for tannins.

D. To add 0.5 mL of 1% vanillin (w/v), in methanol, and 1 mL of hydrochloric acid to 2 mL of the extract obtained in *Identification*, test **B**. The development of dark-gray color indicates a positive reaction for condensed tannins.

E. To add 10 mL of 2 M acetic acid and 5 mL of lead acetate SR to 5 mL of the extract obtained in *Identification*, test **B**. The appearance of whitish precipitate indicates the presence of tannins.

F. To add small pieces of metal magnesium and 1 mL of hydrochloric acid to 5 mL of extract obtained in test **B**. *Identification*. The appearance of red color indicates the presence of flavonoid aglycones.

PURITY TESTS

Foreign matter (5.4.2.2). At most 8.0% of lignified branches and 2.0% of other foreign matter.

Water (5.4.2.3). At most 11.0%.

Total ashes (5.4.2.4). At most 10.0%.

Sulfated ashes (5.4.2.6). At most 12.0 %.

DETERMINATION OF FOAM INDEX (IE)

To accurately transfer about 1 g of the milled vegetable drug (180 µm) to 50 mL erlenmeyer flask containing boiling water. To keep under moderate boil for 15 minutes. To cool down and filter through cotton to 100 mL volumetric flask. To top up to volume through the filter up to 100 mL. To distribute the decoction obtained in 10 test tubes with lid (16 mm diameter and 16 cm height), in a successive series of 1 mL, 2 mL, 3 mL, until 10 mL, and adjust the volume of liquid in each tube to 10 mL of water. To cover the tubes and vigorously stir for 15 seconds in vertical movements, by stirring twice per second. To let it rest for 15 minutes and measure the foam height. Then, add 1 mL of 2 M hydrochloric acid to each tube. If the foam height of all tubes is below 1 cm, the foam index is less than 100. If the foam height after 10 minutes remains equal to or greater than 1 cm, the dilution of the vegetable material in this tube (A) is the index observed. To calculate the foam index, according to the equation:

$$IE = \frac{1000}{A}$$

where

IE = foam index;

A = volume (mL) of decoction used for dilution in the tube, where foam was observed.

The IE for decoction should be at least 100.

DOSAGE

Total flavonoids

To proceed as described in *Visible absorption spectrophotometry* (5.2.14). To prepare the solutions as described below.

Stock solution: to accurately weigh about 0.4 g of powdered drug (250 µm) and transfer to 200 mL erlenmeyer flask and add 40 mL of 60% ethanol (v/v). To put it in water bath at 60 °C for 10 minutes by frequently stirring. To cool down and filter through cotton to 100 mL volumetric flask. To put back the insoluble residue and cotton to same erlenmeyer flask, add 40 mL of 60% ethanol (v/v) and put it again in the water bath for 10 minutes by frequently stirring. To filter the solution through cotton to volumetric flask as previously described. To top up with 60% ethanol (v/v).

Sample solution: to volumetrically transfer 5 mL of *Stock solution* to a 100 mL round bottom flask and evaporate to dryness in a rotary evaporator. To solubilize the residue in 8 mL of mixture of methanol solution and glacial acetic acid (10:100)

and transfer to a 25 mL volumetric flask To wash the round bottom flask with 3 mL of mixture of methanol solution and glacial acetic acid (10:100) and transfer to the volumetric flask as previously described. To add 10 mL of *Reagent solution*. To put it in the ice bath for 10 minutes, to

cool down. Do not allow it to freeze. To top up to volume with anhydrous acetic acid. To immediately put it in ice bath. To remove 10 minutes before the spectrophotometric reading.

Blank solution: to volumetrically transfer 5 mL of *Stock solution* to a 100 mL round bottom flask and evaporate to dryness in a rotary evaporator. To solubilize the residue in 8 mL of mixture of methanol solution and glacial acetic acid (10:100) and transfer to the 25 mL volumetric flask. To wash the round bottom flask with 3 mL of mixture of methanol solution and glacial acetic acid (10:100) and transfer to the volumetric flask as previously described. To add 10 mL of anhydrous formic acid. To put it in the ice bath for 10 minutes, to cool down. Do not allow it to freeze. To top up to volume with anhydrous acetic acid. To immediately put it in ice bath. To remove 10 minutes before the spectrophotometric reading.

Reagent solution: 2.5% boric acid (w/v) and 2% oxalic acid (w/v) in anhydrous formic acid. To solubilize by heating in fume hood.

To measure the absorbance of the *Sample solution* after 30 minutes, at a wavelength of 410 nm. To calculate the percentage of total flavonoids content expressed in hyperoside. To consider the specific absorptivity of hyperoside equal to 405. To calculate the percentage of total flavonoids content according to the equation:

$$H = \frac{Abs \times 1,235}{m}$$

where

H = total flavonoid content expressed in hyperoside;

Abs = absorbance of the *Sample solution*;

m = mass of drug (g) considering the determination of water.

PACKAGING AND STORAGE

In tightly closed container, protected from light and heat.

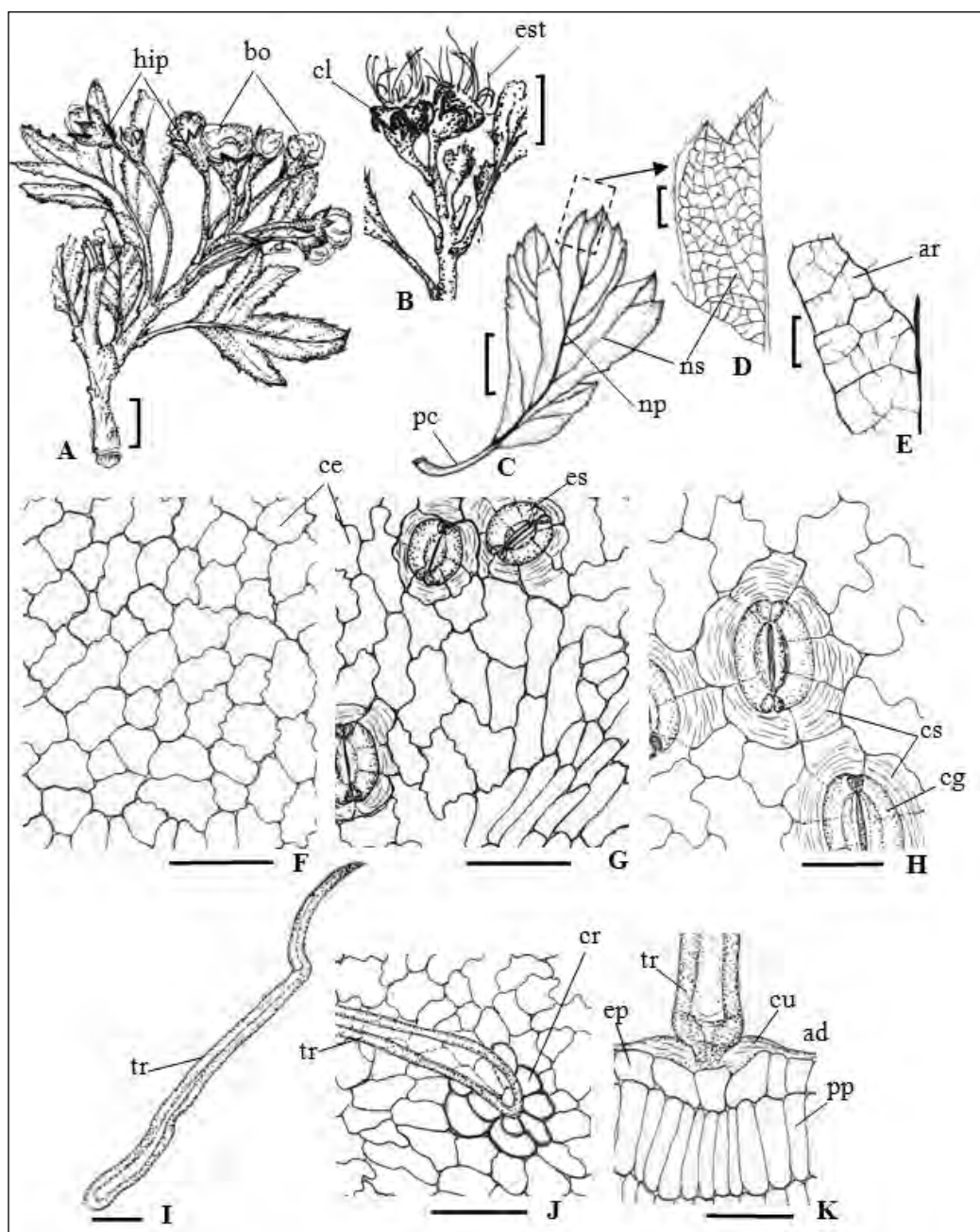


Figure 1 - Macroscopic and microscopic aspects in *Crataegus* spp.

Additional information of **Figure 1**. Scales correspond to 0,5 mm, in **A, B, and C**; 1 mm, in **D**; 0.5 mm, in **E**; 50 µm, in **F, G, I, and J**; 25 µm, in **H and K**.

A — general appearance of a branch in the pre-anthesis phase: hypanthium (hip); bud (bo). **B** — partial detail of a branch after the fall of the corolla: calice (cl), stamens (est). **C** — general appearance of a leaf: petiole (pc); midrib (np), secondary rib (ns). **D** — partial detail of leaf venation highlighted in **C**: secondary rib (ns). **E** — partial detail of areola and vascular endings: areola (ar). **F and G** — frontal view of the leaf adaxial and lower faces, respectively: common epidermal cell (ce); stomata (es). **H** — detail of stomata: guard-cell (cg); subsidiary cell (cs). **I** — leaf tector trichomes: trichome (tr). **J and K** - details of the insert of trichomes in front and transversal view, respectively: trichomes (tr); rosette cells (cr); epidermis (ep), cuticle (cu); adaxial face (ad); palisade parenchyma (pp).

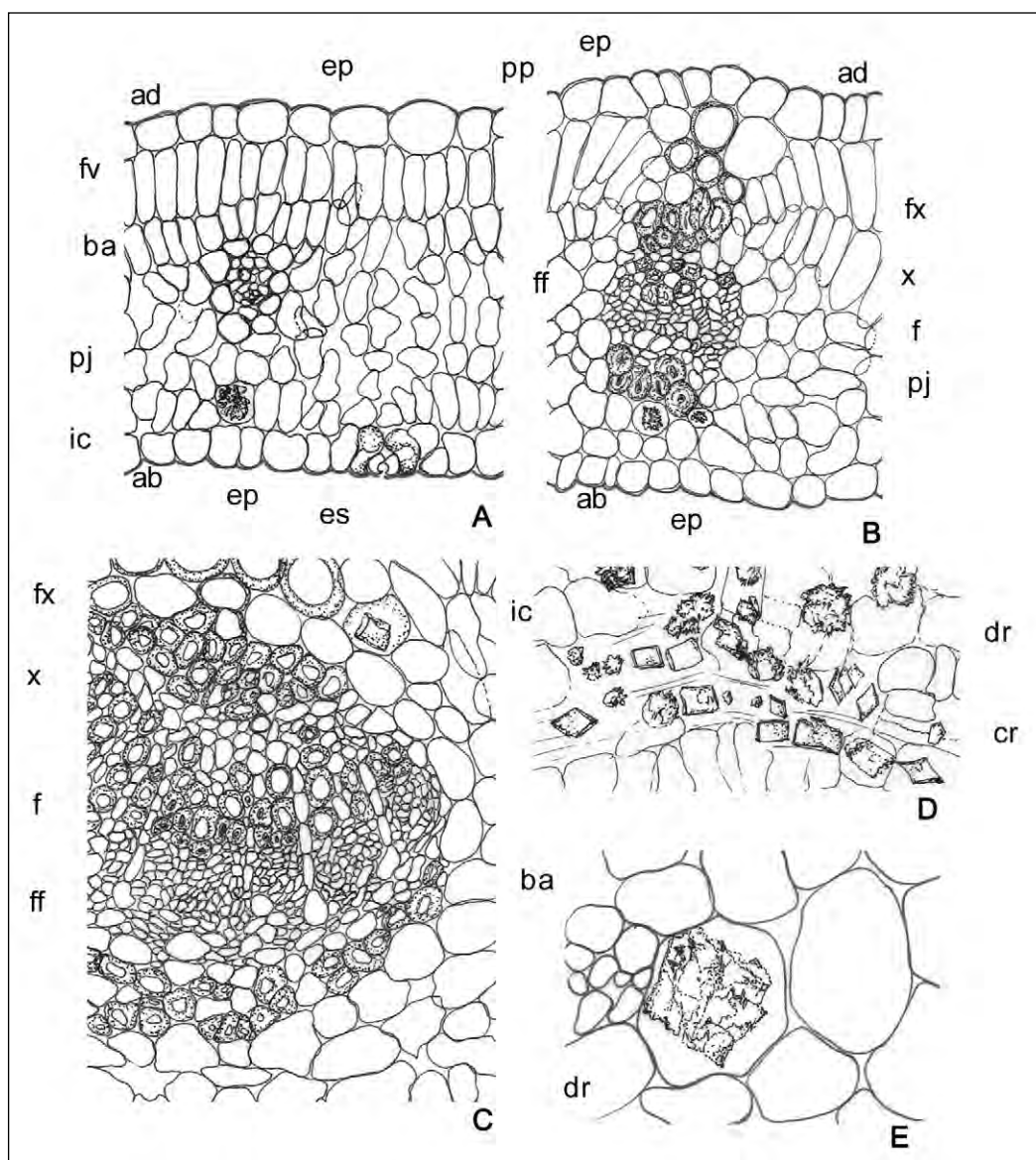


Figure 2 - cross sections of the leaf blade in *Crataegus* spp.

Additional information of **Figure 2**. Scales correspond to 50 μ m in **A**, **B**, **C**, and **D**; in **E**, 25 μ m.

A — detail of median mesophyll with a tertiary vascular bundle: lower face (ab); adaxial face (ad); vascular bundle (fv); vascular bundle sheath (ba); spongy parenchyma (pj); crystal idioblast (ic); epidermis (ep); palisade parenchyma (pp); stomata (es). **B** — detail of a secondary vascular bundle near the leaf edge: lower face (ab); adaxial face (ad); epidermis (ep); palisade parenchyma (pp); phloem fibers (ff); xylem fiber (fx); xylem (x), phloem (f); spongy parenchyma (pj); epidermis (ep). **C** — partial detail of vascular bundle of midrib: xylem fiber (fx); xylem (x), phloem (f); phloem fibers (ff); crystal idioblast (ic); vascular bundle sheath (ba). **D** — fragment of the powder showing crystals near the vascular bundles: crystal idioblast (ic); Druze (dr); prismatic crystal (cr). **E** — detail of a Druze in a fragment of powder: Druze (dr).

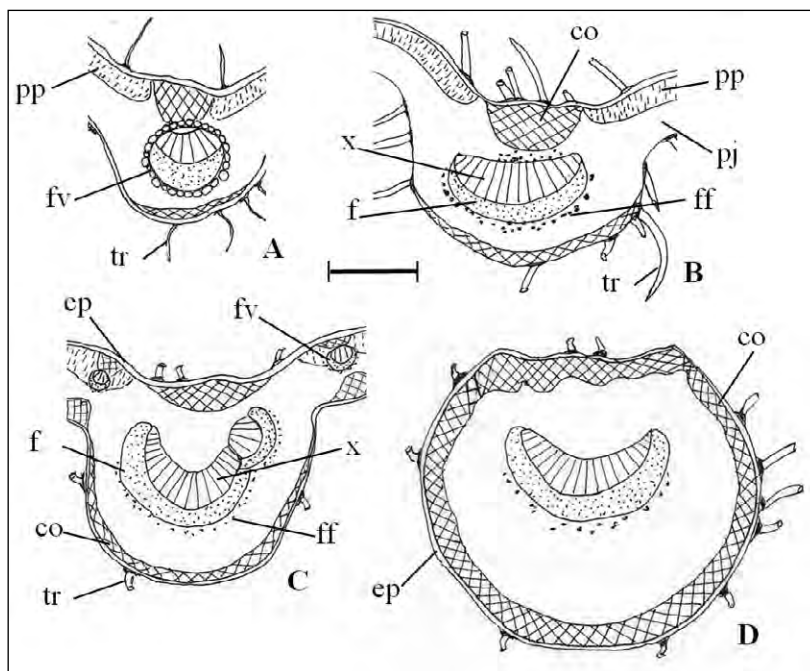


Figure 3 - Diagram of the distribution of tissues in the leaf and petiole, in cross-sections, in *Crataegus* spp.

Additional information of **Figure 3**. Scales correspond to 250 μm in **A**, **B**, **C**, and **D**.

A — apical region of the midrib: palisade parenchyma (pp); vascular bundle (fv); trichomes (tr). **B** — median region of the midrib: xylem (x); phloem (f); collenchyma (co), palisade parenchyma (pp); spongy parenchyma (pj); phloem fibers (ff), trichome (tr). **C** — basal region of the midrib: epidermis (ep); vascular bundle (fv), xylem (x); phloem (f); collenchyma (co), phloem fibers (ff), trichome (tr). **D** — median region of the petiole: collenchyma (co); epidermis (ep).

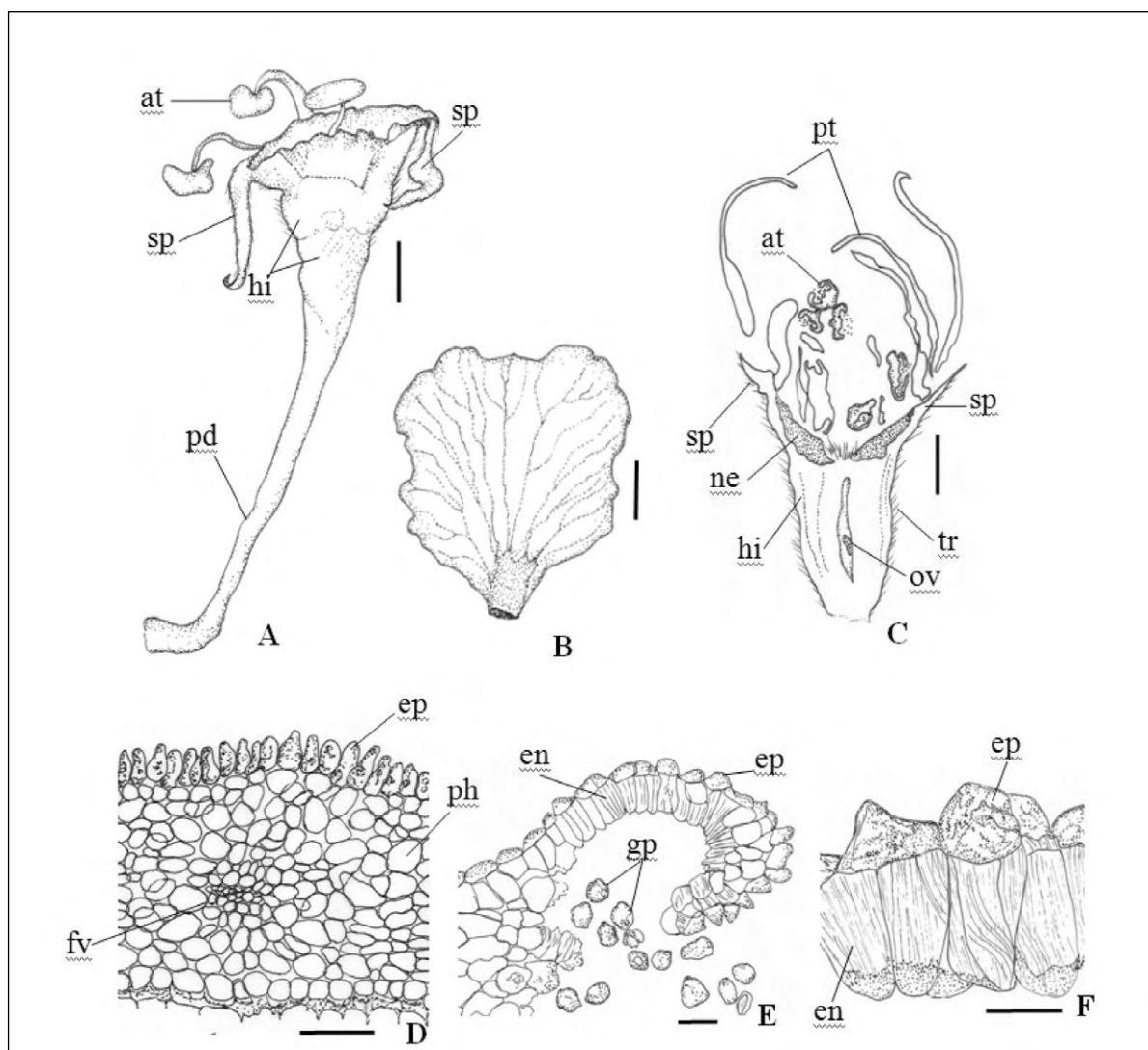


Figure 4 - Macroscopic and microscopic aspects in *Crataegus* spp.

Additional information of **Figure 4**. Scales correspond to 1 mm in **A**, **B**, and **C**, in **D** and **E**, 50 μ m; in **F**, 25 μ m.

A — general appearance of the hypanthium, floral stalk, some sepals and anthers: anther (at); sepal (sp); hypanthium (hi); floral peduncle (pd). **B** — general appearance of a petal. **C** — general appearance of a flower in median longitudinal section: anther (at), petal (pt); sepal (sp); nectary (ne); hypanthium (hi); ovule (ov); trichomes (tr). **D** — partial detail of the base of the petal in cross section: epidermis (ep); homogeneous parenchyma (ph), vascular bundle (fv). **E** and **F** - partial detail of anther and theca wal, respectively, in cross-section: endothecium (en); epidermis (ep); pollen grain (gp).

CURCUMA

Curcumae longae rhizoma

Curcuma longa L. - ZINGIBERACEAE

The vegetable drug consists of the dried rhizomes. It contains at least 2.5% of volatile oil and at least 2.5% of di-cinnamoyl methane derivatives expressed in curcumin ($C_{21}H_{20}O_6$, 368.4)

SCIENTIFIC SYNONYM

Curcuma domestica Valetón

CHARACTERISTICS

Organoleptic characteristics. The drug has faintly aromatic odor, resembling ginger, spicy and slightly bitter taste.

MACROSCOPIC DESCRIPTION

Main oval, oblong, or round rhizomes measuring up to 12 cm in length and up to 5 cm in diameter, elongated and cylindrical side rhizomes, rounded at the ends, measuring from 6 cm to 15 cm long and from 1 cm to 4 cm in diameter, carrying usually small branches. Rhizomes have yellow-brown color, smooth surface, with annular scars from the bases of leaf sheaths, irregular scars from the lateral branches, and small rounded scars in the roots. Brownish lateral roots, paleaceous, striated, from rhizomes. Long down is visible by using lens. Fibrous sheaths can follow the main rhizome. The fracture is smooth, clear, gelatinous, yellow-orange to orange, with scattered light spots, corresponding to the vascular bundles. In cross-section, there are two zones: cortex and central cylinder, separated by endoderm. The cortical region is narrow and more clear, and medulla is well-developed and orange.

MICROSCOPIC DESCRIPTION

In front view, the epidermis has cells of varying shapes and straight and thick walls, with some lipid droplets. The stomata are anomocytic. The down is simple, unicellular to tricellular, long, thick-walled, often obsolete and clear base, rounded, and thick. Bark, viewed by transparency, has a quadrangular to rectangular cells, thick-walled, with lipid droplets. In cross section, the cuticle is thin and smooth. The epidermis is composed of tangentially flattened cells, most of them is tabular, thin walls, and the stomata are located just above the epidermal cells.

Bark consists of few layers of rectangular cells, much larger than the those of epidermis, compact, suberized walls, radially lined with lipid droplets. The last layers of the bark may be collapsed. The cortical parenchyma consists of cells of various shapes and sizes, generally polygonal, voluminous, with clear intercellular spaces. Large starch grains, in many shapes, with well defined lamella and eccentric hilum occur in the cortical parenchyma in large amount. There are oil secretory idioblast dispersed in the cortex,

each one usually consisting of a generally circular secretory cell with a large yellow drop and parenchymal cells arranged radially around this cell. Small collateral vascular bundles, cells containing phenolic compounds, and small lipid droplets are also common in this region. The endoderm is substantially continuous and consists of small and flattened cells in different shapes, with thin walls. The central cylinder is well developed, with parenchymal cells and cells containing phenolic compounds and lipid droplets. In cells of the central cylinder there is smaller quantity of starch grains and larger amount of secretory idioblast. Small vascular bundles of annular distribution occur along the endoderm and well developed bundles, randomly distributed and large number, occurring more internally.

MICROSCOPIC DESCRIPTION OF POWDER

The powder meets all the requirements for the species, unless the macroscopic characters. Microscopic observation of the powder becomes clearer when chloral hydrate is used. Characteristics: dark yellow color; fragments of the epidermis with down; in front view, the isolated down or its portion; fragments of epidermis with stomata in front view; fragments of epidermis showing cells with lipid droplets; fragments of the epidermis showing scar of down, in front view; fragments of epidermis and cortex viewed by transparency in front view; fragments of epidermis and bark, in cross section; fragments of bark in oblique view; fragments of bark, in cross section; fragments of bark and cortical parenchyma, in cross section; parenchymal cells, isolated or grouped; fragments of parenchyma, in cross section; fragments of reserve parenchyma with cells filled with starch grains in cross section; parenchymal cells, isolated, full of starch grains in section cross; starch grain mass; grain starch, isolated and/or grouped; portions of grouped vessel elements with scalariform thickening in longitudinal section; portions of isolated vessel elements with crosslinked thickening in longitudinal section; portions of vessel elements with crosslinked thickening in longitudinal section, associated with parenchyma cells, in cross section; portions of vessel elements with crosslinked thickening and helical thickening in longitudinal section;

IDENTIFICATION

A. To stir 0.5 g of freshly pulverized sample with 5 mL of ethanol for 5 minutes, and filter. To drip the filtrate droplets on filter paper, which should become yellow. Then, moisten the paper with drops of a saturated boric acid solution. The color changes to orange-red. Further addition of ammonium hydroxide causes the development of a dark blue color.

B. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF₂₅₄ silica gel with 250 µm thickness as support and a mixture of chloroform, ethanol, and glacial acetic acid (95:5:0.5) as mobile phase. To apply to the plate, as band, 10 µl of each of the solutions freshly prepared, as described below.

Solution (1): to stir for 30 minutes 0.5 g of freshly pulverized sample with 5 mL of methanol, centrifuge for 10 minutes at 2500 rpm. To filter it.

Solution (2): to dissolve 5 mg of curcumin SQR, demethoxycurcumin SQR, and bis-demethoxycurcumin SQR in 5 mL of methanol.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (365 nm). The region of the chromatogram obtained with *Solution (2)*, when examined under ultraviolet light (365 nm), has a green fluorescent spot, in the median part, corresponding to demethoxycurcumin (R_f about 0.6); in the upper third, there is a spot related to curcumin also with green fluorescent color (R_f about 0.7); in the lower third, there is a spot with the same color as those observed in R_f 0.7 and 0.6, corresponding to bis-demethoxycurcumin (R_f about 0.4). The same spots should be viewed in the region of the chromatogram obtained with *Solution (1)*, and they should correspond in position to those obtained with *Solution (2)*.

C. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF₂₅₄ with 250 µm thickness as support and a mixture of toluene and ethyl acetate (97:3) as mobile phase. To separately apply to the plate, as band, 10 µl of *Solution (1)*, described in test **B. Identification** and 10 µl of *Solution (3)* freshly prepared, as described below.

Solution (3): to dissolve 10 mg of thymol in 10 mL of methanol.

To develop the chromatogram. To remove the plate, and let it drying. To nebulize the plate with vanillin sulfuric SR.

The region of the chromatogram obtained with *Solution (3)*, after development with vanillin sulfuric SR, presents in the middle part of the plate a reddish spot corresponding to thymol (R_f about 0.6). In the *Solution (1)* no spot is observed with R_f corresponding to that observed for the *Solution (3)*. In the chromatogram obtained for the *Solution (1)* violet spot can be observed corresponding to zingiberene (R_f about 0.8). At the bottom of the chromatogram, other violet (top) and red (bottom) spots can be viewed near the point of application.

PURITY TESTS

Water (5.4.2.3). At most 12.0%.

Total ashes (5.4.2.4). At most 8.0%.

DOSAGE

Volatile oils

To proceed as described in *Determination of volatile oils in vegetable drugs (5.4.2.7)*. To use 500 mL round bottom flask containing 200 mL of water as distillation liquid and 0.5 mL of xylene (to put into the graduated tube). To pulverize the sample (500 µm) and immediately proceed to the determination in 5 g of the powdered drug. To distill for 4 hours.

Dicinnamoyl methane derivative

To put 10 mg of the sample into a 50 mL beaker, add 6 mL of glacial acetic acid, and heat in a water bath at 90 °C for 60 minutes. To add 0.2 g of boric acid and 0.2 g of oxalic acid and heat in water bath (90 °C) for 10 minutes. To cool down and dilute with glacial acetic acid in a 10 mL volumetric flask. To transfer 1 mL of the solution to a 100 mL volumetric flask and top up with glacial acetic acid. To measure the absorbance at 530 nm immediately after preparation, by using glacial acetic acid to adjust zero. To use 2350 as specific absorbance value of curcumin. To calculate the dicinnamoyl methane derivative content expressed as curcumin according to the equation:

$$DC\% = \frac{0,0426 \times A}{m}$$

where

$DC\%$ = dicinnamoyl methane derivative content (%,

w/w);

A = absorbance measured;

m = mass of sample (g) considering the water content.

PACKAGING AND STORAGE

In tightly closed container, protected from light and heat.

portions of isolated vessel elements, with helical thickening, in longitudinal section; isolated lipid droplets.

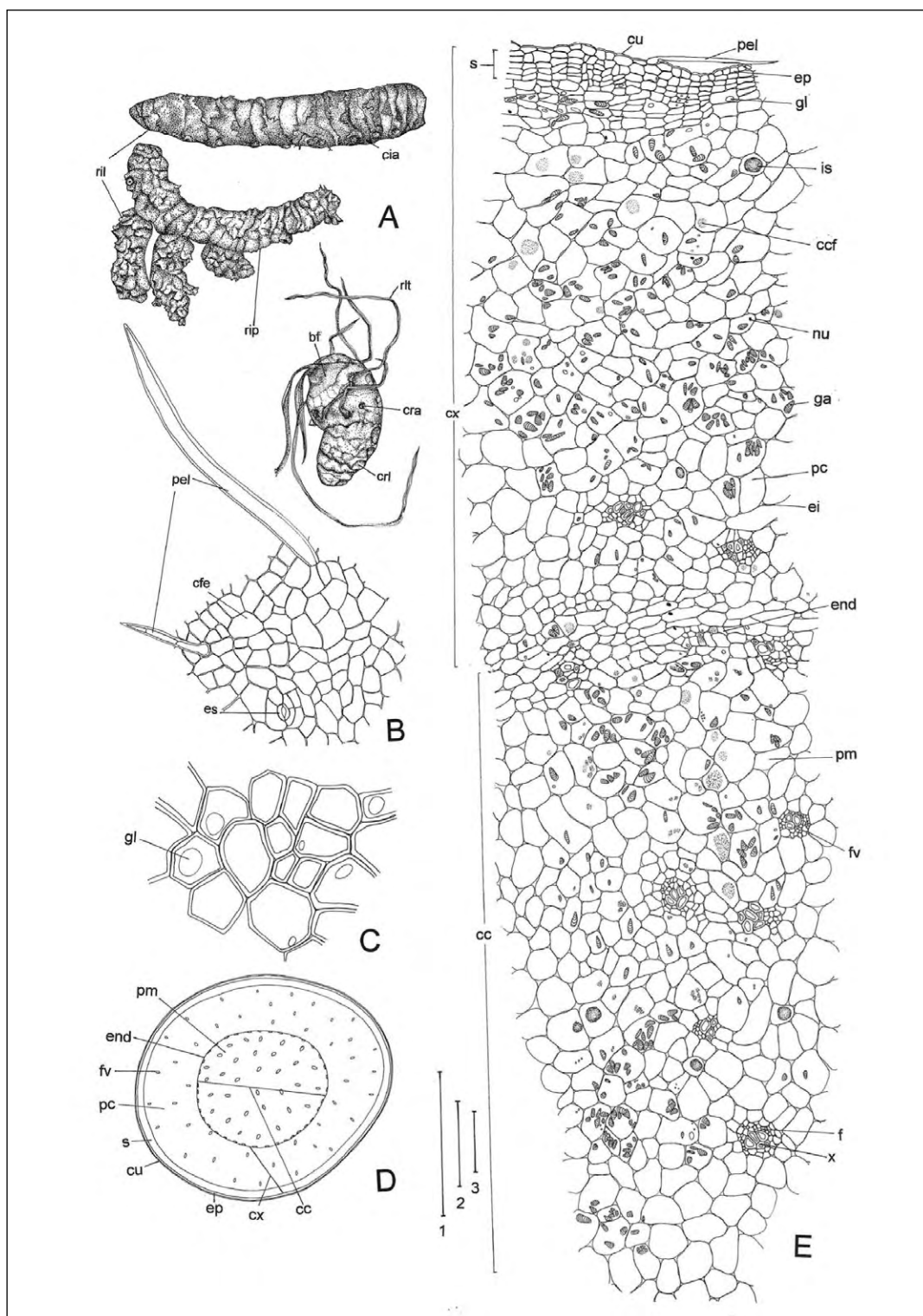


Figure 1 — Macroscopic and microscopic aspects in *Curcuma longa* L.

Additional information of **Figure 1**. Scales correspond to 5 cm in **A** (rule 1); in **B**, **C**, and **E**, 100 μ m (rule 2); in **D**, 1.0 mm (rule 3).

A - general aspects of rhizomes: leaf sheath (bf); annular scar from the base of the leaf sheath (cia); scar with lateral branching (crl); root scar (cra); lateral rhizome (ril), main rhizome (rip), lateral root (rlt). **B** - detail of portion of the epidermis, in front view: fundamental cell of the epidermis (cfe); stomata (es), down (pel). **C** - detail of portion of the bark in front view: lipid droplet (gl). **D** - scheme of the rhizome in cross section: central cylinder (cc); cuticle (cu), cortex (cx), endoderm (end); epidermis (ep), vascular bundle (fv); cortical parenchyma (pc); medullary parenchyma (pm); bark (s). **E** - detail of the portion of the rhizome in cross section: central cylinder (cc); cell containing phenolic compound (ccf); cuticle (cu), cortex (cx), intercellular space (ei); endoderm (end); epidermis (ep), phloem (f); vascular bundle (fv), starch grain (ga), lipid droplet (gl); secretory idioblast (is); nucleus (nu), down (pel), cortical parenchyma (pc); medullary parenchyma (pm); bark (s); xylem (x).

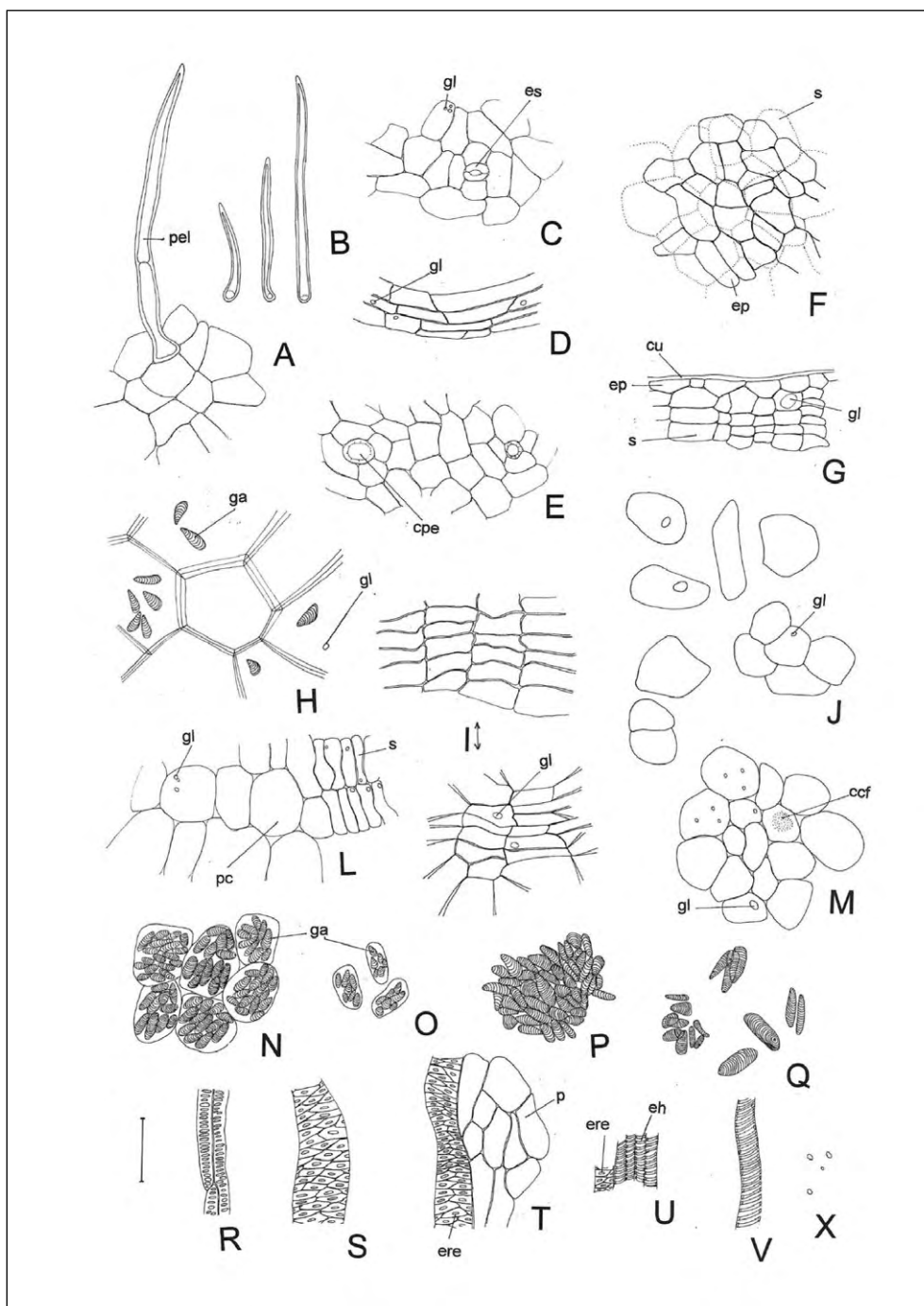


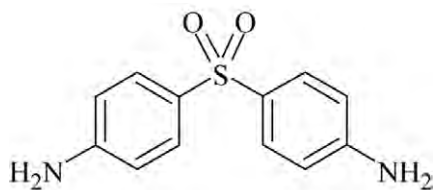
Figure 2 — Microscopic aspects of powder in *Curcuma longa* L.

Additional information of **Figure 2**. Scales correspond to 100 µm.

A - fragment of the epidermis with down in front view: down (pel). **B** - isolated down. **C** - fragment of epidermis with stomata in front view: stomata (es), lipid droplet (gl). **D** - fragment of epidermis in front view: lipid droplet (gl). **E** - fragment of epidermis with down scar in front view: down scar (cpe). **F** - fragment of epidermis and cortex, viewed by transparency, in front view: epidermis (ep); bark (s). **G** - fragment of epidermis and bark, in cross-section: cuticle (cu); epidermis (ep), lipid droplet (gl); bark (s). **H** - fragment of bark, in oblique view: starch grain (ga); lipid droplet (gl). **I** - fragment of bark, in cross-section: lipid droplet (gl). **J** - parenchyma cells, isolated or grouped: lipid droplet (gl). **L** - fragment of bark and cortical parenchyma in cross section: lipid droplet (gl); cortical parenchyma (pc); bark (s). **M** - fragment of parenchyma in cross section: cell containing phenolic compound (ccf), lipid droplet (gl). **N** - fragment of reserve parenchyma with cells filled with starch grains in cross section: starch grain (ga). **O** - isolated parenchymal cells filled with starch grains in cross section: starch grain (ga). **P** - mass of starch grains. **Q** - starch grains, isolated and/or grouped. **R** - portions of grouped vessel elements with scalariform thickening in longitudinal section. **S** - portion of isolated vessel element with crosslinked thickening in longitudinal section. **T** - portion of vessel element with crosslinked thickening in longitudinal section, associated with parenchymal cells in cross-section: vessel element with crosslinked thickening (ere); parenchyma (p). **U** - portions of vessel elements with crosslinked thickening and helical thickening in longitudinal section: vessel element with helical thickening (eh); vessel element with crosslinked thickening (ere). **V** - portion of isolated vessel element with helical thickening in longitudinal section. **X** - isolated lipid droplets.

DAPSONE

Dapsonum



$C_{12}H_{12}N_2O_2S$; 248.30
dapsone; 02 686 4,4'-Sulfonyl bis benzenamine
[80-08-0]

It contains at least 99.0% and at most 101.0% of $C_{12}H_{12}N_2O_2S$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline powder, white or slightly yellowish, odorless, and slightly bitter taste.

Solubility. Practically insoluble in water, freely soluble in acetone, and slightly soluble in ethanol. Freely soluble in diluted mineral acids.

Physical and chemical constants.

Melting range (5.2.2): 175 °C to 181 °C.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the dapsone SQR spectrum similarly prepared.

B. The ultraviolet absorption spectra (5.2.14), from 200 nm to 400 nm, of a 0.0005% solution (w/v), in methanol, exhibits maximum at 260 nm and 295 nm and minima at 232 nm and 268 nm, similar to those observed in the spectrum of a similar solution of dapsone SQR.

C. To proceed as described in *Related substances*. The principal spot obtained with *Solution (4)* corresponds in position, color, and intensity to that obtained with *Solution (5)*.

D. It responds to the reactions of primary aromatic amines (5.3.1.1).

PURITY TESTS

Related substances. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using G silica gel as support and a mixture of acetone and chloroform (1:1) as mobile phase. To separately apply to the plate 10 µL of each of the solutions prepared in methanol.

Solution (1): 1% mg/mL solution (w/v) of the sample.

Solution (2): 0.01% solution (w/v) of the sample.

Solution (3): 0.002% solution (w/v) of the sample.

Solution (4): 0.1% solution (w/v) of the sample.

Solution (5): 0.1% dapsone SQR solution (w/v).

To develop the chromatogram. To remove the plate, dry at room temperature, and firstly nebulize with 0.5% sodium nitrite solution (w/v) in 0.1 M hydrochloric acid. To wait for 5 minutes and nebulize with N-(1-naphthyl) ethylenediamine dihydrochloride SR. Any spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (2)* (1.0%) and not more than any two secondary spots are more intense than that obtained with *Solution (3)* (0.2 %).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 100 °C - 105 °C for 3 hours. At most 1.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To employ one of the methods described below.

A. To proceed as described by *Diazotization titrations (5.3.3.1)*, by using *Method 1* or *Method 2*. To use 0.25 g of the sample. Each mL of 0.1 M SV sodium nitrite is equal to 12.415 mg of $C_{12}H_{12}N_2O_2S$.

B. To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. To weigh 50 mg of the sample, add 40 mL of ethanol, and put it in an ultrasound for 10 minutes. To stir for 30 minutes and top up to 100 mL with the same solvent. To use suitable filter paper. To successively dilute in ethanol to obtain 0.0005% concentration (w/v). To prepare a standard solution of dapsone SQR at similar concentration by using the same solvent. To measure the absorbances of the resulting solutions at 295 nm, by using ethanol to adjust zero. To calculate the content of $C_{12}H_{12}N_2O_2S$ in the sample from the readings obtained.

PACKAGING AND STORAGE

In tightly closed, opaque containers.

LABELLING

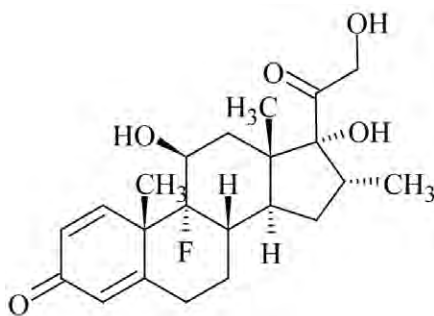
To comply with the legislation in force.

THERAPEUTIC CLASS

Chemotherapy.

DEXAMETHASONE

Dexamethasonum



$C_{22}H_{29}FO_5$; 392.46
dexamethasone; 02817
(11 β , 16 α)-9-Fluorine-11,17,21-trihydroxy-16-methylpregna-1, 4 -
diene-3,20-dione
[50-02-2]

It contains at least 97.0% and at most 102.0% of $C_{22}H_{29}FO_5$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white or almost white powder.

Solubility. Practically soluble in water, slightly soluble ethanol, and sparingly soluble in methylene chloride.

Physical and chemical constants.

Melting range (5.2.2): 255 °C with decomposition.

Specific rotation (5.2.8): +72° to 80°, in relation to the desiccated substance. To determine in 1% solution (w/v) in dioxane.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the dexamethasone SQR spectrum similarly prepared.

B. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using silica gel with fluorescent indicator of maximum intensity at 254 nm as a support and a mixture of water-saturated butanol, toluene, and ethyl ether (5:10:85) as mobile phase. To separately apply to the plate 5 μ L of each of the solutions freshly prepared, as described below.

Solution (1): to dissolve 10 mg of the sample in a mixture of methanol and methylene chloride (1:9), in 10 mL volumetric flask, and top up with the same solvent.

Solution (2): to dissolve 25 mg of dexamethasone SQR in a mixture of methanol and methylene chloride (1:9), in 25 mL volumetric flask, and top up with the same solvent.

Solution (3): to dissolve 10 mg of betamethasone SQR in *Solution (2)* and top up to 10 mL with the same solution.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*. To nebulize with ethanolic sulfuric acid solution. To heat at 120 °C for 10 minutes or spots occur. To let it cool down. To examine in daylight and under 365 nm ultraviolet light. The principal spot obtained with *Solution (1)* corresponds in position, daylight color, fluorescence to 365 nm ultraviolet light, and dimensions to the principal spot obtained with *Solution (2)*. The test is valid only if the *Solution (3)* shows two spots that can not be fully separated.

To dissolve 2 mg of the sample in 2 mL of sulfuric acid. Light red-brown color is developed within 5 minutes. To add the 10 mL water solution and mix. The color disappears.

PURITY TESTS

Related substances. B. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 254 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with phenyl group chemically bonded to the porous silica particles (5 to 10 μ m), kept at room temperature; flow of *Mobile phase* of 1 mL/min.

Formate buffer pH 3.6: To transfer 1.32g of ammonium formate to a 1000 mL volumetric flask, add water, and stir to dissolve it. To adjust the pH 3.6 with formic acid.

Mobile phase: mixture of *Formate buffer pH 3.6* and acetonitrile (67:33). To make the corrections as required.

Solution (1): To dissolve 180 mg of sample and dilute to 100 mL with acetonitrile. To transfer 33 mL of this solution to a 100 mL volumetric flask top up with *Formate buffer pH 3.6*, and mix it.

Procedure: to inject 10 μ L of *Solution (1)*. To calculate the percentage of each impurity according to the portion of dexamethasone by using the equation:

$$100(ri/rs)$$

ri is the peak response for each impurity, and *rs* is the sum of the response related to all peaks: not more than 1.0% of any individual impurity is found, and not more than 2% of total impurities are found. The column efficiency is equal to or greater than 5000 theoretical plates.

Loss on drying (5.2.9). To determine in 0.5 g of the sample. To desiccate in an oven at 105 °C for 3 hours. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 0.25 g of the sample. At most 0.2%.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. To accurately weigh about 0.1 g of the sample and dissolve in ethanol. To top up the volume to 100 mL with the same solvent. To transfer 2.0 mL of the solution to 100 mL volumetric flask and top up with ethanol. To prepare a dexamethasone standard solution in ethanol at the same final concentration. To measure the absorbances of the resulting solutions at 238.5 nm, by using ethanol to adjust zero. To calculate the content of $C_{22}H_{29}FO_5$ in the sample from the readings obtained. Alternatively, make the calculations by taking into account that $A(1\%, 1\text{ cm}) = 394$ at 238.5 nm, in ethanol.

B. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 254 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μm), kept at room temperature; flow of *Mobile phase* of 1.0 mL/min.

Mobile phase: to properly prepare methanol and water (75:25) solution.

Sample solution: To transfer accurately weighed amount of 30 mg of the sample to 100 mL volumetric flask, top up with *Mobile phase*, and mix it.

Standard solution: to dissolve accurately weighed amount of dexamethasone SQR in methanol to obtain a 1 mg/mL solution. To transfer 3 mL of this solution to 10 mL volumetric flask and top up with *Mobile phase*, thus obtaining 0.3 mg/mL solution.

Procedure: to separately inject between 10 μL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_{22}H_{29}FO_5$ from the sample, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Anti-inflammatory

DEXAMETHASONE ELIXIR

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_{22}H_{29}FO_5$.

IDENTIFICATION

To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using silica gel as support and a mixture of chloroform, acetone, and glacial acetic acid (80:40:1) as mobile phase. To separately apply to the plate 5 μL of each of the solutions freshly prepared, as described below.

Solution (1): 0.1 mg/mL dexamethasone solution in the sample.

Solution (2): 0.1 mg/mL dexamethasone SQR solution in mixture of methanol and methylene chloride (1:1).

To develop the chromatogram. To remove the plate. To evaporate the solvent. To examine under ultraviolet light (254 nm). The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

pH (5.2.19). 2.7 to 4.0.

Determination of the alcohol (5.3.3.8). 3.8% to 5.7%. N-propyl alcohol as internal standard.

BIOLOGICAL SAFETY TEST

Counting of the total viable micro-organisms (5.5.3.1.2). It complies with the test.

Survey and identification of pathogens (5.5.3.1.3). It complies with the test.

DOSAGE

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 254 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μm), at room temperature; flow of *Mobile phase* of 1.0 mL/min.

Mobile phase: to properly prepare methanol and water (75:25) solution.

Sample solution: solution equivalent to 0.1 mg/mL of dexamethasone; if necessary, dilute with *Mobile phase*.

Standard solution: to dissolve accurately weighed amount of dexamethasone SQR in *Mobile phase* to obtain a 0.1 mg/mL concentration.

Procedure: to separately inject 10 µL of *Sample* and *standard solutions*, record the chromatograms, and measure the area under the peaks. To calculate the amount of $C_{22}H_{29}FO_5$ in the sample, from the responses from the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

DIAZEPAM TABLETS

It contains at least 92.5 % and at most 107.5 % of the amount stated of $C_{16}H_{13}ClN_2O$.

IDENTIFICATION

A. The ultraviolet absorption spectrum (5.2.14) ranging from 230 nm to 350 nm, of sample solution obtained in method.

A. *A.*, *Dosage*, exhibits maxima at 242 and 284 nm, similar to those observed in the standard solution spectrum.

B. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF_{254} silica gel as support and a mixture of ethyl acetate and hexane (50:50) as mobile phase. To separately apply to the plate 2 µL of each of the solutions as described below.

Solution (1): to stir with ethanol amount of powdered tablets sufficient to prepare a solution containing 0.02% (w/v) of diazepam and filter it.

Solution (2): to prepare 0.02% diazepam SQR solution (w/v) in ethanol.

To develop the chromatogram. To remove the plate, dry it at room temperature, and examine under ultraviolet light (254 nm). The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

C. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method **B.** corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

Procedure for content uniformity. To transfer each tablet to a 100 mL volumetric flask, add 5 mL of water, stir, and wait for 15 minutes to disintegrate. To proceed as described in method **A.** *Dosage*, from "To add 70 mL of 0.1 M methanolic sulfuric acid.."To add 70 mL of 0.1 M hydrochloric acid..."

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 900 mL

Equipment: baskets, 100 rpm *Time:* 45 minutes

Procedure: after testing, remove aliquot from the dissolution medium and dilute, if necessary, with 0.1 M hydrochloric acid, to obtain suitable concentration. To measure the absorbances at 284 nm (5.2.14), by using the same solvent to adjust zero. To calculate the amount of $C_{16}H_{13}ClN_2O$ dissolved in the medium, by comparing the readings obtained with 0.002 % diazepam SQR solution (w/v) prepared in 0.1 M hydrochloric acid.

Tolerance: not less than 75% (Q) of the stated amount of $C_{16}H_{13}ClN_2O$ dissolves within 45 minutes.

PURITY TESTS

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF_{254} silica gel as support and a mixture of ethyl acetate and hexane (50:50) as mobile phase. To separately apply to the plate 20 µL of *Solution (1)* and 5 µL of *Solution (2)* freshly prepared, as described below.

Solution (1): to stir amount of powdered tablets equivalent to 50 mg of diazepam with 5 mL of ethanol and filter.

Solution (2): to dilute 1 volume of *Solution (1)* to 50 volumes with ethanol.

To develop the chromatogram. To remove the plate, dry it at room temperature, and examine under ultraviolet light (254 nm). No secondary spot obtained with *Solution (1)* is more intense than the principal spot obtained with *Solution (2)* (2%).

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To weigh and pulverize 20 tablets. To transfer amount of powder equivalent to 10 mg of diazepam to a 100 mL volumetric flask, add 5 mL of methanol, mix it, and let it rest for 15 minutes. To add 70 mL of 0.1 M hydrochloric acid, mechanically stir for 15 minutes, and top up with the same solvent. To filter it. To successively

dilute to 0.002% concentration (w/v) by using 0.1 M hydrochloric acid as solvent.

To prepare standard solution under the same conditions. To measure the absorbances of the solutions at 284 nm, by using 0.1 M hydrochloric acid to adjust zero. Take a reading of the solutions within 30 minutes. To calculate the amount of $C_{16}H_{13}ClN_2O$ in the tablets, from the readings obtained.

To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 254 nm; 150 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μ m), kept at 30 °C; flow of *Mobile phase* of 0.8 mL/min.

Mobile phase: mixture of water, acetonitrile, and methanol (4:4:2).

Sample solution: To weigh and pulverize 20 tablets. To transfer amount of powder equivalent to 10 mg of diazepam to a 50 mL volumetric flask, add 40 mL of *Mobile phase*, and put it in ultrasound for 5 minutes. To mechanically stir for 5 minutes. To top up with the same solvent, make it homogeneous, and filter it. To transfer 5 mL of the filtrate to a 50 mL volumetric flask and top up with *Mobile phase*, thus obtaining 20 μ g/mL solution.

Standard solution: to dissolve accurately weighed amount of diazepam SQR in *Mobile phase* to obtain a 0.2 mg/mL solution. To transfer 5 mL of this solution to 50 mL volumetric flask and top up with *Mobile phase*, thus obtaining 20 μ g/mL.

To inject replicates of 20 μ L of the *Standard solution*. The column efficiency is greater than 5000 theoretical plates. The tailing factor should not be greater than 2.0. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2.0 %.

Procedure: to separately inject 20 μ L of *Sample* and *standard solutions*, record the chromatograms, and measure the area under the peaks. To calculate the amount of $C_{16}H_{13}ClN_2O$ in the tablets, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

DIAZEPAM, INJECTABLE SOLUTION

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_{16}H_{13}ClN_2O$.

IDENTIFICATION

The test C. may be omitted if the tests A. and B. are conducted. The identification test A. can be omitted if the tests B. and C. are conducted.

A. The ultraviolet absorption spectrum (5.2.14) ranging from 200 nm to 400 nm, of sample solution obtained in *Dosage method A.*, exhibits absorption maximum at 368 nm and intensity similar to those observed in the standard solution spectrum.

B. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using G silica gel as support and a mixture of chloroform and methanol (10:1) as mobile phase. To separately apply to the plate 10 μ L of each of the solutions freshly prepared, as described below.

Solution (1): to dilute the injectable solution in methanol to obtain a 1 mg/mL solution.

Solution (2): to dissolve accurately weighed amount of diazepam SQR in methanol to obtain a 1 mg/mL solution.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (365 nm). To nebulize with 10% sulfuric acid (w/v) in absolute ethanol and heat the plate at 105 °C for 10 minutes. The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

C. Proceed as described in *High performance liquid chromatography* (5.2.17.4). The retention time of the main peak in the chromatogram of the *Sample solution* obtained in method **B.** *Dosage* corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

pH (5.2.19). 6.2 to 7.0.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

Bacterial endotoxins (5.5.2.2). At most 11.6 UE/ mg of diazepam.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To transfer volume of injectable solution equivalent to 10 mg of diazepam to a separatory funnel and add 20 mL of phosphate buffer pH 7.0. To extract with 4 portions of chloroform (20 mL each), by passing the extracts in 5 g of anhydrous sodium sulfate. To

gather the extracts in a 100 mL volumetric flask and top up to volume with chloroform. To make it homogeneous. To evaporate the aliquot of 10 mL under a stream of nitrogen to dryness. To dissolve the residue in 25 mL of 0.05 M methanolic sulfuric acid. To prepare a standard solution under the same conditions of the sample solution. To measure the absorbance of the sample solution and standard solution at 368 nm, by using

0.05 M methanolic sulfuric acid to adjust zero. To calculate the amount of $C_{16}H_{13}ClN_2O$ in the injectable solution, from the readings obtained. Alternatively, make the calculations by taking into account that $A(1\%, 1\text{ cm}) = 151$ at 368 nm.

To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 254 nm; 300 mm column and 3.9 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μm), flow of *Mobile phase* of 1.4 mL/min.

Mobile phase: to prepare a filtered and degassed methanol and water (65:35) solution.

Internal standard solution: to prepare a *p*-tolualdehyde solution containing about 0.3 $\mu\text{L/mL}$ in methanol.

Sample solution: To accurately transfer a volume of injectable solution equivalent to 10 mg of diazepam to a 50 mL volumetric flask. To transfer 10 mL of *Internal standard solution* to *Sample solution* and dilute with methanol to final volume. To make it homogeneous.

Standard solution: to dissolve an amount accurately weighed of diazepam SQR in methanol and dilute with the same solvent, to obtain a 1 mg/mL solution. To transfer 5.0 mL of this solution to a 25 mL volumetric flask, add 5 mL of *Internal standard solution*, dilute with methanol to the final volume, and make it homogeneous, to obtain a 0.2 mg/mL diazepam SQR solution.

To inject replicates of 10 μL of the *Standard solution*. The tailing factor for the diazepam peak is not greater than 2.5, the resolution between the *p*-tolualdehyde and diazepam peaks is not less than 3.5, and the standard deviation for injection replicates is not greater than 2.0%.

Procedure: to separately inject 10 μL of *Sample* and *standard solutions*, record the chromatograms, and measure the area under the main peaks. The relative retention times are about 0.5 to *p*-tolualdehyde and 1.0 to diazepam. To calculate the amount of $C_{16}H_{13}ClN_2O$ in the injectable solution, from the responses obtained for the *Sample solution* and *Standard solutions* by using the equation below.

$$50C/V(R_u/R_s)$$

where C is the concentration in mg/mL of diazepam SQR in the *Standard solution*; V is the volume in mL of injectable solution taken; R_u and R_s are the ratios of the responses for

diazepam peak related to *p*-tolualdehyde obtained from the *Sample solution* and *Standard solution*, respectively.

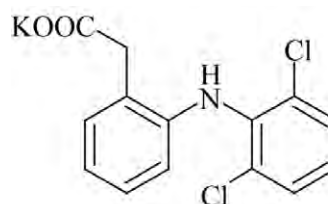
PACKAGING AND STORAGE

In type I glass containers, protected from light.

LABELLING

To comply with the legislation in force.

DICLOFENAC POTASSIUM Diclofenacum kalicum



$C_{14}H_{10}Cl_2KNO_2$; 334.24

diclofenac potassium; 02929

Potassium salt of 2 - [(2,6-dichlorophenyl) amino] benzeneacetic acid (1:1)

[15307-81-0]

It contains at least 99.0% and at most 101.0% of $C_{14}H_{10}Cl_2KNO_2$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or slightly yellow, crystalline powder; slightly hygroscopic.

Solubility. Slightly soluble in water, freely soluble in methanol, soluble in ethanol, very slightly soluble in acetone.

Physical and chemical constants. *Melting range* (5.2.2): 295 °C to 300 °C with decomposition.

IDENTIFICATION

The identification tests B., C, and D. may be omitted if the tests A. and E. are conducted. The identification test A. can be omitted if the tests B., C., D., and E. are carried out.

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the diclofenac potassium SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14), from 200 nm to 350 nm, 0.001% solution (w/v) prepared in 0.1 M sodium hydroxide, has absorption maximum at 218 and 275 nm, similar to those observed in the spectrum of a similar solution of diclofenac potassium SQR.

C. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF₂₅₄ silica gel as support and a mixture of ammonium hydroxide, methanol, ethyl acetate (10:10:80) as mobile phase. To separately apply to the plate 5 µL of each of the solutions freshly prepared, as described below.

Solution (1): to dissolve 25 mg of the sample in methanol and top up to 5 mL with the same solvent.

Solution (2): to dilute 25 mg of diclofenac potassium SQR in methanol and top up to 5 mL with the same solvent.

Solution (2): to dilute 10 mg of indomethacin SQR with *Solution (2)* and top up to 2 mL with methanol.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*. The test is valid only if the chromatogram obtained with *Solution (3)* presents two clearly separated spots.

D. To dissolve about 10 mg of sample in 10 mL of ethanol. To add 0.2 mL of a mixture of 0.6% potassium ferricyanide (w/v) and 0.9% ferric chloride (w/v) (1:1), freshly prepared, to 1 mL of this solution. Let it rest for 5 minutes. To protect it from light. To add 3 mL of 0.1 M hydrochloric acid, let it rest for 15 minutes. To protect it from light. Blue color is developed, and precipitate is produced.

E. To dissolve 0.5 g of sample in 10 mL of water. To add 2 mL of diluted hydrochloric acid, stir for 1 hour, and filter it under vacuum. To neutralize with 5 M sodium hydroxide. It responds to the reaction 2 of the potassium ion (5.3.1.1).

PURITY TESTS

Appearance of solution. The 5% solution (w/v) in methanol is clear (5.2.25) and colorless (5.2.12), and the ultraviolet absorbance of the solution determined at 440 nm is not greater than 0.05.

pH (5.2.19). 7.0 to 8.5. To determine in 1% aqueous solution (w/v).

Related substances. To proceed as described in *Dosage method B*. To prepare *Solutions (1)* and *(2)* as described below.

Diluent: mixture of water and methanol (30:70).

Solution (1): to transfer 50 mg of the sample to 100 mL volumetric flask, dilute with *Diluent*, and top up with the same solvent.

Solution (2): to transfer 2 mL of *Solution (1)* to 100 mL volumetric flask and top up with *Diluent*. To transfer 1 mL of this solution to 10 mL volumetric flask and top up with the same solvent.

Procedure: to separately inject 20 µL of *Solutions (1)* and *(2)*, record the chromatograms, and measure the areas under the peaks. No secondary peak obtained with *Solution (1)* shows the area greater than the area under the main peak obtained in the chromatogram with *Solution (2)* (0.2%). The sum of all areas of all peaks, other than the main peak, in the chromatogram of *Solution (1)* is not greater than 2.5 times the area under the main peak obtained in the chromatogram of *Solution (2)* (0.5%). In the chromatogram of *Solution (1)*, disregard any peak whose area is smaller than 0.25 times the area under the main peak in the chromatogram obtained with *Solution (2)*.

Heavy metals (5.3.2.3). To determine in 2 g of the sample. To incinerate at 500 °C - 600 °C. If the residue does not appear completely white after the incineration, add enough amount of hydrogen peroxide to solubilize it. To heat until to obtain total evaporation. To repeat the procedure to obtain total white residue and proceed as described in *Method III*. At most 0.001% (10 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 100°C - 105 °C for 3 hours. At most 0.5%.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. To accurately dissolve about 0.3 g of the sample in 30 mL of glacial acetic acid. To titrate with 0.1 M SV perchloric acid, to potentiometrically determine the endpoint. Each mL of 0.1 M SV perchloric acid is equal to 33.424 mg of C₁₄H₁₀Cl₂KNO₂.

B. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 254 nm; 250 mm column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 mm), flow of *Mobile phase* of 1.0 mL/min.

Phosphate buffer pH 2.5: to mix equal volumes of 0.05% phosphoric acid (w/v) and 0.08% monobasic sodium phosphate (w/v). If necessary, adjust the pH to 2.5 with phosphoric acid.

Mobile phase: mixture of *Phosphate buffer pH 2.5* and methanol (30:70).

Diluent: mixture of water and methanol (30:70).

Sample solution: to dissolve accurately weighed amount of the sample in *Diluent* to obtain a 40 µg/mL solution.

Standard solution: to dissolve accurately weighed amount of diclofenac potassium SQR in *Diluent* to obtain a 40 µg/mL solution.

Resolution solution: to transfer 2.0 mg of diethyl phthalate, 10.0 mg of diclofenac potassium SQR, and 1.0 mg of 1 - (2,6-dichlorophenyl) -1,3-dihydro-2H-indol-2-one (*Impurity A*) to a 200 mL volumetric flask, by adding *Diluent*, and make it homogeneous.

To inject replicates of 20 µL of the *Resolution solution*. The relative retention times are about 0.5 to diethyl phthalate, 0.7 to *Impurity A*, and 1 to diclofenac potassium. The resolution between diethyl phthalate and *Impurity A* is not less than 4.0, and between *Impurity A* and diclofenac potassium is not less than 6.5.

The relative standard deviation for the replicate areas for the peaks recorded is not greater than 2.0%.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_{14}H_{10}Cl_2KNO_2$ from the sample, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Anti-inflammatory

DICLOFENAC POTASSIUM TABLETS

It contains at least 90.0% and at most 110.0% of the amount stated of $C_{14}H_{10}Cl_2KNO_2$. The tablets may be sugar-coated or film-coated; in this case, they must not meet the hardness and friability tests.

IDENTIFICATION

A. To weigh and pulverize the tablets. To use amount of powder equivalent to 0.15 g of diclofenac potassium, and add 0.5 mL of glacial acetic acid and 15 mL of methanol. To put it in ultrasound for 15 minutes and mechanically stir for 1 minute. To filter and collect the filtrate with 15 mL of water. To filter the solid formerd under reduced pressure, wash with 4 portions of 5 mL of water and dry it at 105 °C for 2 - 3 hours. To solubilize 50 mg of diclofenac potassium SQR in 5 mL of methanol, add 0.5 mL of glacial acetic acid, 15 mL of water, and stir. To filter the solid formerd under reduced pressure, wash with 4 portions of 5 mL of water and dry it at 105 °C for 2 - 3 hours. The infrared absorption spectrum (5.2.14) of the residue, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the diclofenac potassium SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14) ranging from 200 nm to 350 nm, of sample solution obtained in *Dosage* method A., exhibits absorption maximum at 218 nm and 275 nm, similar to those observed in the standard solution spectrum.

C. To proceed as described in test **C. Identification**, in the monograph *Diclofenac potassium*. To prepare *Solution (1)* as described below.

Solution (1): to weigh and pulverize the tablets. To transfer amount of powder equivalent to 50 mg of diclofenac potassium to 10 mL volumetric flask and add 7 mL of methanol. To put it in ultrasound for 15 minutes and top up with the same solvent. To make it homogenous and filter it.

D. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method **B.** corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: phosphate buffer pH 6.8 + 0.05, 900 mL

Equipment: blades, 40 rpm *Time:* 60 minutes

Procedure: after testing, remove aliquot from the dissolution medium and dilute, if necessary, with phosphate buffer pH 6.8 + 0.05, to obtain suitable concentration. To measure the absorbances at 276 nm (5.2.14), by using the same solvent to adjust zero. To calculate the amount of $C_{14}H_{10}Cl_2KNO_2$ dissolved in the medium, by comparing the readings obtained with 0.005 % diclofenac potassium SQR solution (w/v) prepared in phosphate buffer pH 6.8 + 0.05.

Tolerance: not less than 80% (Q) of the stated amount of $C_{14}H_{10}Cl_2KNO_2$ dissolves within 60 minutes.

PURITY TESTS

Related substances. To proceed as described in method **B. Dosage**, in the monograph *Diclofenac potassium*. To prepare *Solutions (1)* and *(2)* as described below.

Diluent: mixture of water and methanol (30:70).

Solution (1): to weigh and pulverize the tablets. To transfer amount of powder equivalent to 50 mg of diclofenac potassium to 50 mL volumetric flask and add 30 mL of *Diluent*. To put it in ultrasound for 15 minutes and top up with the same solvent, thus obtaining a 1 mg/mL solution. To make it homogenous and filter it.

Solution (2): to transfer 2 mL of *Solution (1)* to 100 mL volumetric flask and top up with *Diluent*. To transfer 1 mL of this solution to 10 mL volumetric flask and top up with *Diluent*, thus obtaining a 2 µg/mL solution. To make it homogeneous.

Procedure: to separately inject 20 µL of *Solutions (1)* and *(2)*, record the chromatograms, and measure the areas under the peaks. No secondary peak obtained with *Solution (1)* should show the area greater than the area of the main peak obtained in the chromatogram with *Solution (2)* (0.2%). The sum of all areas of all peaks, other than the main peak, in the chromatogram of *Solution (1)* should not be greater than 2.5 times the area of the main peak obtained in the chromatogram of *Solution (2)* (0.5%). In the chromatogram of *Solution (1)*, discard any peak whose area is smaller than 0.25 times the area of the main peak obtained in the chromatogram of *Solution (2)*.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. To weigh and pulverize the tablets. To transfer amount of powder equivalent to 50 mg of diclofenac potassium to 100 mL volumetric flask, add 70 mL of 0.1 M sodium hydroxide, put it in ultrasound for 15 minutes, and top up with the same solvent. To filter it. To transfer 5 mL of this solution to 50 mL volumetric flask, top up with the same solvent, and make it homogeneous, to obtain a 50 µg/mL solution. To prepare standard solution under the same conditions. To measure the absorbances of the solutions at 276 nm, by using 0.1 M sodium hydroxide to adjust zero. To calculate the amount of $C_{14}H_{10}Cl_2KNO_2$ in the tablets, from the readings obtained.

To proceed as described in method **B. Dosage**, in the monograph *Diclofenac potassium*. To prepare the *Sample solution* as described below.

Sample solution: To weigh and pulverize 20 tablets. To transfer amount of powder equivalent to 25 mg of diclofenac potassium to 25 mL volumetric flask and add 15 mL of *Diluent*. To put it in ultrasound for 15 minutes and top up with the same solvent. To transfer 1 mL of this solu-

tion to 25 mL volumetric flask and top up with *Diluent*, thus obtaining 40 µg/mL solution. To make it homogeneous.

Procedure: to separately inject 20 µL of *Sample* and *standard solutions*, record the chromatograms, and measure the area of the peaks. To calculate the amount of $C_{14}H_{10}Cl_2KNO_2$ in the tablets, from the responses obtained for the *Sample* and *standard solutions*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

DIPHOSPHATE, CHLOROQUINE TABLETS

It contains at least 93.0 % and at most 107.0 % of the amount stated of $C_{18}H_{26}ClN_3 \cdot 2H_3PO_4$.

IDENTIFICATION

The identification test A. may be omitted if the tests B. and C. are conducted.

A. To weigh and pulverize the tablets. To transfer amount equivalent to 0.1 g of chloroquine diphosphate to a separatory funnel and dissolve it in 10 mL of water. To add 2 mL of 2 M sodium hydroxide and extract with 2 portions of chloroform (20 mL each). To combine the organic extracts, wash with water, dry with anhydrous sodium sulfate, and evaporate to dryness. The infrared absorption spectrum (**5.2.14**) of the residue, dissolved in 2 mL of chloroform, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the chloroquine diphosphate SQR spectrum similarly prepared.

B. To weigh and pulverize the tablets. To transfer amount of powder equivalent to 0.1 g of chloroquine diphosphate to 100 mL volumetric flask. To add 70 mL of water, put it in ultrasound for 10 minutes, and top up with the same solvent (use this solution for tests **B.** and **C.**, *Identification*). To filter it. To successively dilute with water to a 0.001% concentration (w/v). The ultraviolet absorption spectrum (**5.2.14**) of the resulting solution, ranging from 200 nm to 400 nm, exhibits maxima and minima only in the same wavelength of a similar chloroquine diphosphate SQR solution. The ratio between the absorbance values measured at 343 nm and 329 nm is between 1.00 and 1.15.

C. To add 5 mL of picric acid SR1 to 20 mL of the first solution obtained in test **B.**, *Identification*. Yellow precipitate is formed. To filter and wash the precipitate with water

until the last washing becomes colorless. To dry over silica gel. The resulting residue melts at 205 °C - 210 °C.

D. The solution obtained in test **B.**, *Identification* responds to the reactions of the phosphate ion (**5.3.1.1**).

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL *Equipment:* blades, 100 rpm

Time: 45 minutes

Procedure: after testing, remove aliquot from the dissolution medium, filter, and dilute with water, to obtain suitable concentration. To measure the absorbances of the solutions at 343 nm (**5.2.14**), by using the same solvent to adjust zero. To calculate the amount of $C_{18}H_{26}ClN_3 \cdot 2H_3PO_4$ dissolved in the medium, by comparing the readings obtained with 0.002 % chloroquine diphosphate SQR solution (w/v) prepared in the same solvent.

Tolerance: not less than 75% (Q) of the stated amount of $C_{18}H_{26}ClN_3 \cdot 2H_3PO_4$ dissolves within 45 minutes.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Titrimetric methods in non-aqueous medium* (**5.3.3.5**). To weigh and pulverize 20 tablets. To dissolve the amount of powder equivalent to 0.5 g of chloroquine diphosphate in 20 mL of *M* sodium hydroxide. To quantitatively transfer to a 250 mL separatory funnel and extract with 4 portions of chloroform (25 mL each). To gather the chloroform extracts and evaporate in a water bath to 10 mL. To add 40 mL of acetic anhydride and titrate with 0.1 *M* SV perchloric acid, to potentiometrically determine the endpoint. Each mL of 0.1 *M* SV perchloric acid is equal to 25.794 mg of $C_{18}H_{26}ClN_3 \cdot 2H_3PO_4$.

B. To proceed as described in *Ultraviolet absorption spectrophotometry* (**5.2.14**). To weigh and pulverize 20 tablets. To transfer amount of powder equivalent to 0.8 g of chloroquine diphosphate to 200 mL volumetric flask and add 100 mL of water. To mechanically stir for 10 minutes and top up with the same solvent. To make it homogeneous. To filter it by discarding the first 50 mL of the filtrate. To

transfer 50 mL of the filtrate to a separatory funnel and add 5 mL of 6 *M* ammonium hydroxide. To stir and extract with 5 portions of chloroform (25 mL each). To gather the chloroform extracts and wash with 10 mL of water. To wash the aqueous phase with 10 mL of chloroform. To evaporate the chloroform extracts combined in a water bath to 10 mL. To add 50 mL of 0.1% hydrochloric acid (v/v) and continue to evaporate until the chloroform odor is no longer perceptible. To transfer the resulting solution to a 200 mL volumetric flask, by washing the walls of the flask with 0.1% hydrochloric acid (v/v) and top up the volume with the same solvent.

To successively dilute in 0.1% hydrochloric acid (v/v) to the 0.001% concentration (w/v). To prepare a standard solution at the same concentration by using 0.1% hydrochloric acid (v/v) as solvent. To measure the absorbances of the resulting solutions at 343 nm, by using the same solvent to adjust zero. To calculate the amount of $C_{18}H_{26}ClN_3 \cdot 2H_3PO_4$ in the tablets, from the readings obtained.

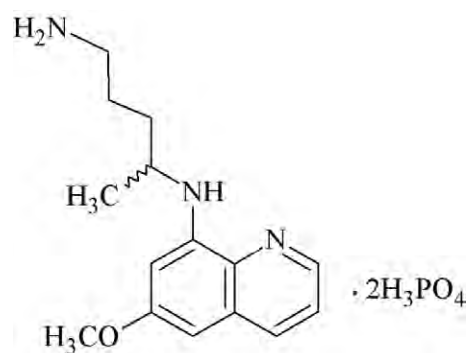
PACKAGING AND STORAGE

In tightly closed containers, protected from light, at monitored temperature.

LABELLING

To comply with the legislation in force.

DIPHOSPHATE, PRIMAQUINE Primaquini diphosphas



$C_{15}H_{21}N_3O \cdot 2H_3PO_4$; 455.34
primaquine diphosphate; 07367
Phosphate of *N*⁴-(6-methoxy-8-quinolinyl) -1,4-pentane diamine (2:1)
[63-45-6]

It contains at least 98.0% and at most 102.0% of $C_{15}H_{21}N_3 \cdot 2H_3PO_4$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, orange, and odorless powder.

Solubility. Practically insoluble in chloroform, ethanol, and ethyl ether; soluble in water.

Physical and chemical characteristics. *Melting range (5.2.2):* 197 °C to 198 °C.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the primaquine diphosphate SQR spectrum similarly prepared.

B. The residue obtained by ignition of the sample responds to the reactions of the phosphate ion (5.3.1.1), however, the precipitate obtained by adding silver nitrate SR is white and that one obtained by adding ammonium molybdate SR is yellow.

PURITY TESTS

pH (5.2.19). 2.5 to 3.5. To determine in 1% aqueous solution (w/v).

Related substances. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 261 nm; 200 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (10 µm), kept at room temperature; flow of *Mobile phase* of 3 mL/min.

Mobile phase: mixture of *n*-hexane, chloroform, methanol, and concentrated ammonia solution (45:45:10:0.1).

Solution (1): to accurately dissolve about 50 mg of primaquine diphosphate SQR in water and dilute to 5 mL with the same solvent. To add 0.2 mL of concentrated ammonia solution to 1 mL of this solution and mix with 10 mL of *Mobile phase*. To use the clear lower layer.

Solution (2): to accurately dissolve about 50 mg of the sample in water and dilute to 5 mL with the same solvent. To add 0.2 mL of concentrated ammonia solution to 1 mL of this solution and mix with 10 mL of *Mobile phase*. To use the clear lower layer.

Solution (3): to dilute 3 mL of *Solution (2)* to 100 mL with *Mobile phase*.

Solution (4): to dilute 1 mL of *Solution (2)* to 10 mL with *Mobile phase*. To dilute 1 mL of the resulting solution to 50 mL with the *Mobile phase*.

Procedure: to separately inject 20 µL of each solution. Then, record the chromatograms at least twice the retention time of the main peak. The sum of the areas of all the peaks obtained with *Solution (2)*, except the solvent peak's one, is not greater than the area under the main peak obtained with *Solution (3)* (3%). To disregard the peaks with

area less than 0.1 times the area under the main peak in the chromatogram obtained with *Solution (4)* (0.2%). The test is valid only if the chromatogram obtained with *Solution (1)* has, before the main peak, a peak whose area has approximately 6% of the primaquine peak; the resolution between the two peaks is at least 2.0 and in the chromatogram obtained with *Solution (4)*, the signal/noise ratio is greater than 5.

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 105 °C for 2 hours. At most 1.0%.

DOSAGE

To employ one of the methods described below.

To proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. To accurately weigh about 0.2 g of the sample and dissolve it in 40 mL of glacial acetic acid, by gently heating it. To titrate with 0.1 M SV perchloric acid, to potentiometrically determine the endpoint. Each mL of 0.1 M SV perchloric acid is equal to 22.767 mg of $C_{15}H_{21}N_3O_2 \cdot 2H_3PO_4$.

PACKAGING AND STORAGE

In amber, airtight closed vials, sealed, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antimalarial.

PRIMAQUINE, DIPHOSPHATE TABLETS

It contains at least 93.0 % and at most 107.0 % of the amount stated of $C_{15}H_{21}N_3O_2 \cdot 2H_3PO_4$.

IDENTIFICATION

A. To pulverize the tablets and transfer about 60 mg of primaquine to a separatory funnel. To add 10 mL of water and 2 mL of 2 M sodium hydroxide, then extract it with 2 portions of chloroform (20 mL each) for 10 minutes. To filter through filter paper containing anhydrous sodium sulfate; evaporate to dryness and dissolve the residue in 2 mL of chloroform. The infrared absorption spectrum (5.2.14) of the resulting solution has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the primaquine diphosphate SQR spectrum similarly prepared.

B. To dissolve amount of tablets finely pulverized containing the equivalent to 25 mg of primaquine diphosphate in 10 mL of water and filter. The filtrate, after neutralization

with 2 mL of 2 *M* nitric acid, responds to the reactions of the phosphate ion (5.3.1.1).

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 *M* hydrochloric acid, 900 mL
Equipment: blades, 100 rpm *Time:* 45 minutes

Procedure: after testing, remove aliquot from the dissolution medium, filter, and proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 254 nm; 300 mm column and 3.9 mm inner diameter, packed with octadecylsilane group chemically bonded to porous silica or ceramic particles (3 mm to 10 mm), flow of *Mobile phase* of 2.0 mL/min.

Sodium 1-pentane sulfonate aqueous solution: to add about 961 mg of sodium 1-pentane sulfonate and 1 mL of glacial acetic acid to 400 mL of water and make it homogeneous.

Mobile phase: filtered and degassed mixture of methanol and *Sodium 1-pentane sulfonate aqueous solution* (60:40).

Standard solution: to dissolve accurately weighed amount of primaquine diphosphate SQR in 0.1 *M* hydrochloric acid, to obtain a 0.003% solution (w/v).

Sample solution: after testing, remove aliquot from the dissolution medium, filter, and dilute, if necessary, with dissolution medium, to obtain suitable concentration.

Procedure: to separately inject 20 mL of filtered *Standard solution* and *Sample solution*, whose concentrations are already known and dissolved in the dissolution medium, record the chromatograms, and measure the areas under the peaks. To calculate the amount of $C_{15}H_{21}N_3O_2 \cdot 2H_3PO_4$ dissolved in the medium from the responses obtained from *Sample* and *standard solutions*.

Tolerance: not less than 80% (Q) of the stated amount of $C_{15}H_{21}N_3O_2 \cdot 2H_3PO_4$ dissolves within 45 minutes.

PURITY TESTS

Water (5.2.20.1). At most 4%.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To proceed as described in *Titrations in non-aqueous medium* (5.3.3.5). To weigh and finely pulverize 20 tablets. To dissolve amount of powder equivalent to 0.15 g of primaquine diphosphate in 20 mL of water. To quantitatively transfer it to a 250 mL separatory funnel. To add 5 mL of 2 *M* sodium hydroxide and extract with 4 portions of chloroform (25 mL each). To gather the chloroform extracts and evaporate to obtain 10 mL. To add 40 mL of glacial acetic acid and titrate with 0.1 *M* SV perchloric acid, to potentiometrically determine the endpoint. Each mL of 0.1 *M* SV perchloric acid is equal to 22.768 mg of $C_{15}H_{21}N_3O_2 \cdot 2H_3PO_4$.

PACKAGING AND STORAGE

In airtight closed containers, protected from light.

LABELLING

To comply with the legislation in force.

DIGOXIN TABLETS

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_{41}H_{64}O_{14}$.

IDENTIFICATION

A. To proceed as described in *Related substances*, in the monograph *Digoxin*, by using the following solutions.

Solution (1): to weigh and pulverize the tablets. To transfer amount of powder equivalent to 0.5 mg of digoxin in a centrifuge tube and add 2 mL of mixture of chloroform and methanol (2:1). To stir for 10 minutes and centrifuge it. To decant and use the clear supernatant.

Solution (2): 0.25 mg/mL digoxin SQR solution in a mixture of chloroform and methanol (2:1).

To develop the chromatogram. The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method **B.** corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

Procedure for content uniformity. To transfer each tablet to a 10 mL volumetric flask containing 7 mL of a mixture of ethanol and water (1:1) and wait for total disintegration of the tablet. To put it in ultrasound for 30 minutes and top up with the same solvent. To make it homogenous and filter it. To proceed as described in *Dosage* method **B**. To prepare *Standard solution* at the same concentration as *Sample solution*.

These precautions are taken to prevent contamination by metal particles coming from cleaning materials.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 500 mL

Equipment: baskets, 120 rpm

Time: 60 minutes

Standard solution: to transfer amount equivalent to 25 mg of digoxin SQR to a 500 mL volumetric flask and dissolve a small amount of ethanol. To top up with 80% ethanol (v/v) and make it homogeneous. To transfer an aliquot of 10 mL of this solution to a 100 mL volumetric flask and top up with 80% ethanol (v/v). To transfer aliquots of this solution to a 50 mL volumetric flask, to prepare a standard curve equivalent to 20%, 40%, 60%, 80%, and 100% of the amount stated of digoxin in 500 mL, and top up the volume with *Dissolution medium*.

Procedure: after testing, remove aliquot from the dissolution medium, filter through filter (porosity less than 0.8 mm), and discard the first 10 mL. To transfer to individual vials with lid, in duplicate, 1 mL of sample solution, 1 mL of standard curve, and 1 mL of *Dissolution medium* to prepare blank and quickly add the following reagents: 1 mL of 0.2% ascorbic acid solution (w/v) in methanol, 5 mL of hydrochloric acid, and 1 mL of methanolic hydrogen peroxide. To stir after adding each reagent. To close the vials after 2 hours and measure the fluorescence of solutions at an excitation wavelength at 372 nm and emission wavelength at 485 nm. To check the stability of the fluorometer and

read the fluorescence again for the solutions in the standard curve. By using the sample blank, correct the readings and analyze the results by plotting the fluorescence standard curve in relation to the percentage of dissolution.

Tolerance: not less than 80% (Q) of the stated amount of $C_{41}H_{64}O_{14}$ dissolves within 60 minutes.

Should performance of the stage E_2 (5.1.5) be required, the acceptance criteria for average of 12 units is equal to or greater than Q and no unit has results lower than Q - 5%.

Attention. The dissolution tanks should be successively washed before the test with hydrochloric acid, water, and ethanol. Then, carefully dry them.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Visible absorption spectrophotometry* (5.2.14). To weigh and pulverize 20 tablets. To use amount of powder equivalent to 1.25 mg of digoxin, add 3 mL of water, and stir. To let it rest for 10 minutes by occasionally stirring. To add 25 mL of glacial acetic acid, by stirring for 1 hour, and filter it. To transfer 4 mL of the filtrate to a 25 mL volumetric flask and add 1 mL of dimethyl sulfoxide. To top up to volume with xanthidrol as reagent, make it homogeneous, and let it rest for 4 hours protected from light. To prepare a standard solution under similar conditions, by using the same solvents. To prepare the blank by using the same solvents. To measure the absorbances of the resulting solutions at 545 nm, by using the blank to adjust zero. To calculate the amount of $C_{41}H_{64}O_{14}$ in the tablets, from the readings obtained.

B. To proceed as described in *High performance liquid chromatography* (5.2.17.4). To proceed as described in *Dosage* method **B**, in the monograph *Digoxin*. To prepare the *Sample solution* as described below.

Sample solution: To weigh and pulverize 20 tablets. To accurately transfer amount of powder equivalent to 1 mg of digoxin to a 25 mL volumetric flask. To add 15 mL of mixture of ethanol and water (1:1) and put it in ultrasound for 30 minutes. To top up to volume and make it homogeneous, thus obtaining a 40 mL/mL solution.

Procedure: to separately inject 20 mL of *Sample* and *standard solutions*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of $C_{41}H_{64}O_{14}$ in the tablets, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.



DIOXIDE, SILICON

Silica

SiO₂; 60.08
silicon dioxide; 09428 Silica
[7631-86-9]

It contains at least 99.0% and at most 100.5% of SiO₂ in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White, amorphous, fine, and hygroscopic powder.

Solubility. Practically insoluble in water and mineral acids, except for hydrofluoric acid. Insoluble in ethanol and other organic solvents. Soluble in hot alkalis.

IDENTIFICATION

To transfer about 5 mg of sample to a platinum crucible. To mix with about 200 mg of anhydrous potassium carbonate. To incinerate to obtain incandescence for 10 minutes and cool down. To dissolve the melt substance in 2 mL of distilled water, heat, if necessary, and slowly add 2 mL of ammonium molybdate SR. An intense yellow color is developed.

PURITY TESTS

pH (5.2.19). 4.0 to 8.0. To determine in 5 % solution (w/v).

Arsenic (5.3.2.5). To use the *Method I*. To transfer 4 g of sample to a platinum crucible, add 5 mL of nitric acid and 35 mL of hydrofluoric acid, and evaporate it in a water bath. To cool down. To add 5 mL of perchloric acid, 10 mL of hydrofluoric acid, 10 mL of sulfuric acid and evaporate it on a heating plate. A dense smoke is observed. To carefully cool down and transfer to a 100 mL beaker by using a few milliliters of hydrochloric acid. To evaporate to dryness and cool down. To add 5 mL of hydrochloric acid, dilute with water to approximately 40 mL, and heat to dissolve any residue. To cool down, transfer to a 100 mL volumetric flask, and top up with water. To use 25 mL of this solution and proceed as described in *Limit test for arsenic*. At most 0.0003% (3 ppm).

Chlorides (5.3.2.1). To boil 5 g of the sample in 50 mL of water under reflux for 2 hours, cool down, and filter it. To use 7 mL of the filtrate and 2 mL of 0.01 M hydrochloric acid standard. To proceed as described in *Limit test for chlorides*. At most 0.1% (1000 ppm).

Heavy metals (5.3.2.3). To use *Method I*.

To transfer 16.7 mL of the solution obtained in the test for *Arsenic* to a 100 mL beaker and neutralize with ammonium hydroxide by using litmus paper as indicator. To adjust the pH to 3 - 4 by using 6 M acetic acid. To filter through rapid filter paper. To wash with water to filtrate volume reaches

40 mL. To proceed as described in *Limit test for heavy metals*, by using 2 mL of *Lead standard solution (10 ppm Pb)*. At most 0.003% (30 ppm).

Sulfates (5.3.2.2). To use 10 mL of the filtrate from the test for *Chlorides* and 10 mL of 0.005 M hydrochloric acid standard. To proceed as described in *Limit test for chlorides*. At most 0.5% (5000 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 145 °C for 4 hours. At most 5%.

Sulfated ashes (5.2.10). To accurately incinerate about 1 g of the sample previously desiccated at 1000 °C for 1 hour. At most 8.5%.

DOSAGE

To accurately transfer about 1 g of the sample to a platinum crucible, incinerate at 900 °C for 1 hour, cool down in a desiccator, and weigh it. To carefully moisten with water and add about 10 mL of hydrofluoric acid in small quantities. To evaporate to dryness in water bath and cool down. To add 10 mL of hydrofluoric acid and 0.5 mL of sulfuric acid and evaporate to dryness. To slowly increase the temperature to volatilize the acid. To incinerate at 900 °C. To cool down in desiccator and weigh it. Each 1 g of the residue is equal to 1 g of SiO₂.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

CATEGORY

Pharmacotechnical adjuvant.

DIPYRONE, TABLETS

It contains at least 95.0 % and at most 105.0 % of the amount stated of C₁₃H₁₆N₃NaO₄S.H₂O.

IDENTIFICATION

A. To weigh and pulverize 20 tablets. To add 0.5 g of the powder and a few drops of concentrated hydrogen peroxide. A blue color is developed, which will quickly disappear and turning to intense red (strongly exothermic reaction).

B. To mix 0.5 g of the powdered tablets with a few drops of 10% potassium persulfate (w/v). An intense yellow color is developed after 5 minutes of reaction.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 500 mL

Equipment: blades, 50 rpm

Time: 45 minutes

Procedure: after testing, remove aliquot from the dissolution medium, filter, and dilute in 0.1 M hydrochloric acid, to obtain suitable concentration. To measure the absorbances of the solutions at 258 nm, by using the same solvent to adjust zero. To calculate the amount of $C_{13}H_{16}N_3NaO_4S \cdot H_2O$ dissolved in the medium, by comparing the readings obtained with dipyrone SQR solution at concentration already known, prepared in the same solvent.

Tolerance: not less than 70% (Q) of the stated amount of $C_{13}H_{16}N_3NaO_4S \cdot H_2O$ dissolves within 45 minutes.

DOSAGE

To weigh and pulverize 20 tablets. To weigh amount of powder equivalent to 0.35 g of $C_{13}H_{16}N_3NaO_4S \cdot H_2O$ and quantitatively transfer to the erlenmeyer flask. To add 25 mL of water and 5 mL of glacial acetic acid and stir to obtain homogeneous dispersion. To titrate with 0.05 M SV iodine at a temperature below 15 °C by using 1 mL of starch SI as an indicator. Each mL of 0.05 M SV iodine is equal to 17.570 mg of $C_{13}H_{16}N_3NaO_4S \cdot H_2O$.

PACKAGING AND STORAGE

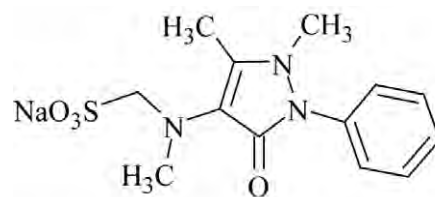
In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

DIPYRONE, SODIUM, MONOHYDRATE

Dipyroneum natricum monohydricum



$C_{13}H_{16}N_3NaO_4S \cdot H_2O$; 351.35

sodium dipyrone monohydrate; 09564

Sodium salt of 1 - [(2,3-dihydro-1,5-dimethyl-3-oxo-2-phenyl-1H-pyrazol-4-yl) methylamino] methanesulfonic acid hydrate (1:1:1)

[5907-38-0]

It contains at least 99.0% and at most 101.0% of $C_{13}H_{16}N_3NaO_4S$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, almost white, odorless powder.

Solubility. Soluble in water and methanol; sparingly soluble in ethanol; practically insoluble in ethyl ether, acetone, benzene, and chloroform.

IDENTIFICATION

A. To add 2 mL of 30% hydrogen peroxide (w/w) to 2 mL of oral solution. Blue color is developed, which disappears rapidly and turns to intense red.

B. To add 2 mL of 10% potassium persulfate (w/v) to 2 mL of oral solution. An intense yellow color is developed.

PURITY TESTS

Appearance of solution. To dissolve 2.5 g of the sample in carbon dioxide-free water and top up the volume to 50 mL with the same solvent. The solution is clear (5.2.25). Immediately after preparation, compare 5 mL of the solution with 5 mL of *Color standard solution* described below. The solution is not more intense than the color standard solution (5.2.12).

Color standard solution: to mix 0.75 mL of the Solution (1), 0.25 mL of the Solution (2), 0.25 mL of the Solution (3), and 48.75 mL of the Solution (4).

Solution (1): to dissolve 4.51 g of ferric chloride with 3.2 mL of M hydrochloric acid and top up with water to 100 mL.

Solution (2): to dissolve 6.5 g of cobaltous chloride with 3 mL of 6 M hydrochloric acid and top up with water to 100 mL.

Solution (3): to dissolve 6.242 g of cupric sulfate pentahydrate with water and top up to 100 mL.

Solution (4): 1% hydrochloric acid (w/v).

Acidity or alkalinity. To add 0.1 mL of phenolphthalein SI to 5 mL of the solution obtained in *Appearance of solution*. The color of the solution does not change. At most 0.1 mL of 0.02 M sodium hydroxide in relation to white is spent to become pink color.

Chloroform-soluble impurities. To weigh 1 g of the sample, add 10 mL of chloroform, and let it rest for 30 minutes. To filter and wash twice with 5 mL of chloroform. To evaporate in a water bath and dry at 105 °C to obtain constant weight. At most 0.5%.

Heavy metals (5.3.2.3). To proceed as described in *Method I*. At most 0.002% (20 ppm).

Sulfates (5.3.2.2). At most 0.1% (1000 ppm).

Loss on drying (5.2.9). To determine in 0.25 g of the sample. To desiccate in an oven at 105 °C to obtain constant weight. At least 4.9% and at most 5.3%.

DOSAGE

To accurately weigh about 0.35 g of the sample and dissolve in 50 mL of water. To add 3 mL of 6% acetic acid (v/v) and titrate with 0.05 M SV iodine at a temperature below 20 °C by using starch SI. Each mL of 0.05 M SV iodine is equal to 16.67 mg of $C_{13}H_{16}N_3NaO_4S$.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Analgesic and antipyretic.

DIPYRONE, ORAL SOLUTION

It contains at least 95.0 % and at most 110.0 % of the amount stated of $C_{13}H_{16}N_3NaO_4S.H_2O$.

IDENTIFICATION

A. To add 2 mL of 30% hydrogen peroxide (w/w) to 2 mL of oral solution. Blue color is developed, which disappears rapidly and turns to intense red.

B. To add 2 mL of 10% potassium persulfate (w/v) to 2 mL of oral solution. An intense yellow color is developed.

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

pH (5.2.19). 5.5 to 7.0.

Dripping test (5.1.8). Dipyrone oral solution packaged with dosage delivery devices complies with the test.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To transfer volume of oral solution equivalent to 5 g of $C_{13}H_{16}N_3NaO_4S.H_2O$ to a 200 mL volumetric flask. To top up with water and make it homogeneous. To transfer 10 mL of the solution to an erlenmeyer flask, add 50 mL of water and 5 mL of glacial acetic acid, and make it homogeneous. To titrate with 0.05 M SV iodine at a temperature below 15 °C by using starch SI as an indicator. Each mL of 0.05 M SV iodine is equal to 17.57 mg of $C_{13}H_{16}N_3NaO_4S.H_2O$.

PACKAGING AND STORAGE

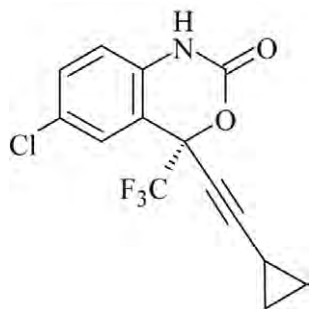
In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

EFAVIRENZ

Efavirenzum



$C_{14}H_9ClF_3NO_2$; 315.67

efavirenz; 03308

(4*S*)-6-Chloro-4-(2-cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-2*H*-3,1-benzoxazin-2-one
[154598-52-4]

It contains at least 98.0% and at most 102.0% of $C_{14}H_9ClF_3NO_2$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white or almost white, and odorless powder.

Solubility. Practically insoluble in water, soluble in methanol and dichloromethane.

Physical and chemical constants.

Melting range (5.2.2): 136 °C to 141 °C.

Specific rotation (5.2.8): -86° to -98°, in relation to the desiccated substance. To determine in 0.3% solution (w/v) in methanol.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the efavirenz SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14), from 200 nm to 350 nm, of 0.001% solution (w/v), in methanol, exhibits maxima at 206 nm, 247 nm, and 293 nm similar to those observed in the spectrum of a similar solution of efavirenz SQR.

C. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in *Dosage* corresponds to that of main peak of *Standard solution*.

PURITY TESTS

Related substances. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 250 nm;

150 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the cyan group (5 µm), kept at 30 °C; flow of *Mobile phase* of 1.5 mL/min.

Eluent A: mixture of water, methanol, and trifluoroacetic acid (90:10:0.05).

Eluent B: mixture of water, methanol, and trifluoroacetic acid (10:90:0.05).

Mobile phase: to use the elution gradient described below

Time (minutes)	Eluent A (% v/v)	Eluent B (% v/v)	Condition
0-16	60 → 50	40 → 50	Linear gradient
16-23	50 → 35	50 → 65	Linear gradient
23-28	35 → 30	65 → 70	Linear gradient
28-29	30 → 20	70 → 80	Linear gradient
29-31	20	80	Isocratic
31-32	20 → 60	80 → 40	Linear gradient

To balance the column for 30 minutes according to the initial conditions. To perform sample blank correction by using the gradient described before injecting the *Solutions* (1), (2), and (3). At the end of each sample blank correction, rebalance the column for at least 8 minutes before starting again.

Diluent: mixture of water and acetonitrile (1:1).

Solution (1): 500 µg/mL solution of the sample in *Diluent*.

Solution (2): 500 µg/mL efavirenz SQR solution in *Diluent*.

Solution (3): to dilute *Solution (2)* with *Diluent* so as to obtain 1.25 µg/mL efavirenz SQR solution.

To inject replicates of 35 µL of *Solution (2)*. The column efficiency is greater than 30000 theoretical plates/meter. To inject replicates of 35 µL of *Solution (3)*. The relative standard deviation for replicate areas of the peaks recorded is not greater than 5.0 %. To inject replicates of 35 µL of *Solution (1)*. The relative retention times are about 0.93 to (4*S*)-6-chloro-4-[(1-*E*) cyclo propylene thenyl]-1,4-dihydro-4 - (trifluoromethyl)-2*H*-3, 1 - benzoxazin-2-one (trans-alkene impurity), if present, and 1.0 to efavirenz. The resolution between the peaks is greater than 1.7.

Procedure: to separately inject 35 µL of each solution, record the chromatograms, and measure the areas under the peaks. To calculate the percentage of trans-alkene impurity, if present in the sample, according to the equation:

$$1.1 \times 100 \times (C_{S3} \cdot At / C_{S1} \cdot Ae)$$

where

1.1 = factor to quantify trans-alkene impurity,

C_{S1} = the sample concentration in mg/mL in *Solution (1)*;

C_{S3} = SQR efavirenz concentration in mg/mL in *Solution (2)*;

A_t = area under the peak corresponding to the trans-alkene impurity in the chromatogram obtained with *Solution (1)*; A_e = area under the peak corresponding to efavirenz in the chromatogram obtained with *Solution (3)*.

At most 0.15% of trans-alkene impurity. The sum of the areas of all the peaks obtained with *Solution (1)*, except those corresponding to efavirenz and trans-alkene impurity, is not greater than the area under the main peak obtained with *Solution (3)* (0.5% for other impurities). To disregard the peaks related to solvent.

Heavy metals (5.3.2.3). To use *Method III*. At most 0.002% (20 ppm).

Loss on drying (5.2.9). To determine in 2 g of the sample. To desiccate in an oven at 60°C under reduced pressure for 3 hours. At most 1.0%.

Water (5.2.20). At most 0.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.2%.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. To accurately weigh about 50 mg of the sample and dissolve in methanol. To top up the volume to 50 mL with the same solvent. To successively dilute in methanol to the 0.001% concentration (w/v). To prepare a standard solution at similar concentration by using the same solvent. To measure the absorbances of the sample and standard solutions at 247 nm, by using methanol to adjust zero. To calculate the content of $C_{14}H_9ClF_3NO_2$ in the sample from the readings obtained.

B. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 252 nm; 250 mm chromatograph column and 4 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μ m), kept at room temperature; flow of *Mobile phase* of 1.0 mL/min.

Mobile phase: to prepare an isocratic system with mobile phase consisting of acetonitrile, water, and orthophosphoric acid (70:30:0.1).

Diluent: mixture of acetonitrile, water, and orthophosphoric acid (70:30:0.1).

Sample solution: to accurately transfer about 40 mg of the sample to a 100 mL volumetric flask. To top up with *Diluent* and make it homogeneous. To transfer 5 mL of this solution to a 100 mL volumetric flask and top up with *Diluent*, thus obtaining 20 μ g/mL solution.

Standard solution: to accurately transfer about 40 mg of efavirenz SQR to a 100 mL volumetric flask.

To top up with *Diluent* and make it homogeneous. To transfer 5 mL of this solution to a 100 mL volumetric flask and top up with *Diluent*, thus obtaining 20 μ g/mL solution.

Procedure: to separately inject 20 μ L of *Standard solution* and *Sample solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_{14}H_9ClF_3NO_2$ from the sample, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antiretroviral.

EEFAVIRENZ TABLETS

It contains at least 90.0% and at most 110.0 % of the amount stated of $C_{14}H_9ClF_3NO_2$.

IDENTIFICATION

A. To weigh and pulverize the tablets. To triturate amount of powder equivalent to 0.3 g of efavirenz with 10 mL of ethyl ether for 1 minute. To filter through a sintered glass funnel, by applying vacuum, if required. To wash with 2 portions of ethyl ether (5 mL each) and combine the ether extracts in a 50 mL beaker. To evaporate under a stream of air at room temperature. To desiccate the residue in an oven at 60 °C for 30 minutes and cool down at room temperature. To add 3 mL of heptane, heat in a water bath at 70 °C, and dissolve the residue by using a spatula. To cover the beaker with a watch glass and cool down in an ice bath at -10 °C for 5 minutes. Then, let it rest at room temperature for 25 minutes. To filter under vacuum, wash the residue with 3 portions of heptane (2 mL each) and finely divide the residue by using a spatula, and keep vacuum for 10 minutes. To desiccate in an oven at 80°C under reduced pressure for 6 hours. The residue responds to test **A.**, *Identification*, in the monograph *Efavirenz*.

B. The residue obtained in test **A.**, *Identification*, melts around 138 °C.

C. To weigh and pulverize the tablets. To transfer amount of powder equivalent to 0.1 g of efavirenz to a 100 mL volumetric flask. To add 70 mL of methanol, put it in ultrasound for 5 minutes, and top up with the same solvent. To filter, by discarding the first few milliliters of the filtrate

and dilute with methanol to obtain 0.002% concentration (w/v).

The ultraviolet absorption spectrum (5.2.14), from 200 nm to 350 nm, exhibits maxima at 206 nm, 247 nm, and 293 nm similar to those observed in the spectrum of a similar solution of efavirenz SQR.

D. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method **B.** corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test. Within 60 minutes.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 1% sodium lauryl sulfate (w/v), 900 mL.

Equipment: blades, 100 rpm

Time: 45 minutes

Procedure: after testing, remove aliquot from the dissolution medium and dilute, if necessary, with dissolution medium, to obtain suitable concentration. To measure the absorbances at 247 nm (5.2.14), by using the *Dissolution medium* to adjust zero. To calculate the amount of $C_{14}H_9ClF_3NO_2$ dissolved in the medium, by comparing the readings obtained with that of 0.0012% efavirenz SQR solution (w/v) with *Dissolution medium*.

Tolerance: not less than 80% (Q) of the stated amount of $C_{14}H_9ClF_3NO_2$ dissolves within 45 minutes.

PURITY TESTS

Related substances. To proceed as described in *Related substances*, in the monograph *Efavirenz*. To prepare *Solution (1)* as described below.

Solution (1): to weigh and pulverize the tablets. To transfer amount of powder equivalent to 0.25 g of efavirenz to a 100 mL volumetric flask. To add 70 mL of *Diluent* and put it in ultrasound for 5 minutes. To top up with the same sol-

vent, make it homogeneous, and let it rest for 15 minutes. To filter, if necessary, and dilute with the same solvent to obtain a 250 µg/mL solution. At most 0.15% for trans-alkene impurity and 1.0% for other impurities.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. To weigh and pulverize 20 tablets. To transfer amount of powder equivalent to 0.15 g of efavirenz to a 100 mL volumetric flask and add 70 mL of absolute ethanol. To put it in ultrasound for 5 minutes. To filter, if necessary, and dilute to 0.0075% concentration (w/v) by using the same solvent. To prepare standard solution under the same conditions. To measure the absorbances of the resulting solutions at 293 nm, by using absolute ethanol to adjust zero. To calculate the content of $C_{14}H_9ClF_3NO_2$ in the sample from the readings obtained.

To proceed as described in *High performance liquid chromatography (5.2.17.4)*. To proceed as described in *Dosage* method **B.**, in the monograph *Efavirenz*. To prepare the *Sample solution* as described below.

Sample solution: To weigh and pulverize 20 tablets. To transfer amount of powder equivalent to 40 mg of efavirenz to a 100 mL volumetric flask, add 40 mL of *Diluent*, and put it in ultrasound for 15 minutes. To top up with the same solvent, make it homogeneous, and filter it. To transfer 5 mL to 100 mL volumetric flask and top up with *Diluent*, thus obtaining 20 µg/mL solution.

Procedure: to separately inject 20 µL of *Sample and standard solutions*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of $C_{14}H_9ClF_3NO_2$ in the tablets, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

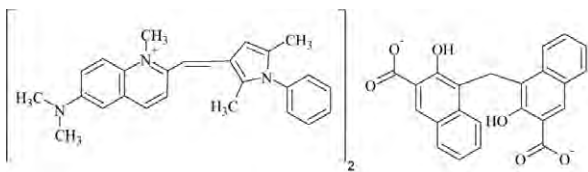
In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

EMBonATE, PYRVINIUM

Pyrvinii embonas



$(C_{26}H_{28}N_3)_2 \cdot C_{23}H_{14}O_6$; 1151.40

pyrvinium embonate; 03346

4,4'-methylenebis [3-hydroxy-2-naphthalene] of
6-(dimethylamino)-2-[2-(2,5-dimethyl-1-phenyl-1H-pyrrol-3-yl)ethenyl]-1-methyl-quinolinium (1:2)
[3546-41-6]

It contains at least 96.0% and at most 104.0% of $(C_{26}H_{28}N_3)_2 \cdot C_{23}H_{14}O_6$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. Crystalline, light orange or orange-red to almost black powder.

Solubility. Practically insoluble in water and ethyl ether, slightly soluble in chloroform and methoxyethanol, very slightly soluble in methanol. Freely soluble in glacial acetic acid.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the pyrvinium embonate SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14) ranging from 200 nm to 800 nm, of sample solution obtained in *Dosage*, exhibits absorbance maxima around 358 nm and 505 nm. The ratio between the absorbance values measured is between 1.93 and 2.07.

PURITY TESTS

Water (5.2.20). To determine in 0.2 g of the sample, by using a mixture of 10 mL of methanol and 10 mL of chloroform as solvent. At most 6.0%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.5%.

DOSAGE

Note: to use low actinism vials for solutions and protect solutions from direct light. To perform dosage without prolonged interruptions.

To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. To accurately weigh about 0.25 g of the sample and dissolve in 125 mL of glacial acetic acid. To top up to 250 mL with methanol and make it homogeneous. To successively dilute in methanol to the 0.001% concentration (w/v). To prepare a standard solution at similar concentration by using the same solvents. To measure the absorbance of the resulting solutions at 505 nm, by using methanol to adjust zero. To calculate the content of $(C_{26}H_{28}N_3)_2 \cdot C_{23}H_{14}O_6$ in the sample from the readings obtained.

PACKAGING AND STORAGE

In airtight, opaque containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Anthelmintic (oxiurose).

DILL

Anethi fructus

Anethum graveolens L. - APIACEAE

The vegetable drug consists of fruit, which are diachenes (schizocarp) containing at least 2.0% of volatile oil.

CHARACTERISTICS

Organoleptic characteristics. It has aromatic odor and typical taste.

MACROSCOPIC DESCRIPTION

The fruit is an oval diachene divided into two mericarps dorsally compressed from 0.30 cm to 0.60 cm long and 0.12 cm to 0.30 cm wide, brown to light brown, with two stylopodiums and apex of retrorse stylet. In the dissection, the mericarps are usually separated, as a rule, they are not accompanied by carpophorus; remainder of stylopodiums and calice may occur. Each mericarp has two margined edges prolonged in an enclosed wing, large, membranous, lighter, yellowish, and three dorsal, longitudinal, filiform edges, light brown to yellowish, slightly elevated, but evident, all primary. The commissural face is flattened and slightly concave by desiccation, and shows clearly the line of carpophorus. The cuticle of each mericarp is covered with an epicuticular wax formed by short filaments randomly arranged. These filaments are more dense in the commissural face, which makes it whitish. In cross section, the mericarp is plano-convex, and six elliptical secretory canals are visible, four of them are large and narrow, arranged in the dorsal portion, and two, more rarely large, in the commissural or ventral face. There are vascular bundles in each dorsal edge. Those corresponding to the wings

are slightly larger than the other. The endosperm is oily and concave on the commissural face.

MICROSCOPIC DESCRIPTION

In cross section, the mericarp, dorsally flattened, shows three primary, dorsal, narrow edges and two primary, lateral, elongated edges. The exocarp is composed of striated cuticle, consisting of wax filaments randomly arranged and a colorless layer of epidermal, flattened cells with thin walls, except for the outer periclinal, which is thicker.

The mesocarp is externally composed of a few layers of flattened, parenchyma cells, with thinner walls compared with those of the inner layers, which show obvious pits. In the mesocarp region, corresponding to the primary edges, both marginal and dorsal, there are fiber strands with some vascular elements that sometimes are visible. The fiber strands corresponding to the dorsal edges are less developed than those corresponding to the marginal winged edges. In the region between the edges and the commissural region, there are secretory canals, schizogenous, elliptical, and narrow-elongated in tangential direction. The secretory epithelium is composed of tangentially flattened cells with thick walls. In the secretory portion of the canal, facing the epicarp and just below it, there are thick-walled sclereids, square or rectangular, with numerous and conspicuous pits. The innermost portion of mesocarp comprises 2-3 layers of brownish-yellow cells, very flat tangentially, thick-walled, if compared with the epicarp. The endocarp comprises a layer of lignified cells. Between the pericarp and the seed, in the commissural face, there is a chamber next to raphe. Usually associated with the endocarp, there is the coat, composed of a single layer of cells, usually collapsed, brown color and thin walls. The endosperm is abundant, composed of thick-walled cells, the outer one are more elongated and completely filled with starch grains. Spherical oil droplets and various crystals of calcium oxalate of different shapes are also present. The innermost layers of this tissue have a polyhedral shape and generally fewer starch grains. Cells with starch grains, when subjected to lugol, become reddish, and oil drops, isolated on material, are yellow to orange. The oil droplets can occupy most of the cell volume.

MICROSCOPIC DESCRIPTION OF POWDER

The powder meets all the requirements for the species, unless the macroscopic characters. Characteristics: brown color; portions of the epidermis of the epicarp, whose cells have cuticle covered with wax filaments arranged haphazardly; portions of mesocarp with polygonal to rectangular cells with clear pit walls; portions of endocarp with cells of sinuous walls; small crystals of different shapes, especially druzen-type, occur abundantly and aggregates; isolated crystals in the form of prisms, especially rhombohedral generally larger than the aggregated crystals; grouping of fibers associated with vascular bundles; tracheary elements of helical and/or ringed thickening or occasionally crosslinked or pit; portions of the endosperm consists of thick-walled parenchyma tissue with cells filled with

starch grains; sclereids as described; secretory canals, or their portions with secretory epithelial cells.

IDENTIFICATION

To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ with 250 µm thick as support and a mixture of toluene and ethyl acetate (93:7) as mobile phase. To separately apply to the plate, as band, 10 µL of Solution (1), Solution (2), and Solution (3) freshly prepared, as described below.

Solution (1): to stir for 10 minutes 0.5 g of pulverized drug with 10 mL of methylene chloride. To filter it. To concentrate the filtrate in a water bath at temperature up to 60 °C, to obtain residue. To resuspend the residue in 10 mL of toluene.

Solution (2): to dilute 2 µL of volatile oil obtained in *Dosage for volatile oils* in 1 mL of toluene.

Solution (3): to dilute 2 µL of carvone in 1 mL of toluene.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). One of the principal spots obtained with *Solution (1)* corresponds in position and intensity to that obtained with *Solution (3)* associated with carvone (R_f about 0.60). The chromatogram also provides an intense spot with R_f about 0.80, corresponding to dilapiol. To nebulize the plate with vanillin sulfuric SR and put it in an oven at 100 °C - 105 °C for 5 minutes. The spot corresponding to carvone has pink color, and that corresponding to dilapiol has brown color.

PURITY TESTS

Foreign matter (5.4.2.2). At most 2.0%.

Water (5.4.2.3). At most 11.0%.

Total ashes (5.4.2.4). At most 7.0%.

DOSAGE

Volatile oils

To proceed as described in *Determination of volatile oils in vegetable drugs* (5.4.2.7). To use 250 mL flask containing 100 mL of water as distillation liquid and 0.5 mL of xylene. To pulverize the dried fruits to obtain coarse powder. To immediately proceed to determine the volatile oil from 25 g of the powdered drug. To distill for 4 hours.

Carvone

To employ one of the methods described below.

A. To prepare the solutions as described below.

Solution (1): to transfer to glass jars (approximately 150 mm x 25 mm) 1.5 g of volatile oil after its extraction and

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add 10 mL of *Solution (2)*, previously prepared, as described below.

Solution (2): to dissolve 7 g of hydroxylamine hydrochloride in 90 mL of 90% ethanol (v/v) and gently heat it, if necessary. To add 1.6 mL of dimethyl yellow SI and *M* potassium hydroxide in 90% ethanol (v/v) in sufficient amount to produce yellow color without forming a precipitate in the bottom of the flask, and dilute with 90% ethanol (v/v) to obtain 100 mL.

To titrate the *Solution (1)* with *M* potassium hydroxide diluted in 90% ethanol (v/v) to pink color becomes intense yellow. To place the tube in a water bath at 70 °C - 80 °C, and neutralize with *M* potassium hydroxide in 90% ethanol (v/v) at every 5 minutes. After 40 minutes, complete the titration to obtain yellow color as in *Solution (2)*. To repeat the procedure by using as a standard color to determine the endpoint of titration the solution titrated by adding 0.5 mL of *M* potassium hydroxide in 90% ethanol (v/v). To calculate the carvone content for the second determination. Each ml of *M* potassium hydroxide in ethanol 90% (v/v) is equal to 151.4 mg of carvone, $C_{10}H_{14}O$.

The volatile oil must have at least 43.0% of carvone.

B. To proceed as described in *Gas chromatography (5.2.17.5)*. To use chromatograph equipped with flame ionization detector, by using mixture of nitrogen, synthetic air, and hydrogen (1:1:10) as the auxiliary gas to flame detector; 30 m long capillary column and 0.25 mm inner diameter, filled with poly (dimethyl/diphenyl siloxane), with a film thickness of 0.25 µm; column temperature from 60 °C to 300 °C, at 3 °C per minute (total: 80 minutes); injector temperature at 220 °C; detector temperature at 250 °C; helium

at 80 kPa as carrier gas and flow of carrier gas 1 mL/minute.

Sample solution: to dilute the volatile oil, obtained in *Dosage for volatile oils*, in ethyl ether (2:100).

Procedure: to inject 1 µL of *Sample solution* into the gas chromatograph, by using 1:50 flow division. The carvone and dilapiol have linear retention times (Kovats index) of 1236 and 1615, respectively. The relative concentrations are obtained by integrating manually or electronically.

To calculate the Kovats index (IK), according to the equation:

$$IK = 100 \times n + \frac{100 \times (tr_z - tr_z)}{(tr_{z+1} - tr_z)}$$

where

n = number of carbon atoms in the alkane with lower molecular weight,

tr_x = retention time of compound "x" (intermediate to tr_z and tr_{z+1});

tr_z = retention time of the alkane with 'n' carbons;

tr_{z+1} = retention time of the alkane with 'n+1' carbons.

The volatile oil must have at least 30.0% of carvone and 30.0% of dilapiol.

PACKAGING AND STORAGE

In tightly closed container, protected from light and heat.



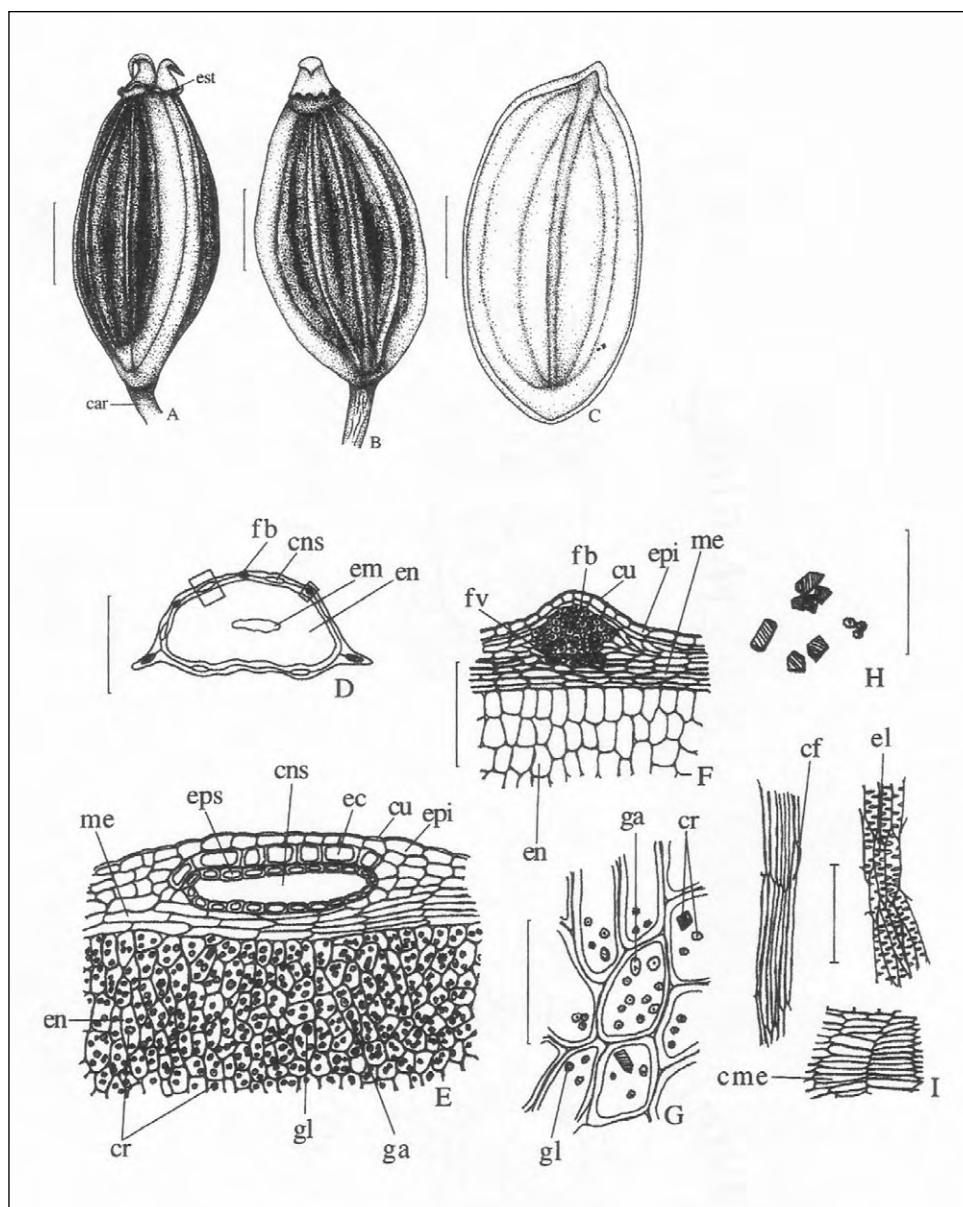


Figure 1 - Macroscopic and microscopic aspects in *Anethum graveolens* L.

Additional information of **Figure 1**. Scales correspond to: in A, B, C, D, to 1 mm; in E, F, I to 100 µm; in G and H, to 50 µm.

A - diachene in side view: carpophorus (car); stylopodium (est). **B** - mericarp in front view. **C** - view of the commissural face of the mericarp. **D** - general appearance of mericarp in cross-section: fibers (fb); secretory canal (cns); embryo (em); endosperm (en). **E** - detail of cross section of mericarp, as noted in **D**: mesocarp (me), endosperm (en); crystals (cr); lipid droplet (gl); starch grain (ga); secretory epithelium (eps); secretory canal (cns); sclereid (c); cuticle (cu); epicarp (epi). **F** - detail of cross-section of the mericarp in the dorsal edge, as indicated in **D**: vascular bundle (fv); fibers (fb); cuticle (cu); epicarp (epi), mesocarp (me), endosperm (en). **G** - detail of endosperm cells: starch grain (ga); lipid droplet (gl); crystals (cr). **H** - crystal of different shapes. **I** - details of powder: fiber strand (cf); detail of tracheary elements in longitudinal view (el); detail of mesocarp cells with thick walls (cme).

MEDICAL TAPE

It consists of tissue from various sources uniformly coated on one side by a pressure sensitive adhesive layer.

The tape has an adhesive flat surface, which is uniform and lump-free; it also has neutral reaction and is free of toxic or irritating substances. The opposite side of the adhesive mixture can be coated with a thin layer of water-proof substances. In general, it is rolled in continuous strips of various sizes. The tape must be free of impurities and contamination.

CHARACTERISTICS

Dimension. To determine the length of the medical tape. The result must be not less than 98% of length stated on the label. To determine the width of medical tape at 5 different points along its length. The average of the results should not have a difference greater than 1.6 mm wide according to the label.

Tensile strength (5.7.1). To determine the tensile strength of the tape after unrolling and be a minimum of 4 hours in a standard atmosphere of $65 \pm 2\%$ RH at $21^\circ\text{C} \pm 1.1^\circ\text{C}$, by using a pendulum type device. To proceed as described in *Tensile strength*. The average with three determinations in strips of 2.5 cm wide must be not less than 20 kg.

Adhesion to the surface. From the sample made of fabric, cut a strip of 2.54 cm wide and approximately 15 cm long. To apply pressure equivalent to 850 g in one end of the tape (surface equal to 12.90 cm^2 - 2.54 cm wide x 5.08 cm long) against a clean glass, plastic or stainless steel. To exert pressure twice by using a rubber roller at 30 cm/minute. To adjust the tape and surface temperature at 37°C (5.7.1) and immediately conduct the test as described in *Tensile strength*. To use a pendulum type device and the breakage must be in parallel to the surface. The average value of at least 10 tests should be at least 18 kg.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). Applicable when the medical tap is considered sterile. It complies with the test.

PACKAGING AND STORAGE

In tightly closed packaging, protected from light and excessive heat.

The medical tape, when stated sterile or sterilized, should be packaged in order to protect its sterility against contamination.

LABELLING

To comply with the legislation in force.

ESPINHEIRA SANTA Mayteni folium

Maytenus ilicifolia Mart. ex Reissek - CELASTRACEAE; 09912

The vegetable drug consists of dried leaf containing at least 2.0 % of total tannins, expressed as pyrogallol ($\text{C}_6\text{H}_6\text{O}_3$; 126.11), from which at least 2.8 mg/g is equivalent to epicatechin ($\text{C}_{15}\text{H}_{14}\text{O}_6$; 290.3).

CHARACTERISTICS

Organoleptic characteristics. The dried leaves are odorless, slightly bitter, and astringent.

MACROSCOPIC DESCRIPTION

Simple, whole leaves, oval-lanceolate when young, becoming elliptical-lanceolate with maturation. Blade with 2.1 cm - 9.0 cm (rarely 15.0 cm) long and 1.0 cm - 3.1 cm (rarely 7.0 cm) wide, coriaceous to subcoriaceous, glabrous, with mucronate apex, obtuse to acute base, pinnate, with prominent midrib on the lower face. Venation is mixed craspedodromus-type, with secondary ribs at an acute angle relative to the principal one, ending at the edge of the blade, or branching off near it, or towards edge, where they meet up with the following one, by forming arcs. In the leaf margin, in both secondary ribs and those from them, together with the marginal rib, forming pointed projections, 9-14 units per leaf, arranged more often in the apical half of the blade. The areolas are predominantly rectangular, with branched endings. Short petiole with 0.2 cm to 0.5 cm in length. In dried samples, the adaxial face of the limb is relatively darker than the lower one (whitish).

MICROSCOPIC DESCRIPTION

Hypostomatic leaves with dorsiventral mesophyll. The stomata are "laterocítico" (1-3 subsidiary cells for each guard-cell), located just above or at the same height of the epidermal cells. The inner thickness of the guard-cells is prominent. Due to the thickness of leaf cuticle on the stomatal pore, there are projections formed, resulting in a stomatal-supra atrium. Other epidermal cells on both sides of the blade are polygonal, various sizes, with straight anticlinal walls, larger on the adaxial face. In cross section, there is single-layered epidermis with thick walls covered with a thickened cuticle layer also forming a cuticular flange, with on average $7.8\text{ }\mu\text{m}$ on adaxial face and $4.8\text{ }\mu\text{m}$ on the opposite face,

ever more prominent in the midrib, where cuticular ornamentations occur in the form of strips and papillae. In epidermal cells, there are small styloids (young leaves) or rectangular prismatic crystals (mature leaves), both of calcium oxalate. The palisade parenchyma is formed by two layers of long and thin cells, in typical palisade, or even by 2-3 layers of cubic or slightly elongated cells, depending on the sample analyzed. The spongy parenchyma is formed by 6-9 layers of cells with short brachiform ex-

pansions with wide intercellular spaces, more compressed toward the lower region. In the mesophyll, cells containing phenolic compounds, isolated or grouped, are common, especially those belonging to the palisade, in addition to styloid and prismatic crystals of small dimensions. In the midrib, biconvex in cross-section, there are 3 - 4 layers of angular collenchyma near the adaxial face and 2 - 3 on the opposite side, which positively react to ferric chloride SR (phenolic substances). The vascular bundle of the midrib is unique, collateral in open arc, surrounded by a sheath of parenchyma cells with thin walls, and fiber calottes on both poles of conductive tissue, also present in the small bundles. The arrangement of tissue in the vascular bundle is not constant and may vary with the portion of the blade and degree of maturation of the organ. Phloem has rhombic crystals of calcium oxalate, sclerids, and cells containing phenolic compounds. Fibers that accompany it have thick cell wall with simple pits. Mature leaves can have bicollateral or concentric vascular bundle (anficrival), always surrounded by sclerenchyma. In the region of the leaf margin, the vascular bundle, which constitutes the marginal rib, is surrounded by 250-280 very thick fiber wall. The petiole has a circular to plano-convex outline, in cross section, and toward the distal portion of the leaf occur side fins and a slight convexity in the adaxial portion. The epidermis of the petiole is uniseriate, covered with a thick cuticle layer. Both epidermal cells and underlying cell layers have small crystals of calcium oxalate and dense content, brown color, which positively react to ferric chloride SR. The parenchyma has thickened cellulose, collenchymatous, and may contain styloids similar to the blade and prismatic crystals of small dimensions. Isolated braquisclereids with a thickened wall and simple pits randomly occur in the fundamental parenchyma. The vascular bundle is single, concentric, cylindrical to slightly concave-convex, surrounded by a sclerenchymatic sheath composed of isolated fibers or in groups from two to many elements. Some parenchyma phloem cells parenchyma beams positively react to ferric chloride SR.

MICROSCOPIC DESCRIPTION OF POWDER

The powder meets all the requirements for the species, unless the macroscopic characters. Characteristics: odorless powder, slightly refreshing;

yellowish-green color; fragments of epidermis with straight periclinal walls, covered with a thick cuticle and containing small styloid or prismatic crystals in abundance; fragments of epidermis with laterocytic stomata; fragments of palisade parenchyma with 2 or 3 cell layers, completely distended or not; fragments of high caliber fiber with simple pits.

IDENTIFICATION

A. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using F_{254} silica gel, with 250 μm thickness as support and a mixture of ethyl acetate, formic acid, and water (90:5:5) as mobile phase. To separately apply to the

plate, as band, 10 μL of *Solution (1)* and 3 μL of *Solution (2)* freshly prepared, as described below.

Solution (1): to accurately weigh about 1 g of milled drug, add 50 mL of methanol, and heat under reflux for 15 minutes. After cooling at room temperature, filter the solution obtained in cotton under reduced pressure, transfer to a 50 mL volumetric flask, and top up to volume with distilled water.

Solution (2): to weigh about 1 mg of epicatechin SQR and dissolve in 1 mL of methanol.

To develop the chromatogram. To remove the plate and dry in fume hood. To examine under ultraviolet light (254 nm). The chromatogram obtained with *Solution (1)* has a burgundy spot at the same height that obtained in the chromatogram of *Solution (2)* (R_f about 0.82). Then, nebulize the plate with vanillin sulfuric SR and put it in oven at 110 °C for 10 minutes. After viewing it, two burgundy spots in *Solution (1)* with R_f about 0.82 to epicatechin and 0.72 to burgundy band, which appears below, should be observed.

B. To add 2 drops of hydrochloric acid SR to 2 mL of extract obtained upon preparing *Solution (1)*, in test A., *Identification*, and drip gelatin SR to precipitation. The appearance of clear precipitate indicates a positive reaction for total tannins.

C. To add 10 mL of water and 2-4 drops of 1% ferric chloride solution (w/v), in ethanol, to 2 mL of the extract obtained in *Solution (1)*, in test A., *Identification*. The development of dark-gray color indicates a positive reaction for total tannins.

D. To add 0.5 mL of 1% vanillin (w/v), in methanol, and 1 mL of hydrochloric acid to 2 mL of the extract obtained in *Solution (1)*, in test A., *Identification*. The development of dark-gray color indicates a positive reaction for condensed tannins.

E. To add 10 mL of 2 M acetic acid and 5 mL of lead acetate SR to 5 mL of the extract obtained in *Solution (1)*, in test A., *Identification*. The appearance of whitish precipitate indicates the presence of tannins.

PURITY TESTS

Foreign matter (5.4.2.2). At most 2%.

Water (5.4.2.3). At most 12%.

Total ashes (5.4.2.4). At most 8%.

Sulfated ashes (5.4.2.6). At most 12 %.

DETERMINATION OF FOAM INDEX (IE)

To accurately transfer about 1 g of the milled vegetable drug (180 μm) to 50 mL erlenmeyer flask containing boiling water. To keep under moderate boil for 15 minutes. To

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cool down and filter through cotton to 100 mL volumetric flask. To top up to volume through the filter up to 100 mL. To distribute the decoction obtained in 10 test tubes with lid (16 mm diameter and 16 cm height), in a successive series of 1 mL, 2 mL, 3 mL, until 10 mL, and adjust the volume of liquid in each tube to 10 mL of water. To cover the tubes and vigorously stir for 15 seconds in vertical movements, by stirring twice per second. To let it rest for 15 minutes and measure the foam height. Then, add 1 mL of 2 M hydrochloric acid to each tube. If the foam height of all tubes is below 1 cm, the foam index is less than 100. If the foam height after 10 minutes remains equal to or greater than 1 cm, the dilution of the vegetable material in this tube (A) is the index observed. To calculate the foam index, according to the equation:

To volumetrically transfer 2 mL of this solution, 1 mL of phosphotungstic molybdate reagent, and 10 mL of distilled water to a 25 mL volumetric flask and top up with a 29% sodium carbonate solution (w/v). To determine the absorbance at 760 nm (A_1) after 30 minutes by using distilled water to adjust zero.

Sample solution for polyphenols not adsorbed by hide powder: to obtain 10 mL of the filtrate, add 0.1 g of hide powder SQR and mechanically stir in 125 mL erlenmeyer flask for 60 minutes. To use filter paper. To dilute 5 mL of the filtrate in 25 mL volumetric flask with distilled water. To volumetrically transfer 2 mL of this solution, 1 mL of phosphotungstic molybdate reagent, and 10 mL of distilled water to a 25 mL volumetric flask and top up with a 29% sodium carbonate solution (w/v). To determine the absorbance at 760 nm (A_2) after 30 minutes by using distilled water to adjust zero.

Standard solution: just before using it, dissolve 50 mg of pyrogallol in a 100 mL volumetric flask with distilled water. To volumetrically transfer 5 mL of this solution to a 100 mL volumetric flask and top up with distilled water. To volumetrically transfer 2 mL of this solution, 1 mL of phosphotungstic molybdate reagent, and 10 mL of distilled water to a 25 mL volumetric flask and top up with a 29% sodium carbonate solution (w/v). To determine the absorbance at 760 nm (A_3) after 30 minutes by using distilled water to adjust zero.

To calculate the content of tannin (dried drug), in %, expressed as pyrogallol, according to the equation:

$$IE = \frac{1000}{A}$$

where

A = volume (mL) of decoction used for dilution in the tube, where foam was observed.
The foam index is at least 250.

DOSAGE

Total tannins

Note: to perform all the extraction and dilution operations protected from the light.

To prepare the solutions as described below.

Stock solution: to weigh 0.750 g of the pulverized drug (250 μ m) and transfer to a 250 mL erlenmeyer flask with ground mouth. To add 150 mL of distilled water. To heat it in water bath at 60 °C for 30 minutes. To cool down with running water and transfer to a 250 mL volumetric flask. To wash the erlenmeyer flask and transfer the washings with all the content of vegetable drug to the same volumetric flask. To top up to volume with distilled water. To decant and filter the supernatant liquid by using filter paper. To discard the first 50 mL of the filtrate.

Sample solution for total polyphenols: to dilute 5 mL of the filtrate in 25 mL volumetric flask with distilled water.

$$TT = \frac{62,5 \times (A_1 - A_2) \times m_2}{A_3 \times m_1}$$

where

A_1 = absorbance of Sample solution for total polyphenols;
 A_2 = absorbance of Sample solution for polyphenols not adsorbed by hide powder;
 A_3 = absorbance of Standard solution;
 m_1 = mass of the sample used in the test (g) by considering the determination of water;
 m_2 = mass of pyrogallol (g).

Epicatechin

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 210 nm; pre-column packed with silica chemically bonded to the octadecylsilane group, 250 mm column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μ m); 250 mm column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μ m); flow of *Mobile phase* of 0.8 mL/min.

Eluent A: mixture of water and 0.05% trifluoroacetic acid (v/v).

Eluent B: mixture of acetonitrile and 0.05% trifluoroacetic acid (v/v).

Mobile phase gradient: to adopt the gradient system described in the following table:

<i>Time (minutes)</i>	<i>Eluent A (%)</i>	<i>Eluent B (%)</i>	<i>Elution</i>
0 - 13	82 → 75	18 → 25	Linear gradient
13 - 16	75 → 66	25 → 34	Linear gradient
16 - 20	66 → 58	34 → 42	Linear gradient
20 - 23	58 → 35	42 → 65	Linear gradient
23 - 25	35 → 82	65 → 18	Linear gradient
25 - 28	82	18	isocratic

Sample solution: to accurately weigh about 5 g of pulverized vegetable drug (250 µm) in a 100 mL round bottom flask and ground mouth, add 50 mL of distilled water, and put it under reflux for 15 minutes. After cooling down at room temperature, filter under reduced pressure the solution obtained. To extract the filtrate with 3 portions of 50 mL of ethyl acetate in a separatory funnel (250 mL). To let it rest at -18 °C for 5 minutes, to obtain complete separation of the phases. To gather the organic phases. To filter through filter paper containing 5 g of anhydrous sodium sulfate under reduced pressure. To evaporate the organic phase in a rotary evaporator under reduced pressure to obtain the residue. To resuspend the residue with 5 mL of mixture of methanol and water (2:8). To extract in extraction cartridge in solid phase, packed with silica chemically bonded to the octadecylsilane group (55 µm, 70 Å), previously packed with 8 mL of methanol and water (2:8) to a 100 mL flask. To elute 10 mL of methanol and water (2:8) to the same flask and top up the volume (S₁) with methanol and water (2:8). To volumetrically transfer 5 mL of S₁ to 25 mL volumetric flask and top up with methanol and water (1:1) (S₂). To filter S₂ (PTFE membrane, porosity 0.5 µm) and inject it into the chromatograph.

Epicatechin standard solution: to dissolve accurately weighed amount of epicatechin SQR in mixture of methanol and water (1:1) to obtain 0.4 mg/mL solution.

Solution for epicatechin analytical curve: to dilute aliquots of 50 µL, 200 µL, 350 µL, 500 µL, and 600 µL of the *Epicatechin standard solution* in 2 mL volumetric flask with methanol and water (1:1) to obtain of 10 µg/mL, 40 µg/mL, 70 µg/mL, 100 µg/mL, and 120 µg/mL concentrations.

Procedure: to separately inject 20 µL of solutions for analytical and *Sample solution* curves in quintuplicate, record the chromatograms, and measure the areas under the peaks. The relative retention time is about 8.0 minutes to epicatechin. To calculate the epicatechin content from the sample, from the linear equation obtained with analytical curve for the standard. The result is the mean of the determinations in mg/g of vegetable drug, according to the equation:

$$EC = \frac{VLR \times 500}{1000 \times m}$$

where

EC = epicatechin;

VLR = value obtained in (µg/mL) of epicatechin/mL in S₂, from the linear equation;

500 = dilution factor;

1000 = conversion factor from µg to mg;

m = mass (g) of vegetable drug, by considering the water determination.

PACKAGING AND STORAGE

In tightly closed container, protected from light and heat.

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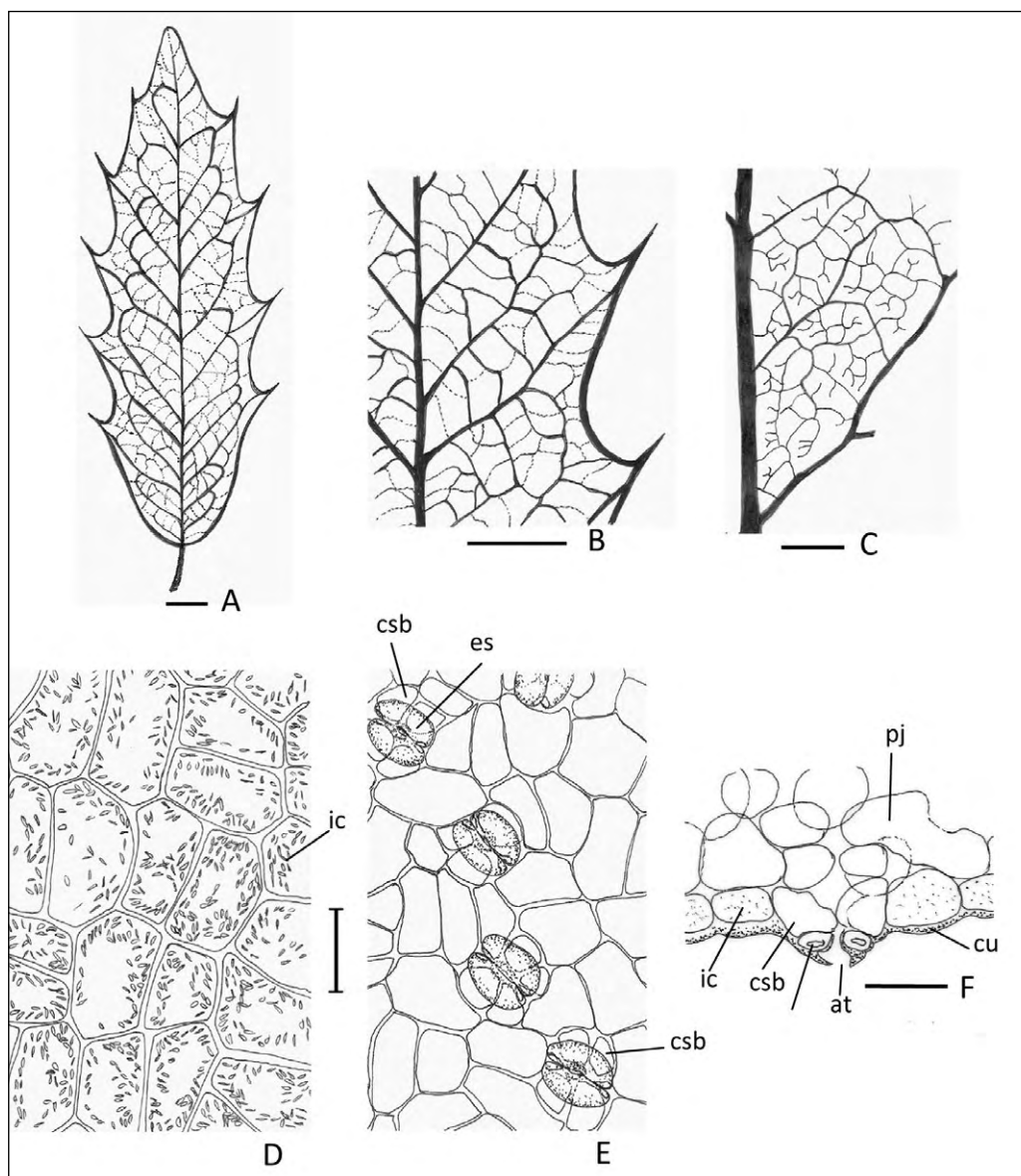


Figure 1 - Macroscopic and microscopic aspects in *Maytenus ilicifolia* Mart. ex Reissek

Additional information of **Figure 1**. Scales correspond to 5 mm in **A**, and **B**; in **C**, 1 mm; in **D**, **E**, and **F**, 30 μ m.

A - general appearance of the leaf blade. **B** - detail of leaf venation on the adaxial face, in front view. **C** - detail of portion of the leaf blade, on the adaxial face, in front view, showing the areolae and xylem endings: areola (ar). **D and E** - partial detail of the epidermis facing the adaxial and lower face, respectively, in front view: crystal idioblast (ic); subsidiary cell (csb); stomata (es). **F** - partial detail of leaf blade, in cross section, showing a stoma: spongy parenchyma (pj); cuticle (cu); stomatal-supra atrium (at); guard-cell (cg); subsidiary cell (csb); crystal idioblast (ic).

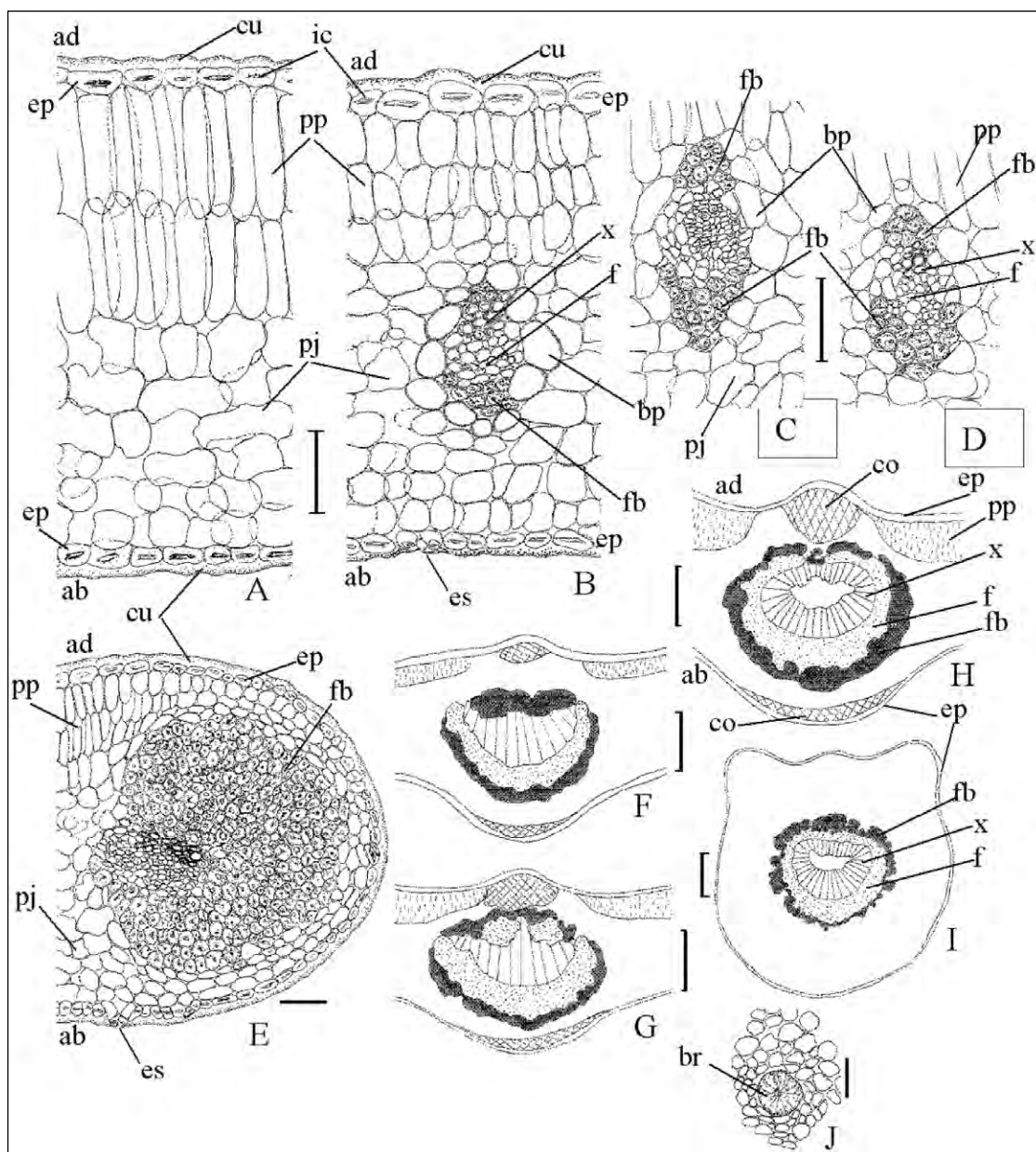


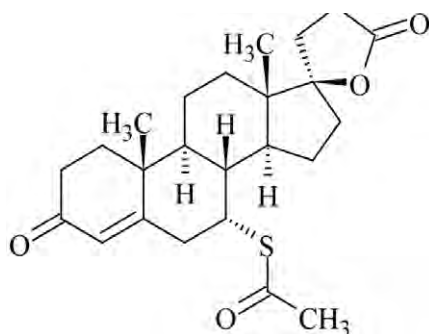
Figure 2 - Microscopic aspects in *Maytenus ilicifolia* Mart. ex Reissek

Additional information of **Figure 2**. Scales correspond to 50 µm, in **A and B**; in **C and D**, 75 µm; in **E**, 35 µm, in **F**, 120 µm; in **G**, 180 µm, in **H and I**, 200 µm; and **J**, 50 µm.

A and B — partial details of mesophyll from different samples, in cross-section: adaxial face (ad), lower face (ab); epidermis (ep), cuticle (cu); crystal idioblast (ic); palisade parenchyma (pp); spongy parenchyma (pj); xylem (x), phloem (f); parenchymatous sheath (bp); fibers (fb); stomata (es). **C and D** — detail of a secondary vascular bundle in the basal and middle portion of the leaf blade, respectively, in cross-section: fibers (fb); parenchymatous sheath (bp); spongy parenchyma (pj); palisade parenchyma (pp); xylem (x), phloem (f). **E** — detail of leaf edge, in cross section, showing the marginal rib: adaxial face (ad), lower face (ab); epidermis (ep); palisade parenchyma (pp); spongy parenchyma (pj); fibers (fb); stomata (es). **F, G, and H** — schemes of the general appearance of the middle portion of the midrib, in cross section, showing variations in the distribution of phloem, xylem, and fibers: adaxial face (ad), lower face (ab), collenchyma (co); epidermis (ep); palisade parenchyma (pp); xylem (x), phloem (f); fibers (fb). **I** — scheme of general appearance of the petiole in cross-section: epidermis (ep); fiber (fb), xylem (x); phloem (f). **J** — detail of a braquisclereid of the petiole in cross-section: braquisclereid (br).

SPIRONOLACTONE8

Spironolactonum



$C_{24}H_{32}O_4S$; 416.57
 spironolactone; 03561
 γ -lactone acid (7 α , 17 α) -7 - (acetylthio)-17-hydroxy-3-oxopregn-4-ene-21-carboxylic acid
 [52-01-7]

It contains at least 97.0% and at most 103.0% of $C_{24}H_{32}O_4S$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, light beige to yellowish brown powder. Stable to air.

Solubility. Practically insoluble in water, freely soluble in benzene and chloroform, soluble in ethyl acetate and absolute ethanol, sparingly soluble in methanol.

Physical and chemical constants.

Specific rotation (5.2.8): -33° to -37° , in relation to the desiccated substance. To determine in 1% solution (w/v) in chloroform.

Melting range (5.2.2): 198°C to 207°C with decomposition. It can occasionally show preliminary melting at about 135°C followed by re-solidification.

IDENTIFICATION

The identification test **A.** may be omitted if the tests **B.** and **C.** are conducted.

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated at 5% chloroform solution (w/v), has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the spironolactone SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14) of the sample solution obtained in method A., Dosage, ranging from 200 nm to 400 nm, exhibits maxima and minima only in the same wavelength of a similar spironolactone SQR solution.

The respective absorptivity, measured at the wavelength of maximum absorbance around 238 nm, does not differ by more than 3%.

C. To dissolve 100 mg of the sample in a mixture of 10 mL of water and 2 mL of sodium hydroxide SR. To boil the mixture for 3 minutes, cool down, and add 1 mL of glacial acetic acid and 1 mL of lead acetate SR. Brown to black lead sulfide precipitate is formed.

PURITY TESTS

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel as support and butyl acetate as mobile phase. To separately apply to the plate 5 μL of each of the solutions freshly prepared, as described below.

Solution (1): to dissolve 0.2 g of the sample in chloroform and top up to 10 mL with the same solvent.

Solution (2): to dilute 0.5 mL of *Solution (1)* to 50 mL with chloroform.

To develop the chromatogram. To remove the plate, and let it drying. To nebulize with sulfuric acid/methanol SR, heat the plate at 105°C for 10 minutes, and immediately examine. Any secondary spot in the chromatogram obtained with *Solution (1)* (2%), other than the principal spot, is not more intense than that obtained with *Solution (2)* (1.0%).

Mercapto compounds. To stir 2 g of the sample with 30 mL of water, filter, then add 3 mL of starch SI to 15 mL of the filtrate and titrate with 0.005 M SV iodine. To perform sample blank correction and adjust it as required. At most 0.10 mL of 0.005 M SV iodine is consumed.

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 105°C for 2 hours. At most 0.5%.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To accurately weigh about 50 mg of the sample and dissolve in methanol. To top up the volume to 250 mL with the same solvent. To successively dilute in methanol to the 0.001% concentration (w/v). To prepare a standard solution at similar concentration by using the same solvent. To measure the absorbances of the resulting solutions at 238 nm, by using methanol to adjust zero. To calculate the content of $C_{24}H_{32}O_4S$ in the sample from the readings obtained.

B. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 230 nm; 150 mm chromatograph column and 3.9 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group

(5 µm), kept at room temperature; flow of *Mobile phase* of 1 mL/minute.

Mobile phase: filtered and degassed mixture of methanol and water (60:40).

Sample solution: To transfer about 50 mg of the sample to a 100 mL volumetric flask and top up with a mixture of acetonitrile and water (50:50). To make it homogeneous. To transfer 2 mL of this solution to a 10 mL volumetric flask and top up with a mixture of acetonitrile and water (50:50), thus obtaining 100 µg/mL concentration.

Standard solution: to dissolve accurately weighed amount of spironolactone SQR in mixture of acetonitrile and water (50:50), to obtain 500 µg/mL solution. To successively dilute the mixture of acetonitrile and water (50:50) to obtain a 100 µg/mL solution.

Procedure: to separately inject 20 µL of *Sample and standard solutions*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of C₂₄H₃₂O₄S in the sample, from the responses from the *Standard solution* and *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

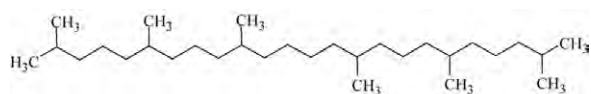
LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Diuretic.

SQUALANE Squalanum



C₃₀H₆₂; 42281
squalane; 09701
2,6,10,14,18,22-hexamethyl tetracosane
[111-01-3]

DESCRIPTION

Physical characteristics. Oily, colorless, and clear liquid.

Solubility. Practically insoluble in water, soluble in acetone, freely soluble in hexane, and slightly soluble in ethanol. Miscible with oils.

Physical and chemical constants.

Relative density (5.2.5): 0.807 to 0.810.

Refractive index (5.2.6): 1.4510 to 1.4525.

IDENTIFICATION

The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium chloride, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the squalane SQR spectrum similarly prepared.

PURITY TESTS

Index of acidity (5.2.29.7). At most 0.2.

Index of saponification (5.2.29.8). At most 2.0.

Index of Iodine (5.2.29.10). At most 4.0.

Chromatographic Purity. To proceed as described in *Gas chromatography (5.2.17.5)*. To use chromatograph equipped with flame ionization detector, 30 m long capillary column and 0.25 mm inner diameter, filled with polydimethylsiloxane, with a film thickness of 0.25 µm; column temperature to be kept at 260 °C for 3 minutes, then rises by 6 °C per minute to 290 °C; injector temperature at 280 °C and the detector temperature at 300 °C; use nitrogen as carrier gas; flow of carrier gas of 2 mL/minute.

Sample solution: to prepare a 1.5% sample solution (w/v).

Standard solution: to prepare a 1.5% squalane SQR solution (w/v).

Procedure: to separately inject 1 µL of *Sample and standard solutions*, record the chromatograms, and measure the area under the peaks. The sum of the areas under the secondary peaks, except the main peak's one, is not greater than 3.0% of the total area of the peaks obtained. Solvent peaks should not be included in the calculations.

PACKAGING AND STORAGE

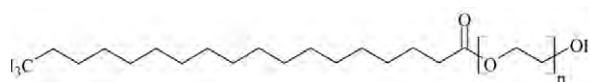
In tightly closed containers, protected from light, at 8 °C to 15 °C.

LABELLING

To comply with the legislation in force and state the origin (plant or animal) on the label.

CATEGORY

Adjuvant.

MACROGOL STEARATE 40**Macrogoli stearas 40**
 $C_{18}H_{36}O_2 \cdot (C_2H_4O)_n$, 09890

 α -(1-oxo octadecyl)- ω -hydroxypoly (oxy-1,2-ethanediyl)
[9004-99-3]
DESCRIPTION

Physical characteristics. Scaly, white solid.

Solubility. Slightly soluble in water, soluble in ethanol, ethyl ether, and acetone, insoluble in mineral and vegetable oils.

IDENTIFICATION

The infrared absorption spectrum (5.2.14) of the sample, dispersed in mineral oil, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the polyoxyl 40 SQR spectrum similarly prepared.

PURITY TESTS

Freezing temperature (5.2.4). 37 °C to 47 °C.

Index of acidity (5.2.29.7). At most 2.0.

Index of saponification (5.2.29.8). 25 to 35.

Index of Hydroxyl (5.2.29.12). 25 to 40.

Water (5.2.20). At most 3.0%.

Heavy metals (5.3.2.3). To use *Method III*. At most 0.001%.

Free polyethylene glycol. To accurately weigh 6 g of the sample and transfer to a 500 mL separatory funnel containing 50 mL of ethyl acetate. To completely dissolve and add 50 mL of 29% sodium chloride (w/v), vigorously stir for 2 minutes, and let it rest for 15 minutes. Should the separation be incomplete, carefully put the separatory funnel in steam bath in short time intervals. To repeat this procedure as often as necessary to ensure total phase separation. To cool down and transfer the lower aqueous phase to a 500 mL separatory funnel, extract the upper phase again with 50 mL of 29% sodium chloride solution (w/v), by repeating the procedure described above. To add 50 mL of ethyl acetate to the second separatory funnel containing aqueous phases, vigorously stir for 2 minutes, and let it rest for 15 minutes. To separate the lower aqueous phase to a third 500 mL separatory funnel and extract with 2 portions of chloroform (50 mL each), by stirring for 2 minutes each time.

To repeat the steam bath procedure to obtain total phase separation. To transfer portions of chloroform to a 150 mL beaker and evaporate to apparent dryness in the steam bath. To add 15 mL of chloroform to the residue and filter, by collecting the filtrate in a 150 mL beaker. To wash the filter with small portions of chloroform, by collecting in the same 150 mL beaker from where the filtrate was collected. To evaporate so as chloroform or ethyl acetate odor to not be smelt. To desiccate in a vacuum oven at 60 °C for 1 hour. To cool down in desiccator and weigh it. At least 17% and at most 27% of free polyethylene glycols.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

CATEGORY

Pharmacotechnical adjuvant, surfactant.

STEVIA
Steviae folium

Stevia rebaudiana (Bertoni) Bertoni - ASTERACEAE

The vegetable drug consists of dried leaves, containing at least 12.0% of total carbohydrate and 4.0% of stevioside ($C_{38}H_{60}O_{18}$; M 804.87).

CHARACTERISTICS

Organoleptic characteristics. Faint odor, sweet taste at the beginning of chewing, bitter at the end.

MACROSCOPIC DESCRIPTION

Simple leaves, up to 6.0 cm long and up to 2.5 cm wide, dark green in the adaxial face and lighter in the lower face, brittle when dry, opposite arrangement, alternate only when near the inflorescence, membranous, spatulate to lanceolate, sessile, acute apex, attenuated base, and serrated margin from basal third towards leaf apex, with three longitudinal ribs, the main one is more developed. Actinodromous venation. The leaf is covered with tector trichomes on both sides. Flowers, when present, white, all the same, assembled in clusters and protected by a 5 - 6 bract cover. Clusters are grouped in terminal corymbiform panicles. When present, fruit is achene-type, 4 or 5 longitudinal angles and pilose surface, accompanied by papus composed of a single row of bristles.

MICROSCOPIC DESCRIPTION

The leaf epidermis, in front view, exhibits cell of sinous walls more pronounced in the lower face. In the region of the ribs, cells are elongated with periclinal straight walls.

Various anomocytic stomata in the lower face. Multicellular uniseriate tector trichomes, two types are found throughout the leaf blade surface, on both faces, the biggest ones are enlarged at the base, with acute apex, and basal cells with voluminosity, small ones with uniform diameter from the base to the apex, this being less thin. Glandular occur throughout the length of the blade, on both sides, located in the small depressions of the epidermis, with multicellular and uniseriate pedicel, unicellular and rounded head. In some places of the epidermis, there are epicuticular strips. In cross section, the blade is dorsiventrally arranged and amphistomatic with stomata located at the same level or slightly above the epidermal cells. The inner periclinal and anticlinal walls that surround the stomatal pore are thickened. The palisade parenchyma consists of 1-2 layers. When there are 2 layers, these cover half the thickness of the blade. The spongy parenchyma has various strata, irregularly arranged. The secondary vascular bundles are collateral, surrounded by a chlorophyllated parenchymatous sheath. The main rib, in cross section, is most prominently in the lower face. Epidermal cells in this region are isodiametric, and collenchyma is lacunar. The vascular system is represented by a collateral vascular bundle, partially covered with sclerenchyma fibers next to xylem and phloem, in the form of calotte. In cross section, the leaf base shows semicircular open shape, slightly concave in the adaxial face and convex in the lower one. The epidermis has polyhedral to quadrangular cells with ornamented cuticle. The stomata are located above the level of other epidermal cells and occur only at the edges. The collenchyma consists of 1-2 layers of cells on both faces. The fundamental parenchyma fills in most part of this region, and chlorenchyma does the same on the edges. The vascular system consists of 5-7 collateral vascular bundles, the central is the largest one and the others gradually diminish to the most peripheral area.

MICROSCOPIC DESCRIPTION OF POWDER

The powder meets all the requirements for the species, unless the macroscopic characters. Characteristics: fragments of epidermis with cells of sinuous anticlinal walls and anomocytic stomata; fragments of the ribs with elongated epidermal cells, glandular and tector trichomes as described above.

IDENTIFICATION

To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using F_{254} silica gel, with 250 μm thickness as support and a mixture of ethyl acetate, methanol, and glacial acetic acid (60:40:5) as mobile phase.

To separately apply to the plate, as band, 10 μL of *Solution* (1) and 5 μL of *Solution* (2) freshly prepared, as described below.

Solution (1): to weigh about 0.25 g of ground leaves and put it in a round bottom flask. To add 10 mL of a mixture of water and ethanol (1:1). To heat under reflux for 1 hour. To filter it by using filter paper. To transfer the filtrate to a

10 mL volumetric flask, cool down, and top up to volume with mixture of water and ethanol (1:1). To dilute 50 mL of the resulting solution with 150 mL of methanol.

Solution (2): to dissolve accurately weighed amount of stevioside in methanol, to obtain a 1 mg/mL solution.

To develop the chromatogram. To remove the plate, and let it drying. To nebulize with anisaldehyde SR and to heat in an oven at 100 °C - 110 °C for 5 minutes. The main spot obtained with *Solution* (1) corresponds in position, color, and intensity to that obtained with *Solution* (2), R_f about 0.50. The spot corresponding to stevioside has fleeting green color.

PURITY TESTS

Foreign material (5.4.2.2). At most 2.0%.

Water (5.4.2.3). At most 13.0%.

Total ashes (5.4.2.4). At most 9.5%.

DOSAGE

Total carbohydrates

Concentrated sample solution: to weigh 2 g of ground stevia leaf. To extract by infusion with 80 mL of hot water three times and filter it. To gather the filtrate and top up to 250 mL. To transfer 5 mL of the extract to 50 mL volumetric flask and top up with water.

Sample solution: to transfer 0.6 mL of *Concentrated sample solution* to test tube and add 0.6 mL of 5% phenol (w/v) and 3 mL of sulfuric acid. Let it rest for 10 minutes.

Blank solution: to transfer 0.6 mL of water to test tube and add 0.6 mL of 5% phenol (w/v) and 3 mL of sulfuric acid. Let it rest for 10 minutes.

Standard solution: to transfer 0.6 mL of 0.01% glucose standard (w/v) in water to test tube and add 0.6 mL of 5% phenol (w/v) and 3 mL of sulfuric acid. Let it rest for 10 minutes.

To measure the absorbance of the sample solution and standard solution at 490 nm (5.2.14), by using *Blank solution* to adjust zero. To calculate the total carbohydrate from the sample according to the equation:

$$TC = 1,125 \times D \times \frac{As}{Ap}$$

where

TC = carbohydrate content in%;

D = 10;

As = absorbance of the Sample solution measured;

Ap = absorbance of standard solution measured;

Stevioside

To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 206 nm; pre-column packed with silica chemically bonded to the octadecylsilane group; 150 mm column and 3.9 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μm); kept at room temperature; flow of *Mobile phase* of 1.0 mL/min.

Eluent A: mixture of acetonitrile and water (20:80).

Eluent B: acetonitrile.

Mobile phase gradient: to adopt the linear gradient system described in the following table:

Time (minutes)	Eluent A (%)	Eluent B (%)
0	100	0
4	70	30
7	0	100

Sample solution: to accurately transfer about 0.4 g of ground and dried drug to a round bottom flask. To add 10 mL of a mixture of water and ethanol (1:1). To heat at 100 °C under reflux for 1 hour. To cool down the extract at room temperature with cold running water. To filter the ex-

tract through filter paper under vacuum, by washing the frame with a small amount of water. To transfer the filtrate to a 10 mL volumetric flask and top up to volume with mixture of water and ethanol (1:1). To dilute 50 μL of the resulting solution with 950 μL of mixture of acetonitrile and water (20:80).

Stock standard solution: to dissolve accurately weighed amount of stevioside in methanol, to obtain a 1 mg/mL solution. To gently warm, if necessary.

Analytical curve: to dilute 500 μL of *Stock standard solution* by half, so as to obtain a 0.50 mg/mL solution. To successively dilute the previous solution in methanol, to obtain 0.25 mg/mL, 0.125 mg/mL, 0.0625 mg/mL, 0.032 mg/mL, and 0.016 mg/mL concentrations. To inject the 6 concentrations obtained.

Procedure: to separately inject 10 μL of Solutions for *Analytical curve* and *Sample solution*. To record the chromatograms and measure the areas under the peaks. The retention time is approximately 4.6 minutes to stevioside. To calculate the stevioside content from the sample, from the linear equation obtained with analytical curve.

PACKAGING AND STORAGE

In tightly closed container, protected from light and heat

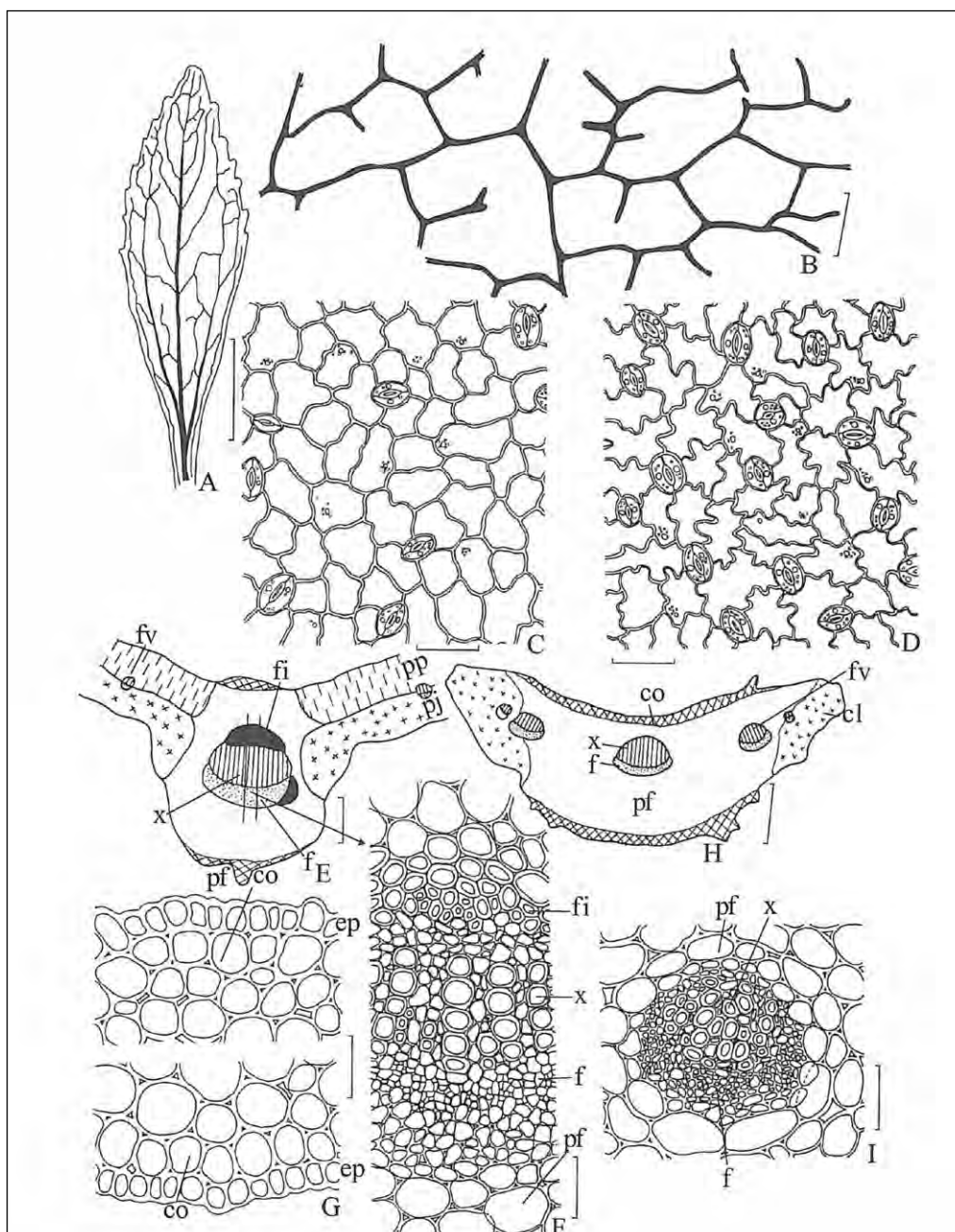


Figure 1 - Macroscopic and microscopic aspects in *Stevia rebaudiana* (Bertoni) Bertoni

Additional information of **Figure 1**. Scales correspond to 1 cm in **A**; in cm **B**, **E**, and **H**, to 250 μm ; in **C**, **D**, **G**, **F**, and **I**, to 50 μm .

A - general appearance of the leaf; **B** - detail of leaf venation; **C** - detail of the epidermis in adaxial face, in front view, showing stomata, **D** - detail of the epidermis in the lower face, in front view, showing stomata and cells with highly sinuous walls; **E** - scheme of cross section of the leaf blade at midrib showing collateral bundle: phloem (f); fibers (fi); vascular bundle (fv); fundamental parenchyma (pf); palisade parenchymal (pp); spongy parenchyma (pj); xylem (x). **F** — detail of the vascular bundle of the midrib in cross section as shown in **E**: phloem (f); fibers (fi); fundamental parenchyma (pf), xylem (x). **G** — detail of epidermis and collenchyma in the midrib toward the adaxial face and detail of epidermis and collenchyma in the midrib toward the lower face: collenchyma (co); epidermis (ep). **H** — scheme of cross-section of leaf blade at the base of the midrib showing collateral bundle: chlorenchyma (cl); collenchyma (co), phloem (f); vascular bundle (fv), xylem (x). **I** — detail of the vascular bundle at the basal region of the leaf blade: phloem (f); fundamental parenchyma (pf), xylem (x).

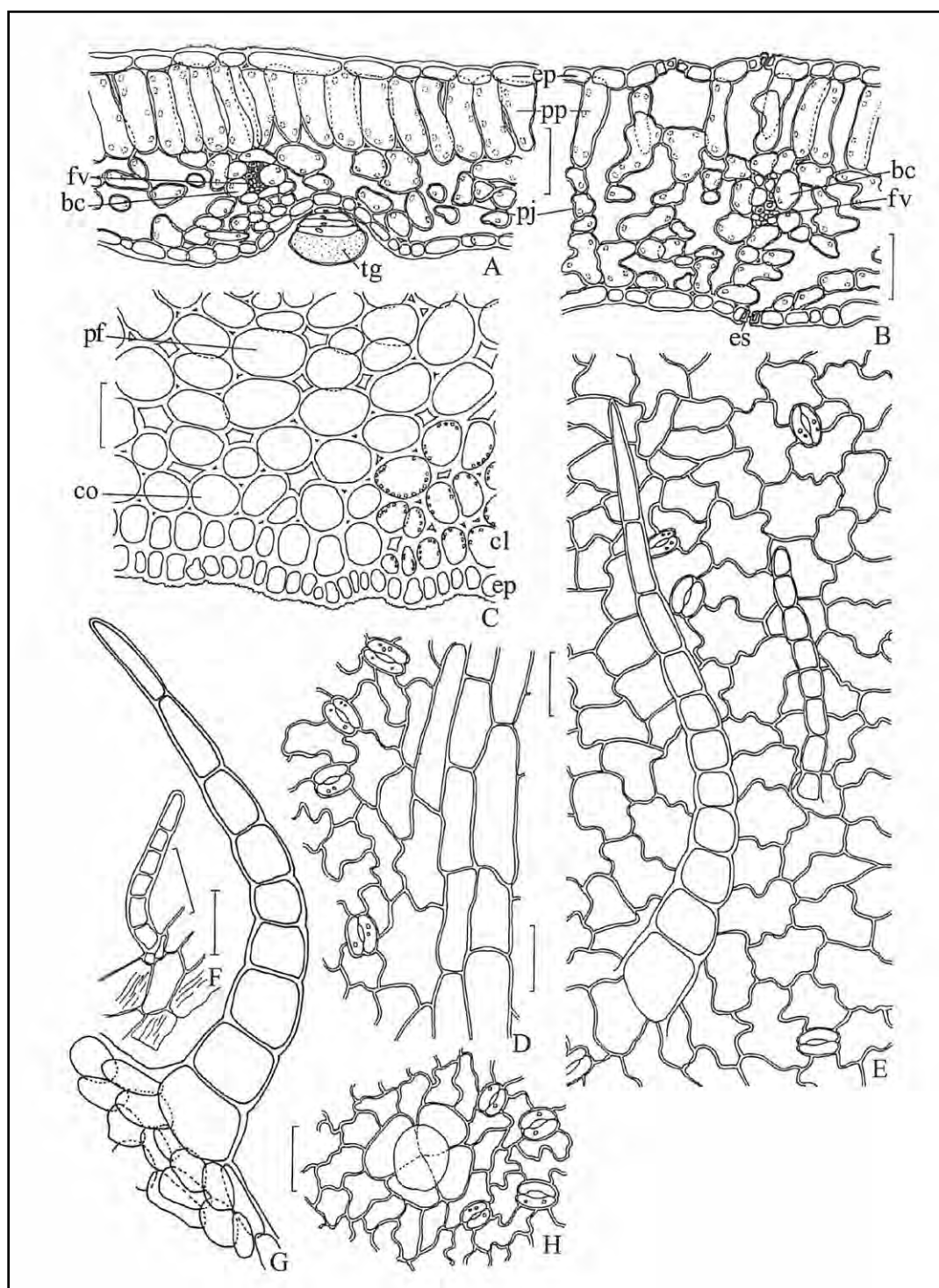


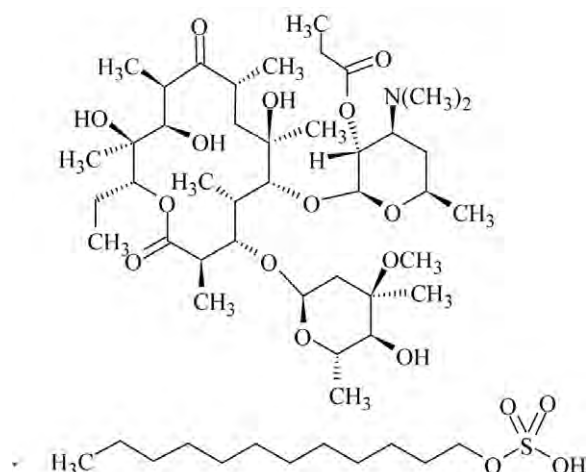
Figure 2 - Microscopic aspects in *Stevia rebaudiana* (Bertoni) Bertoni

Additional information of **Figure 2**. Scales correspond to 50 μ m in A-H.

A — detail of cross section of the leaf blade: epidermis (ep); vascular sheath with chloroplasts (bc); vascular bundle (fv); spongy parenchyma (pj); palisade parenchyma (pp); glandular trichomes (tg). **B** — detail of cross section of the leaf blade: stomata (es); epidermis (es); vascular sheath with chloroplasts (bc); vascular bundle (fv); spongy parenchyma (pj); palisade parenchyma (pp). **C** — fragment of the basal portion of the leaf blade in cross section at the midrib level showing epicuticular strips: epidermis (ep); chlorenchyma (cl); collenchyma (co); fundamental parenchyma (pf). **D** - fragment of epidermis in front view showing stomata and morphological variability of cells, **E** - fragment of the epidermis, in front view, showing stomata and tector trichomes, **F** - tector trichomes and fundamental epidermal cells showing epicuticular strips; **G** - tector trichomes with extended basal cell; **H** - fragment of the epidermis, in front view, highlighting stomata and glandular trichomes.

ESTOLATE, ERYTHROMYCIN

Erythromycini stoles



$C_{40}H_{71}NO_{14} \cdot C_{12}H_{26}O_4S$; 1056.39

erythromycin estolate; 03494

Dodecyl sulphate 2'-erythromycin propanoate (1:1)
[3521-62-8]

It shows minimum potency of 610 UI of erythromycin estolate ($C_{40}H_{71}NO_{14} \cdot C_{12}H_{26}O_4S$), per milligram, relative to the anhydrous substance.

DESCRIPTION

Physical characteristics. White crystalline powder.

Solubility. Practically insoluble in water, soluble in ethanol, acetone, and chloroform. Practically insoluble in diluted hydrochloric acid.

Physical and chemical constants.

Melting range (5.2.2): 135 °C to 138 °C with decomposition.

PURITY TESTS

pH (5.2.19). 4.5 to 7.0. To determine in 1% aqueous suspension (w/v).

Related substances. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using G silica gel, as support, and a mixture of 15% ammonium acetate (w/v) with pH 7.0, ethanol, and chloroform (1:15:85) as mobile phase. To separately apply to the plate 10 µL of each of the solutions freshly prepared, as described below.

Solution (1): 4 mg/mL solution of the sample in acetone.

Solution (2): to dilute 2.5 mL of *Solution (1)* in 10 mL of acetone.

Solution (3): 1 mg/mL erythromycin estolate SQR solution in acetone.

Solution (4): to dissolve 10 mg of erythromycin estolate SQR and 10 mg of erythromycin ethylsuccinate in 10 mL of acetone.

Solution (5): 80 mg/mL erythromycin SQR solution in acetone.

To develop the chromatogram. To remove the plate, and let it drying. To nebulize with anisaldehyde SR and heat at 110 °C for 5 minutes. Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (5)* (2.0%).

Water (5.2.20.1). To use 20 mL of methanol containing 10% of imidazole in the titration vial. At most 4.0%.

Sulfated ashes (5.2.10). To determine in 0.5 g of the sample. At most 0.5%.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the erythromycin estolate SQR spectrum similarly prepared.

B. The principal spot of the chromatogram of *Solution (2)* obtained with *Related substances* corresponds in position, color, and intensity to that obtained with *Solution (3)*. The test is valid only if the chromatogram obtained with *Solution (4)* presents two clearly separated spots.

C. To suspend 3 mg of the sample in 2 mL of *M* sulfuric acid. To add 0.1 mL of 10% methylthioninium chloride (w/v), 2 mL of chloroform, and mix it. The chloroform layer becomes blue.

D. To dissolve 10 mg of the sample in 5 mL of hydrochloric acid. Let it rest for 20 minutes. The yellow color develops.

DOSAGE

To proceed as described in *Microbiological test of antibiotics (5.5.3.3.1)* by using plate cylinders with the agar diffusion method.

Micro-organism: *Micrococcus luteus* ATCC 9341.

Culture media: culture medium number 1, to maintain the micro-organism; sterile saline solution to the standardization of the inoculum; and culture medium number 11, to the base layer and inoculum preparation.

Sample solution: to dissolve amount of sample equivalent to 50 mg of erythromycin in 20 mL of methanol. To dilute to 50 mL with 0.1 M Potassium phosphate buffer, sterile, pH 8.0 (*Solution 2*). To keep it at 60 °C for 3 hours. To filter it. To successively dilute to obtain 0.30 µg/mL, 0.60

µg/mL, and 1.2 µg/mL concentrations, by using the 0.1 M Potassium phosphate buffer, sterile, pH 8.0 (Solution 2).

Standard solution: to accurately weigh about 50 mg of erythromycin estolate SQR, quantitatively transfer to a 50 mL volumetric flask by using 20 mL of methanol. To stir and top up with 0.1 M Potassium phosphate buffer, sterile, pH 8.0 (solution 2). To successively dilute to obtain 0.30 µg/mL, 0.6 µg/mL e 1.2 µg/mL concentrations, by using the 0.1 M Potassium phosphate buffer, sterile, pH 8.0 (Solution 2).

Procedure: to add 20 mL of culture medium number 11 in each plate, wait to solidify, add 5 mL of 1.5% inoculum, and proceed as described in *Microbiological test of antibiotics (5.5.3.3.1)*, by adding 0.2 mL of freshly prepared solutions to the cylinders. To calculate the potency of the sample, in UI, of erythromycin estolate per mg, from the potency of the standard and responses obtained with the *Standard and sample solutions*.

PACKAGING AND STORAGE

In airtight closed containers, protected from light, at temperature below 30 °C.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antimicrobial.

ESTOLATE, ERYTHROMYCIN TABLETS

It contains erythromycin estolate equivalent to at least 90.0% and at most 120.0% of the amount stated of erythromycin (C₃₇H₆₇NO₁₃). The tablets must be coated.

IDENTIFICATION

To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using silica gel (0,25 mm) as support and a mixture of methanol and chloroform (85:15) as mobile phase. To separately apply to the plate 3 µL of each of the solutions freshly prepared, as described below.

Solution (1): to weigh and pulverize the tablets. By using the powder, prepare a solution equivalent to 20 mg/ml erythromycin in methanol.

Solution (2): to use erythromycin estolate SQR to obtain a 20 mg/ml erythromycin solution in methanol.

To develop the chromatogram. To remove the plate, and let it drying. To nebulize with a mixture of ethanol, anisaldehyde, and sulfuric acid (90:5:5). To heat the plate at 100 °C for 10 minutes. The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity

to that obtained with *Solution (2)*. Erythromycin is like a purple to black spot.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Disintegration test (5.1.4.1). Within 30 minutes. To use simulated gastric fluid instead of water.

Uniformity of dosage units (5.1.6). It complies with the test. PURITY TESTS

Water (5.2.20.1). To use 20 mL of methanol containing 10% of imidazole in the titration vial. At most 5.0%.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To proceed as described in *Microbiological test of antibiotics (5.5.3.3.1)* by using cylinders with the agar diffusion method. To prepare the Sample solution as described below.

Sample solution: To weigh and pulverize the tablets. To use amount of powder equivalent to 0.5 g of erythromycin and transfer to 500 mL volumetric flask. To dilute in 200 mL of methanol and mechanically stir for 10 minutes. To add 100 mL of 0.1 M Potassium phosphate buffer, sterile, pH 8.0 (Solution 2) and mechanically stir for 10 minutes. To top up the volume with the same solvent and filter it.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

ESTOLATE, ERYTHROMYCIN ORAL SUSPENSION

Erythromycin estolate oral suspension is a mixture of erythromycin estolate with one or more coloring, flavoring, buffer, sweetener, and preservative agents. It contains erythromycin estolate equivalent to at least 90.0% and at most 115.0% of the amount stated of erythromycin (C₃₇H₆₇NO₁₃).

IDENTIFICATION

To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using silica gel (0.25 mm) as support and a mixture of methanol and chloroform (85:15) as mobile phase. To separately apply to the plate 3 µL of each of the solutions freshly prepared, as described below.

Solution (1): to transfer volume of oral suspension equivalent to 20 mg of erythromycin to a separatory funnel. To add 15 mL of 0.02 M sodium hydroxide and mix it. To add 2 g of sodium chloride and 25 mL of chloroform and stir for 3 minutes. To separate the chloroform phase by passing it through a small amount of anhydrous sodium sulphate previously washed with chloroform. To collect the chloroform extract. To wash the sodium sulfate by using 5 mL of chloroform. To evaporate the organic phase to dryness by using a rotary evaporator. To dissolve the residue in 1 mL of methanol.

Solution (2): to transfer amount of erythromycin estolate SQR equivalent to 20 mg of erythromycin to a separatory funnel and extract as described in *Solution (1)*.

To develop the chromatogram. To remove the plate, allow to air dry, and nebulize with a mixture of ethanol, anisaldehyde, and sulfuric acid (90:5:5). To heat the plate at 100 °C for 10 minutes. The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*. Erythromycin is like a purple to black spot.

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

pH (5.2.19). 3.5 to 6.5.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To proceed as described in *Microbiological test of antibiotics* (5.5.3.3.1) by using cylinders with the agar diffusion method.

Micro-organism: *Micrococcus luteus* ATCC 9341.

Standard solution: to dissolve accurately weighed amount of erythromycin SQR in methanol to obtain a 10 mg/mL solution. To quantitatively dilute with 0.1 M Potassium phosphate buffer, sterile, pH 8.0 (*Solution 2*) to 1 mg/mL concentration. To successively dilute with 0.1 M Potassium phosphate buffer, sterile, pH 8.0 (*Solution 2*) to obtain solutions at the concentration range appropriate for the standard curve.

Sample solution: to transfer bubble-free volume of oral suspension equivalent to 0.25 g of erythromycin to a 250 mL volumetric flask. To add 100 mL of methanol and stir for 10 minutes. To top up to volume with 0.1 M Potassium phosphate buffer, sterile, pH 8.0 (*Solution 2*) and heat up to 60 °C for 3 hours, cool down, and filter it. To successively dilute with 0.1 M Potassium phosphate buffer, sterile, pH 8.0 (*Solution 2*) to obtain solutions at the concentration range appropriate for the standard curve.

Procedure: to add 20 mL of culture medium number 11 in each plate, wait to solidify, add 5 mL of seeded medium number 11, and proceed as described in *Microbiological agar diffusion test*. To calculate the amount, in mg, of erythromycin ($C_{37}H_{67}NO_{13}$), in the oral suspension, from the potency of the standard and responses obtained for the *Sample and standard solutions*.

PACKAGING AND STORAGE

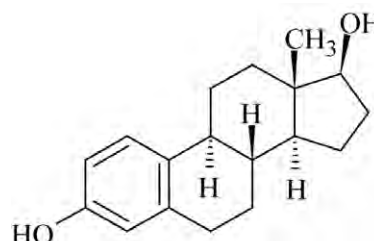
In tightly closed containers.

LABELLING

To comply with the legislation in force.

ESTRADIOL

Estradiolum



$C_{18}H_{24}O_2$; 272.38
estradiol; 03595
(17β)-Estra-1,3,5(10)-triene-3,17-diol [50-28-2]

It contains at least 97.0% and at most 103.0% of $C_{18}H_{24}O_2$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White to yellowish-white powder.

Solubility. Practically insoluble in water, soluble in acetone and dioxane, freely soluble in ethanol, slightly soluble in vegetable oil and sparingly soluble in methylene chloride. Soluble in alkalis.

Physical and chemical constants.

Melting range (5.2.2): 173 °C to 179 °C.

Specific rotation (5.2.8): +76 to +83. To determine in 1% aqueous solution (w/v).

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the estradiol SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14), from 200 nm to 400 nm, 0.0005% solution (w/v) in ethanol has absorption maximum at 280 nm, similar to those observed in the spectrum of a similar solution of estradiol SQR.

PURITY TESTS

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.5%.

Water (5.2.20.1). At most 3.5%.

DOSAGE

To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 205 nm; 300-205 mm ultraviolet column, 300 mm column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at room temperature; flow of *Mobile phase* of 1 mL/minute.

Mobile phase: acetonitrile and water (55:45).

Sample solution: To transfer accurately weighed amount of 100 mg of the sample to 250 mL volumetric flask and top up with methanol. To transfer 10 mL to 200 mL volumetric flask, add 5 mL of *Internal standard solution*, top up with water, and make it homogeneous.

Standard solution: to dissolve accurately weighed amount of estradiol SQR and estrone SQR in methanol to obtain 0.4 mg/mL and 0.24 mg/mL solutions. To transfer 10 mL of the solution and 5 mL of *Internal standard solution* to a 200 mL volumetric flask. To add 100 mL of methanol and top up with water to obtain 20 µg/mL estradiol SQR solution.

Internal standard solution: To transfer 300 mg of ethylparaben to 500 mL volumetric flask, top up with methanol, and make it homogenous.

To inject replicates of 20 µL of the *Standard solution*. The retention times are about 0.7 to internal standard, 1.3 to estrone, and 1.0 to estradiol. The resolution between estradiol and estrone peaks is greater than 2.0. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2.0 %.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks.

To calculate the content of $C_{18}H_{24}O_2$ from the sample, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Hormone.

DATURA Stramonii folium

Datura stramonium L. - SOLANACEAE

The drug consists of leaves of *Datura stramonium* L. and its diversities. It contains at least 0.25% of total alkaloids calculated as hyoscyamine ($C_{17}H_{23}NO_3$, 289.37) in relation to the dry drug.

CHARACTERISTICS

Organoleptic characteristics. The leaf has an unpleasant odor, nauseous and slightly salty taste.

MACROSCOPIC DESCRIPTION

Ovate or ovate-triangular leaf blade, lobed toothed, acuminate apex and asymmetrical base, dark brownish green to dark grayish green, twisted and shrunken due to drying, thin and fragile, with 15.0 cm to 20.0 cm long, and 10.0 cm to 8.0 cm wide. Pinnate venation, with 4-5 secondary alternate ribs, concave on the adaxial face and prominent on the lower face. Short petiole. Young pubescent leaves on the ribs.

MICROSCOPIC DESCRIPTION

The leaf blade is amphistomatic and dorsiventral symmetry. The epidermis, in front view, shows polygonal cells with sinuous and thick anticlinal walls, and stomata of anisocytic type, rarely anomocytic, more abundant on the lower face. The glandular and tector trichomes are more abundant on the lower face and ribs. The tector trichomes are multicellular, uniseriate, conical, formed by 2-5 elongated cells with walls finely verrucous; glandular trichomes are generally shortly pedicel with apical ovoid or club-shaped gland, formed by 2-7 cells. The epidermis, in cross section, is uniseriate and covered with a smooth and thin cuticle. The mesophyll consists of palisade pa-

renchyma composed of a layer of cells and spongy parenchyma.

Between the two parenchyma there is at least one layer of idioblast containing crystals of calcium oxalate in the form of druze. The vascular bundles are bicollateral.

MICROSCOPIC DESCRIPTION OF POWDER

The powder meets all the requirements for the species, unless the macroscopic characters. Characteristics: fragments of epidermis with cells of slightly sinuous anticlinal walls and smooth cuticle; fragments of epidermis with anisocytic and anomocytic stomata more frequent in the lower epidermis; conical tector trichomes, multicellular, uniseriate and short club-shaped glandular trichomes; fragments of mesophyll in cross section; fragments of ringed spiral and vessel elements; fragments of parenchyma with numerous idioblasts containing crystals (Druze-type).

IDENTIFICATION

A. To stir 1 g of pulverized sample with 10 mL of 0.05 M sulfuric acid for 2 minutes, and filter. To add 1 mL of concentrated ammonia solution and 5 mL of water to the filtrates. To carefully stir with 15 mL of peroxide-free ethyl ether to avoid formation of emulsion. To dry the ether phase over anhydrous sodium sulfate. To filter to a porcelain capsule and evaporate the solvent to dryness in a water bath. To add 2 mL of acetone and add dropwise 3% potassium hydroxide (w/v) in 96% ethanol (v/v). The intense violet color develops.

B. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel as support and a mixture of concentrated ammonia solution, water, and acetone (3:7:90) as mobile phase. To separately apply to the plate, as band, 10 µL and 20 µL of the following solutions, respectively:

Solution (1): To add 10 mL of 0.05 M sulfuric acid to 1 g of the pulverized sample, stir for 15 minutes, and filter it. To wash the filter with 0.05 M sulfuric acid to obtain 25 mL of filtrate. To add 1 mL of concentrated ammonia solution to the filtrate and stir twice with 10 mL of peroxide-free ethyl ether each time. To separate by centrifugation, if necessary. To gather the ether layers, dry over anhydrous sodium sulfate, filter, and evaporate to dryness in a water bath. To dissolve the residue in 0.5 mL of methanol.

Solution (2): to dissolve 50 mg of hyoscyamine sulfate in 9 mL of methanol. To dissolve 15 mg of scopolamine hydrobromide in 10 mL of methanol. To mix 3.8 mL of hyoscyamine sulfate solution and 4.2 mL of scopolamine hydrobromide solution and top up with 10 mL of methanol.

To develop the chromatogram. To dry the plate at 110 °C - 105 °C for 15 minutes. To allow to cool down and pulverize with about 10 mL of potassium iodobismuthate SR2

over a plate (200 mm side) to orange or brown bands appear over yellow background. The bands of the chromatograms obtained with *Solution*

(1) are similar on the position (hyoscyamine in the lower third and scopolamine in the upper third of the chromatograms) and color, to the chromatograms obtained with *Solution (2)*. The size of the bands of the chromatograms obtained with *Solution (1)* is not larger than that of the bands of the chromatograms obtained with the same volume of *Solution (2)*. Secondary bands may appear weak especially in the center of the chromatogram with 20 µL of *Solution (1)* or near the point of application of the chromatogram obtained with 10 µL of *Solution (1)*. To pulverize with sodium nitrite SR until the layer becomes transparent. To examine after 15 minutes. The color of the bands corresponding to hyoscyamine in the chromatograms obtained with the *Solution (2)* and the chromatograms obtained with *Solution (1)* are brown and become reddish-brown, but they not change to blue-gray (atropine).

PURITY TESTS

Foreign matter (5.4.2.2). At most 3% of stems having a diameter greater than 5 mm.

Water (5.2.20.2). At most 12.0%.

Total ashes (5.4.2.4). At most 20%.

Insoluble ashes (5.4.2.5). At most 4%.

DOSAGE

Total alkaloids

To weigh about 10 g of pulverized sample (180 µm) and moisten with 5 mL of ammonium hydroxide. To add 10 mL of 96% ethanol (v/v) and 30 mL of peroxide-free ethyl ether carefully mixed. To transfer the mixture to a percolator, if necessary, by using an extraction solution. To macerate for 4 hours and percolate the mixture with a mixture of chloroform and peroxide-free ethyl ether (1:3) until complete extraction of alkaloids. To evaporate to dryness 1 mL of leachate and dissolve the residue in 0.25 M sulfuric acid. Then, check the absence of alkaloids with mercury alkali potassium iodide SR. To reduce the volume of leachate to 50 mL and transfer to a separatory funnel with the aid of peroxide-free ethyl ether. To add peroxide-free ethyl ether to the liquid obtained 2.5 times the volume of the percolator to obtain a liquid of density lower than water. To extract the solution at least three times, by using 20 mL of 0.25 M sulfuric acid solution each time. To separate by centrifugation the phases, if necessary, and transfer the acid phase to another separatory funnel. To alkalize the acid phase with ammonium hydroxide to pH between 8.0 and 9.0 and extract with chloroform three times, with aliquots of 30 mL. To gather the chloroform phases and remove the residual water, by adding 4 g of anhydrous sodium sulfate. Let it rest

for 30 minutes by occasionally stirring. To remove the chloroform phase and wash the remaining sodium sulfate with three aliquots of 10 mL of chloroform. To gather the chloroform extracts and evaporate to dryness in a water bath. To heat the residue in oven at 100 °C - 105 °C for 15 minutes. To dissolve the residue in 5 mL of chloroform, add 20 mL of 0.01 *M* SV sulfuric acid, and remove the chloroform by evaporation in a water bath.

To titrate the acid in excess with a 0.02 *M* SV sodium hydroxide solution by using methyl red as indicator. To calculate the percentage of total alkaloids, expressed as hyoscyamine, according to the equation:

$$\% \text{ alcaloides} = \frac{57,88 \times (20 - n)}{(100 - d) \times m}$$

where

d = loss on drying expressed in percentage;

n = number of ml of 0.02 *M* sodium hydroxide spent;

m = mass of the test portion in g.

PACKAGING AND STORAGE

In tightly closed containers, protected from light and heat.

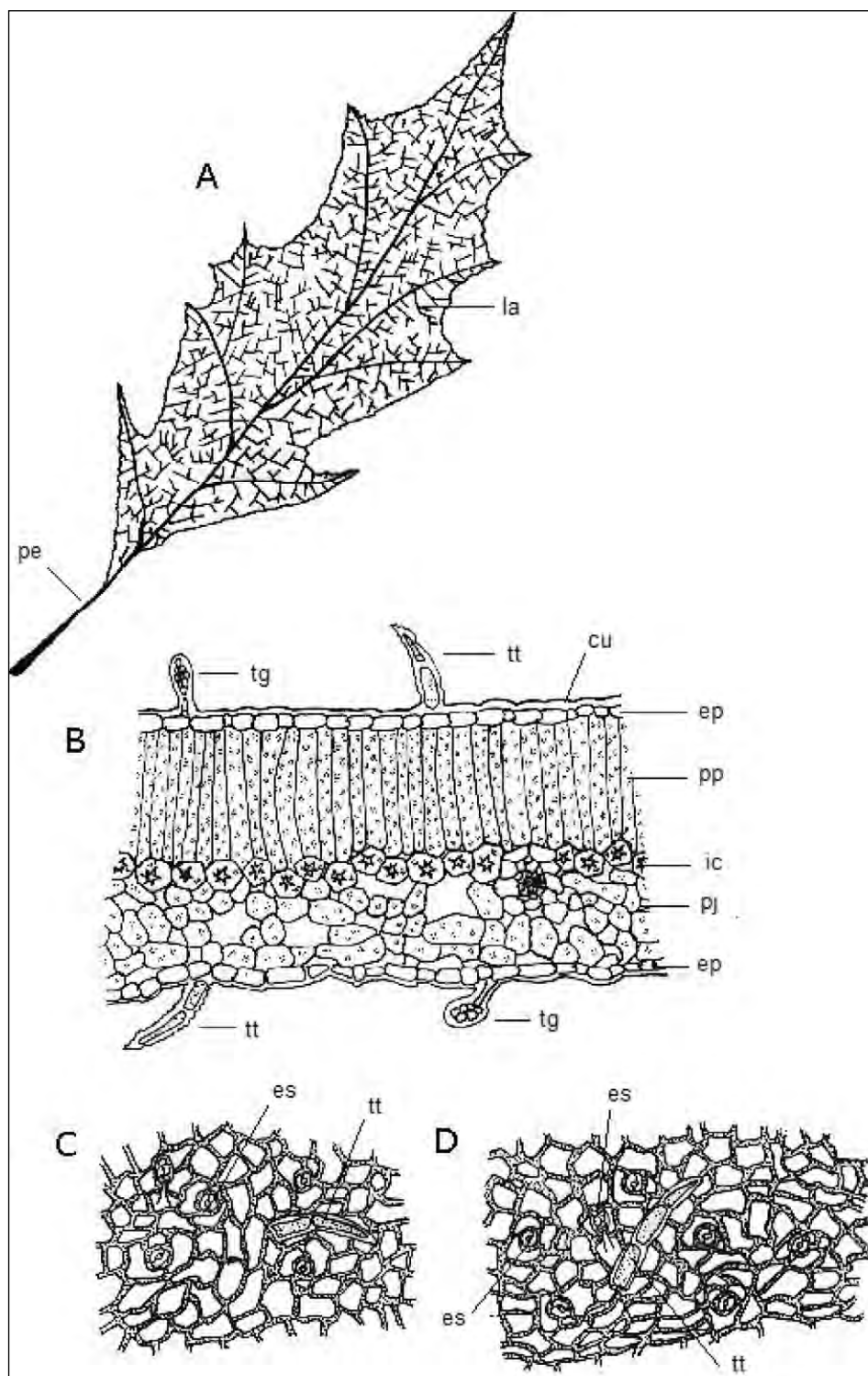


Figure 1 - Macroscopic and microscopic aspects of *Datura stramonium* L.

Additional information of Figure 1.

A — Schematic representation of the leaf, in front view: blade (la), petiole (pe). **B** - details of leaf blade in cross-section: cuticle (cu); epidermis (ep); idioblast containing druses of calcium oxalate (ic); spongy parenchyma (pj); glandular trichomes (tg); tector trichomes (tt). **C** — detail of the portion of the epidermis facing the adaxial side, in front view: stomata (es); tector trichomes (tt). **D** — detail of the portion of the epidermis facing the lower side, in front view: stomata (es); tector trichome.

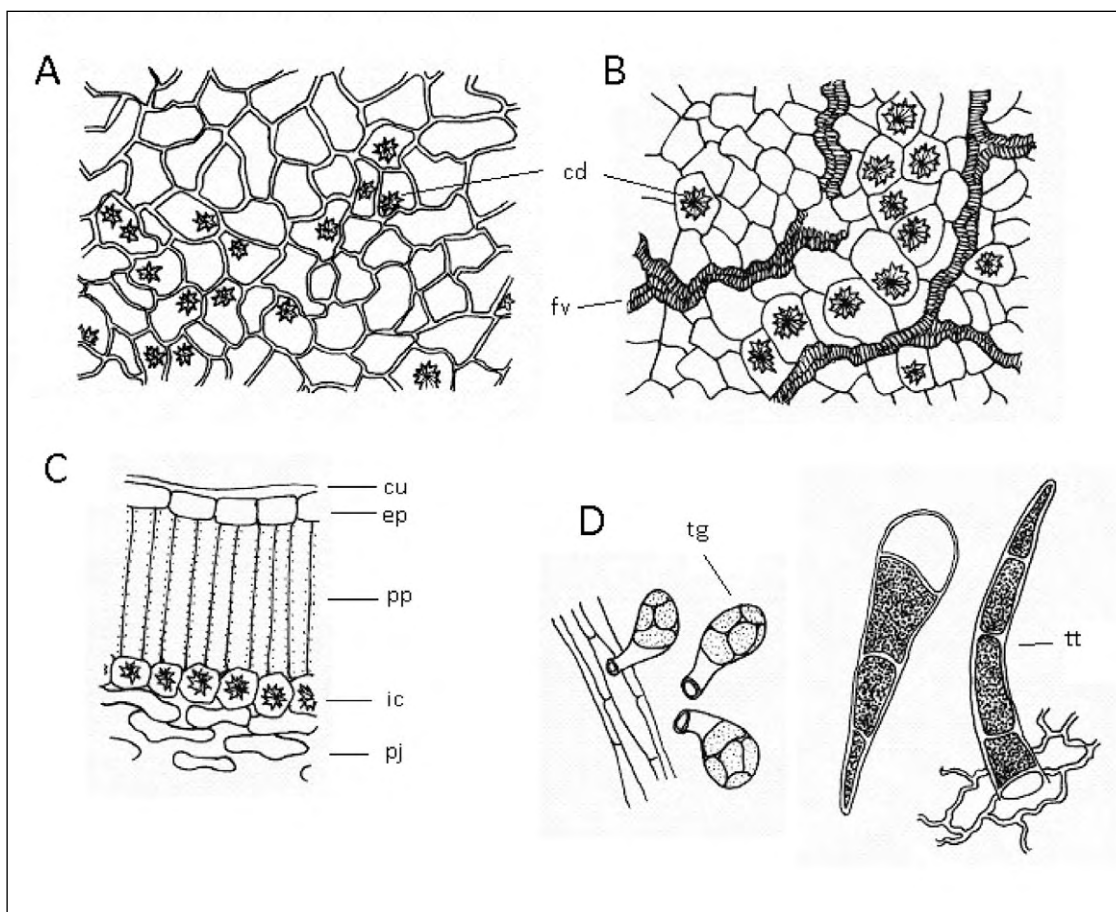


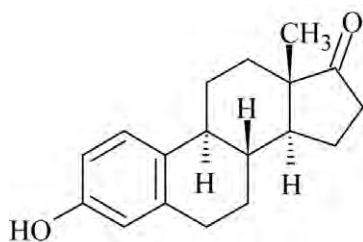
Figure 2 - Microscopic aspects of *Datura stramonium* L.

Additional information of **Figure 2**.

A - D - Schematic representation of the powder. **A** - fragment of the epidermis in front view, in the adaxial face, showing crystals by transparency: Druze-type crystal. **B** - fragment of the epidermis in front view, in the lower face, showing crystals and portions of vessel elements by transparency: Druze-type crystal (cd); vascular bundle (fv). **C** — fragment of portion of the mesophyll, in cross-section: cuticle (cu); epidermis (ep); palisade parenchyma (pp); crystal idioblast (ic); spongy parenchyma (pj). **D** — trichomes or their portions, isolated: glandular trichomes (tg); tector trichomes (tt).

ESTRONE

Estronum



$C_{18}H_{22}O_2$; 270.37

estrone; 03630

3-hydroxyestra-1, 3,5 (10)-trien-17-one
[53-16-7]

It contains at least 97.0% and at most 103.0% of $C_{18}H_{22}O_2$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White to yellowish white powder or small yellowish-white crystals.

Solubility. Practically insoluble in water, soluble in ethanol, methanol, acetone, and vegetable oils. Sparingly soluble in fixed alkalis.

Physical and chemical constants.

Melting range (5.2.2): 258°C to 262°C.

Specific rotation (5.2.8): +158° to +165°, in relation to the desiccated substance. To determine in 1% solution (w/v) in dioxane.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only

at the same wavelength, with the same relative intensities to those observed in the estrone SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14), from 200 nm to 400 nm, 0.005% solution (w/v) in ethanol heated in water bath and cooled down at room temperature, has absorption maximum similar to those observed in the spectrum of a similar solution of estrone SQR.

C. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

PURITY TESTS

Loss on drying (5.2.9). To determine in 1 g of the sample in oven at 105 °C for 3 hours. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.5%.

DOSAGE

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 280 nm; 150 mm column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at room temperature; flow of *Mobile phase* of 1 mL/minute.

Mobile phase: acetonitrile and 0.05 M monobasic potassium phosphate (50:50).

Sample solution: to dissolve accurately weighed amount of the sample in methanol, to obtain a 0.2 mg/mL solution. To transfer 5 mL of this solution to a 25 mL volumetric flask and top up with *Mobile phase*, thus obtaining 40 µg/mL solution.

Standard solution: to dissolve accurately weighed amount of estrone SQR in methanol to obtain a 0.2 mg/mL solution. To transfer 5 mL of this solution to a 25 mL volumetric flask and top up with *Mobile phase*, thus obtaining 40 µg/mL solution.

To inject replicates of 20 µL of the *Standard solution*. The column efficiency should be greater than 1500 theoretical plates/meter. The tailing factor is not greater than 2.0. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2.0 %.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_{18}H_{22}O_2$ from the sample, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

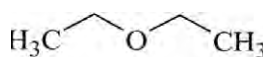
To comply with the legislation in force.

THERAPEUTIC CLASS

Hormone.

ETHYL ETHER

Aether ethylicus



C₄H₁₀O; 74.12
ethyl ether; 03663
1,1'-oxybisethane
[60,29,7]

DESCRIPTION

Physical characteristics. Clear, colorless, volatile, highly flammable liquid.

Solubility. Soluble in water, miscible with ethanol, methylene chloride, and fatty oils.

IDENTIFICATION

A. It complies with *Relative density* test (5.2.5).

B. It complies with the test for Determination of distillation range (5.2.3).

PURITY TESTS

Acidity. To put 10 mL of ethanol, 2 mL of distilled water, 0.5 mL of phenolphthalein SI in vial with ground lid. Then, add 0.02 M sodium hydroxide solution to obtain persistent pink color after stirring for 30 seconds. To exactly add 25 mL of the sample, close, and gently stir. Then, add the 0.02 M sodium hydroxide solution to obtain a persistent pink color after stirring for 30 seconds. No more than 0.4 mL of 0.02 M sodium hydroxide should be spent to neutralize the ethyl ether.

Relative density (5.2.5). 0.714 to 0.716.

Peroxides. To put 10 mL of the sample and 1 mL of 10% potassium iodide solution (w/v), freshly prepared, into a 25 mL beaker with ground lid, and stir. The mixture shall be protected from light for 1 hour. Liquids must be colorless.

Distillation range (5.2.3). *Do not distill if the sample fails the test for peroxides.* The sample completely distills between 34 °C and 35 °C. To perform the test by using appropriate heating device. To proceed with caution to avoid heating the flask above the liquid level.

Nonvolatile residue. To spontaneously evaporate 50 mL in tared porcelain dish. To desiccate the residue in an oven at 105 °C for 1 hour, cool down, and weigh it. The weight of the residue should not exceed 1 mg (0.003 %).

Aldehydes. To put 20 mL of ethyl ether in a separatory funnel and add 7 mL from a mixture of 1 mL of mercuric alkali potassium iodide SR and 17 mL of saturated sodium chloride solution. To close and vigorously stir for 10 sec-

onds. Let it rest for 1 minute. The aqueous layer should not be turbid.

Strange odor. To apply 5 mL of the sample on a filter-paper disk (80 cm diameter). To spontaneously evaporate. After volatilization of the sample no strange odor related to ethyl ether must be smelt.

PACKAGING AND STORAGE

In tightly closed containers, protected from light, at 8 °C to 15 °C.

LABELLING

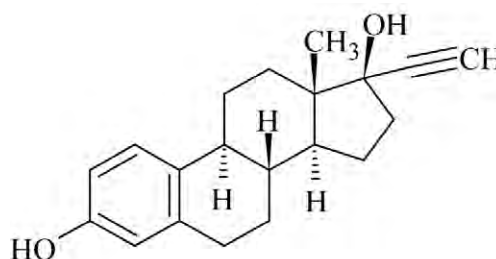
The label shall state the name and concentration of the non-volatile antioxidant occasionally used.

CATEGORY

Solvent.

ETHINYLESTRADIOL

Ethinylestradiolum



C₂₀H₂₄O₂; 296.40
ethinylestradiol; 03699
(17α)-19-norpregna-1, 3,5 (10)-trien-20-yne-3,17-diol
[57-63-6]

It contains at least 97.0% and at most 102.0% of C₂₀H₂₄O₂, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white or yellowish-white, odorless powder.

Solubility. Practically insoluble in water, soluble in acetone, chloroform, dioxane, ethanol, and ethyl ether. Soluble in diluted alkaline solutions.

Physical and chemical constants.

Melting range (5.2.2): 180 °C to 186 °C. It shows polymorph appearance with melting range at 142 °C - 146 °C.

Specific rotation (5.2.8): -28,0° to -29,5°, in relation to the desiccated substance. To determine in 0.4 % solution (w/v) in pyridine.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the ethinylestradiol SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14), from 200 nm to 400 nm, 0.005% solution (w/v) in ethanol has absorption maximum at 281 nm, similar to those observed in the spectrum of a similar solution of ethinylestradiol SQR. The values of A (1%, 1 cm) at 281 nm, calculated in relation to the desiccated substance, do not differ more than 3.0%.

C. The principal spot in the chromatogram of *Solution (2)* in the *Related substances* corresponds in position, color, and intensity to that obtained with the *Solution (3)*.

D. To dissolve 1 g of the sample in 1 mL of sulfuric acid inside test tube. Orange-red color is developed, which under ultraviolet light at 365 nm, has green fluorescence. To add 10 mL of water. Violet color is developed, and precipitate with similar color is produced.

PURITY TESTS

Appearance of solution. The 2% solution (w/v) in ethanol is clear (5.2.25).

Related substances. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using G silica gel as support and a mixture of ethanol and toluene (10:90) as mobile phase. To separately apply to the plate 5 µL of each of the solutions freshly prepared, as described below.

Solution (1): to dissolve 0.2 g of the sample in a mixture of methanol and chloroform (10:90). To dilute to 10 mL with the same solvent to obtain 20 mg/mL solution.

Solution (2): 1 mg/mL solution of the sample in a mixture of methanol and chloroform (10:90).

Solution (3): to dissolve 25 g of ethinylestradiol SQR in a mixture of methanol and chloroform (10:90). To dilute to 25 mL with the same diluent to obtain 1 mg/mL solution.

Solution (4): to dissolve 10 mg of estrone SQR in a mixture of methanol and chloroform (10:90). To dilute to 10 mL with the same solvent. To dilute 2 mL of this solution to 10 mL with the same solvent, thus obtaining a 0.2 mg/mL solution.

Solution (5): to dilute 1 mL of the *Solution (2)* to 5 mL with a mixture of methanol and chloroform (10:90), thus obtaining a 0.2 mg/mL solution.

To develop the chromatogram. To remove the plate, allow it to dry in a current of air, and heat it for 10 minutes. To nebulize the hot plate with methanolic sulfuric acid SR. To

heat the plate again at 110 °C for 10 minutes and examine under ultraviolet light (365 nm). Any spot related to estrone in the chromatogram obtained with *Solution (1)* is not more intense than that obtained in the chromatogram with *Solution (4)* (1%). Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot and the spot related to estrone, is not more intense than that in the chromatogram obtained with *Solution (5)* (1%).

Loss on drying (5.2.9). To determine in 0.5 g of the sample in an oven at 105 °C for 3 hours. At most 1.0%.

DOSAGE

To employ one of the methods described below.

A. To accurately weigh about 0.2 mg of the sample. To dissolve in 40 mL of tetrahydrofuran and add 5 mL of 10% silver nitrate (w/v). To titrate with 0.1 M SV sodium hydroxide, to potentiometrically determine the endpoint. To perform sample blank correction and adjust it as required. Each mL of 0.1 M SV sodium hydroxide is equal to 29.64 mg of $C_{20}H_{24}O_2$.

B. To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. To accurately weigh about 50 mg of the sample, dissolve in ethanol, and top up to 100 mL with the same solvent. To dilute with ethanol to the 0.01% concentration (w/v). To prepare a standard solution at similar concentration by using the same solvent. To measure the absorbances of the resulting solutions at 281 nm, by using ethanol to adjust zero. To calculate the content of $C_{20}H_{24}O_2$ in the sample from the readings obtained.

C. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 280 nm; 150 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (3 µm to 10 µm), at room temperature; flow of *Mobile phase* of 1 mL/min.

Mobile phase: mixture of acetonitrile and water (1:1).

Sample solution: To exactly transfer about 25 mg of the sample to a 25 mL volumetric flask, dissolve, and top up the volume with *Mobile phase*. To transfer 10 mL to 50 mL volumetric flask, dilute, and top up with *Mobile phase*, thus obtaining 0.2 mg/mL solution.

Standard solution: To exactly dissolve about 10 mg of ethinylestradiol SQR in *Mobile phase* and dilute to 50 mL, thus obtaining a 0.2 mg/mL solution.

Procedure: to separately inject 25 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_{20}H_{24}O_2$ from the sample, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

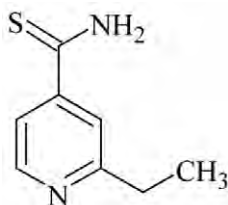
To comply with the legislation in force.

THERAPEUTIC CLASS

Contraceptive.

ETHIONAMIDE

Ethionamidum



$C_8H_{10}N_2S$; 166.24
ethionamide; 03704
2-Ethyl-4-pyridine carbothioamide
[536-33-4]

It contains at least 98.0% and at most 102.0% of $C_8H_{10}N_2S$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Yellow crystalline powder or small yellow crystals, with sulfide odor, mild to moderate.

Solubility. Practically insoluble in water, soluble in methanol, slightly soluble in ethanol, sparingly soluble in propylene glycol, chloroform, and ethyl ether.

Physical and chemical constants.

Melting range (5.2.2): 158 °C to 164 °C.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated for 18 hours on silica gel, under reduced pressure, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the thiamine ethionamide SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14) of the resulting solution from the method **B.**, ranging from 220 nm to 350 nm, exhibits maxima and minima only in the same wavelength of a similar ethionamide SQR solution.

C. To dissolve 10 mg of the sample in 5 mL of methanol and add 5 mL of 0.1 M silver nitrate. Dark brown precipitate is formed.

PURITY TESTS

pH (5.2.19). 6.0 to 7.0. To determine in 1% aqueous suspension (w/v).

Related substances. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF₂₅₄ silica gel as support and a mixture of chloroform and methanol (90:10) as mobile phase. To apply separately to the plate 10 µl of each of the solutions as described below.

Solution (1): 20 mg/mL solution of the sample in acetone.

Solution (2): 0.1 mg/mL solution of the sample in acetone.

Solution (3): 0.04 mg/mL solution of the sample in acetone.

To develop the chromatogram. To remove the plate, allow to air dry, and examine under ultraviolet light (254 nm). Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (2)* (0.5%) and not more than a secondary spot obtained with *Solution (1)* is more intense than that obtained with *Solution (3)* (0.2 %).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 105 °C for 3 hours. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.2%.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Titrations in non-aqueous medium (5.3.4.5)*. To dissolve 0.25 g of the sample in 50 mL of glacial acetic acid and titrate with 0.1 M SV perchloric acid, to potentiometrically determine the endpoint. To perform sample blank correction and adjust it as required. Each mL of 0.1 M SV perchloric acid is equal to 16.624 mg of $C_8H_{10}N_2S$.

B. To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. To accurately weigh about 50 mg of the sample and dissolve in methanol. To top up the volume to 100 mL with the same solvent. To successively dilute in methanol to the 0.001% concentration (w/v). To prepare a standard solution of ethionamide SQR at similar concentration by using the same solvent. To measure the absorbances of the resulting solutions at 290 nm, by using methanol to adjust zero. To calculate the content of $C_8H_{10}N_2S$ in the sample from the readings obtained.

PACKAGING AND STORAGE

In tightly closed containers, in dry and cool place.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antibacterial (tuberculostatic).

ETHIONAMIDE, TABLETS

It contains at least 95.0 % and at most 110.0 % of the amount stated of $C_8H_{10}N_2S$. Tablets must be sugar-coated.

IDENTIFICATION

A. To weigh and pulverize the tablets. To stir amount of powder equivalent to 0.125 g of ethionamide with 25 mL of ethyl ether for 2-3 minutes. To filter and evaporate the filtrate at room temperature. To dry the residue over silica gel under reduced pressure. The infrared absorption spectrum (5.2.14) of the residue, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the ethionamide SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14), ranging from 220 nm to 350 nm, of sample solution obtained in *Dosage* exhibits absorption maximum at 290 nm, similar to those observed in the standard solution spectrum.

C. To weigh and pulverize the tablets. To stir amount of powder equivalent to 1 g of ethionamide with 50 mL of methanol and filter through filter paper. To evaporate the filtrate to dryness in steam bath. The resulting residue melts at 155 °C - 164 °C.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Disintegration test (5.1.4.1). To use 0.1 M hydrochloric acid. Within 30 minutes.

Uniformity of dosage units (5.1.6). It complies with the test.

Procedure for content uniformity. To transfer each tablet to a 100 mL volumetric flask containing 60 mL of methanol and wait for total disintegration of the tablet. To put it in ultrasound for 10 minutes, mechanically stir for 15 minutes, and top up with the same solvent. To filter and discard the first 20 mL. To successively dilute to 0.001% concentration (w/v) by using methanol as solvent. To prepare standard solution under the same conditions. To measure the absorbances at 290 nm (5.2.14), by using methanol to adjust zero.

To calculate the amount of $C_8H_{10}N_2S$ in the tablets, from the readings obtained.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 900 mL

Equipment: baskets, 100 rpm

Time: 45 minutes

Procedure: after testing, remove aliquot from the dissolution medium and dilute, if necessary, with 0.1 M hydrochloric acid, to obtain suitable concentration. To measure the absorbances at 274 nm (5.2.14), by using the same solvent to adjust zero. To calculate the amount of $C_8H_{10}N_2S$ dissolved in the medium, by comparing the readings obtained with 0.001 % ethionamide standard solution (w/v) prepared in 0.1 M hydrochloric acid.

DOSAGE

To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To weigh and pulverize 20 tablets. To transfer amount of powder equivalent to 50 mg of ethionamide to 100 mL volumetric flask and add 80 mL of methanol. To put it in ultrasound for 10 minutes, mechanically stir for 15 minutes, and top up with the same solvent. To filter and discard the first 20 mL. To transfer 2 mL of the filtrate to 100 mL volumetric flask, top up with methanol, and make it homogenous. To prepare standard solution under the same conditions. To measure the absorbances of the solutions at 290 nm, by using methanol to adjust zero. To calculate the amount of $C_8H_{10}N_2S$ in the tablets, from the readings obtained.

PACKAGING AND STORAGE

In tightly closed containers.

Tolerance: not less than 75% (Q) of the amount stated of $C_8H_{10}N_2S$ dissolves within 45 minutes.

LABELLING

To comply with the legislation in force.

HUMAN BLOOD CLOTTING FACTOR IX, LYOPHILISATE

Fator IX Coagulationis Sanguinis Humanus Cryodesiccatus

The Blood Clotting Factor IX, lyophilisate, is the plasma protein fraction containing Factor IX of human blood clotting obtained by a method which allows the separation of Factor IX from the other Human Prothrombin Complex Factors (Factors II, VII, and X). It is prepared from human plasma according to the monograph *Human Plasma for Fractionating*. Preparation, reconstituted according to the label, is not less than 20 IU of Factor IX per milliliter.

PRODUCTION

The preparation method should be developed to maintain the functional integrity of Factor IX, thus minimizing the activation of any Clotting factor (to limit the thrombogenic potential) and must include one or more steps that proof to eliminate or inactivate known and unknown infectious agents. If substances are added to the viral inactivation at production step, a purification procedure must be validated to demonstrate that the concentration of these substances have been reduced to an acceptable level, and such residues do not compromise the safety of the preparation. The specific activity should not be less than 50 IU for Factor IX per mg of total protein prior to any addition of a protein stabilizer.

The fraction containing Factor IX is solubilized in an appropriate diluent. Heparin, antithrombin, and other auxiliary substances can be added as a stabilizer. No antimicrobial preservative should be added. The solution is filtered through a sterilizing filter and aseptically distributed in the final vials and frozen immediately. Then, they are lyophilisate, and vials are closed by vacuum or inert gas.

The regularity of the production method is evaluated by appropriate analytical procedures throughout development studies, among which we have:

- determination of factor IX;
- determination of Activated clotting factor;
- determination of the activity of Clotting factors II, VII, and X, which is not greater than 5% of the activity of Factor IX.

IDENTIFICATION

The preparation to be examined must be reconstituted according to the label, immediately before the identification test (except water and solubility tests).

A. To subject the sample to precipitation tests, by using a suitable range of animal sera. The test is conducted by using specific sera containing plasma proteins from animal that are commonly used in the country to prepare biological products. The sample contains human proteins and does

not precipitate with specific sera containing plasma proteins from other animal species.

B. The determination of the clotting activity of the Factor IX contributes to identify the sample.

CHARACTERISTICS

Appearance. Friable, white or pale yellow powder or solid.

pH (5.2.19). The pH of the sample is between 6.5 and 7.5.

Osmolality (5.2.28). At least 240 mosmol/kg.

Solubility. To add volume of diluent according to the label to the content of a sample container and gently stir for 10 minutes at room temperature. There is total dissolution, thus forming a clear solution or slightly opalescent and colorless solution.

PHYSICAL AND CHEMICAL TESTS

Water. To determine by an appropriate method, such as *Determination of water by semi-micro method (5.2.20.3)*, *Loss on drying (5.2.9)* or by *Infrared absorption spectrophotometry (5.2.14)*. At most 2.0%.

BIOLOGICAL SAFETY TEST

Pyrogens (5.5.2.1). Sample complies with the test. To inject into each rabbit, per kilogram of body weight, volume of solution of the reconstituted sample corresponding to at least 30 IU of Factor IX and at most 50 IU of Factor IX.

Sterility (5.5.3.2.1). Sample complies with the test.

Toxicity (5.5.2.3). Sample complies with the test.

DOSAGE

Blood Clotting Factor IX

To proceed as described in *Determination of Blood Clotting Factor IX (5.5.1.4)*. The specific activity is neither less than 80% nor more than 125% of the stated activity. The confidence interval ($P = 0.95$) of the activity does not exceed 80% - 125%.

Activated Clotting Factors

To proceed as described in *Determination of activated clotting factors (5.5.1.8)*. If necessary, dilute the sample to obtain a solution containing 20 IU of Factor IX per milliliter. For each dilution, the clotting time is not less than 150 seconds.

Heparin

Should heparin be added throughout production step, determine the amount according to the monograph *Determination of heparin in the clotting factors (5.5.1.1)*. The



sample does not contain more than the amount of heparin stated on the label and not more than 0.5 IU of heparin per international unit of Factor IX.

Total protein

If necessary, dilute the reconstituted preparation with a 0.9% sodium chloride solution (w/v) to obtain a solution containing about 15 mg of protein in 2 mL. To put 2.0 mL of this solution into a round bottom centrifuge tube. To add 2 mL of 7.5% sodium molybdate solution (w/v) and 2 mL of a mixture of nitrogen-free sulfuric acid and water (1:30). To stir and centrifuge for 5 minutes. The supernatant liquid must be decanted, thus allowing the tube to be dry on a filter paper. To determine the nitrogen in the residue by the method *Determination of nitrogen by the Kjeldahl method* (5.3.3.2). To calculate the protein content, and its result should be multiplied by 6.25. This method may not be applicable to certain products, especially those with no protein stabilizer, such as albumin. To use another validated method for the dosage of protein.

STORAGE

Protected from light.

LABELLING

The label states at least the number of international units of Factor IX per vial; the amount of protein in each vial; the name and amount of any added substance, including heparin, where applicable; the name and volume of diluent needed to reconstitute the preparation; the storage conditions; the expiration date; the transmission of infectious agents cannot be totally excluded when administering human blood or plasma medicinal products (the latter can be alternatively stated on the text of package insert.).

HUMAN BLOOD CLOTTING FACTOR VII, LYOPHILISATE

Factor VII Coagulationis Humanus Cryodesiccatus

Human clotting blood Factor VII, lyophilized, is a plasma protein fraction containing Factor VII (a glycoprotein single-chain derivative), and may also contain small amounts of its activated form (two chains derivative or Factor VIIa) as well as Factors II, IX, and X, Protein C, and Protein S. It is prepared from human plasma according to the monograph *Human Plasma for Fractionating*.

Preparation, reconstituted according to the label, is not less than 15 IU of Factor VII per milliliter.

The preparation method should be developed to maintain the functional integrity of Factor VII, thus minimizing the activation of any Clotting factor (to limit the thrombogenic potential) and must include one or more steps that proof to eliminate or inactivate known and unknown infectious

agents. Should substances be added to the viral inactivation at production step, a purification procedure must be validated to demonstrate that the concentration of these substances have been reduced to an acceptable level, and such residues do not compromise the safety of the preparation. The specific activity should not be less than 2 IU for Factor VII per mg of total protein prior to any addition of a protein stabilizer.

The fraction containing Factor VII is dissolved in an appropriate diluent. Heparin, antithrombin, and other auxiliary substances can be added as a stabilizer.

No antimicrobial preservative should be added. The solution is filtered through a sterilizing filter and aseptically distributed in the final vials and frozen immediately. Then, it is lyophilisate, and vials are closed by vacuum or inert gas.

Regularity of production method is demonstrated regarding the activities of Factors II, IX, and X of the preparation expressed in international units and in relation to the activity of Factor VII.

Regularity of production method is demonstrated regarding the activity of Factor VIIa of the preparation. The activity of Factor VIIa can be determined with a soluble recombinant Tissue Factor that does not activate the Factor VII into Factor VIIa, but it has specific cofactor function of Factor VIIa: after incubating the mixture of soluble recombinant Tissue Factor and phospholipids with a dilution of the sample and Factor VII deficient plasma, gather calcium chloride and determine the clotting time; the clotting time is inversely proportional to the activity of Factor VIIa of the sample.

IDENTIFICATION

To reconstitute the sample according to the label immediately before performing the identification, test (except for solubility and water), and dosage.

A. To subject the sample to precipitation tests with a suitable series of specific sera to the various species. The test should be performed with specific plasma protein sera from each of the domestic species commonly used in the preparation of organic products. It is demonstrated that the preparation contains human proteins and negative sera results with specific plasma protein sera from other species.

B. The determination of Factor VII contributes to identify the preparation.

CHARACTERISTICS

Appearance. Friable, white, pale yellow, green, or blue powder or solid.

pH (5.2.19). The pH of the sample is between 6.5 and 7.5.

Osmolality (05.02.28). The osmolality of the sample is at least 240 mosmol/kg.

Solubility. To add volume of diluent according to the label to the content of a sample container, according to the temperature recommended, and gently stir for at most 10 minutes. The sample completely dissolves to form a clear or slightly opalescent solution that can be colored.

PHYSICAL AND CHEMICAL TESTS

Water. To determine by an appropriate method, such as *Determination of water by semi-micro method (5.2.20.3)*, *Loss on drying (5.2.9)* or *Infrared absorption spectrophotometry (5.2.14)*. At most 2%.

BIOLOGICAL SAFETY TEST

Pyrogens (5.5.2.1). To inject into each rabbit, per kilogram of body weight, volume of sample corresponding to at least 30 IU of Factor VII. It complies with the test.

Sterility (5.5.3.2.1). It complies with the test.

Toxicity (5.5.2.3). It complies with the test.

DOSAGE

Blood Clotting Factor VII

To proceed as described in *Determination of Blood Clotting Factor VII (5.5.1.5)*. The specific activity is neither less than 80% nor more than 125% of the stated activity. The confidence interval ($P = 0.95$) of the activity does not exceed 80% - 125%.

Activated Clotting Factors

To proceed as described in *Determination of activated clotting factors (5.5.1.8)*. The clotting time is not less than 150 seconds for each dilution,

Heparin

Should heparin be added throughout production step, determine the amount according to the monograph *Determination of heparin in the clotting factors (5.5.1.1)*. The sample does not contain more than the amount of heparin stated on the label and not more than 0.5 IU of heparin per international unit of Factor VII.

Total protein

If necessary, dilute the reconstituted preparation with a 0.9% sodium chloride solution (w/v) to obtain a solution containing about 15 mg of protein in 2 mL. To put 2.0 mL of this solution into a round bottom centrifuge tube. To add 2 mL of 7.5% sodium molybdate solution (w/v) and 2 mL of a mixture of nitrogen-free sulfuric acid and water (1:30). To stir and centrifuge for 5 minutes. The supernatant liquid must be decanted, thus allowing the tube to be dry on a

filter paper. To determine the nitrogen in the residue by the method *Determination of nitrogen by the Kjeldahl method (5.3.3.2)*. To calculate the protein content, and the result should be multiplied by 6.25.

Thrombin

If the sample contains heparin, determine the current amount, as per *Determination of heparin in the clotting factors (5.5.1.1)*, and neutralize it by adding protamine sulfate (10 µg of protamine sulfate neutralizes 1 IU of heparin). To use 2 test tubes and mix equal volumes of reconstituted sample and 0.3% fibrinogen solution (w/v) inside each one. To keep one of them at 37 °C for 6 hours and another one at room temperature for 24 hours. In a third tube, mix a volume of fibrinogen solution with a volume of human thrombin solution containing 1 IU per milliliter, and put it in the tube, in a water bath at 37 °C. No clotting occurs in the sample tubes. Clotting occurs within 30 seconds in the tube containing thrombin.

STORAGE

Protected from light.

LABELLING

The label states at least: the number of international units of Factor VII per vial; the amount of protein in each vial; the name and amount of any added substance, including heparin, where applicable; the name and volume of diluent needed to reconstitute the preparation; the storage conditions; the expiration date; the transmission of infectious agents can not be totally excluded when administering human blood or plasma medicinal products (the latter can be alternatively stated on the text of package insert.).

HUMAN BLOOD CLOTTING FACTOR VIII, LYOPHILISATE

Factor VIII Coagulationis Sanguinis Humanus Cryodesiccatus

Human clotting blood Factor VIII, lyophilized, is a plasma protein fraction containing a glycoprotein called clotting Factor VIII and

depending on the purification method, variable amounts of von Willebrand factor. It is prepared from a mixture of plasma taken from healthy donors.

Preparation, reconstituted according to the label, is not less than 20 IU of Factor VIII:C per milliliter.

The method of preparation includes two or more steps of viral inactivation, which demonstrate elimination or inactivation of known viral infectious agents. If substances are used to inactivate the virus during the production, the subsequent purification process must demonstrate by validation that the concentration of the inactivating substances is

eliminated or reduced to levels acceptable by the rules and any residues are not likely to pose a risk to patients.

The specific activity should not be less than 1 IU for Factor VIII:C per mg of total protein prior to any addition of a protein stabilizer. The lyophilized Factor VIII is dissolved in diluent specified by the manufacturer. Auxiliary substances can be added, such as a stabilizer. No antimicrobial preservative should be added. The solution is filtered so as to provide retention of bacteria, and then aseptically distributed in the final containers and immediately frozen. Then, it is lyophilisate, and containers are closed by vacuum or inert gas.

Validation applied to products with an indication of having an activity of von Willebrand factor. In products intended for the treatment of Von Willebrand's disease, it has been demonstrated that the manufacturing process produces a substance with constant composition with respect to von Willebrand factor. This composition can be demonstrated in several ways. For example, the number and the various multimers of von Willebrand Factor may be determined by agarose gel electrophoresis (approximately 1% agarose) in the presence of sodium dodecyl sulfate (SDS), with or without Western blot analysis, by using a mixture of common human plasma as reference. The multimeric profile view can be performed by an immunoenzymatic technique and quantitative densitometry evaluation or other appropriate methods.

Products with flakes or particles after reconstitution. If tiny particles or flakes remain after reconstituted preparation, during the validation study, it must be shown that the potency is not significantly influenced after filtration of the preparation.

IDENTIFICATION

It complies with the test *Human blood clotting Factor VIII, lyophilized*, described in *Dosage*.

CHARACTERISTICS

pH (5.2.19). 6.5 to 7.5.

Osmolality (05.02.28). At least 240 mosmol/kg.

Solubility. To add volume of diluent according to the label to the container containing the sample, according to the temperature recommended, and gently stir for at most 10 minutes. The sample completely dissolves to form a clear or slightly opalescent and colorless or slightly yellowish solution. In case of tiny particles or flakes after reconstitution, reconstitute and filter the preparation according to the label. The filtered solution is clear or slightly opalescent.

PHYSICAL AND CHEMICAL TESTS

Water. To determine by using one of the following methods: *Determination of water by semi-micro method*

(5.2.20.3), *Loss on drying (5.2.9)* or *Infrared absorption spectrophotometry (5.2.14)*. At most 2.0%.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

Pyrogens (5.5.2.1). It complies with the test. To inject into each rabbit, per kilogram of body weight, volume corresponding to at least 30 IU of Factor VIII:C.

DOSAGE

Hepatitis B Surface Antigens

To proceed as described in *Immunochemical methods (5.6)*. To examine the reconstituted sample. No Hepatitis B surface antigen is found.

Human von Willebrand factor

To employ one of the methods described below.

A. To proceed as described in *Determination of Human Von Willebrand Factor (5.5.1.2)*.

B. To determine the activity of ristocetin cofactor. To prepare appropriate dilutions of the reconstituted sample and reference preparation by using 0.9% sodium chloride solution (w/v) and 5% human albumin (w/v) as diluent. Whenever make a preparation, add an appropriate amount of a mixture containing stabilized human platelets and ristocetin A. To mix it on a glass slide with gentle circular motions for 1 minute. To let it rest for 1 minute and perform the reading of result in dark background and side lighting. The last dilution with a clearly visible agglutination will indicate the title of the sample. Diluent should be used as negative evidence. The specific activity is at least 60% and at most 140% of the activity approved for the product.

Appearance. friable, white or slightly yellow, hygroscopic powder or solid.

Human blood clotting Factor VIII, lyophilisate

To proceed as described in *Determination of Blood Clotting Factor VIII, lyophilized (5.5.1.7)*. The specific activity is neither less than 80% nor more than 120% of the stated activity. It complies with the test.

Anti-A and anti-B hemagglutinins

To proceed as described in *Determination of Anti-A and Anti-B hemagglutinin (5.5.1.9)*. To dilute the reconstituted sample with a 0.9% sodium chloride solution (w/v) to a 3 IU/mL concentration. The dilutions to 1/64 have no signs of agglutination. It complies with the test.

Total protein

To proceed as described in *Determination of nitrogen by the Kjeldahl method* (5.3.3.2). If necessary, dilute the reconstituted preparation with a 0.9% sodium chloride solution (w/v) to obtain a solution containing about 15 mg of protein in 2 mL. To add 2 mL of this solution, 2 mL of 7.5% sodium molybdate solution (w/v), and 2 mL of a mixture of nitrogen-free sulfuric acid and water (1:30) to a round bottom centrifuge tube. To stir and centrifuge for 5 minutes. The supernatant liquid must be decanted, thus allowing the tube to be dry on a filter paper. To multiply the result by 6.25 to determine the protein content. This method may not be applicable to certain products, especially those with no protein stabilizer, such as albumin. To use another validated method for the dosage of protein.

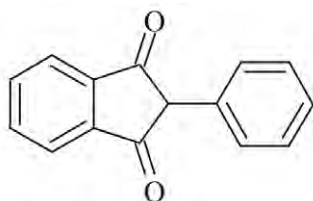
STORAGE

Protected from light.

LABELLING

To comply with the legislation in force. The label indicates the following: the number of international units of Factor VIII:C and, if appropriate, von Willebrand Factor; the amount of protein in each vial; the name and amount of any added substance; the name and volume of the liquid needed to reconstitute the preparation; the storage conditions; the expiration date; the transmission of infectious agents can not be totally excluded when administering human blood or plasma medicinal products (the latter can be alternatively stated on the text of package insert.).

PHENINDIONE Phenindionum



$C_{15}H_{10}O_2$; 222.24
phenindione; 03938
2-Phenyl-1H-indene-1,3 (2H)-dione [83-12-5]

It contains at least 98.0% and at most 100.5% of $C_{15}H_{10}O_2$ in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, almost odorless and tasteless powder, or silky, white or slightly yellowish crystals.

Solubility. Insoluble in water, freely soluble in chloroform, sparingly soluble in ethanol and ethyl ether.

Physical and chemical constants.

Melting range (5.2.2): 148 °C to 151 °C, with decomposition. IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the phenindione SQR spectrum similarly prepared.

B. To dissolve 0.1 g of the sample in 30 mL of ethanol by heating, if necessary. To cool down, transfer to a 50 mL volumetric flask, and top up with the same solvent. To successively dilute to 0.0004% (w/v) with 0.1 M sodium hydroxide. The ultraviolet absorption spectrum (5.2.14) of this solution, ranging from 200 nm to 400 nm, exhibits maxima at 278 nm and 330 nm. The absorbance at 248 nm is 0.54 and at 330 nm is 0.16.

PURITY TESTS

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF_{254} silica gel as support and 0.02% butylated hydroxytoluene (w/v) in a mixture of ethyl acetate and glacial acetic acid (20:4) as mobile phase. To separately apply to the plate 10 μ L of each of the solutions freshly prepared, as described below.

Solution (1): 10 mg/mL solution of the sample in methylene chloride.

Solution (2): to dilute 1 mL of *Solution (1)* to 50 mL with methylene chloride.

Solution (3): to dilute 2.5 mL of *Solution (2)* to 10 mL with methylene chloride.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than the main spot obtained with *Solution (2)* (2.0%). No more than a secondary spot obtained in the chromatogram with *Solution (1)* is more intense than the principal spot obtained with *Solution (3)* (0.5 %).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 105 °C for 2 hours. At most 1.0%.

Sulfated ashes (5.2.10). At most 0.1 %.

DOSAGE

To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To accurately weigh about 50 mg of the sample, dissolve in 0.1 M sodium hydroxide, and dilute to 250 mL with the same solvent. To successively dilute in 0.1 M sodium hydroxide to the 0.0004% concentration (w/v). To measure the absorbances of the resulting solutions at 278 nm, by using 0.1 M sodium hydroxide to adjust zero. To calculate the content of $C_{15}H_{10}O_2$ in the sample by

considering that $A(1\%, 1\text{ cm}) = 1310$, at 278 nm, in 0.1 M sodium hydroxide.

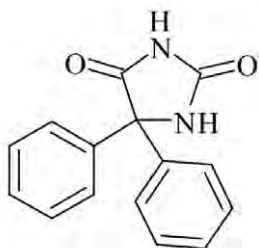
PACKAGING AND STORAGE

In tightly closed containers, protected from light.

THERAPEUTIC CLASS

Anticoagulant.

PHENYTOIN Phenytoinum



$C_{15}H_{12}N_2O_2$; 252.27

phenytoin; 03953

5,5-Diphenyl-2,4-imidazolidinedione
[57-41-0]

It contains at least 98.0% and at most 102.0% of $C_{15}H_{12}N_2O_2$ in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white or almost white powder.

Solubility. Practically insoluble in water, soluble in hot ethanol, sparingly soluble in chloroform, cold ethanol, and ethyl ether.

Physical and chemical constants.

Melting range (5.2.2): 295 °C to 298 °C.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the phenytoin SQR spectrum similarly prepared.

B. The principal spot in the chromatogram of *Solution (2)* in the *Related substances* corresponds in position, color, and intensity to that obtained with the *Solution (3)*.

C. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage method B* corresponds to that of main peak of *Standard solution*.

D. To solubilize 10 mg of the sample in 1 mL of water and 50 µL of concentrated ammonia solution. To heat to start boiling. To add 50 µL of 5% cupric sulfate pentahydrate (w/v) in 2 M ammonia. To stir it. Pinkish precipitate is produced.

PURITY TESTS

Acidity or alkalinity. To accurately weigh about 1 g of the sample. To add 45 mL of water and heat to boiling for 2 minutes. To cool down and filter it. To wash the filter with carbon dioxide-free water. To gather the washings in 50 mL volumetric flask and top up with carbon dioxide-free water. To make it homogeneous. To pipette 10 mL of the resulting solution to erlenmeyer flask. To add 0.15 mL of methyl red SI. To titrate with 0.01 M hydrochloric acid to obtain red color. At most 0.5 mL of titrant is spent to change color. To pipette 10 mL of the original solution to erlenmeyer flask. To add 0.15 mL of bromothymol blue SI. To titrate with 0.01 M sodium hydroxide to obtain blue color. At most 0.5 mL of titrant is spent to change color.

Related substances. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF₂₅₄ silica gel as support and a mixture of dioxane and hexane (30:75) as mobile phase. Before the test, wash the plate with mobile phase and allow to air dry. To separately apply to the plate 10 µL of each of the solutions freshly prepared, as described below.

Solution (1): 40 mg/mL solution of the sample in mixture of acetone and methanol (50:50).

Solution (2): 2 mg/mL solution of the sample in mixture of acetone and methanol (50:50).

Solution (3): 2 mg/mL phenytoin SQR solution in mixture of acetone and methanol (50:50).

Solution (4): 80 µg/mL benzophenone solution in mixture of acetone and methanol (50:50).

Solution (5): 80 µg/mL benzyl solution in mixture of acetone and methanol (50:50).

Solution (6): 0.4 mg/mL solution of the sample in mixture of acetone and methanol (50:50).

Solution (7): mixture of 1 mL of *Solution (4)* and 1 mL of *Solution (5)*.

Procedure: To develop the chromatogram. To remove the plate, and allow it to dry in a current of cold air for 2 minutes. To examine under ultraviolet light (254 nm). Any spot related to benzophenone or benzyl in the chromatogram obtained with *Solution (1)* is not more intense than the spots obtained with *Solution (4)* and *Solution (5)* (0.2%). Any spot in the chromatogram obtained with *Solution (1)*, other than the spots related to phenytoin, benzophenone, or benzyl, is not more intense than that in the chromatogram obtained with *Solution (6)* (1%). The test is valid only if

the chromatogram obtained with *Solution (7)* shows two principal spots clearly separated.

Heavy metals (5.3.2.3). To use *Method III*. To determine in 2 g of the sample. To use 2 mL of the *Lead standard solution (10 ppm Pb)*. At most 0.001% (10 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 105 °C to obtain constant weight. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. To accurately weigh about 0.2 g of the sample, transfer to a 250 mL erlenmeyer flask, and dissolve in 50 mL of dimethylformamide. To titrate with 0.1 M SV sodium methoxide, to potentiometrically determine the endpoint. To perform sample blank correction and adjust it as required. Each mL of 0.1 M SV sodium methoxide is equal to 25.227 mg of $C_{15}H_{12}N_2O_2$.

B. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 220 nm; 250 mm column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at room temperature; flow of *Mobile phase* of 1.5 mL/minute.

Mobile phase: mixture of methanol and water (55:45).

Sample solution: to accurately weigh 0.1 g of the sample and transfer to a 100 mL volumetric flask. To dissolve in methanol, top up with the same solvent, and make it homogeneous. To transfer 10 mL of the resulting solution to 100 mL volumetric flask, top up with *Mobile phase*, and make it homogeneous.

Standard solution: to dissolve accurately weighed amount of phenytoin SQR in *Mobile phase*, by using ultrasound, to obtain a 0.1 mg/mL solution.

Resolution solution: to prepare a solution containing approximately 1.5 mg/mL of benzoin in *Mobile phase*. To mix 1 mL of the solution obtained with 9 mL of *Standard solution* and make it homogeneous.

To inject 20 µL of *Resolution solution*. The relative retention times are about 0.75 to phenytoin and 1.0 to benzoin. The resolution between the benzoin and phenytoin peaks is not less than 1.5. To inject replicates of 20 µL of *Standard solution*. The relative standard deviation for replicate areas of the peaks recorded is not greater than 1.0 %. The tailing factor for the phenytoin peak is not greater than 1.5.

Procedure: to separately inject 20 µL of *Standard solution* and *Sample solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_{15}H_{12}N_2O_2$ from the sample, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Anticonvulsant.

PHENYTOIN TABLETS

It contains at least 95.0 % and at most 105.0 % of the amount stated of $C_{15}H_{12}N_2O_2$.

IDENTIFICATION

The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: Tris buffer 0.05 M pH 9.0, 900 mL

Equipment: blades, 100 rpm

Time: 120 minutes

Procedure: immediately after testing, remove aliquot from the dissolution medium and filter it by discarding the first few milliliters. To pipette 10 mL of the filtrate and transfer to 50 mL volumetric flask To top up to volume with *Mobile phase*, according to *Dosage*, and make it homogeneous. To prepare the standard exactly weighing about 75 mg of phenytoin SQR. To transfer to a 25 mL volumetric flask, dissolve in methanol, and top up with the same solvent. To pipette 1 mL of the resulting solution, transfer to 50 mL volumetric flask, and top up with *Dissolution medium*. To



pipette 10 mL of the previous solution and dilute to 25 mL with *Mobile phase*. To proceed as described in *Dosage*.

Tolerance: not less than 70% (Q) of the amount stated of $C_{15}H_{12}N_2O_2$ dissolves within 120 minutes.

DOSAGE

To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 254 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μ m), kept at room temperature; flow of *Mobile phase* of 1.5 mL/min.

Mobile phase: mixture of water, methanol, acetonitrile, 1% triethylamine (v/v), and acetic acid (500:270:230:5:1).

Sample solution: to weigh and pulverize 20 tablets. To transfer amount of powder equivalent to 50 mg of phenytoin to a 100 mL volumetric flask, add about 70 mL of *Mobile phase*, and put it in ultrasound for 10 minutes. To top up with the same solvent and make it homogeneous.

Standard solution: to accurately weigh about 50 mg of phenytoin SQR and transfer to a 100 mL volumetric flask. To dissolve in at most 5 mL of methanol in ultrasound. To top up to volume with *Mobile phase* and make it homogeneous.

To inject replicates of 25 μ L of the *Standard solution*. The column efficiency should be greater than 6500 theoretical plates/meter. The tailing factor is not greater than 1.5. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2.0 %.

Procedure: to separately inject 25 μ L of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks.

To calculate the amount of $C_{15}H_{12}N_2O_2$ in the tablets, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

PHENYTOIN SODIUM INJECTABLE SOLUTION

It contains at least 95.0 % and at most 105.0 % of the amount stated of $C_{15}H_{11}N_2NaO_2$.

IDENTIFICATION

A. The principal spot in the chromatogram of *Solution (1)* in the *Related substances* corresponds in position, color, and intensity to that obtained with the *Solution (2)*.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

C. It responds to the reactions of sodium ion (5.3.1.1).

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

pH (5.2.19). 10.0 to 12.3.

PURITY TESTS

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF_{254} silica gel as support and a mixture of dioxane and hexane (30:75) as mobile phase. Before the test, wash the plate with mobile phase and allow to air dry. To separately apply to the plate 5 μ L of each of the solutions freshly prepared, as described below.

Solution (1): to dilute the injectable solution in methanol to obtain a 20 mg/mL phenytoin sodium solution.

Solution (2): 20 mg/mL phenytoin sodium SQR solution in methanol.

Solution (3): 0.1 mg/mL benzophenone solution in ethanol.

Solution (4): 0.1 mg/mL benzyl solution in ethanol.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). Any spot related to benzophenone or benzyl in the chromatogram obtained with *Solution (1)* is not

more intense than the spots obtained with *Solution (3)* and *Solution (4)* (0.5%).

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

Bacterial endotoxins (5.5.2.2). At most 0.35 UE/ mg of phenytoin sodium.

DOSAGE

To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 254 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5

μm), kept at room temperature; flow of *Mobile phase* of 1.5 mL/min.

Mobile phase: mixture of methanol and water (55:45).

Sample solution: to transfer a volume of injectable solution equivalent to 0.25 g of phenytoin sodium to a 100 mL volumetric flask. To top up to volume with *Mobile phase* and make it homogeneous. To transfer to a 50 mL volumetric flask. To top up to volume with *Mobile phase* and make it homogeneous.

Standard solution: to dissolve accurately weighed amount of phenytoin sodium SQR in *Mobile phase* and dilute to obtain a 0.25 mg/mL solution.

To inject replicates of 20 μL of the *Standard solution*. The tailing factor is not greater than 2.0. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2.0 %.

Procedure: to separately inject 20 μL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of $\text{C}_{15}\text{H}_{11}\text{N}_2\text{NaO}_2$ in the injectable solution, from the responses, from the *Standard solution* and *Sample solution*.

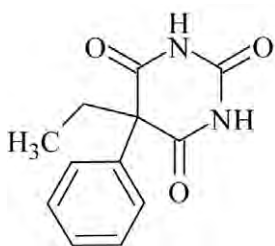
PACKAGING AND STORAGE

In type I glass containers, protected from light at a temperature below 25 °C.

LABELLING

To comply with the legislation in force.

PHENOBARBITAL Phenobarbitalum



$\text{C}_{12}\text{H}_{12}\text{N}_2\text{O}_3$; 232.24

phenobarbital; 03960

5-Ethyl-5-phenyl-2, 4,6 (1*H*, 3*H*, 5*H*)-pyrimidinetrione
[50-06-6]

It contains at least 99.0% and at most 101.0% of $\text{C}_{12}\text{H}_{12}\text{N}_2\text{O}_3$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White, crystalline powder, or colorless, odorless crystals.

Solubility. Very slightly soluble in water, freely soluble in ethanol, slightly soluble in ethyl ether. Soluble in carbonates and diluted hydroxides.

Physical and chemical constants.

Melting range (5.2.2): 174 °C to 178 °C.

IDENTIFICATION

The identification test A may be omitted if the tests B., C., D., E. and F are conducted. The identification tests C. and D. can be omitted if the tests A., B., E., and F are carried out.

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the phenobarbital SQR spectrum similarly prepared.

B. A. The ultraviolet absorption spectrum (5.2.14) ranging from 200 nm to 400 nm, of sample obtained in *Dosage*, method **B.**, exhibits absorption maximum and minimum similar to those observed in the standard solution spectrum.

C. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF_{254} silica gel as support and a mixture of 13.5 *M* ammonia, ethanol, and chloroform (5:15:80) as mobile phase. To separately apply to the plate 10 μL of each of the solutions freshly prepared, as described below.

Solution (1): 1 mg/mL solution of the sample in ethanol.

Solution (2): 1 mg/mL phenobarbital SQR solution in ethanol.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

D. The relationship between the retention times of the main peak and internal standard peak, in the chromatogram of the sample solution, obtained in method **C.** *Dosage*, corresponds to the relationship between the retention times of the main peak and internal standard peak in the chromatogram of the standard solution.

E. It responds to the reaction of barbiturates with no substituent in nitrogen (5.3.1.1). To use 1 mg/mL solution in methanol.

F. To stir 0.1 g of the sample with 4 mL of 0.1 *M* sodium hydroxide and 1 mL of water and filter it. To add 2 mL of the filtrate, 0.25 mL of 0.2 *M* mercury chloride. White precipitate is produced, which is dissolved by adding 5 mL of 6 *M* ammonium hydroxide.

PURITY TESTS

Appearance of solution. The 10% solution (w/v) in a mixture of 2 M sodium hydroxide and water (2:3) keeps clear (5.2.25).

Acidity. To boil 1 g of sample, for 2 minutes, in 50 mL of water. To cool down and filter it. To add 0.15 mL of methyl red SI to 10 mL of the filtrate. Solution becomes yellow-orange. To titrate with 0.1 M SV sodium hydroxide. At most 0.1 mL of 0.1 M SV sodium hydroxide is required to produce a clear yellow color.

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel as support and a mixture of 13.5 M ammonia, ethanol, and chloroform (5:15:80) as mobile phase. To separately apply to the plate 20 µL of each of the solutions freshly prepared, as described below.

Solution (1): to dissolve 0.1 g of the sample in ethanol and top up to 10 mL with the same solvent.

Solution (2): to dilute 0.5 mL of *Solution (1)* to 100 mL with ethanol.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). To nebulize with mercury diphenyl carbazone SR, air dry, and nebulize with ethanolic potassium hydroxide SR freshly prepared. To heat the plate at 105 °C for 5 minutes and examine immediately. Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (2)* (0.5%).

Loss on drying. (5.2.9). To desiccate in an oven at 105°C for 2 hours. At most 1%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To employ one of the methods described below.

A. To accurately weigh about 0.4 g of sample, transfer to 125 mL erlenmeyer flask, and by dropping 6 drops of thymolphthalein SI dissolve it in 50 mL of ethanol previously neutralized with 0.1 M SV sodium hydroxide. To titrate with 0.1 M SV sodium hydroxide. Each mL of 0.1 M SV sodium hydroxide is equal to 23.220 mg of C₁₂H₁₂N₂O₃.

B. To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To accurately weigh about 50 mg of the sample, transfer to a 50 mL volumetric flask, dissolve in 5 mL of ethanol, and top up with borate buffer pH 9.6. To successively dilute to obtain 0.001% concentration (w/v) by using the same solvent. To prepare a standard solution at the same concentration by using the same solvent and phenobarbital SQR instead of sample. To measure the absorbance of

the resulting solutions at 240 nm, by using borate buffer pH 9.6 to adjust zero. To calculate the content of C₁₂H₁₂N₂O₃ in the sample from the readings obtained.

C. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 254 nm; 250 mm column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), flow of *Mobile phase* of 1.2 mL/min.

Acetate buffer pH 4.5: to dissolve about 6.6 g of sodium acetate trihydrate and 3 mL of glacial acetic acid in 1000 mL of water and adjust, if necessary, with glacial acetic acid to pH 4.5 ± 0.1.

Mobile phase: mixture of *Acetate buffer pH 4.5* and methanol (56:44).

Diluent: mixture of *Acetate buffer pH 4.5* and methanol (1:2).

Internal standard solution: to dissolve accurately weighed amount of caffeine SQR in *Diluent* to obtain a 0.6 mg/mL solution.

Sample solution: to accurately transfer about 60 mg of the sample to a 100 mL volumetric flask. To add 10 mL of *Internal standard solution* and about 60 mL of *Diluent*. To put it in the ultrasound bath for 15 minutes. To top up to volume with *Diluent*.

Standard solution: to accurately transfer about 60 mg of phenobarbital SQR to a 100 mL volumetric flask. To add 10 mL of *Internal standard solution* and about 60 mL of *Diluent*. To put it in the ultrasound bath for 15 minutes and top up with *Diluent*, to obtain a solution containing 0.6 mg/mL of phenobarbital.

To inject replicates of 20 µL of the *Standard solution*. The relative retention times are about 0.4 to caffeine and 1.0 to phenobarbital. The tailing factor is less than 2.0.

The resolution between phenobarbital and caffeine should not be less than 1.2. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2.0%.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the phenobarbital and caffeine peaks. To calculate the content of C₁₂H₁₂N₂O₃ from the sample, from the responses obtained for phenobarbital/caffeine with *Sample solution* and *Standard solution*.

Uniformity of dosage units (5.1.6). It complies with the test.

Procedure for content uniformity. To transfer each tablet to a 100 mL volumetric flask containing 5 mL of water and wait for total disintegration of the tablet. To add 5 mL of ethanol and 60 mL of borate buffer pH 9.6. To put it in ultrasound for 15 minutes. To mechanically stir for 15 minutes and top up

with buffer. To proceed as described in *Dosage*, method **A.**, from “To make it homogeneous and filter.”

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Anticonvulsant, hypnotic, sedative.

PHENOBARBITAL TABLETS

It contains at least 90.0% and at most 110.0% of the amount stated of $C_{12}H_{12}N_2O_3$.

IDENTIFICATION

A. To weigh and pulverize the tablets. To transfer amount of powder equivalent to 60 mg of phenobarbital to a beaker, add 50 mL of chloroform, make it homogeneous, and filter. To evaporate to dryness. To dry the residue in an oven at 105 °C for 2 hours. The residue responds to test **A.**, *Identification*, in the monograph *Phenobarbital*.

B. The ultraviolet absorption spectrum (5.2.14) ranging from 200 nm to 400 nm, of sample solution obtained in *Dosage* method **A.**, exhibits maximum and minimum similar to those observed in the standard solution spectrum.

C. The relationship between the retention times of the main peak and internal standard peak, in the chromatogram of the *Sample solution*, obtained in method **B.** *Dosage*, corresponds to the relationship between the retention times of the main peak and internal standard peak in the chromatogram of the *Standard solution*.

D. The residue obtained in test **A.**, *Identification*, melts at 174 °C - 178 °C.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL

Equipment: blades, 50 rpm

Time: 45 minutes

Procedure: after testing, remove aliquot from the dissolution medium and dilute with borate buffer pH 9.6, to obtain suitable concentration. To measure the absorbances at 240 nm (5.2.14), by using the same solvent to adjust zero. To calculate the amount of $C_{12}H_{12}N_2O_3$ dissolved in the medium, by comparing the readings obtained with 0.001 % phenobarbital SQR solution (w/v) prepared in borate buffer pH 9.6.

Tolerance: not less than 75% (Q) of the amount stated of $C_{12}H_{12}N_2O_3$ dissolves within 45 minutes.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in the *Ultraviolet absorption spectrophotometry* (5.2.14). To weigh and pulverize 20 tablets. To transfer amount of powder equivalent to 50 mg of phenobarbital to a 50 mL volumetric flask, dissolve in 5 mL of ethanol, and add 35 mL of borate buffer pH 9.6. To put it in ultrasound for 15 minutes and top up with buffer. To make it homogenous and filter it. To successively dilute with the same solvent to a 0.001% concentration (w/v). To prepare a standard solution at the same concentration by using the same solvent. To measure the absorbance of the resulting solutions at 240 nm wavelength, by using borate buffer pH 9.6 to adjust zero. To calculate the amount of $C_{12}H_{12}N_2O_3$ in the tablets, from the readings obtained.

B. By *High performance liquid chromatography* (5.2.17.4). To proceed as described in *Dosage*, method **C.**, in the monograph *Phenobarbital*. To prepare the *Sample solution* as described below.

Sample solution: to weigh and pulverize 20 tablets. To transfer amount of powder equivalent to 60 mg of phenobarbital to a 50 mL volumetric flask, add 4 mL of *Internal standard solution* and 30 mL of *Diluent*. To put it in ultrasound for 15 minutes. To mechanically stir for 15 minutes, top up with *Diluent*, make it homogeneous, and filter it.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the phenobarbital and caffeine peaks. To calculate the amount of $C_{12}H_{12}N_2O_3$ in the tablets, from the responses obtained for phenobarbital/caffeine with *Sample solution* and *Standard solution*.



PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

PHENOBARBITAL ORAL SOLUTION

It contains at least 90.0% and at most 105.0% of the amount stated of $C_{12}H_{12}N_2O_3$. It contains suitable stabilizers and alkalizing.

IDENTIFICATION

A. To transfer volume of oral solution equivalent to 0.4 g of phenobarbital to a 125 mL separatory funnel containing 20 mL of water, add 5 mL of *M* sodium hydroxide, make it homogeneous, and extract with 2 portions (10 mL each) of chloroform, by discarding the organic layer. Add 5 mL of 3 *M* hydrochloric acid and extract with 2 portions (25 mL each) of chloroform, and filter by collecting the organic extracts to a beaker. To evaporate to dryness. To dry the residue in an oven at 105 °C for 2 hours. The residue responds to test A., *Identification*, in the monograph *Phenobarbital*.

A. The relationship between the retention times of the main peak and internal standard peak, in the chromatogram of the *Sample solution*, obtained in *Dosage*, corresponds to the relationship between the retention times of the main peak and internal standard peak in the chromatogram of the *Standard solution*.

B. The residue obtained in test A., *Identification*, melts at 174 °C - 178 °C (5.2.2).

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

pH (5.2.19). 9.2 to 10.2.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To proceed as described in *Dosage*, method C., in monograph *Phenobarbital*. To prepare the *Sample solution* as described below.

Sample solution: to transfer volume of oral solution equal to 0.6 g of phenobarbital to a 100 mL volumetric flask and

top up with *Diluent*. To transfer 10 mL to 50 mL volumetric flask, add 4 mL of *Internal standard solution*, and top up with *Diluent*.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the phenobarbital and caffeine peaks. To calculate the amount of $C_{12}H_{12}N_2O_3$ in the oral solution, from the responses obtained for phenobarbital/caffeine with *Standard solution* and *Sample solution*.

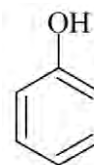
PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

PHENOL
Phenolum



C_6H_6O ; 94.11
phenol; 03968
Phenol
[108-95-2]

It contains at least 99.0% and at most 100.5% of C_6H_6O , in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. Acicular, colorless crystals or white crystalline mass, typical odor, corrosive, irritant to mucous membranes and skin, deliquescent. It gets darker if exposed to air and light.

Solubility. Soluble in water, very soluble in ethanol, ethyl ether, chloroform, glycerol, and fixed and volatile oils, insoluble in petroleum ether.

Physical and chemical constants.

Freezing temperature (5.2.4). at least 39 °C.

IDENTIFICATION

A. To add a drop of 5% ferric chloride aqueous solution (w/v) to 5 mL of 2% aqueous solution (w/v) of the sample. The violet color develops. To add 10 mL of 90% ethanol (v/v). Color becomes yellow.

B. To add bromine water SR to 1% aqueous solution (w/v) of the sample. White precipitate is produced, which rapidly dissolves, but becomes permanent after adding excess reagent.

PURITY TESTS

Appearance of solution. The solution of 1 g of the sample in 15 mL of water is clear (5.2.25).

Acidity. To add a drop of methyl orange SI to 5 mL of a solution of 1 g of the sample in 15 mL of water. Yellow color is produced.

Water (5.2.20.1). At most 0.5%.

Evaporation residue. To accurately weigh about 5 g of the sample, evaporate in a water bath, and dry at 105 °C for 1 hour. The mass of the residue is less than 2.5 mg.

DOSAGE

To accurately weigh about 0.2 g of the sample, dissolve in water, and top up the volume to 100 mL with the same solvent. To transfer 25 mL of the solution to an erlenmeyer flask with lid and add 50 mL of 0.05 M SV bromine and 5 mL of hydrochloric acid. To cover it and occasionally stir for 20 minutes. To protect it from light for 15 minutes. To add 5 mL of 20% potassium iodide solution (w/v) and gently stir. To titrate with 0.1 M SV sodium thiosulfate. To add 3 mL of starch SI solution and 10 mL of chloroform when the color of the solution remains slightly yellowish. To continue the titration by vigorously stirring to blue color fades out. To perform sample blank correction and adjust it as required. Each mL of 0.05 M SV bromine is equal to 1.569 mg of $C_{16}H_{17}O_5S$.

PACKAGING AND STORAGE

In airtight closed containers, protected from light.

LABELLING

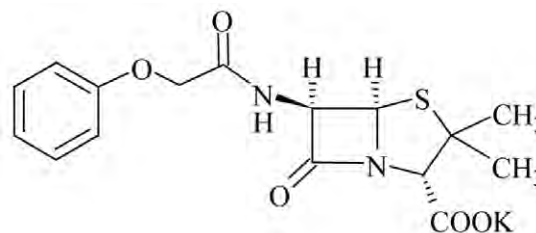
To comply with the legislation in force.

CATEGORY

Antiseptic, preservative, and antipruritic.

PHENOXYMETHYLPENICILLIN POTASSIUM

Phenoxymethylpenicillinum kalicum



$C_{16}H_{17}KN_2O_5S$; 388.48

phenoxymethylpenicillin potassium; 03996

Potassium salt of (2*S*, 5*R*, 6*R*) -3,3-dimethyl-7-oxo-6-[(phenoxycarbonyl) amino]-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid (1:1) [132-98-9]

It has content between 1380 IU and 1610 IU of phenoxymethylpenicillin ($C_{16}H_{18}N_2O_5S$) per milligram, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White, crystalline, odorless powder.

Solubility. Very soluble in water, very slightly soluble in ethanol, insoluble in acetone.

Physical and chemical constants.

Specific rotation (5.2.8): +220° to +235°, in relation to the desiccated substance. To determine in 1% solution (w/v) in carbon dioxide-free water.

IDENTIFICATION

The identification tests **B.** and **C.** may be omitted if the tests **A.** and **D.** are conducted. The identification test **A.** can be omitted if the tests **B.**, **C.**, and **D.** are carried out.

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the phenoxymethylpenicillin potassium SQR spectrum similarly prepared.

B. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using H silica gel, as support, and a mixture of acetone and 15.4% ammonium acetate (w/v) with pH 5.0 adjusted with glacial acetic acid (30:70) as mobile phase. To separately apply to the plate 1 µL of each of the solutions freshly prepared, as described below.

Solution (1): 5 mg/mL solution of the sample in water.

Solution (2): 5 mg/mL phenoxymethylpenicillin potassium solution SQR in water.

Solution (3): solution containing 5 mg/mL of benzylpenicillin potassium SQR and 5 mg/mL of phenoxymethylpenicillin potassium SQR in water.

To develop the chromatogram. To remove the plate, allow to air dry, and expose it to iodine vapor to appear the spots. To examine under visible light. The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*. The test is valid only if the chromatogram obtained with *Solution (3)* presents two clearly separated spots.

C. To transfer 2 mg of the sample to a test tube. To moisten with 0.05 mL of water and add 2 mL of the mixture of 2 mL of formaldehyde solution with 100 mL of sulfuric acid. To stir the tube. The reddish-brown color develops. To immerse the tube in a water bath for 1 minute. The reddish-brown color gets darker.

D. It responds to the reactions of potassium ion (5.3.1.1).

PURITY TESTS

pH (5.2.19). 4.0 to 7.5. To determine in 3% aqueous solution (w/v).

Limit for phenoxyacetic acid. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 254 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at room temperature; flow of *Mobile phase* of 1 mL/min.

Phosphate buffer pH 6.6: to transfer 250 mL of 0.2 M monobasic potassium phosphate and 82 mL of 0.2 M sodium hydroxide to a 1000 mL volumetric flask. To top up with water and make it homogeneous.

Mobile phase: mixture of water, acetonitrile, and glacial acetic acid (65:35:1). To make the corrections as required.

Standard solution: 0.1 mg/mL phenoxyacetic acid solution in *Phosphate buffer pH 6.6*.

Sample solution: 20 mg/mL solution of the sample in *Phosphate buffer pH 6.6*. To use the solution on the same day.

To inject replicates of 20 µL of the *Standard solution*. The tailing factor is not greater than 1.5. The relative standard deviation for replicate areas under the peaks recorded is not greater than 2.0 %.

Procedure: to separately inject 20 µL of *Sample and standard solutions*, record the chromatograms, and measure the area under the peaks. At most 0.5% of phenoxyacetic acid.

Limit for 4-hydroxy phenoxymethylpenicillin. To use the chromatogram obtained in *Dosage* and calculate the percentage of 4-hydroxy phenoxymethylpenicillin in the sample by using the equation:

$100r_h/rs$ where r_h is the area under the 4-hydroxy phenoxymethylpenicillin peak and r_s is the sum of the areas under the 4-hydroxy phenoxymethylpenicillin and phenoxymethylpenicillin peaks. At most 5.0%.

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 105 °C. At most 1.5%.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Microbiological test of antibiotics (5.5.3.3.1)* by using cylinders with the agar diffusion method.

Micro-organism: *Staphylococcus aureus* ATCC 6538P.

Culture media: culture medium number 1, to maintain the micro-organism and inoculum preparation; culture medium number 2, to the base layer.

Sample solution: to dissolve accurately weighed amount of the sample in 1% *Potassium phosphate buffer, sterile, pH 6.0 (Solution 1)* to obtain a 100 IU/mL solution. To successively dilute to obtain 0.2 UI/mL, 0.4 UI/mL, and 0.8 UI/mL concentrations, by using 1% *Potassium phosphate buffer, sterile, pH 6.0 (Solution 1)* to top up the volume.

Standard solution: to dissolve accurately weighed amount of phenoxymethylpenicillin potassium in 1% *Potassium phosphate buffer, sterile, pH 6.0 (Solution 1)* to obtain a 100 IU/mL solution. To successively dilute to obtain 0.2 UI/mL, 0.4 UI/mL, and 0.8 UI/mL concentrations, by using 1% *Potassium phosphate buffer, sterile, pH 6.0 (Solution 1)* to top up the volume.

Procedure: to add 21 mL of culture medium number 2 in each plate, wait to solidify, add 4 mL of 1% inoculum, and proceed as described in *Microbiological test of antibiotics (5.5.3.3.1)*, by adding 0.2 mL of freshly prepared solutions to the cylinders. To calculate the potency of the sample, in IU, of phenoxymethylpenicillin, per mg, from the potency of the standard and responses obtained with the *Standard and sample solutions*.

B. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 254 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at room temperature; flow of *Mobile phase* of 1 mL/min.

Mobile phase: mixture of water, acetonitrile, and glacial acetic acid (650:350:5.75). To make the corrections as required.

Sample solution: to dissolve accurately weighed amount of the sample in *Mobile phase* to obtain a 2.5 mg/mL solution.

Standard solution: to dissolve accurately weighed amount of phenoxymethylpenicillin potassium SQR in *Mobile phase* to obtain a 2.5 mg/mL solution.

Resolution solution: to prepare a solution in *Mobile phase* containing approximately 2.5 mg of benzylpenicillin potassium and 2.5 mg of phenoxymethylpenicillin per milliliter.

To inject replicates of 10 µL of the *Resolution solution*. The relative retention times are about 0.8 to benzylpenicillin and 1.0 to phenoxymethylpenicillin. The resolution between benzylpenicillin and phenoxymethylpenicillin peaks should not be less than 3.0. The relative standard deviation for replicate areas of the peaks recorded is not greater than 1.0 %.

Procedure: to separately inject 10 µL of the *Sample and standard solutions*, record the chromatograms, and measure the area under the higher phenoxymethylpenicillin peak and any peak whose relative retention time of the main peak of phenoxymethylpenicillin is approximately 0.4. To calculate the phenoxymethylpenicillin content in IU ($C_{16}H_{18}N_2O_5S$) per milligram, from the sample, from the responses obtained for the sum of the areas under the peaks with *Standard solution* and *Sample solution*.

PACKAGING AND STORAGE

In airtight closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antibiotic.

FIBRINOGEN, HUMAN, LYOPHILISATE Fibrinogenum Humanum Cryodesiccatus

Lyophilized human fibrinogen contains the soluble fraction of human plasma, which is transformed into fibrin by adding thrombin. Fibrinogen should be obtained from *Human Plasma for Fractionating*. The preparation may contain additives, such as salts, buffers, or stabilizers. The preparation reconstituted with the volume of diluent on the label must contain at least 10 g/L of fibrinogen.

The method of preparation comprises one or more steps that demonstrate to eliminate infectious agents known; it has been shown that residues in the final product of substances may be used in the process for the viral inactivation

or further purification processes properly validated have no adverse effect on patients.

No antibiotic should be added to plasma, and preparation should contain no antimicrobial preservative.

The method of preparation should be such that the specific activity (fibrinogen content in relation to the total protein content) is not less than 80%.

Should protein stabilizer be added to the preparation (e.g., human albumin), it must comply with the requirements for the specific fibrinogen activity before adding stabilizer.

During the fractionation of human plasma, fibrinogen and albumin can be simultaneously obtained. The determination of the specific albumin activity should be determined by a suitable immunochemical method, and quantity determined shall be subtracted from the total protein for the calculation of specific activity.

IDENTIFICATION

To reconstitute the sample according to the label, perform precipitation tests with various specific sera of different species. It is recommended that testing be performed in specific sera of plasma proteins of each domestic animal, commonly used for the preparation of biological products. The sample should contain human proteins and it gives negative results to specific sera with plasma protein from other species. The dosage of the sample contributes to identify the preparation.

CHARACTERISTICS

Appearance. Friable powder or solid, white or pale yellow.

pH (5.2.19). 6.5 to 7.5.

Osmolality (5.2.28). The Osmolality of the reconstituted sample is at least 240 mosmol/kg.

Solubility. To add the volume of diluent, according to the label, to the content of the vial. Fibrinogen dissolves within 30 minutes at 20 °C - 25 °C, thus resulting a almost colorless and slightly cloudy preparation.

Stability of preparation. After reconstitution of the preparation at 20 °C - 25 °C, let it rest. No signs of ice should appear over 60 minutes following the reconstitution.

PHYSICAL AND CHEMICAL TESTS

Water. To determined by using one of the following methods: *Determination of water by semi-micro method (5.2.20.3)*, *Loss on drying (5.2.9)* or *Infrared absorption spectrophotometry (5.2.14)*. At most 2.0%.



BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

Pyrogens (5.5.2.1). To inject into each rabbit, per kilogram of body weight, volume corresponding to at least 30 mg of fibrinogen calculated in relation to the amount given on the label. It complies with the test.

Toxicity (5.5.2.3). It complies with the test.

DOSAGE

Hepatitis B Surface Antigens

To examine the reconstituted sample as described in *Immunochemical methods* (5.6). No Hepatitis B Surface Antigens must be detected.

Fibrinogen

To mix 0.2 mL of reconstituted sample with 2 mL of appropriate buffer (pH 6.6 - 6.8) containing a sufficient amount of thrombin (around 3 IU/mL) and calcium (0.05 mol/L). To keep the mixture at 37 °C for 20 minutes, separate the precipitate by centrifugation (5000 g for 20 minutes), and carefully wash with 0.9% sodium chloride solution (w/v). To determine the nitrogen content by *Determination of nitrogen by the Kjeldahl method* (5.3.3.2) and calculate the amount of fibrinogen (coagulable proteins) by multiplying the result by 6.0. The content is at least 70.0% and at most 130.0% of the amount given on the label. Kits to quantitatively determine fibrinogen in citrated plasma, manual or automated, by the clot formation method, are also available in the market. These kits are based on an optimum amount of bovine thrombin, which is added to a 1:10 diluted plasma. The coagulation time measured should be inversely related to the fibrinogen concentration in the sample tested. These kits must be registered by the competent authority and duly authenticated in accordance with the standards of the National Committee for Clinical Standards: Collection Transport and Processing of Blood Specimens for Coagulation Testing and Performance of Coagulation Assays. 1991. NCCLS Document H21 - A2 can be used in hemotherapy units that produce cryoprecipitate from fresh frozen plasma, whose need is to quantify plasma fibrinogen.

STORAGE

Protected from light.

LABELLING

The label must state the amount of fibrinogen contained in the bottle; the name and volume of diluent to be used, to reconstitute the preparation, where appropriate; the name and amount of protein stabilizer used in the preparation.

TAPE

It consists of tissue and/or film uniformly coated on one side by a pressure sensitive adhesive layer.

CHARACTERISTICS

Dimensions. To determine the length of the tape. At least 98.0% of the stated value. To determine the width at 5 points evenly spaced long the center line of the tape and calculate the average. At least 95.0% of the stated value.

Tensile strength (5.7.1). To determine the tensile strength of the tape after unrolling and store a minimum of 4 hours in a standard atmosphere of $65 \pm 2\%$ RH at 21 ± 1.1 °C, by using a pendulum type device. To proceed as described in *Tensile strength*. The tape made from fabric must have tensile strength of at least 20.41 kg by 2.54 cm wide. The tape made from polymeric film should present tensile strength of at least 3 kg by 2.54 cm wide.

Adhesion to the surface. From the sample made of fabric, cut a strip of 2.54 cm wide and approximately 15 cm long. To apply pressure equivalent to 850 g in one end of the tape (surface equal to 12.90 cm² - 2.54 cm wide x 5.08 cm long) against a clean glass, plastic or stainless steel. To exert pressure twice by using a rubber roller at 30 cm/minute. To adjust the tape and surface temperature at 37 °C (5.7.1) and immediately conduct the test as described in *Tensile strength*. To use a pendulum type device and the breakage must be in parallel to the warping and surface. The average value of at least 10 tests should be at least 18 kg.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). Applicable when the tape is considered sterile. It complies with the test.

PACKAGING AND STORAGE

In tightly closed packaging, protected from light and excessive heat.

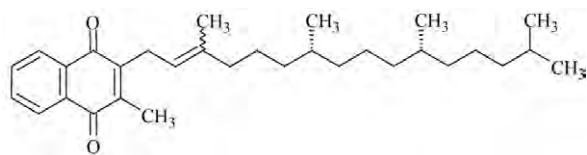
The tape, when stated sterile or sterilized, should be packaged in order to protect its sterility against contamination.

LABELLING

To comply with the legislation in force.

PHYTOMENADIONE

Phytomenadionum



$C_{31}H_{46}O_2$; 450.70

phytomenadione; 04060

2-Methyl-3-[(2*E*, 7*R*, 11*R*) -3,7,11,15-tetramethyl-2-hexadecen-1-yl] -1,4-naftalenodiona
[84-80-0]

CPhytomenadione is a mixture of *E* and *Z* isomers containing at least 97.0% and at most 103.0% of $C_{31}H_{46}O_2$. It contains at most 21.0% of *Z* isomer.

DESCRIPTION

Physical characteristics. Clear, yellow to amber, very viscous, oily, odorless or almost odorless liquid. It is stable in air, but decomposes by exposure to light.

Solubility. Practically insoluble in water; soluble in absolute ethanol, benzene, chloroform, ethyl ether, and vegetable oils; slightly soluble in ethanol.

Physical and chemical constants. *Refractive index* (5.2.6): 1.523 to 1.526.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the thin film has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the phytomenadione SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14) ranging from 200 nm to 400 nm, of 0.001% solution (w/v) in n-hexane, exhibits maxima and minima only at the same wavelength of phytomenadione SQR solution similarly prepared.

PURITY TESTS

Acidity or alkalinity. The 5% solution (v/v) in absolute ethanol is neutral to litmus paper.

Menadione. To mix about 20 mg of the sample with 0.5 mL of mixture of equal volumes of methanol and ammonia SR. To add a drop of ethyl cyanoacetate and gently stir. No blue or purple color develops.

Limit for (Z)-phytomenadione. To proceed as described in *Dosage*. To calculate the (Z)-phytomenadione content in the sample by using the following equation:

$$100 \times a_z / (a_z + a_e),$$

where

a_z = area under the (Z)-phytomenadione peak obtained with *Sample solution*;

a_e = area under the (E)-phytomenadione peak obtained with *Sample solution*.

At most 21.0%.

DOSAGE

To proceed as described in *High performance liquid chromatography* (5.2.17.4). To protect solutions from direct light. To use chromatograph equipped with an ultraviolet detector at 254 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica (5 μ m), kept at 30 °C; flow of *Mobile phase* of 1.0 mL/min.

Mobile phase:

mixture of n-hexane and n-amyl alcohol (2000:1.5).

Internal standard solution: to dissolve accurately weighed amount of cholesteryl benzoate in *Mobile phase* to obtain a 2.5 mg/mL solution.

Sample solution: to exactly transfer about 60 mg of the sample to a 50 mL volumetric flask and dissolve in 20 mL of *Mobile phase*. To top up with the same solvent and make it homogeneous. To transfer 4 mL of the solution to 50 mL volumetric flask, top up with *Mobile phase*, and make it homogeneous. To transfer 10 mL of resulting solution and 7 mL of *Internal standard solution* to 25 mL volumetric flask, top up with *Mobile phase*, and make it homogeneous.

Standard solution: to accurately transfer about 60 mg of phytomenadione SQR to a 50 mL volumetric flask and dissolve in 20 mL of *Mobile phase*. To top up with the same solvent and make it homogeneous. To transfer 4 mL of the solution to 50 mL volumetric flask, top up with *Mobile phase*, and make it homogeneous. To transfer 10 mL of resulting solution and 7 mL of *Internal standard solution* to 25 mL volumetric flask, top up with *Mobile phase*, and make it homogeneous.

To inject replicates of 50 μ L of the *Standard solution*. The relative retention times are about 0.7 to cholesteryl benzoate, 0.9 to (Z)-phytomenadione, and 1.0 to (E) - phytomenadione. The resolution between (Z)-phytomenadione and (E) - phytomenadione is less than 1.5. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2.0 %.

Procedure: to separately inject 50 μ L of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_{31}H_{46}O_2$ in the sample from the responses obtained for the ratio (area of (Z)-phytomenadione + area of (E)-phytomenadione)/area of cholesterol benzoate with *Standard solution* and *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

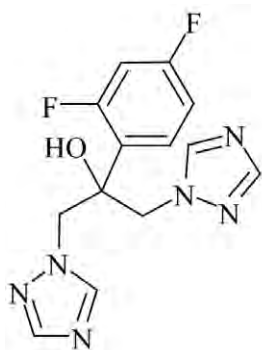
To comply with the legislation in force.

THERAPEUTIC CLASS

Anti-hemorrhagic.

FLUCONAZOLE

Fluconazolum



$C_{13}H_{12}F_2N_6O$; 306.27

fluconazole; 04109

α -(2,4-difluorophenyl)- α -(1H-1,2,4-triazol-1-ylmethyl)-
1H-1,2,4-triazole-1-ethanol
[86386-73-4]

It contains at least 98.0% and at most 102.0% of $C_{13}H_{12}F_2N_6O$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or almost white, odorless powder.

Solubility. Sparingly soluble in water; freely soluble in methanol; soluble in ethanol and acetone; slightly soluble in isopropyl alcohol and chloroform; sparingly soluble in toluene. Soluble in diluted mineral acid solutions and alkalis.

Physical and chemical constants.

Melting range (5.2.2): 138 °C to 140 °C.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the fluconazole SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14), from 200 nm to 400 nm, 0.02% solution (w/v) of the sample, in 0.1 M sodium hydroxide, has absorption maximum at 261 nm, similar to those observed in the spectrum of a similar solution of fluconazole SQR.

C. To dissolve 50 mg of sample in 1 mL of acetone. To add by stirring 5-8 drops of 5% chromic acid (w/v). A precipitate is produced within 5 seconds.

D. To add 3 mL of 1% ferric chloride (w/v), in glacial acetic acid, to 50 mg of sample, and stir. To carefully add M sulfuric acid on walls of the tube. Color must be orange and yellow.

PURITY TESTS

Appearance of solution. The 5% solution (w/v) in methanol is clear (5.2.25) and colorless (5.2.12).

Iron (5.3.2.4). To dissolve 0.5 g of the sample in 5 mL of ethanol. To add 5 mL of water, make it homogeneous, and proceed as described in *Limit test for iron, Method III*. At most 0.002% (20 ppm).

Heavy metals (5.3.2.3). To use the residue obtained in the test *Sulfated ashes* and proceed as described in *Method III*, from "to add 4 mL of 6 M hydrochloric acid..." To use 2.5 mL of *Lead standard solution (10 ppm Pb)* to *Standard preparation*. At most 0.0025% (25 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 105 °C for 3 hours. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. To accurately dissolve about 0.1 g of the sample and dissolve in 50 mL of glacial acetic acid. To titrate with 0.1 M SV perchloric acid. To potentiometrically determine the endpoint or use methylrosanilinium chloride SI as an indicator. To perform sample blank correction and adjust it as required. Each mL of 0.1 M SV perchloric acid is equal to 15.314 mg of $C_{13}H_{12}F_2N_6O$.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antifungal.

FLUCONAZOLE, CAPSULES

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_{13}H_{12}F_2N_6O$.

IDENTIFICATION

A. To stir amount of powder equivalent to 10 mg of fluconazole with 20 mL of methanol. To filter and evaporate the filtrate to dryness. The residue responds to test **A**, *Identification*, in the monograph *Fluconazole*.

B. To transfer amount of powder equivalent to 0.1 g of fluconazole to a 100 mL volumetric flask. To add 70 mL of 0.1 M hydrochloric acid, leave it in ultrasound for 10 minutes, top up with the same solvent, make it homogeneous, and filter it. To dilute in 0.1 M hydrochloric acid to the 0.02% concentration (w/v). The ultraviolet absorption spectrum (5.2.14), from 200 nm to 400 nm, 0.02% solution (w/v) in 0.1 M hydrochloric acid, has absorption maximum at 261 nm, similar to those observed in the spectrum of a similar solution of fluconazole SQR.

C. To dissolve amount of powder equivalent to 0.25 g of the sample in 5 mL of acetone and filter. To add by stirring 5-8 drops of 5% chromic acid (w/v). A precipitate is produced within 5 seconds.

D. To add 3 mL of 1% ferric chloride (w/v), in glacial acetic acid, to an amount of powder equivalent to 50 mg of fluconazole and stir. To carefully add M sulfuric acid on walls of the tube. Color must be orange and yellow.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test. To proceed as described in *Dosage* method **A**.

DISSOLUTION TEST

Dissolution medium: 0.1 M hydrochloric acid, 900 mL

Equipment: baskets, 100 rpm

Time: 30 minutes

Procedure: after testing, remove aliquot from the dissolution medium, filter, and measure the absorbances of the solutions at 261 nm (5.2.14) by using 0.1 M hydrochloric acid to adjust zero. To calculate the amount of $C_{13}H_{12}F_2N_6O$ dissolved in the medium, by comparing the readings obtained with that of 0.02 % fluconazole SQR solution (w/v) prepared in the same solvent.

Tolerance: not less than 80% (Q) of the amount stated of $C_{13}H_{12}F_2N_6O$ dissolves within 30 minutes.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. To weigh 20 mg capsules, remove the content, and weigh them again. To make the content of the capsules homogeneous. To transfer amount of powder equivalent to 0.1 g of fluconazole to a 100 mL volumetric flask. To add 70 mL of 0.1 M hydrochloric acid, leave it in ultrasound for 10 minutes, top up with the same solvent, make it homogeneous, and filter it. To dilute in 0.1 M hydrochloric acid to the 0.02% concentration (w/v). To prepare a standard solution at the same concentration by using the same solvent. To measure the absorbances of the resulting solutions at 261 nm, by using 0.1 M hydrochloric acid to adjust zero. To calculate the amount of $C_{13}H_{12}F_2N_6O$ in the capsules, from the readings obtained.

B. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 260 nm; 150 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at 30 °C; flow of *Mobile phase* of 0.8 mL/min.

Mobile phase: mixture of water and acetonitrile (78:22).

Sample solution: To weigh the capsules, remove the content, and weigh them again. To make the content of the capsules homogeneous. To transfer amount of powder equivalent to 50 mg of fluconazole to a 100 mL volumetric flask. To add 70 mL of *Mobile phase* and put it in ultrasound for 10 minutes. To top up with the same solvent, make it homogeneous, and filter it.

Standard solution: to dissolve accurately weighed amount of fluconazole SQR in *Mobile phase* to obtain a 0.5 mg/mL solution.

To inject replicates of 20 µL of the *Standard solution*. The relative standard deviation for the replicate areas for the peaks recorded is not greater than 2.0%.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of $C_{13}H_{12}F_2N_6O$ in the capsules from the responses obtained from *Sample and standard solutions*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.



FLUNITRAZEPAM TABLETS

It contains at least 92.5 % and at most 107.5 % of the amount stated of $C_{16}H_{12}FN_3O_3$.

IDENTIFICATION

A. The ultraviolet absorption spectrum (5.2.14) ranging from 200 nm to 400 nm, of sample solution obtained in *Dosage*, exhibits maxima and minima similar to those observed in the standard solution spectrum.

B. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF_{254} silica gel as support and a mixture of nitromethane, ethyl ether, n-heptane, and 25% ammonia (v/v) (30:60:15:2.5) as mobile phase. To separately apply to the plate 10 μ L of each of the solutions freshly prepared, as described below.

Solution (1): to weigh and pulverize the tablets. To use amount of powder equivalent to 20 mg of flunitrazepam, add 20 mL of methanol, stir, and filter it.

Solution (2): 1 mg/mL flunitrazepam SQR solution in methanol.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). To nebulize with a 10% sodium hydroxide solution (w/v). The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

Procedure for content uniformity. To individually weigh and transfer each tablet to a centrifuge tube, add 5 mL of water, and put it in ultrasound to disintegrate the tablet. To add 25 mL of methanol, put it in ultrasound for 3 minutes, and stir for 10 minutes. To centrifuge for 10 minutes and filter the supernatant through a 0.45 μ m membrane. To successively dilute in methanol to the 0.0016% concentration (w/v). To proceed as described in *Dosage*, from "To prepare a standard solution..." To calculate the amount of $C_{16}H_{12}FN_3O_3$ in each tablet, from the readings obtained.

DISSOLUTION TEST (5.1.5)

Dissolution medium: simulated gastric fluid, 900 mL

Equipment: blades, 50 rpm

Time: 45 minutes

To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 279 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octylsilane group (5 μ m), at room temperature; flow of *Mobile phase* of 1.5 mL/min.

Mobile phase: to transfer 670 mL of 0.05 M monobasic potassium phosphate to 1000 mL volumetric flask and top up to volume with acetonitrile. To adjust the pH to 6.2 with 0.25 M potassium hydroxide solution.

Sample solution: after testing, remove aliquot from the dissolution medium, filter through 0.45 μ m membrane, cool down, and dilute with *Dissolution medium*, to obtain 1.1 μ g/mL concentration.

Standard solution: to accurately weigh about 22 mg of flunitrazepam SQR, transfer to a 250 mL volumetric flask, add 100 mL of methanol, and put it in ultrasound to total dissolution. To top up with the same solvent and make it homogeneous. To successively dilute in *Dissolution medium* in order to obtain 1.1 μ g/mL solution.

Procedure: to separately inject 100 μ L of *Sample and standard solutions*, record the chromatograms, and measure the area under the peaks. To calculate the amount of $C_{16}H_{12}FN_3O_3$ dissolved in the medium from the responses obtained from *Sample and standard solutions*.

Tolerance: not less than 85% (Q) of the amount stated of $C_{16}H_{12}FN_3O_3$ dissolves within 45 minutes.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

to weigh and pulverize 20 tablets. To accurately transfer amount of powder equivalent to 16 mg of flunitrazepam to a 100 mL volumetric flask. To add 10 mL of water and stir to total dissolution. To top up to volume with methanol, stir in a magnetic stirrer for 20 minutes, and filter through 0.45 μ m membrane. To successively dilute in methanol to the 0.0016% concentration (w/v). To prepare a standard solution at the same concentration by using the same solvent. To measure the absorbances of the resulting solutions at 309 nm (5.2.14), by using methanol to adjust zero. To calculate the amount of $C_{16}H_{12}FN_3O_3$ in the tablets, from the readings obtained.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

FLUNITRAZEPAM INJECTABLE SOLUTION

It contains at least 95.0 % and at most 105.0 % of the amount stated of $C_{16}H_{12}FN_3O_3$. Sterile water solution for injection or another suitable solvent.

IDENTIFICATION

A. The ultraviolet absorption spectrum (5.2.14) ranging from 200 nm to 400 nm, of sample solution obtained in *Dosage*, exhibits maxima and minima similar to those observed in the standard solution spectrum.

B. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ 60 silica gel as support and a mixture of chloroform, methanol, and 25% ammonium hydroxide (90:10:1) as mobile phase. To separately apply to the plate 10 µL of each of the solutions freshly prepared, as described below. To protect it from direct light.

Solution (1): to dilute volume of the injectable solution in ethanol, to obtain about 0.5 mg/mL solution.

Solution (2): 0.5 mg/mL flunitrazepam SQR solution in ethanol.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). To nebulize with potassium iodide and bismuth subnitrate SR. The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*. Due to excipients, other spots may appear.

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

pH (5.2.19). 3.9 to 4.7.

BIOLOGICAL SAFETY TEST

Sterility test (5.5.3..2.1). It complies with the test.

Pyrogens (5.5.2.1). It complies with the test. To inject 0.5 mL/ kg by using 0.2 mg/mL flunitrazepam in saline.

DOSAGE

To transfer volume of injectable solution equivalent to 0.2 g of flunitrazepam to a 200 mL volumetric flask, dissolve, and top up to volume with methanol.

To successively dilute in methanol to the 0.0015% concentration (w/v). To prepare a standard solution at the same

concentration by using the same solvent. To measure the absorbances of the resulting solutions at 309 nm (5.2.14), by using methanol to adjust zero. To calculate the content of $C_{16}H_{12}FN_3O_3$ in the sample from the readings obtained.

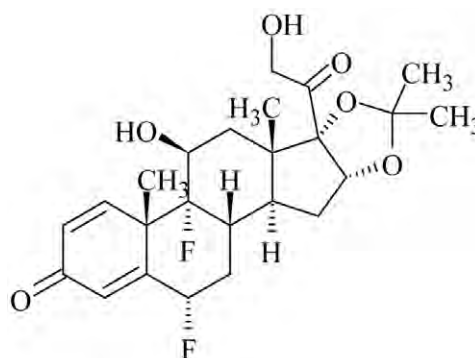
PACKAGING AND STORAGE

In type I glass containers, protected from light.

LABELLING

To comply with the legislation in force.

FLUOCINOLONE ACETONIDE Fluocinoloni acetonidum



C₂₄H₃₀F₂O₆; 452.49

fluocinolone acetonide; 04159 (6α,11β,16α)-6,9-difluoro-11, 21 - dihydroxy-16, 17 - [(1-methylethylidene) bis (oxy)]-pregna-1,4-diene-3,20-dione [67-73-2]

It contains at least 98.0% and at most 102.0% of $C_{24}H_{30}F_2O_6$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white or almost white powder. It shows polymorphism.

Solubility. Practically insoluble in water, soluble in acetone, ethanol, and methanol; slightly soluble in chloroform.

Physical and chemical constants.

Specific rotation (5.2.8): +98° to +108°, in relation to the desiccated substance. To determine in 1% solution (w/v) in methanol.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same

relative intensities to those observed in the fluocinolone acetonide SQR spectrum similarly prepared. Should variations be observed, separately dissolve the sample and standard in ethanol, evaporate the solvent, and trace new spectra with residues.

B. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF₂₅₄ silica gel as support and a mixture of nitromethane, methylene chloride, and methanol (50:50:1) as mobile phase. To separately apply to the plate 5 µL of each of the solutions freshly prepared, as described below.

Solution (1): 5 mg/mL solution of the sample in acetone.

Solution (2): 5 mg/mL fluocinolone acetonide SQR solution in acetone.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

PURITY TESTS

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 105 °C for 3 hours. At most 1.0% to anhydrous form and at most 8.5% to hydrated form.

DOSAGE

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 254 nm; 100 mm chromatograph column and 4.5 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at room temperature; flow of *Mobile phase* of 1 mL/min.

Mobile phase: mixture of water, acetonitrile, and tetrahydrofuran (77:13:10).

Sample solution: to transfer accurately weighed 20 mg of sample to a 100 mL volumetric flask, dissolve in 23 mL of mixture of acetonitrile and tetrahydrofuran (13:10), top up to volume with water, and make it homogeneous.

Standard solution: to transfer accurately weighed 20 mg of fluocinolone acetonide SQR to a 100 mL volumetric flask, dissolve in 23 mL of mixture of acetonitrile and tetrahydrofuran (13:10), top up to volume with water, and make it homogeneous.

To inject replicates of 20 µL of the *Standard solution*. The column efficiency is greater than 3000 theoretical plates. The relative standard deviation for the replicate areas for the peaks recorded is not greater than 3.0%. The flow of *Mobile phase* can be adjusted so that the retention time of the fluocinolone acetonide peak is between 9 and 13 minutes.

Procedure: to separately inject 20 µL of Sample and standard solutions, record the chromatograms, and measure the area under the peaks.

To calculate the content of C₂₄H₃₀F₂O₆ from the sample, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

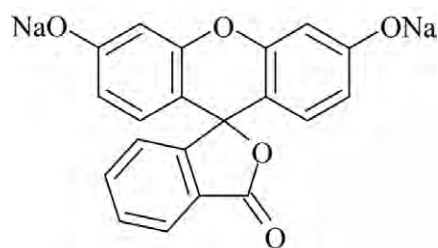
LABELLING

To comply with the legislation in force. Label must indicate whether the fluocinolone acetonide form is anhydrous or hydrated.

THERAPEUTIC CLASS

Anti-inflammatory, steroid.

FLUORESCEIN SODIUM Fluoresceinum natrium



C₂₀H₁₀Na₂O₅; 376.27

fluorescein sodium; 04167

Sodium salt of 3', 6'-dihydro-spiro [isobenzofuran-1 (3H), 9'-[9H] xanthen]-3-one (2:1) [518-47-8]

It contains at least 90.0% and at most 102.0% of C₂₀H₁₀Na₂O₅, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. Fine, orange-red, odorless, hygroscopic powder.

Solubility. Freely soluble in water; slightly soluble in ethanol; practically insoluble in hexane and methylene chloride.

IDENTIFICATION

A. The 0.05% aqueous solution (w/v) shows yellowish-green fluorescence. The fluorescence fades out by adding 0.1 mL of 2 M hydrochloric acid and fades in by adding 0.2 mL of 2 M sodium hydroxide.

B. To add a drop of 0.05% solution (w/v) in filter paper. Yellow spot is produced. When exposed to bromine vapor for 1 minute, followed by ammonia vapors, it turns intense pink color.

C. To calcine 0.1 g of sample in a porcelain crucible. To dissolve the residue in 5 mL of water and filter it. Solution responds to the reactions of sodium ion (5.3.1.1).

PURITY TESTS

pH (5.2.19). 7.0 to 9.0. To determine in 2% aqueous solution (w/v).

Acriflavine. To dissolve 10 mg of sample in 5 mL of water and add a few drops of 10% aqueous sodium salicylate solution (w/v). No precipitate is produced.

Zinc. To dissolve 0.1 g of the sample in 10 mL of a saturated sodium chloride solution. To add 2 mL of 3 M hydrochloric acid, vigorously stir, and filter it. To add 1 mL of potassium ferrocyanide SR. No turbidity is produced.

Water (5.2.20.1). At most 17.0%.

DOSAGE

To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.15). To accurately weigh about 0.1 g of the sample and dissolve in water. To quantitatively dilute with the same solvent to obtain 1 µg/mL solution. To transfer 3 mL to a 100 mL volumetric flask, add 20 mL of borate buffer pH 9.0, and top up with water, to obtain 0.03 µg/mL solution. For the preparation of the standard solution, dissolve accurately weighed amount of diacetyl fluorescein SQR in 10 mL of ethanol in a 100 mL volumetric flask. To add 2 mL of 2.5 M sodium hydroxide and heat it in a boiling water bath for 20 minutes, by stirring it. To cool down and top up with water. To quantitatively dilute in water to obtain 1 µg/mL sodium fluorescein solution. To transfer 3 mL to a 100 mL volumetric flask, add 20 mL of borate buffer pH 9.0, and top up with water, to obtain 0.03 µg/mL standard solution. To measure the fluorescence intensities from resulting solutions by using the fluorimeter with excitation and emission wavelength at 485 nm and 515 nm, respectively. To calculate the content of $C_{20}H_{10}Na_2O_5$ in the sample from the readings obtained. Each mg of diacetyl fluorescein is equivalent to 0.9037 mg of sodium fluorescein ($C_{20}H_{10}Na_2O_5$).

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Diagnostic agent.

FLUORIDE, SODIUM

Natrii fluoridum

NaF; 41.99

sodium fluoride; 04170

Sodium fluoride

[7681-49-4]

It contains at least 98.0% and at most 102.0% of NaF, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White, odorless powder.

Solubility. Soluble in water, practically insoluble in ethanol.

IDENTIFICATION

A. To transfer 1 g of the sample to a platinum crucible in a hood and add 15 mL of sulfuric acid. To cover with a clear and polished glass and heat it in a water bath for 1 hour. To remove the glass cover, rinse with water, and dry. The glass surface is marked.

B. The 4% solution (w/v) responds to the reactions of sodium ion (5.3.1.1).

PURITY TESTS

Acidity or alkalinity. To dissolve 2 g of sample in 40 mL of water in a platinum capsules, add 10 mL of saturated potassium nitrate solution, cool down the solution at 0°C, and add 3 drops of phenolphthalein SI. If solution becomes pink, no more than 0.5 mL of 0.05 M sulfuric acid is required to indicator changes color. If solution remains colorless, no more than 2 mL of 0.1 M sodium hydroxide are required for persistent pink color for 15 seconds.

Fluorosilicate. To heat to boiling the neutralized solution obtained in *Acidity or alkalinity* and titrate, still hot, with 0.1 M SV sodium hydroxide to obtain permanent pink color. At most 1.5 mL of 0.1 M SV sodium hydroxide is required.

Chlorides (5.3.2.1). To dissolve 0.3 g of the sample in 20 mL of water and add 0.2 g of boric acid, 1 mL of nitric acid SR, and 1 mL of 0.1 M silver nitrate. Any resulting turbidity is not more intense than that of a standard prepared with 1 mL of 0.001 M hydrochloric acid by using the same reagents. At most 0.012% (120 ppm).

Heavy metals (5.3.2.3). To transfer 1 g of the sample to a platinum capsule or crucible, add 1 mL of water and 3 mL of sulfuric acid. To heat in the hood at a temperature as low as possible, until all sulfuric acid has been expelled.

To dissolve the residue in 20 mL of water and neutralize with ammonium hydroxide by using phenolphthalein SI. To add 1 mL of glacial acetic acid, dilute with water to 45



mL, and filter. To proceed as described in *Method I*, by using 30 mL of the filtrate. At most 0.003% (30 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample in oven at 150 °C for 4 hours. At most 1.0%.

DOSAGE

To proceed as described in *Titration in non-aqueous medium (5.3.3.5)*. To accurately dissolve about 80 mg of the sample, add 5 mL of acetic anhydride, 20 mL of glacial acetic acid, and gently warm it to obtain total dissolution. To cool down and add 20 mL of dioxane. To add 3 drops of methylrosanilinium chloride SI and titrate with 0.1 M SV perchloric acid to obtain emerald green color. To perform sample blank correction and adjust it as required. Each mL of 0.1 M SV perchloric acid is equal to 4.199 mg of NaF.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Dental prophylaxis.

FLUORIDE, SODIUM ORAL SOLUTION

It contains at least 90.0 % and at most 110.0 % of the amount stated of NaF.

IDENTIFICATION

A. To transfer 0.1 mL of oral solution to test tube, add 0.1 mL of mixture of alizarin SI and 0.1% zirconyl nitrate (w/v) in 7 M hydrochloric acid (1:1). Yellow color develops.

B. If necessary, reduce the volume of oral solution by heating in a water bath until to obtain about 10 mg of sodium per milliliter. Resulting solution responds to the reactions of sodium ion (5.3.1.1).

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

pH (5.2.19). 5.5 to 7.0.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To potentiometrically determine by using fluoride ion selective electrode.

Fluoride buffer: to add 57 mL of glacial acetic acid, 58 g of sodium chloride, and 4 g of 1,2-cyclohexylene-dinitrile-tetraacetic (CDTA) to 500 mL of water. In a cold water bath, slowly add, by stirring, a concentrated sodium hydroxide SR solution pH between 5.3 - 5.5. To transfer to a 1000 mL volumetric flask, top up with water, and make it homogeneous.

Sample solution: to dilute oral solution in water by a factor of 100 times.

Standard solution: to prepare a reference stock solution of 100 mg/L fluoride in water by using analytical grade sodium fluoride. To construct the analytical curve with reference fluoride solutions at the following concentrations: 0.25 mg/L, 0.5 mg/L, 1.0 mg/L, 2.5 mg/L, and 5.0 mg/L.

Procedure: to add 10 mL of *Fluoride buffer* to each 10 mL of *Sample solution* or *Standard solution*. To measure the potency of solutions under magnetic stirring. To construct a graph and list the concentration of fluoride of the patterns on the ordinate (on a logarithmic scale $\log_{10}$) with measurements of the potency differences on the abscissa (on normal scale). To determine the fluoride concentration in mg/L. Each mg of fluoride is equivalent to 2.21 mg of NaF.



PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

FLUORIDE, STANNOUS

Stannosi fluoridum

SnF₂; 156.71
stannous fluoride; 09827
Stannous fluoride
[7783-47-3]

It contains at least 71.2% tin ion (Sn²⁺), at least 22.3%, and at most 25.5% of fluoride, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White powder.

Solubility. Freely soluble in water.

IDENTIFICATION

A. To dissolve 0.25 g of sample in 25 mL of water. In a test tube, add 2 mL of calcium chloride SR on 5 mL of sample solution. There is a white precipitate of calcium fluoride.

B. To use the same sample solution of test A., *Identification*. To add 2 drops of 0.1 M silver nitrate on 2 drops of sample solution. No black precipitate is produced.

C. To add 2 drops of mercury chloride SR on a drop of sample solution. White precipitate is produced. By adding an excess of sample solution, a black precipitate is formed.

PURITY TESTS

pH (5.2.19). 2.8 to 3.5. To determine in 0.4 % solution (w/v) freshly prepared.

Substances insoluble in water. To transfer 5 g of sample to a beaker, add 100 mL of water, and stir the solution for 3 minutes or until total dissolution occurs. To filter in crucible with mass already known and previously tared, wash with 1% ammonium fluoride (w/v) and water. To dry the residue for 4 hours at 105 °C, cool down, and weigh it. The weight of the residue does not exceed 0.2%.

Antimony.

Sample solution: to transfer 1 g of stannous fluoride to a 50 mL volumetric flask, dissolve in 6 M hydrochloric acid, and top up to 50 mL with the same solvent.

Standard solution: to transfer 55 mg of potassium antimony tartrate to a 200 mL volumetric flask, dissolve in water, and top up to 200 mL with the same solvent. To transfer 5 mL of this solution to a 500 mL volumetric flask, dissolve in 6 M hydrochloric acid, and top up with the same solvent.

Rhodamine B solution: to dissolve 20 mg of rhodamine B in 200 mL of 0.5 M hydrochloric acid.

To pipette 5 mL of *Sample solution* and *Standard solution* to different separatory funnels, add 15 mL of hydrochloric acid, 1 g of ceric sulfate, and let it rest for 5 minutes, by occasionally stirring. To add 500 mg of hydroxylamine hydrochloride and stir for 1 minute. To transfer 15 mL of isopropyl ether to the solution, stir for 30 seconds, add 7 mL of water and stir again. To cool down in water bath at room temperature for 10 minutes, stir for 30 seconds, let it rest to phase separation occurs and discard the aqueous phase.

To add 20 mL of *Rhodamine B solution*, stir for 30 seconds, and discard the aqueous phase. To decant the ether phase and centrifuge, if necessary, to obtain a clear solution. To determine the absorbance of the solutions obtained from the *Sample solution* and *Standard solution* at 550 nm maximum wavelength. The absorbance of the *Sample solution* does not exceed the absorbance of the *Standard solution* (0.005%). To perform sample blank correction.

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 105 °C for 4 hours. At most 0.5%.

DOSAGE

Stannous ion

0.1M Potassium iodide-iodate solution: to dissolve 3.567 g of potassium iodate, previously desiccated at 110 °C to constant weight, in 200 mL of water containing 1 g of sodium hydroxide and 10 g of potassium iodide, into 1000 mL volumetric flask. To top up the volume to 1000 mL with water. To titrate this solution to standardize by using metallic tin, analytical grade (99.5% purity), and dissolve in hydrochloric acid. Each mL of 0.1 M potassium iodide-iodate is equal to 5.936 mg of Sn.

To accurately weigh about 250 mg of stannous fluoride and dissolve in 300 mL of 3 M hydrochloric acid (freshly boiled). To turn the bottle containing the sample while passing flow of inert gas (oxygen-free) through surface of the liquid. To cool down at room temperature. To add 5 mL of potassium iodide SR and 3 mL of starch SI solution, then titrate with 0.1 M potassium iodide-iodate in inert atmosphere. Each mL of 0.1 M potassium iodide-iodate is equal to 5.936 mg of Sn²⁺.



Fluoride ion

Buffer solution: to dissolve 57 mL of glacial acetic acid, 58 g of sodium chloride, and 4 g of 1,2-cyclohexylene-dinitrile-tetraacetic into 500 mL of water. To adjust the pH to 5.25 ± 0.25 with sodium hydroxide and top up to 1000 mL with water.

Sample solution: to transfer 100 mg of stannous fluoride to a 250 mL volumetric flask. To add 50 mL of water, vigorously stir for 5 minutes, and top up to 250 mL with water. To transfer 10 mL of this solution to 50 mL volumetric flask and top up with water.

Standard solution: to dissolve an exact amount of sodium fluoride SQR in water to obtain 0.42 mg/mL solution. Each mL of this solution (*Standard solution A*) contains 0.19 mg of fluoride ion ($10^{-2} M$). To transfer 25 mL of this solution to 250 mL volumetric flask and top up with water. This solution, *Standard solution B*, contains 19 µg of fluoride ion per mL ($10^{-2} M$). To transfer 25 mL of this solution to 250 mL volumetric flask and top up with water. This solution, *Standard solution C*, contains 1.9 µg of fluoride ion per mL ($10^{-4} M$).

To pipette 20 mL of each *Standard solution A, B and C*, transfer to different plastic beakers and add 20 mL of *buffer solution* into each one. To determine the potency of each *Standard solution* and *Sample solution*, in mV, by using fluoride ion selective electrode. During the measurements, immerse the electrode into the solution under magnetic stirring and wait to balance to be reached, to record the value of potency. To wash the electrode between measurements with water and carefully dry to avoid damage to the solid membrane of the electrode. To construct an analytical curve by plotting a graph Logarithm of the fluoride ion concentration of (µg/mL) versus potency (mV). To calculate the fluoride ion concentration in the sample solution by intercepting the potency value recorded in the analytical curve. The percentage of fluoride ion is calculated by the equation: $125C/W$, where C is the fluoride ion concentration in the sample, in µg/mL, and W is the sample weight in mg, used.

PACKAGING AND STORAGE

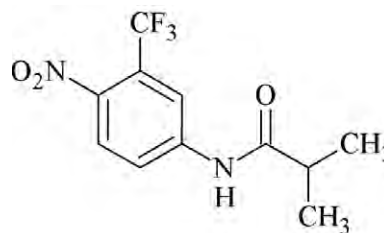
In tightly closed containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Dental prophylaxis.

FLUTAMIDE**Flutamidum**

$C_{11}H_{11}F_3N_2O_3$; 276.21

flutamide; 04220

2-Methyl-N [4-nitro-3-(trifluoromethyl) phenyl]

propanamide

[13311-84-7]

It contains at least 98.0% and at most 101.0% of $C_{11}H_{11}F_3N_2O_3$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Yellow, crystalline powder.

Solubility. Practically insoluble in water; freely soluble in acetone and ethanol.

Physical and chemical constants. *Melting range (5.2.2):* 173 °C to 179 °C.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in mineral oil, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the flutamide SQR spectrum similarly prepared.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

PURITY TESTS

Heavy metals (5.3.2.3). To use *Method III*. At most 0.001% (10 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 60 °C for 3 hours. At most 1.0%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1 %.

DOSAGE

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 240 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecyl-

silane group (5 μm), kept at room temperature; flow of *Mobile phase* of 1.0 mL/min.

Mobile phase: mixture of water and acetonitrile (50:50).

Sample solution: to dissolve accurately weighed amount of the sample in *Mobile phase* to obtain a 0.5 mg/mL solution.

Standard solution: to dissolve accurately weighed amount of flutamide SQR in *Mobile phase* to obtain a 0.5 mg/mL solution.

Resolution solution: to prepare a solution in *Mobile phase* containing approximately 0.5 mg of *o*-flutamide SQR per milliliter. To transfer 1 mL of this solution and 1 mL of *Standard solution* to 50 mL volumetric flask and top up with *Mobile phase*, thus obtaining 0.01 mg/mL *o*-flutamide and flutamide solution.

To inject replicates of 20 μL of the *Resolution solution*. The relative retention times are about 1.0 to flutamide and 1.4 to *o*-flutamide. The tailing factor should not be greater than 2.0. The resolution between flutamide and *o*-flutamide should not be less than 6.0. The relative standard deviation for replicate areas of the peaks recorded is not greater than 1.5 %.

Procedure: to separately inject 20 μL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $\text{C}_{11}\text{H}_{11}\text{F}_3\text{N}_2\text{O}_3$ from the sample, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In containers protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antiandrogen.

FLUTAMIDE TABLETS

It contains at least 95.0 % and at most 105.0 % of the amount stated of $\text{C}_{11}\text{H}_{11}\text{F}_3\text{N}_2\text{O}_3$.

IDENTIFICATION

A. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using G silica gel as support and a mixture of chloroform and ethyl acetate (3:1), as mobile phase. To separately apply to the plate 20 μL of each of the solutions freshly prepared, as described below.

Solution (1): to weigh and pulverize the tablets. To transfer amount of powder equal to 15 mg of flutamide to a 10 mL

volumetric flask and top up with mixture of chloroform and methanol (5:1).

Solution (2): to dissolve about 15 mg of flutamide SQR in 10 mL of a mixture of chloroform and methanol (5:1).

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). The flutamide spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 3% sodium lauryl sulfate aqueous solution (w/v), 900 mL.

Equipment: blades, 75 rpm

Time: 45 minutes

Procedure: after testing, remove aliquot from the dissolution medium, filter, and dilute, if necessary, in 3% sodium lauryl sulfate aqueous solution (w/v), to obtain suitable concentration. To measure the absorbances at 306 nm (5.2.14), by using the same solvent to adjust zero. To calculate the amount of $\text{C}_{11}\text{H}_{11}\text{F}_3\text{N}_2\text{O}_3$ dissolved in the medium, by comparing the readings obtained with that of 0.0025% flutamide SQR solution (w/v) prepared in the same solvent.

Tolerance: not less than 75% (Q) of the amount stated of $\text{C}_{11}\text{H}_{11}\text{F}_3\text{N}_2\text{O}_3$ dissolves within 45 minutes.

DOSAGE

To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 254 nm; 300 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μm), kept at room temperature; flow of *Mobile phase* of 1.0 mL/min.



Mobile phase: mixture of 0.05 M monobasic potassium phosphate and methanol (30:70).

Sample solution: to weigh and pulverize 20 tablets. To transfer amount of powder equivalent to 125 mg of flutamide to a 100 mL volumetric flask and add 60 mL of a mixture of methanol and water (95:5). To mechanically stir for 30 minutes, top up with methanol, make it homogeneous, and filter it. To transfer 10 mL of the filtrate to a 50 mL volumetric flask and dilute in methanol, thus obtaining 0.25 mg/mL flutamide solution.

Standard solution: to dissolve accurately weighed amount of flutamide SQR in methanol to obtain a 0.25 mg/mL solution.

The relative standard deviation for the replicate areas for the peaks recorded is not greater than 2.0%.

Procedure: to separately inject 10 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the area under the peaks. To calculate the amount of $C_{11}H_{11}F_3N_2O_3$ in the tablets, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

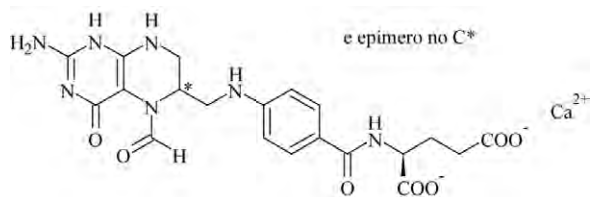
In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

FOLINATE, CALCIUM

Calcii folinas



$C_{20}H_{21}CaN_7O_7$; 511.50
calcium folinate; 00197

Calcium Salt of *N*-[4 - [[(2-amino-5-formyl-1,4,5,6,7,8-hexahydro-4-oxo-6-pteridiny) methyl] amino] benzoyl] - L-glutamic acid (1:1)
[1492-18-8]

It contains at least 95.0% and at most 105.0% of $C_{20}H_{21}CaN_7O_7$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. Amorphous or crystalline, white or yellowish, odorless powder.

Solubility. Soluble in water; practically insoluble in ethanol and acetone.

Physical and chemical constants

Specific rotation (5.2.8): +14.4° to +18.0°, in relation to the anhydrous substance. To determine in 2.5% solution (w/v) in carbon dioxide-free water.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the calcium folinate SQR spectrum similarly prepared.

B. It responds to the reaction 2 of the calcium ion (5.3.1.1).
PURITY TESTS

Appearance of solution. The 2.5% aqueous solution (w/v) is clear (5.2.25) and yellow. To measure the absorbance of the solution at 420 nm, by using water to adjust zero. The absorbance should be less than 0.60.

pH (5.2.19). 6.8 to 8.0. To determine in 2.5% aqueous solution (w/v).

Heavy metals (5.3.2.3). To use *Method III*. At most 0.005% (50 ppm).

Water (5.2.20.3). To determine in 0.1 g of the sample. At most 17%.

DOSAGE

To protect it from direct light. To perform dosage without prolonged interruptions.

To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 254 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), at room temperature; flow of *Mobile phase* of 1.5 mL/min.

Mobile phase: to mix 835 mL of water, 125 mL of acetonitrile, and 15 mL of 25% tetrabutylammonium hydroxide solution (w/v) in methanol. To adjust pH to 7.5 ± 0.1 with 2 M monobasic sodium phosphate and top up to 1000 mL with water.

Diluent: to mix 900 mL of water and 15 mL of 25% tetrabutylammonium hydroxide solution (w/v) in methanol. To adjust pH to 7.5 ± 0.1 with 2 M monobasic sodium phosphate and top up to 1000 mL with water.

Sample solution: to dissolve accurately weighed amount of the sample with *Diluent* to obtain a 0.2 mg/mL solution.

Standard solution: to dissolve accurately weighed amount of calcium folinate SQR with *Diluent* to obtain a 0.2 mg/mL solution.

Resolution solution: to transfer 17.5 mg of folic acid to 100 mL volumetric flask, dissolve in *Diluent*, and top up with the same solvent. To transfer 5 mL of this solution to 25 mL volumetric flask and top up with *Standard solution*. To make it homogeneous.

To inject replicates of 15 µL of the *Resolution solution*. The relative retention times are about 1.0 to calcium folinate and 1.6 to folic acid. The resolution between calcium folinate and folic acid should not be less than 3.6. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2.0%.

Procedure: to separately inject 15 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_{20}H_{21}CaN_7O_7$ from the sample, from the responses obtained for the *Sample and Standard solutions*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antidote for folic acid antagonists.

PHOSPHATE, ALUMINUM Aluminii phophas

AlPO₄; 121.95
AlPO₄ · 1/3H₂O; 127.96
aluminum phosphate; 00200
Aluminum salt of phosphoric acid (1:1)
[7784-30-7]
Aluminum salt of phosphoric acid hydrate (3:3:1)
[1117729-44-8]

It contains at least 94.0% and at most 102.0% of AlPO₄, in relation to the incinerated substance.

DESCRIPTION

Physical characteristics. White or almost white powder containing some friable aggregates.

Solubility. Very slightly soluble in water; practically insoluble in ethanol. Soluble in alkalis and diluted acid solutions.

IDENTIFICATION

A. The solution obtained in *Appearance of solution* responds to the reactions of aluminum ion (5.3.1.1).

B. The solution obtained in *Appearance of solution* responds to the reactions of phosphate ion (5.3.1.1).

PURITY TESTS

Appearance of solution. To transfer 2 g of the sample to a 100 mL volumetric flask, dissolve in 50 mL of hydrochloric acid SR, and top up with the same solvent. The solution is clear (5.2.25) and colorless (5.2.12).

pH (5.2.19). To weigh 2.0 g of the sample and add 50 mL water, by vigorously stirring for 5 minutes. The pH of the solution is between 5.5 and 7.2.

Neutralizing capacity. To pass sufficient amount of sample over a 75 µm tammy. A 0.5 g of the sieved sample, To add 30 mL of *M* hydrochloric acid, previously heated at 37 °C to 0.5 g of the sample sieved. To keep the mixture at 37 °C for 30 minutes, by continuously stirring. After 30 minutes, pH (5.2.19) of the mixture at 37 °C is 2.0 - 2.5.

Chlorides (5.3.2.1). To dissolve 54.4 mg of the sample in 30 mL of 20% nitric acid (w/v) and filter it. To use 15 mL of this solution and 1 mL of 0.01 *M* hydrochloric acid standard solution. At most 1.3% (13000 ppm).

Soluble phosphates. To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To accurately weigh about 5 g of the sample and mix with 125 mL of water. To stir for 2 hours. To filter and wash with 50 mL of water.

To collect the filtrate in a 250 mL volumetric flask and top up to volume with water. To transfer 10 mL of this solution to 100 mL volumetric flask and top up with water, thus obtaining *Solution (1)*. In parallel, transfer 2.86 g of monobasic potassium phosphate to 100 mL volumetric flask, dissolve in water, and top up with the same solvent, thus obtaining *Solution (2)*. To transfer 1 mL of *Solution (2)* to 5 mL volumetric flask and top up with water, thus obtaining *Solution (3)*. To transfer 3 mL of *Solution (2)* to 5 mL volumetric flask and top up with water, thus obtaining *Solution (4)*. To transfer 5 mL of each solution obtained to 25 mL volumetric flask, add 4 mL of *M* sulfuric acid, 1 mL of 1% ammonium molybdate (w/v) in *M* sulfuric acid, 5 mL of water, and 2 mL of a solution containing 0.1 g of 4-methylamino phenol sulfate, 0.5 g of anhydrous sodium sulfite, 20 g of sodium metabisulfite in 100 mL of water. To stir and let it rest for 15 minutes. To top up to volume with water. To measure the absorbance of the resulting solutions at 730 nm. To calculate the concentration of PO₄³⁻ in *Solution (1)* from the calibration curve prepared with *Solutions (2), (3), and (4)*. At most 1.0%.

Sulfates (5.3.2.2). To dissolve 0.4 g of the sample in 30 mL of diluted hydrochloric acid and filter it. To use 15 mL of



this solution and 2.5 mL of 0.005 M sulfuric acid standard solution. At most 0.6% (6000 ppm).

Arsenic (5.3.2.5). To determine in 1 g of the sample. To proceed as described in *Spectrophotometric method, Method I*. At most 0.0001% (1 ppm).

Heavy Metals (5.3.2.3). To use the *Method I*. To dissolve 2 g of the sample in 20 mL of hydrochloric acid SR. To use 10 mL of this solution. At most 0.002% (20 ppm).

Sulfated ashes (5.2.10). To determine in 1 g of the sample. To incinerate the residue at 800 °C - 1000 °C, to obtain constant weight. From 10% to 20%.

DOSAGE

To accurately weigh about 0.4 g of the sample previously incinerated, dissolve in 10 mL of dilute hydrochloric acid and dilute to 100 mL with water. To add 10 mL of 0.1 M SV sodium edetate and 30 mL of mixture of ammonium acetate SR and diluted acetic acid (1:1) to 10 mL of this solution. To boil it for 3 minutes and cool down. To add 25 mL of ethanol and 1 mL of 0.025% dithizone (w/v) in ethanol freshly prepared. To titrate the 0.1 M SV sodium edetate in excess with 0.1 M SV zinc sulfate to change to pink. Each mL of 0.1 M SV disodium edetate is equal to 12.195 mg of AlPO_4 .

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antacid

PHOSPHATE, DIBASIC AMMONIUM Ammonii hydrogenophosphas

$(\text{NH}_4)_2\text{HPO}_4$; 132.06
ammonium phosphate dibasic; 04277
Ammonium salt of phosphoric acid (2:1)
[7783-28-0]

It contains at least 96.0% and at most 102.0% of $(\text{NH}_4)_2\text{HPO}_4$.

DESCRIPTION

Physical characteristics. Crystalline powder, or white or almost white crystals.

Solubility. Freely soluble in water; practically insoluble in acetone and ethanol.

IDENTIFICATION

A. The 5% solution (w/v) responds to the reactions of ammonium ion (5.3.1.1).

B. The 5% solution (w/v) responds to the reactions of phosphate ion (5.3.1.1).

PURITY TESTS

pH (5.2.9). 7.6 to 8.2. To determine in 1% aqueous solution (w/v).

Arsenic (5.3.2.5). To use *Spectrophotometric method, Method I*. To determine in 1 g of the sample. At most 0.0003% (3 ppm).

Chlorides (5.3.2.1). To determine in 1.22 g of the sample. At most 0.03% (300 ppm).

Sulfates (5.3.2.2). To determine in 0.8 g of the sample. At most 0.15% (1500 ppm).

Heavy metals (5.3.2.3). To dissolve 2 g of the sample in 25 mL of water. At most 0.001% (10 ppm).

DOSAGE

To dissolve 0.6 g of the sample in 40 mL of water. To titrate with 0.05 M SV sulfuric acid to pH 4.6 potentiometrically determined. Each mL of 0.05 M SV sulfuric acid is equivalent to 13.206 mg of $(\text{NH}_4)_2\text{HPO}_4$.

PACKAGING AND STORAGE

In nonmetallic containers, tightly closed.

LABELLING

To comply with the legislation in force.

CATEGORY

Buffering agent, sequestrant agent.

PHOSPHATE, DIBASIC CALCIUM, DIHYDRATE

Calcii hydrogenophosphas dihydricus

$\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$; 172.09
calcium phosphate dibasic dihydrate; 04278
Calcium salt of phosphoric acid hydrate (1:1:2)
[7789-77-7]

It contains at least 98.0% and at most 100.5% of $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$.

DESCRIPTION

Physical characteristics. White crystalline powder.

Solubility. It is practically insoluble in water and ethanol. Soluble in diluted solutions of hydrochloric acid and nitric acid; slightly soluble in diluted acetic acid.

IDENTIFICATION

A. To dissolve about 0.1 g of the sample by heating it with a mixture of 5 mL of 3 *M* hydrochloric acid and 5 mL of water. To add dropwise, under stirring, 2.5 mL of 6 *M* ammonium hydroxide and add 5 mL of ammonium oxalate SR. White precipitate is produced.

B. To add 10 mL of ammonium molybdate SR to 10 mL of 1% solution (w/v) of the sample heated with slight excess of nitric acid. Yellow precipitate of ammonium phosphomolybdate is produced.

PURITY TESTS

Barium. To dissolve 2.5 g of the sample in 20 mL of hydrochloric acid SR, filter it, if necessary, and add ammonia SR to form precipitate. To add hydrochloric acid SR to dissolve the precipitate and top up to 50 mL with water. To add 0.5 mL of diluted sulfuric acid to 10 mL of this solution. To add 0.5 mL of water to other aliquot of 10 mL. After 15 min, any opalescence in the aliquot added to the acid is not more intense than that obtained with the aliquot added to water.

Carbonates. To mix 0.5 g of the sample with 5 mL of carbon dioxide-free water and add 1 mL of hydrochloric acid. No effervescence is observed.

Tricalcium and monocalcium phosphate. To dissolve 2 g of the sample in 30 mL of *M* SV hydrochloric acid, add 20 mL of water and 0.05 mL of methyl orange SI.

To titrate the *M* SV hydrochloric acid in excess with *M* SV sodium hydroxide. The consumption of *M* SV hydrochloric acid is between 11 mL and 12.5 mL.

Substances insoluble in acid. To heat 5 g of the sample with a mixture of 40 mL of water and 10 mL of hydrochloric acid to maximum solubilization and top up to 100 mL with water. If an insoluble residue appears, filter, wash with hot water to obtain negative reaction to chlorides. To dry the residue at 105 °C for 1 hour. At most 0.2%.

Arsenic (5.3.2.5). To dissolve 2.5 g of the sample in 20 mL of hydrochloric acid SR. To filter, if necessary, and add ammonia SR to form precipitate. To add hydrochloric acid SR to dissolve the precipitate and top up to 50 mL with water. To use 2 mL of this solution and proceed as described in *Visual method*. At most 0.001% (10 ppm).

Chlorides (5.3.2.1). To dissolve 3.58 g of the sample in a mixture of 7 mL of nitric acid and 20 mL of water. To dilute

to 50 mL with water. To use 15 mL of this solution. At most 0.033% (330 ppm).

Iron (5.3.2.4). To dissolve 2.5 g of the sample in 20 mL of hydrochloric acid SR. To filter, if necessary, and add ammonia SR to form precipitate. To add hydrochloric acid SR to dissolve the precipitate and top up to 50 mL with water. To use 5 mL of this solution and proceed as described in *Method I*. To use 1 mL of *Iron standard solution* (100 ppm Fe). At most 0.04% (400 ppm).

Heavy metals (5.3.2.3). To dissolve as much as possible by heating 1.3 g of the sample in 3 mL of 3 *M* hydrochloric acid, cool down, and dilute to 50 mL with water. To determine in 25 mL of this solution. At most 0.003% (30 ppm).

Sulfates (5.3.2.2). To dissolve 0.8 g of the sample in 20 mL of hydrochloric acid SR. To filter, if necessary, and add ammonia SR to form precipitate. To add hydrochloric acid SR to dissolve the precipitate and top up to 50 mL with water. To use 15 mL of this solution. At most 0.5% (5000 ppm).

Sulfated ashes (5.2.10). To determine in 1 g of the sample. To incinerate the residue at 800 °C - 825 °C, to obtain constant weight. From 24.5% to 26.5%.

DOSAGE

To accurately weigh about 0.3 g of the sample and dissolve in a mixture of 1 mL of 0.3% hydrochloric acid (v/v) and 5 mL of water. To add 25 mL of 0.1 *M* SV disodium edetate and dilute to 200 mL with water. To neutralize with ammonium hydroxide and add 10 mL of ammonium chloride buffer solution pH 10.0 and approximately 50 mg of eriochrome black T. To titrate the disodium edetate in excess with 0.1 *M* SV zinc sulfate to obtain violet color. To perform sample blank correction. Each mL of 0.1 *M* SV disodium edetate is equal to 17.209 mg of CaHPO₄·2H₂O.

PACKAGING AND STORAGE

In nonmetallic containers, tightly closed.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Calcium supplement.

PHOSPHATE, TRIBASIC CALCIUM
Tricalcii phosphas

Ca₃(OH)(PO₄)₃; 502.31
calcium phosphate tribasic; 00203
Calcium phosphate hydroxide
[12167-74-7]



Calcium phosphate tribasic consists of a variable mixture of calcium phosphates. It contains at least 35.0% and at most 40.0% of Ca (40.08).

DESCRIPTION

Physical characteristics. White, odorless, and tasteless powder.

Solubility. It is practically insoluble in water and ethanol. Soluble in diluted solutions of hydrochloric acid and nitric acid. Practically insoluble in acetic acid.

IDENTIFICATION

A. To dissolve 0.1 g of the sample in 5 mL of 25% nitric acid (v/v). Solution responds to the reactions of phosphate ion (5.3.1.1).

B. It responds to the reactions of the calcium ion (5.3.1.1).

PURITY TESTS

Substances insoluble in acid. To dissolve 5 g of the sample in the mixture of 10 mL of hydrochloric acid and 30 mL of water. To filter, wash the residue with water, and dry at 105 °C in an oven to obtain constant weight. The mass of the residue is not greater than 10 mg (0,2 %).

Arsenic (5.3.2.5). To add 0.25 g of the sample to 20 mL of water. To add hydrochloric acid to total dissolution and proceed as described in *Visual method*. At most 0.0004% (4 ppm).

Barium. To weigh 0.5 g of the sample. To add 10 mL of water and 1 mL of nitric acid by stirring. The resulting solution should remain clear after adding 1 mL of calcium sulfate SR.

Iron (5.3.2.4). To dissolve 2.5 g of the sample in 20 mL of diluted hydrochloric acid.

To filter, if the solution is not clear. To slowly add diluted ammonia to form precipitate. To dissolve the precipitate in diluted hydrochloric acid and top up to 50 mL with water. To use 5 mL of this solution and proceed as described in *Method I*. To use 1 mL of *Iron standard solution (100 ppm Fe)*. At most 0.04% (400 ppm).

Heavy metals (5.3.2.3). To dissolve 0.6667 g of the sample in 10 mL of 10% hydrochloric acid (w/v) and heat in a water bath for 5 minutes. To dilute with water to 25 mL and proceed as described in *Method I*. At most 0.003% (30 ppm).

Chlorides (5.3.2.5). To add 0.1 g of the sample to 10 mL of water. To add 1 mL of nitric acid, stir to dissolve it, and dilute to 40 mL with water. To proceed as described in *Limit test for chlorides*. At most 0.35%.

Sulfates (5.3.2.5). To add 0.15 g of the sample to 10 mL of water. To add 1 mL of hydrochloric acid, stir to fully dissolve it, and dilute to 40 mL with water. At most 0.8%.

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 800 °C for 30 minutes. At most 8.0 %.

DOSAGE

To dissolve 0.2 g of the sample in the mixture of 1 mL of hydrochloric acid SR and 5 mL of water. To add 25 mL of 0.1 M disodium edetate and top up to 200 mL with water. To adjust the pH to 10.0 with ammonia and add 10 mL of ammonium chloride buffer pH 10.0. To use eriochrome black T SI as indicator. To titrate the 0.1 M disodium edetate in excess with 0.1 M SV zinc sulfate to indicator changes from blue to violet. Each mL of 0.1 M disodium edetate is equal to 4.008 mg of Ca.

PACKAGING AND STORAGE

In tightly closed containers.

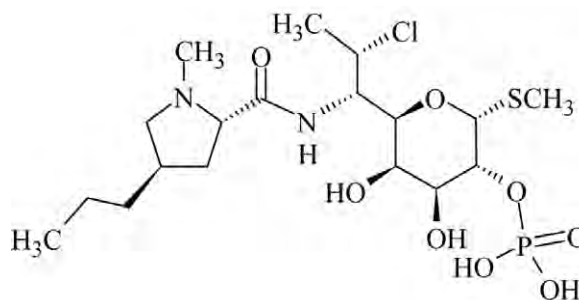
LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Supplement and Pharmacotechnical adjuvant.

PHOSPHATE, CLINDAMYCIN Clindamycini phosphas



$C_{18}H_{33}ClN_2O_5S$; 424.98

$C_{18}H_{34}ClN_2O_8PS$; 504.96

clindamycin; 02229

clindamycin phosphate; 02232

7-Chloro-6,7,8-trideoxy-6-[[[(2S, 4R)-1-methyl-4-propyl-2-pyrrolidinyl] carbonyl] amino]-1-thio-L-threo-α-D-galacto-methyl octo pyranoside
[18323-44-9]

2 - (dihydrogen phosphate) of 7-chloro-6,7,8-trideoxy-6-[[[(2S, 4R)-1-methyl-4-propyl-2-pyrrolidinyl] carbonyl] amino] -1 - thio-L-threo-α-D-galacto-methyl octo pyranoside
[24729-96-2]

It contains at least 95.0% and at most 100.5% of $C_{18}H_{34}ClN_2O_8PS$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White or almost white powder, slightly hygroscopic. It shows polymorphism.

Solubility. Freely soluble in water; very slightly soluble in ethanol; practically insoluble in methylene chloride.

Physical and chemical constants.

Specific rotation (5.2.8): $+115^\circ$ to $+130^\circ$, in relation to the anhydrous substance. To determine at 1% solution (w/v) in water.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the clindamycin phosphate SQR spectrum similarly prepared.

B. To heat for 90 minutes, under reflux, 0.1 g of the sample with a mixture of 5 mL of concentrated sodium hydroxide SR solution and 5 mL water. To cool down. To add 5 mL of nitric acid. To extract with 3 portions (15 mL each) of methylene chloride. To filter the aqueous layer. The filtrate responds to the reactions of phosphate ion (5.3.1.1).

PURITY TESTS

Appearance of solution. To dissolve 1 g of the sample in water. To gently warm, if necessary. To cool down and top up with water to 25 mL. The solution is clear (5.2.25) and colorless (5.2.12).

pH (5.2.19). 3.5 to 4.5. To determine in 1% aqueous solution (w/v).

Related substances. To proceed as described in *Dosage*. To prepare the solutions as described below.

Solution (1): to transfer accurately weighed amount of 75 mg of the sample to 25 mL volumetric flask, top up with *Mobile phase*, and mix it.

Solution (2): to dilute 1 mL of Standard solution to 100 mL with *Mobile phase*.

Procedure: to separately inject 20 μ L of each solution, record the chromatograms, and measure the areas under the peaks. The sum of all the peaks obtained with *Solution (1)*, except the solvent peak's one and main peak's one, is not greater than 2.5 times the area under the main peak obtained with *Solution (2)* (2.5%). The sum of all the peaks obtained with *Solution (1)*, except the solvent peak's one and main peak's one, is not greater than 4 times the area under the main peak obtained with *Solution (2)* (4.0%). To

disregard the peaks with an area less than 10% of the area under the main peak obtained with *Solution (2)*.

Water (5.2.20.1). To determine in 0.25 g of the sample. At most 6.0%.

BIOLOGICAL SAFETY TEST

When the label states that the substance is sterile, the sample complies with Bacterial endotoxins and Sterility tests. Should the substance be indicated to sterilization for sterile preparations, the sample complies with Bacterial endotoxins tests.

Sterility (5.5.3.2.1). It complies with the test. To use the *Membrane filtration method*.

Bacterial endotoxins (5.5.2.2). At most 0.6 UE/ mg of clindamycin phosphate.

DOSAGE

To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 210 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octylsilane group (5 μ m to 10 μ m), kept at room temperature; flow of *Mobile phase* of 1.0 mL/min.

Mobile phase: to mix 200 mL of acetonitrile and 800 mL of 1.36% monobasic potassium phosphate (w/v) previously adjusted to pH 2.5 with phosphoric acid.

Sample solution: to transfer accurately weighed amount of 75 mg of the sample to 25 mL volumetric flask, top up with *Mobile phase*, and mix it.

Standard solution: to dissolve accurately weighed amount of clindamycin phosphate SQR in *Mobile phase* and properly dilute to obtain a 3 mg/mL solution.

Resolution solution: to dissolve 5 mg of lincomycin hydrochloride SQR and 15 mg of clindamycin hydrochloride SQR in 5 mL of *Standard solution* and top up to 100 mL with *Mobile phase*.

To inject replicates of 20 μ L of the *Resolution solution*. The dosage is valid only if the lincomycin hydrochloride peak is separated from the solvent peak. The resolution between clindamycin phosphate and clindamycin hydrochloride peaks is not less than 6.0. To adjust the proportion of acetonitrile in the *Mobile phase*, if necessary. The tailing factor for clindamycin phosphate peak is not greater than 1.5.

To inject replicates of 20 μ L of the *Standard solution*. The relative standard deviation for the replicate areas for the peaks recorded is not greater than 1.0%. To adjust the integrator parameters, if necessary.



Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_{18}H_{34}ClN_2O_8PS$ from the sample, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force. Label must indicate whether the product is sterile or must be sterilized during the process, if substance is intended for parenteral preparations.

THERAPEUTIC CLASS

Antiprotozoal, antibacterial.

PHOSPHATE, CLINDAMYCIN INJECTABLE SOLUTION

It contains at least 90.0 % and at most 105.0 % of the amount stated of $C_{18}H_{33}ClN_2O_5S$.

IDENTIFICATION

A. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF_{254} silica gel as support and a mixture of methanol, toluene, and 18 M ammonia (70:30:1.5) as mobile phase.

To separately apply to the plate 10 µL of each of the solutions freshly prepared, as described below.

Solution (1): to transfer volume of injectable solution equivalent to 50 mg of clindamycin phosphate to a 10 mL volumetric flask, top up to volume with methanol, and make it homogeneous.

Solution (2): 5 mg/mL clindamycin phosphate SQR solution in methanol.

To develop the chromatogram. To remove the plate, and let it drying. To nebulize the plate with diluted potassium iodobismuthate SR. To examine under ultraviolet light (254 nm). The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

pH (5.2.19). 5.5 to 7.0.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

Bacterial endotoxins (5.5.2.2). At most 0.58 UE/ mg of clindamycin phosphate.

DOSAGE

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 210 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), at room temperature; flow of *Mobile phase* of 1.0 mL/min.

Mobile phase: to mix 250 mL of acetonitrile and 750 mL of 1.36% monobasic potassium phosphate (w/v) previously adjusted to pH 2.5 with phosphoric acid.

Sample solution: to dilute volume of the injectable solution in *Mobile phase* to obtain a 0.15 mg/mL clindamycin solution.

Standard solution: 0.18 mg/mL clindamycin phosphate SQR solution in the *Mobile phase*.

Resolution Solution: solution containing 0.12 mg/mL of lincomycin hydrochloride SQR and 0.24 mg/mL of clindamycin phosphate SQR in *Mobile phase*.

To inject replicates of 20 µL of the *Resolution solution*. The resolution between the lincomycin hydrochloride and clindamycin phosphate peaks is not less than 7.7.

The relative standard deviation for the replicate areas for the peaks recorded is not greater than 2.0%.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_{18}H_{33}ClN_2O_5S$ in the injectable solution, from the responses obtained for the *Sample solution* and *Standard solution*. Each mg of $C_{18}H_{34}ClN_2O_8PS$ is equal to 0.8416 mg of $C_{18}H_{33}ClN_2O_5S$.

PACKAGING AND STORAGE

In type I glass containers, protected from light, at 8 °C - 30 °C.

LABELLING

To comply with the legislation in force.

PHOSPHATE, DIBASIC SODIUM

Natrii hydrogenophosphas

Na_2HPO_4 ; 141.96

$\text{Na}_2\text{HPO}_4 \cdot \text{H}_2\text{O}$; 159.97

$\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$; 177.99

$\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$; 268.07

$\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$; 358.14

dibasic sodium phosphate; 00207

dibasic sodium phosphate dihydrate; 00209

dibasic sodium phosphate heptahydrate; 00211

dibasic sodium phosphate dodecahydrate; 00210

Sodium salt of phosphoric acid (2:1)

[7558-79-4]

Disodium salt of phosphoric acid monohydrate

[118830-14-1]

Disodium salt of phosphoric acid dihydrate

[10028-24-7]

Disodium salt of phosphoric acid heptahydrate

[7782-85-6]

Sodium salt of phosphoric acid hydrate (2:1:12)

[10039-32-4]

Dibasic sodium phosphate is anhydrous or contains one, two, seven, or twelve molecules of water of hydration. It contains at least 98.0% and at most 100.5% of Na_2HPO_4 , in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or colorless, odorless powder with saline taste.

Solubility. Freely soluble in water, very slightly soluble in ethanol.

IDENTIFICATION

A. The 3% aqueous solution (w/v) responds to the reactions of phosphate ion (5.3.1.1).

B. The 3% aqueous solution (w/v) responds to the reactions of sodium ion (5.3.1.1).

PURITY TESTS

Insoluble substances. To dissolve amount of the sample equivalent to 5 g of Na_2HPO_4 in 100 mL of hot water, filtering it in tared Gooch crucible, wash the residue with hot water, and dry at 105 °C for 2 hours. The weight of the residue is not greater than 20 mg (0.4%).

Arsenic (5.3.2.5). To use *Spectrophotometric method, Method I*. To determine in solution containing the equivalent to 187.5 mg of Na_2HPO_4 in 35 mL of water. At most 0.0016% (16 ppm).

Chlorides (5.3.2.1). To determine in amount of sample equivalent to 0.6 g of Na_2HPO_4 . At most 0.06% (600 ppm).

Heavy metals (5.3.2.3). To use *Method I*. To dissolve the amount of sample equivalent to 2.1 g of Na_2HPO_4 in 50 mL of water and use 12 mL of this solution for *Preparation sample*. At most 0.002% (20 ppm).

Sulfates (5.3.2.1). To determine in amount of sample equivalent to 0.6 g of Na_2HPO_4 . At most 0.2% (2000 ppm).

Loss on drying (5.2.9). To desiccate in an oven at 130 °C to obtain constant weight. At most 5.0% to anhydrous form, between 10.3% and 12.0% to monohydrate form, between 18.5% and 21.5% to dihydrate form, between 43.0% and 50.0% to heptahydrate form, and between 55.0% and 64.0% to dodecahydrate form.

DOSAGE

To accurately weigh amount of the sample equivalent to 1 g of Na_2HPO_4 and dissolve in 40 mL of water. By using volumetric pipette, add 15 mL of *M* hydrochloric acid. To potentiometrically titrate with *M* SV sodium hydroxide to inflection point close to pH 4.0 and record the volume spent. To continue the titration to second inflection point near pH 8.8. To perform sample blank correction by transferring 15 mL of *M* hydrochloric acid, by using volumetric pipette, and 40 mL of water to erlenmeyer flask and potentiometrically titrating with *M* SV sodium hydroxide. The difference between the volume of *M* SV sodium hydroxide spent in the sample blank correction and the volume spent to titrate the sample to the inflection point pH 4.0 is considered *Volume A*. The difference between the volume *M* SV sodium hydroxide spent between the inflection points pH 8.8 and pH 4.0 is considered *Volume B*. If the *Volume A* is equal to or less than the *Volume B*, each mL of the *Volume A* of *M* SV sodium hydroxide is equivalent to 141.960 mg of Na_2HPO_4 . If *Volume A* is greater than *Volume B*, each mL of (2 *Volume B*) - *Volume A* of *M* SV sodium hydroxide is equivalent to 141.960 mg of Na_2HPO_4 .

PACKAGING AND STORAGE

In nonmetallic containers, tightly closed.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Adjuvant.



PHOSPHATE, MONOBASIC SODIUM

Natrii dihydrogenophosphas

NaH_2PO_4 ; 119,98

$\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$; 138.00

$\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$; 156.01

monobasic sodium phosphate; 00212

monobasic sodium phosphate monohydrate; 09331

monobasic sodium phosphate dihydrate; 00213

Sodium salt of phosphoric acid (1:1)

[7558-80-7]

Monosodium salt of phosphoric acid monohydrate

[10049-21-5]

Sodium salt of phosphoric acid hydrate (1:1:2)

[13472-35-0]

Monobasic sodium phosphate is anhydrous or contains one or two molecules of water of hydration. It contains at least 98.0% and at most 103.0% of NaH_2PO_4 , in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. Crystalline powder or colorless or white crystals, odorless, slightly deliquescent.

Solubility. Freely soluble in water and practically insoluble in ethanol.

IDENTIFICATION

A. The 5% aqueous solution (w/v) responds to the reactions of phosphate ion (5.3.1.1).

B. The 5% aqueous solution (w/v) responds to the reactions of sodium ion (5.3.1.1).

PURITY TESTS

pH (5.2.19). 4.1 to 4.5. To determine in solution containing the equivalent to 1 g of $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ in 20 mL of water.

Insoluble substances. To dissolve amount of the sample equivalent to 10 g of $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ in 100 mL of hot water, filtering it in tared Gooch crucible, wash the residue with hot water, and dry at 105 °C for 2 hours. The weight of the residue is not greater than 20 mg (0.2%).

Aluminum, calcium, and related elements. The solution containing the equivalent to 1 g of $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ in 10 mL of water has no turbidity when the medium is slightly alkalized with 6 M ammonium hydroxide, by using litmus paper pink.

Arsenic (5.3.2.5). To use *Spectrophotometric method, Method I*. To determine in solution containing the equivalent to 0.375 g of $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ in 35 mL of water. At most 0.0008% (8 ppm).

Chlorides (5.3.2.1). To determine in amount of sample equivalent to 2.5 g of $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$. At most 0.014% (140 ppm).

Heavy metals (5.3.2.3). To use *Method I*. To dissolve amount of sample equivalent to 1 g of $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ in 20 mL of water, add 1 mL of 3 M hydrochloric acid, and top up to 25 mL with water. At most 0.002% (20 ppm).

Sulfates (5.3.2.1). To determine in amount of sample equivalent to 0.8 g of $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$. At most 0.15% (1500 ppm).

Water (5.2.20.1). At most 2.0% to anhydrous form, between 10.0% and 15.0% to monohydrate form, and between 18.0% and 26.5% to dihydrate form.

DOSAGE

To accurately dissolve about 2.5 g of sample in 10 mL of cold water and add 20 mL of cold saturated sodium chloride solution. To titrate with M SV sodium hydroxide by using phenolphthalein SI as an indicator and keeping the solution temperature between 10 and 15 °C during the titration. Each mL of M SV sodium hydroxide is equal to 119.98 mg of NaH_2PO_4 .

PACKAGING AND STORAGE

In nonmetallic containers, tightly closed.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Adjuvant.

PHOSPHATE, SODIUM ORAL SOLUTION

Sodium phosphate Oral solution is a solution containing dibasic sodium phosphate and monobasic sodium phosphate or dibasic sodium phosphate and phosphoric acid in purified water. It contains in 100 mL at least 16.2 g and at most 19.8 g of dibasic sodium phosphate

($\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$) and at least 43.2 g and at most 52.8 g of monobasic sodium phosphate ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$).

IDENTIFICATION

A. It responds to the reactions of sodium ion (5.3.1.1).

B. It responds to the reactions of phosphate ion (5.3.1.1).

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

Relative density (5.2.5). 1.333 to 1.366.

pH (5.2.19). Between 4.4 and 5.2.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To pipette 25 mL of the sample, transfer to 500 mL volumetric flask, and top up with water. To transfer 25 mL of this solution to a 250 mL beaker and add 15 mL of 0.5 M sodium hydroxide and 75 mL of water. To potentiometrically titrate the base in excess with 0.5 M SV hydrochloric acid to the first inflection point (pH around 9.2). To record the volume of titrant spent (A). To continue the titration to second inflection point (pH around 4.4) and record the volume (B). To determine the blank, transfer 15 mL of 0.5 M sodium hydroxide to a 250 mL beaker, add 100 mL of water, and titrate with 0.5 M SV hydrochloric acid. To record the volume of 0.5 M SV hydrochloric acid consumed (C) in mL. Each mL of volume (C-A) of 0.5 M hydrochloric acid is equivalent to 69 mg of monobasic sodium phosphate ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$) and each mL of volume (B-C) of 0.5 M SV hydrochloric acid is equivalent to 134.0 mg of dibasic sodium phosphate ($\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$).

PACKAGING AND STORAGE

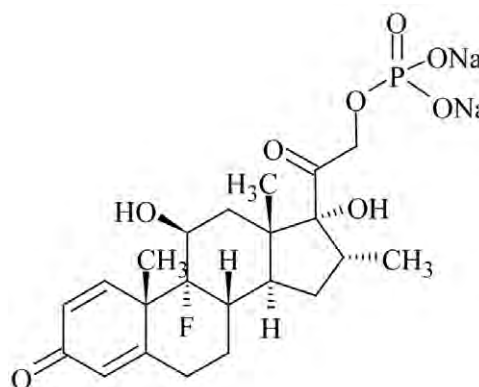
In tightly closed containers.

LABELLING

To comply with the legislation in force.

PHOSPHATE, DISODIUM DEXAMETHASONE

Dexamethasoni natrii phosphas



$\text{C}_{22}\text{H}_{28}\text{FNa}_2\text{O}_8\text{P}$; 516.40

dexamethasone disodium phosphate; 02821

Sodium salt of (11 β , 16 α)-9-fluoro-11,17-dihydroxy-16-methyl-21-(phosphonoxy)-pregna-1,4-diene-3,20-dione (2:1)

[2392-39-4]

It contains at least 97.0% and at most 103.0% of $\text{C}_{22}\text{H}_{28}\text{FNa}_2\text{O}_8\text{P}$, in relation to the anhydrous substance, ethanol-free.

DESCRIPTION

Physical characteristics. White powder, very hygroscopic.

Solubility. Freely soluble in water; very slightly soluble in ethanol and dioxane; insoluble in chloroform.

Physical and chemical constants.

Specific rotation (5.2.8): +75° to +83, in relation to the anhydrous substance, ethanol-free. Determined at 1% solution (w/v) in water.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the dexamethasone disodium phosphate SQR spectrum similarly prepared. If the spectrum obtained in the solid state shows differences, dissolve separately the substance to be examined and dexamethasone disodium phosphate SQR in a minimum volume of ethanol, evaporate, and dry in a water bath. To record new spectra by using the residues.

B. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using HF_{254} silica gel as support and a mixture of chloroform, methanol, and water (180:15:1) as mobile phase. To separately apply to the plate 10 μL of each of the solutions freshly prepared, as described below.

Solution (1): to weigh 20 mg of sample in a centrifuge tube. To add 5 mL of alkaline phosphatase solution, vigorously stir, and let it rest for 30 minutes. To add 5 mL of ethyl acetate, vigorously stir, centrifuge, and use the upper layer.

Solution (2): 3 mg/mL dexamethasone SQR solution in ethyl acetate.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

C. To dissolve 2 mg in 2 mL of sulfuric acid and stir. Yellowish-brown color is developed within 5 minutes. To add 10 mL of water and mix. The color fades out, and the preparation remains clear.

D. The residue obtained as described in *Sulfated ashes (5.2.10)* responds to the reactions of sodium ion and phosphate ion (**5.3.1.1**).

PURITY TESTS

Appearance of solution. The 5% solution (w/v) in carbon dioxide-free water is clear (**5.2.25**).

pH (5.2.19). 7.5 to 10.5. To determine in 1% solution (w/v) in carbon dioxide-free water.

Related substances. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 254 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at room temperature; flow of *Mobile phase* of 1 mL/min.

Mobile phase: to mix 1.36 g of monobasic potassium phosphate and 0.6 g of hexylamine and let it rest for 10 minutes. To dissolve in 182.5 mL of water and add 67.5 mL of acetonitrile. To make the adjustments as required.

Solution (1): to dissolve accurately weighed amount of the sample in *Mobile phase* to obtain a 2.5 mg/mL solution.

Solution (2): to dissolve 2 mg of dexamethasone disodium phosphate SQR and 2 mg of betamethasone sodium phosphate in *Mobile phase* and dilute to 100 mL with the same solvent.

Solution (3): to transfer 1 mL of *Solution (1)* to 100 mL volumetric flask and top up with *Mobile phase*.

To inject 20 µL of *Solution (2)*. The resolution between betamethasone sodium phosphate and dexamethasone disodium phosphate is greater than 2.2.

Procedure: to separately inject 20 µL of *Solution (1)* and *Solution (3)*. Then, record the chromatograms at least

twice the retention time of the main peak. The area under any peak, in relation to the main peak obtained with *Solution (1)*,

is not greater than half of the area under the main peak obtained with *Solution (3)* (0.5%). The sum of the areas under all the peaks obtained with *Solution (1)*, except the solvent peak's one, is not greater than the area under the main peak obtained with *Solution (3)* (1%). To disregard the peaks with an area less than 0.05 times the area under the main peak obtained with *Solution (3)*.

Limit for ethanol. To proceed as described in *Gas chromatography (5.2.17.5)*. To use gas chromatograph equipped with flame ionization detector, 1 m long capillary column and 3.2 mm inner diameter, filled with ethylvinylbenzene-divinylbenzene copolymer, with a film thickness 150 µm - 180 µm; column temperature 150 °C; injector temperature at 250 °C, and the detector temperature at 280 °C. To use nitrogen as a carrier gas, flow of 30 mL/min.

Internal standard solution: to dilute 1 mL of n-propyl alcohol in 100 mL of water.

Sample solution: to dissolve 0.5 g of the sample in 5 mL of *Internal standard solution* and dilute to 10 mL with water.

Standard solution: to dilute 1 mL of ethanol to 100 mL with water. To transfer 2 mL of this solution to 10 mL volumetric flask, add 5 mL of *Internal standard solution*, and top up with water.

Procedure: to separately inject 2 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the percentage of ethanol in the sample. At most 3.0% (w/w) of ethanol.

Limit for phosphate ions. To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. To accurately dissolve about 50 mg of sample in a mixture of 10 mL of water and 5 mL of *M* sulfuric acid. To heat, if required. To add 1 mL of 5% ammonium molybdate solution (w/v) in 0.05 *M* sulfuric acid and 1 mL of 4-methylamino phenol sulfate SR. To top up to 25 mL with water, make it homogeneous, and let it rest for 30 minutes. To prepare a standard solution at the same concentration, by using 5 mL of 0.01433% monobasic potassium phosphate (w/v) instead of sample, and the same solvents. To measure the absorbance of the resulting solutions at 730 nm, by using water to adjust zero. The absorbance of the solution of the sample is not greater than that of the Standard solution. At most 1.0%.

Water (5.2.20.1). The sum of the percentage for the water and ethanol content determined in *Limit for ethanol* is not greater than 16.0%.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.



Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. To accurately weigh about 0.1 g of the sample and dissolve it in water. To top up the volume to 100 mL with the same solvent. To successively dilute in water to the 0.002% concentration (w/v). To prepare a standard solution at the same concentration by using the same solvent and dexamethasone disodium phosphate SQR instead of sample. To measure the absorbance of the resulting solutions at 241.5 nm, by using water to adjust zero. To calculate the content of $C_{22}H_{28}FN_2O_8P$ in the sample from the readings obtained. Alternatively, make the calculations by taking into account that $A(1\%, 1\text{ cm}) = 303$ at 241.5 nm in water.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

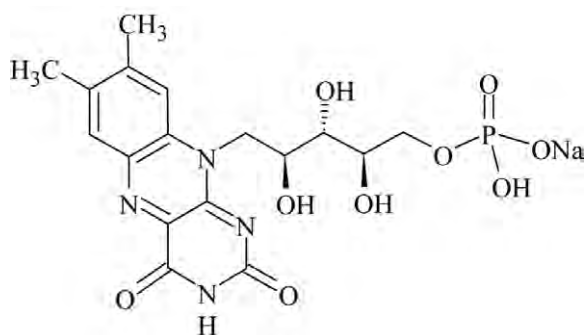
LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Anti-inflammatory, steroid.

PHOSPHATE, RIBOFLAVIN SODIUM Riboflavinum natrii phosphas



$C_{17}H_{20}N_4NaO_9P$; 478.33

$C_{17}H_{20}N_4NaO_9P \cdot 2H_2O$; 514.36

riboflavin phosphate sodium; 07703

Riboflavin 5'-(sodium hydrogen phosphate) dihydrate (1:1)

[130-40-5]

Riboflavin 5'-monosodium salt of (dihydrogen phosphate) dihydrate

[6184-17-4]

Mixture of riboflavin 5'-(sodium hydrogen phosphate) as the main component and other sodium riboflavin monophosphates. It contains at least 73.0% and at most 79.0%

of riboflavin ($C_{17}H_{20}N_4O_6$), in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, yellow or yellowish-orange, hygroscopic powder.

Solubility. Soluble in water, very slightly soluble in ethanol, and practically insoluble in ethyl ether.

Physical and chemical constants.

Specific rotation (5.2.8): $+37^\circ$ to $+42^\circ$. To determine in 1.2% solution (w/v) in 5 M hydrochloric acid.

IDENTIFICATION

A. The ultraviolet absorption spectrum (5.2.14) ranging from 230 nm to 350 nm, of 0.001% solution (w/v) in citro phosphate buffer pH 7.0 exhibits maxima at 266 nm. The absorbance at 266 nm is between 0.58 and 0.64.

B. The retention time of the main peak in the chromatogram of *Solution (1)* obtained in *Related substances* corresponds to that of main peak of *Solution (3)*.

C. To transfer 10 mg of the sample to 100 mL volumetric flask, dissolve in SR sodium hydroxide, and top up the volume with the same solvent. To examine 1 mL of this solution under ultraviolet light (254 nm) for 5 minutes. To add sufficient acetic acid to acidify the solution, by using litmus paper blue as indicator. To stir with 2 mL of methylene chloride. The lower phase of the mixture is yellow fluorescent.

D. To add 10 mL of nitric acid to 0.5 g of the sample and evaporate to dryness in a water bath. To heat the residue to obtain a white color, dissolve in 5 mL of water, and filter it. The filtrate responds to the reactions of sodium ion (5.3.1.1) and phosphate ion (5.3.1.1).

PURITY TESTS

pH (5.2.19). 5.0 to 6.5. To determine in 1% aqueous solution (w/v).

Related substances. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 266 nm; 250 mm column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μ m), kept at room temperature; flow of *Mobile phase* of 2 mL/minute.

Mobile phase: mixture of methanol and 0.735% monobasic potassium phosphate (w/v) (15:85).

Solution (1): to exactly transfer about 0.1 g of the sample to a 100 mL volumetric flask, dissolve in 50 mL of water, and top up the volume with *Mobile phase*. To transfer 4 mL

of this solution to 25 mL volumetric flask and top up with *Mobile phase*.

Solution (2): to accurately dissolve about 60 mg of riboflavin SQR in 1 mL of hydrochloric acid and dilute to 250 mL with water. To transfer 4 mL of this solution to 100 mL volumetric flask and top up with *Mobile phase*.

Solution (3): to accurately dissolve about 0,1 g of riboflavin phosphate sodium SQR in 50 mL of water and dilute to 100 mL with *Mobile phase*. To transfer 4 mL of this solution to 25 mL volumetric flask and top up with *Mobile phase*.

To inject replicates of 100 µL of *Solutions (1) and (3)*. To record the chromatogram until complete elution of the riboflavin peak. Under the chromatographic conditions prescribed, the relative retention times are about 0.2 to riboflavin 3', 4'-diphosphate, 0.3 to riboflavin 3', 5'-diphosphate, 0.5 to riboflavin 4', 5'-diphosphate, 0.7 to riboflavin 3'-monophosphate, 0.9 to riboflavin 4'-monophosphate, 1.0 to riboflavin 5'-monophosphate, and 2.0 to riboflavin. The resolution between riboflavin 4'-monophosphate and riboflavin 5'-monophosphate in the chromatogram obtained with *Solution (3)* is not less than 1.5. The relative standard deviation for replicate areas of riboflavin 5'-monophosphate peak is not greater than 1.5%

Procedure: to separately inject 100 µL of *Solutions (1), (2), and (3)*, record the chromatograms, and measure the areas under the peaks. To calculate the content of free riboflavin and riboflavin diphosphates in the sample from the area under the peaks in the chromatogram obtained with *Solutions (1), (2), and (3)*. At most 6.0% of free riboflavin and at most 6.0% of riboflavin diphosphates in relation to desiccated substance.

Limit for lumiflavin. To stir for 5 minutes 35 mg of sample with 10 mL of alcohol-free chloroform, freshly prepared, and filter. The absorbance (5.2.14) of the resulting solution at 440 nm by using alcohol-free chloroform to adjust zero is at most 0.025.

Limit for free phosphate. To dissolve 0.3 mg of the sample in water and dilute to 100 mL with the same solvent. To transfer 10 mL of this solution to 50 mL volumetric flask. To add 10 mL of ammonium molybdate acid solution and 5 mL of 10% ferrous sulfate in 0.075 M sulfuric acid and make it homogeneous. To prepare a standard solution in a similar manner by using 10 mL of 0.004% monobasic potassium phosphate (w/v), 10 mL of ammonium molybdate acid solution and 5 mL of 10% ferrous sulfate (w/v) in 0.075 M sulfuric acid. To measure the absorbances of the resulting solutions at 700 nm (5.2.14). To use a mixture of 10 mL of water, 10 mL of ammonium molybdate acid solution and 5 mL of 10% ferrous sulfate (w/v) in 0.075 M sulfuric acid to adjust zero. The absorbance of the sample solution is not greater than that of the standard solution. At most 1.0%.

Heavy metals (5.3.2.3). To transfer 2 g of the sample to a silica crucible, add dropwise 2 mL of nitric acid and 0.25 mL of sulfuric acid. To carefully heat until white smoke and ignition of the sample appear. To cool down. To extract the residue with 2 portions (2 mL each) of hydrochloric acid. To evaporate the extract to dryness.

To dissolve the residue in 2 mL of diluted acetic acid and dilute to 20 mL with water. To proceed as described in *Method I*. At most 0.001% (10 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample in oven at 105 °C for 5 hours, under reduced pressure. At most 8.0%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 25.0%.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. To accurately weigh about 0.1 g of the sample and dissolve with 150 mL of water. To add 2 mL of glacial acetic acid and dilute with water to 1000 mL. To transfer 10 mL of this solution to 50 mL volumetric flask, add 3.5 mL of 1.4% sodium acetate (w/v), and top up with water. To measure the absorbance of the solution at 444 nm. To use a mixture of water and 1.4% sodium acetate (w/v) (93:7) to adjust zero. To calculate the content of $C_{17}H_{20}N_4O_6$ in the sample by considering that $A(1\%, 1\text{ cm}) = 328$, at 444 nm.

B. To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.15)*. To accurately dissolve about 50 mg of the sample in 20 mL of pyridine and 75 mL of water and dilute to 1000 mL with water. To prepare a standard solution at similar concentration by using the same solvents. To transfer 10 mL of each solution to 1000 mL volumetric flasks. To add 0.05 M sulfuric acid to adjust pH between 5.9 and 6.1 (approximately 4 mL) and top up with water. To measure the fluorescence intensities from resulting solutions by using the fluorimeter with excitation and emission wavelength at 440 nm and 530 nm, respectively. To calculate the content of $C_{17}H_{20}N_4O_6$ in the sample from the readings obtained.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

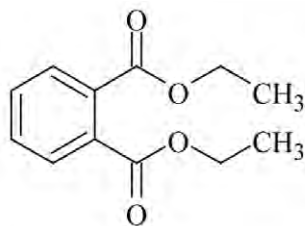
LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Vitamin B component.

PHTHALATE, ETHYL thylis phthalas



$C_{12}H_{14}O_4$; 222.24
ethyl phthalate; 09828
1,2-diethyl ester of 1,2-benzenedicarboxylic acid
[84-66-2]

It contains at least 99.0% and at most 101.0% of $C_{12}H_{14}O_4$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. Oily liquid; colorless or slightly yellowish.

Solubility. Practically insoluble in water, miscible with ethanol and ethyl ether.

Physical and chemical constants.

Relative density (5.2.5): 1.117 to 1.121. To determine at 20 °C.

Refractive index (5.2.6): 1.500 to 1.505. To determine at 20°C.

Boiling point (5.2.3): 295 °C.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed among potassium bromide or sodium chloride plates, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the ethyl phthalate SQR spectrum similarly prepared.

B. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF_{254} silica gel as support and a mixture of heptane and ethyl ether (30:70) as mobile phase. To separately apply to the plate 10 µL of each of the solutions freshly prepared, as described below.

Solution (1): 5 mg/mL solution of the sample in ethyl ether.

Solution (2): 5 mg/mL ethyl phthalate SQR solution in ethyl ether.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). The principal spot obtained with *Solution (1)* corresponds

in position, color, and intensity to that obtained with *Solution (2)*.

C. To add 0.25 mL of sulfuric acid and 50 mg of resorcinol to 0.1 mL of the sample. To heat in water bath for 5 minutes. To cool down, add 10 mL of water and 1 mL of concentrated sodium hydroxide SR solution. Yellow or yellowish-brown and green fluorescence are developed.

PURITY TESTS

Appearance of solution. The examined solution is clear (5.2.25) and is not darker than the following solution (5.2.12). To mix 24 mL of *Ferric chloride base solution*, 6 mL of *Cobaltous chloride base solution*, and 70 mL of 1% hydrochloric acid (w/v). To transfer 12.5 mL of this solution to a 100 mL volumetric flask and top up with 1% hydrochloric acid (w/v).

Acidity. To dissolve 20 g of the sample in 50 mL of ethanol previously neutralized. To add 0.2 mL of phenolphthalein SI and titrate with 0.1 M sodium hydroxide. At most 0.1 mL of 0.1 M sodium hydroxide is spent to neutralize the solution.

Water (5.2.20.1). To determine in 5 g of the sample. At most 0.2%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To accurately weigh about 0.75 g of the sample and transfer to a 250 mL erlenmeyer flask. To add 25 mL of 0.5 M SV ethanolic potassium hydroxide and some glass beads. To heat under reflux in water bath for 1 hour. To add 1 mL of phenolphthalein SI and immediately titrate with 0.5 M SV hydrochloric acid. To perform sample blank correction and adjust it as required. To calculate the volume of 0.5 M SV ethanolic potassium hydroxide used for saponification. Each mL of 0.5 M SV ethanolic potassium hydroxide is equal to 55.560 mg of $C_{12}H_{14}O_4$.

PACKAGING AND STORAGE

In tightly closed full containers, protected from light.

LABELLING

To comply with the legislation in force.

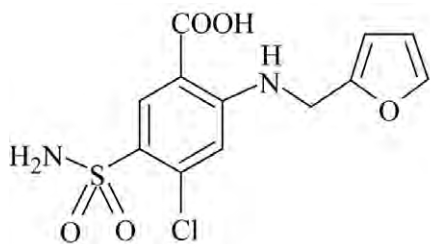
CATEGORY

Pharmaceutical adjuvant.



FUROSEMIDE

Furosemidum



$C_{12}H_{11}ClN_2O_5S$; 330.74

furosemide; 04361

5 - (aminosulfonyl)-4-chloro-2-[(2-furanyl)methyl] amino] benzoic acid

[54-31-9]

It contains at least 98.0% and at most 101.0% of $C_{12}H_{11}ClN_2O_5S$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white or almost white, and odorless powder.

Solubility. Practically insoluble in water, freely soluble in acetone and dimethylformamide, soluble in methanol, slightly soluble in ethyl ether and practically insoluble in chloroform. Freely soluble in alkalis.

IDENTIFICATION

A. To proceed as described in *Infrared absorption spectrum* (5.2.14). The Infrared absorption spectrum of the sample, dispersed in potassium bromide has absorption maximum at the same wavelength, with the same relative intensities to those observed in the furosemide SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14), from 200 nm to 400 nm, 0.0005% solution (w/v) in 0.1 M sodium hydroxide, has absorption maximum at 228, 271, and 333 nm, similar to those observed in the spectrum of a similar solution of furosemide SQR. The absorbances of the solutions at 271 nm do not differ more than 3% when calculated in relation to desiccated substance.

C. To dissolve about 5 mg of sample in 10 mL of methanol. To transfer 1 mL of this solution to reflux flask, add 10 mL of 2 M hydrochloric acid, and proceed to reflux for 15 minutes. To cool down and add 15 mL of M sodium hydroxide and 5 mL of 0.1% sodium nitrite (w/v). To make it homogeneous and wait for 3 minutes. To add 5 mL of 2.5% ammonium sulfamate (w/v), make it homogeneous, add 1 mL of 0.5% N-(1-naphthyl) ethylenediamine (w/v) freshly prepared. The red-violet color develops.

PURITY TESTS

Free aromatic primary amines. To transfer 0.1 g of the sample to a 25 mL volumetric flask by using methanol. To stir, top up with the same solvent, make it homogeneous, and filter it. To pipette 1 mL of the filtrate, transfer to 25 mL volumetric flask, add 3 mL of dimethylformamide, by stirring, 12 mL of distilled water, and 1 mL of M hydrochloric acid. To cool down and add 1 mL of 0.5% sodium nitrite (w/v) by stirring. To let it rest for 5 minutes. To add 1 mL of 2.5% sulfamic acid (w/v) by stirring and let it rest for 3 minutes. Then, add 1 mL of 0.5% N-(1-naphthyl) ethylenediamine (w/v) and dilute to 25 mL with distilled water. At the same time, conduct sample blank correction by substituting 1 mL of methanol for 1 mL of the filtrate. Immediately perform the reading of the absorbance at 530 nm. The absorbance obtained is not greater than 0.20.

Chlorides (5.3.2.1). To add a mixture of 0.2 mL of nitric acid and 30 mL of water to 1 g of the sample. To stir for 5 minutes, let it rest for 15 minutes, and filter it. To use 15 mL of the filtrate. At most 0.02% (200 ppm).

Sulfates (5.3.2.1). To add a mixture of 0.2 mL of acetic acid and 30 mL of water to 2 g of the sample. To stir for 5 minutes, let it rest for 15 minutes, and filter it. To use 15 mL of the filtrate. At most 0.03% (300 ppm).

Heavy metals (5.3.2.3). To use *Method III*. To determine in 1 g of the sample. At most 0.002% (20 ppm).

Loss on drying (5.2.9). To desiccate at 105 °C for 3 hours. At most 1.0%.

Sulfated ashes (5.2.10). At most 0.1 %. **DOSAGE**

To dissolve 0.25 g of the sample in 20 mL of dimethylformamide, add 0.2 mL of 1% bromothymol blue solution (w/v) in dimethylformamide, and titrate with 0.1 M SV sodium hydroxide solution to obtain blue color. To perform sample blank correction and adjust it as required. Each mL of 0.1 M SV sodium hydroxide is equal to 33.07 mg of $C_{12}H_{11}ClN_2O_5S$.

PACKAGING AND STORAGE

In tightly closed, opaque containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Diuretic.

FUROSEMIDE, TABLETS

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_{12}H_{11}ClN_2O_5S$.

IDENTIFICATION

The ultraviolet absorption spectrum (5.2.14) ranging from 220 nm to 320 nm, of final solution obtained in *Dosage*, exhibits maxima at 228 nm and 271 nm.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

Procedure for content uniformity. To individually weigh each tablet and crush. To quantitatively transfer it to 100 mL volumetric flask, add 0.1 M sodium hydroxide, stir, top up with the same solvent, and make it homogeneous. To filter, transfer 1 mL of the filtrate to a 50 mL volumetric flask, and top up with the same solvent. To prepare a standard solution at the same concentration by using the same solvent. To measure the absorbances at 271 nm (5.2.14), by using 0.1 M sodium hydroxide to adjust zero. To calculate the amount of $C_{12}H_{11}ClN_2O_5S$ in the tablet, from the readings obtained. Alternatively, make the calculations by taking into account that $A(1\%, 1\text{ cm}) = 580$ at 271 nm.

DISSOLUTION TEST (5.1.5)

Dissolution medium: phosphate buffer pH 8.6, 900 mL

Equipment: blades, 50 rpm

Time: 60 minutes

Procedure: after testing, remove aliquot from the dissolution medium, filter, and dilute with phosphate buffer pH 5.8 to obtain suitable concentration. To measure the absorbances of the solutions at 271 nm (5.2.14), by using the same solvent to adjust zero. To calculate the amount of $C_{12}H_{11}ClN_2O_5S$ dissolved in the medium, by comparing the readings obtained with that of 0.0008% furosemide SQR solution (w/v) prepared in the same solvent.

Tolerance: not less than 80% (Q) of the amount stated of $C_{12}H_{11}ClN_2O_5S$ dissolves within 30 minutes.

PURITY TESTS

Free primary aromatic amines. To pulverize the tablets, take from the powder the amount equivalent to 0.1 g of fu-

roseamide, and weigh it. To transfer to a 25 mL volumetric flask by using methanol.

To stir, top up with methanol, make it homogeneous, and filter it. To proceed as described in *Free primary aromatic amines*, in the monograph *Furosemide*, from "To pipette 1 mL of the filtrate..." The absorbance obtained is not greater than 0.20.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

to weigh and pulverize 20 tablets. To transfer amount of powder equivalent to 0.2 g of furosemide to a 500 mL volumetric flask, by using 300 mL of 0.1 M sodium hydroxide. To stir for 10 minutes. To top up with the same solvent, make it homogeneous, and filter it. To dilute 5 mL of the filtrate to 250 mL with 0.1 M sodium hydroxide and make it homogeneous. To prepare a standard solution at the same concentration by using the same solvent. To measure the absorbances of the resulting solutions at 271 nm (5.2.14), by using 0.1 M sodium hydroxide to adjust zero. To calculate the content of $C_{12}H_{11}ClN_2O_5S$ in the tablets, from the readings obtained. Alternatively, make the calculations by taking into account that $A(1\%, 1\text{ cm}) = 580$ at 271 nm.

PACKAGING AND STORAGE

In tightly closed, opaque containers.

LABELLING

To comply with the legislation in force.

FUROSEMIDE INJECTABLE SOLUTION

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_{12}H_{11}ClN_2O_5S$.

IDENTIFICATION

A. To transfer volume of injectable solution equivalent to 20 mg of furosemide to a 100 mL volumetric flask, top up to volume with water, and make it homogeneous. To dilute 5 mL of the solution to 100 mL with 0.1 M sodium hydroxide. The ultraviolet absorption spectrum (5.2.14), from 220 nm to 340 nm, of the resulting solution, has maxima at 228 nm and 271 nm, similar to those observed in the spectrum of a similar solution of furosemide SQR.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method **B**, corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

pH (5.2.19). 8.0 to 9.3.

PURITY TESTS

Free aromatic primary amines. To transfer volume of injectable solution equivalent to 40 mg of furosemide to a 10 mL volumetric flask, top up to volume with methanol, make it homogeneous, and filter it. To proceed as described in *Free primary aromatic amines*, in the monograph *Furosemide*, from “To pipette 1 mL of the filtrate...” The absorbance obtained is not greater than 0.20.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

Bacterial endotoxins (5.5.2.2). At most 3.6 UE/ mg of furosemide.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in Ultraviolet *absorption spectrophotometry (5.2.14)*. To pipette volume of injectable solution containing the equivalent to 20 mg of furosemide, transfer to a 100 mL volumetric flask, top up with water, and make it homogeneous. To dilute 5 mL to 100 mL with 0.1 M sodium hydroxide and make it homogeneous. To prepare a standard solution at similar concentration by using the same solvents. To measure the absorbances of the resulting solutions at 271 nm, by using 0.1 M sodium hydroxide to adjust zero. To calculate the amount of $C_{12}H_{11}ClN_2O_5S$ in the injectable solution, from the readings obtained. Alternatively, make the calculations taking into account that $A(1\%, 1\text{ cm}) = 580$ at 271 nm, in 0.1 M sodium hydroxide.

B. To proceed as described in *High performance liquid chromatography (5.2.17.4)*. To protect solutions from di-

rect light. To use chromatograph equipped with an ultraviolet detector at 272 nm; 150 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μm), kept at 30 °C; flow of *Mobile phase* of 1.0 mL/min.

Mobile phase: mixture of water, tetrahydrofuran, and glacial acetic acid (70:30:1).

Diluent: to dilute 22 mL of glacial acetic acid in a mixture of water and acetonitrile (50:50), top up to 1000 mL, and make it homogeneous.

Sample solution: to transfer volume of injectable solution equivalent to 20 mg of furosemide to a 50 mL volumetric flask, top up to volume with *Diluent*, and make it homogeneous.

To transfer 5 mL of this solution to 50 mL volumetric flask, top up with the same solvent, and make it homogeneous.

Standard solution: to dissolve accurately weighed amount of furosemide SQR in *Diluent* and properly dilute to obtain a 40 $\mu\text{g/mL}$ solution.

To inject replicates of 20 μL of the *Standard solution*. The relative standard deviation for the replicate areas for the peaks recorded is not greater than 2.0%.

Procedure: to separately inject 20 μL of *Sample solution* and *Standard solution*, record the chromatograms.

and measure the areas under the peaks. To calculate the content of $C_{12}H_{11}ClN_2O_5S$ in the injectable solution, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In type I glass containers, protected from light.

LABELLING

To comply with the legislation in force.



GAUZE, PETROLATUM

Petrolatum gauze is purified saturated hydrophilic gauze with white petrolatum. It is sterile and may be prepared under aseptic conditions at proportion petrolatum-gauze (60 g - 20 g), by adding melted white petrolatum gauze to hydrophilic, dry, purified, pre-cut gauze at the final size. The weight of the petrolatum gauze is at least 70% and at most 80% in relation to the total weight of petrolatum gauze.

Petrolatum recovered by drainage in *Dosage* has the same features and complies with the tests for *Description* and *Purity*, as described in the monograph *White petrolatum*.

CHARACTERISTICS

The packaged gauze obtained in *Dosage* complies with the tests for *Yarns count*, *Length*, *Width*, and *Weight* described in the monograph *Hydrophilic purified gauze*.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

DOSAGE

To weigh at least 20 units of sample and individually transfer them to warmed glass funnel, by keeping about 75 °C. Petrolatum should melt and drain through the funnel. The drainage can be facilitated by pressing the gauze with a glass rod or porcelain spatula. To wash the gauze over the funnel or porcelain spatula. To wash the gauze over funnel with successive portions of hot 1,1,1-trichloroethane to totally remove petrolatum. To wait the residual solvent to evaporate spontaneously. To keep the gauze in standard atmosphere of 65% ± 2% relative humidity and 21 °C ± 1.1 °C for at least 4h and weigh it. The difference between the two weighings represents the weight of petrolatum.

PACKAGING AND STORAGE

Each unit of petrolatum gauze is individually packaged to maintain its sterility before utilization.

LABELLING

To comply with the specific legislation in force.

GENTIAN

Gentianae rhizoma et radix

Gentiana lutea L. - GENTIANACEAE

The vegetable drug consists of dried, fragmented rhizomes and roots.

CHARACTERISTICS

Organoleptic characteristics. The drug has strong odor and bitter and persistent taste.

MACROSCOPIC DESCRIPTION

Rhizomes and roots are cylindrical pieces of different sizes. In general, rhizomes are larger than the roots, with up to 6 cm diameter. Roots are twisted or curved, with deep longitudinal strips and small and oval scars, coming from secondary branches. Rhizomes are strong; externally they have yellowish-brown to yellowish-gray and longitudinal cracks and numerous annular grooves, marked by rows of small scars. Rhizomes and roots expand considerably in contact with moisture, thus becoming flexible. The fracture is neither farinaceous nor fibrous; it is yellowish with reddish spot. In cross section, rhizome shows the cortical zone clearly demarcated by an outer suberous region with darker lines, which occupies 1/3 of the section. The yellow-brown central cylinder is porous and has fine radial striations.

MICROSCOPIC DESCRIPTION

The felema (bark) cells, in cross section, have thin yellowish-brown walls and are arranged in 4 - 8 layers. The pheloderm is composed of several layers, externally with chollenchyma and internally with parenchyma of cells tangentially elongated containing crystals of calcium oxalate as raphids and lipid droplets. This region gradually merges with the cortical parenchyma. The vascular system is separated from the cortical zone by well developed cambium. In the phloem, there are small groups of sieve tubes, in addition to parenchyma cells. The xylem is predominantly parenchyma and has dispersed vessel elements, with walls showing annular, helical, or crosslinked thickening. The vessel elements are isolated or in small groups; interxylem phloem (phloem included) is dispersed in small groups. The medulla of the rhizome is parenchyma and well developed. In the parenchyma cells, there are oil droplets and acicular crystals or thin prisms of calcium oxalate. Starch is almost completely absent. In the secondary structure, the anatomy of the root is similar to the rhizome.

The bark (including secondary phloem) and secondary xylem are separated by clear cambium and have a porous structure with few parenchyma beams.

MICROSCOPIC DESCRIPTION OF POWDER

The powder meets all the requirements for the species, unless the macroscopic characters. Characteristics: yellowish to yellow-brown; fragments of cells with lipid droplets; prismatic crystals or as raphides and free lipid droplets; fragments containing cambium; fragments containing parenchyma cells; lignified crosslinked, spiral, or scalariform vessels are rarely visible. Fibers and sclerids are absent.

IDENTIFICATION

To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF₂₅₄ silica gel with 250 µm thick as support, as stationary phase, and a mixture of acetone, methylene chloride, and water (70:30:2) as mobile phase. To separately apply to the chromatoplate, as band, 15 µl

- 20 µl of *Sample solution* and 5 µl - 10 µl of *Reference solution* prepared as described below.

Sample solution: To add 20 mL of methanol to 2 g of pulverized drug and stir for 20 minutes. To filter and evaporate the filtrate to dryness under reduced pressure at 50 °C (maximum). To dissolve the residue in 5 mL of methanol, which can contain a sediment.

Reference solution: 0.5% amarogentin solution (w/v) and 0.12% fentiopicroside (w/v) in methanol.

To develop the chromatogram. To remove the chromatoplate and allow it to dry in a current of air for 15 minutes.

To observe under ultraviolet light (254 nm). To nebulize the plate with vanillin sulfuric SR, heat at 100 °C - 105 °C for 5 minutes, and observe under visible light. The amarogentin spot is brownish, with a R_f around 0.50. In the *Sample solution*, there is also brown spots at the lower part and blue-violet spots in the upper part of the chromatogram.

PURITY TESTS

Foreign matter (5.4.2.2). At most 2%.

Water (5.4.2.3). At most 8%.

Total ashes (5.4.2.4). At most 6%.

Bitterness value (5.4.2.12). At most 10000.

To weigh 1 g of the pulverized drug and add 1000 mL of boiling water and put it in the water bath for 30 minutes, by occasionally stirring. To cool down and top up to 1000 mL with distilled water. To vigorously stir and filter it by discarding the first 20 mL of the filtrate.

Matter Extractable With Water

To weigh 5 g of the pulverized drug, add 200 mL of boiling water, and continuously stir for 10 minutes. To cool down and top up to 200 mL with distilled water. To filter it. To evaporate to dryness 20 mL of the filtrate. To dry the residue in an oven at 100 °C - 105 °C to obtain constant weight. The residue should weigh at least 0.165 g.

PACKAGING AND STORAGE

In tightly closed container, protected from light and heat.

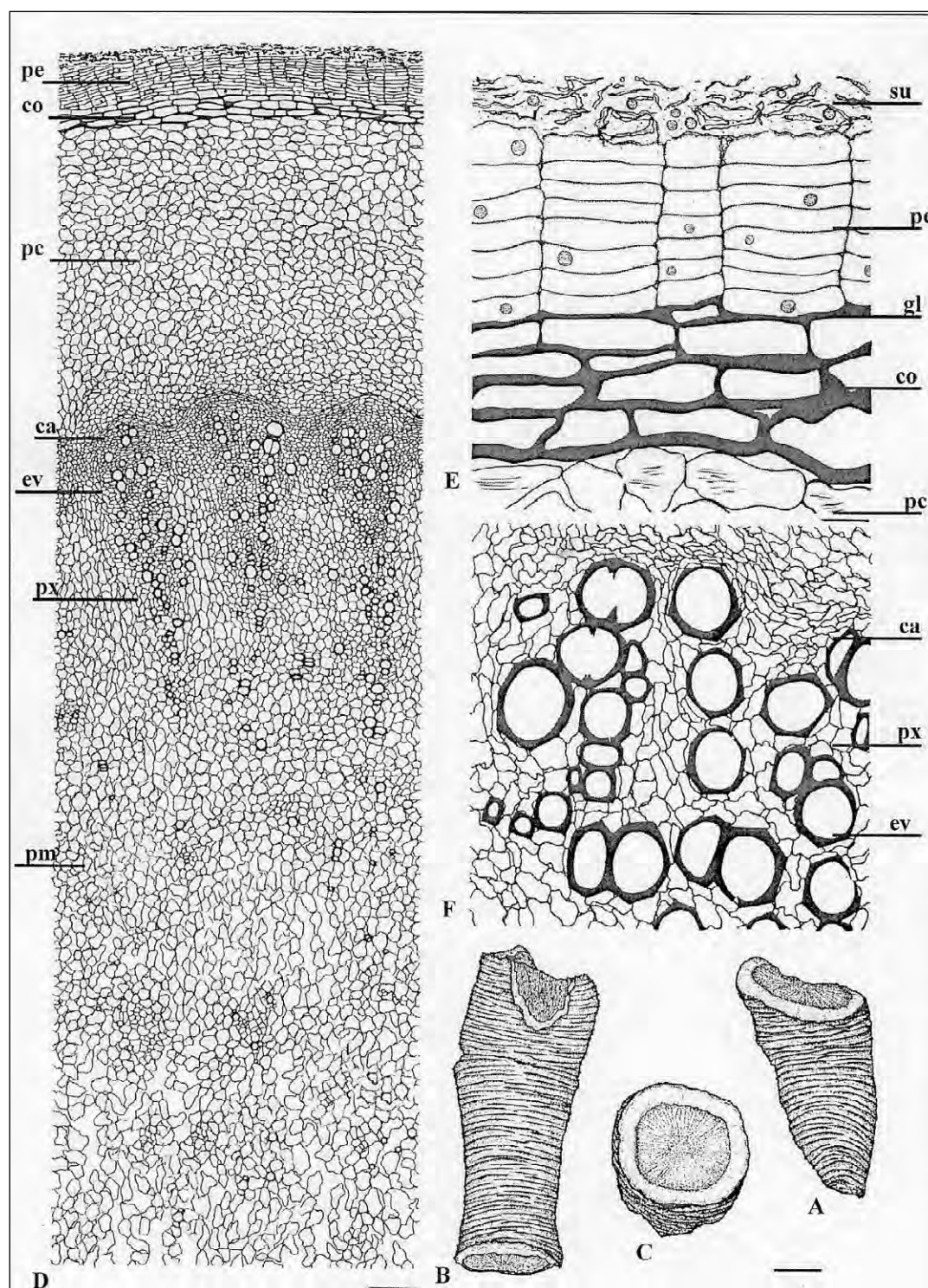


Figure 1 - Macroscopic and microscopic aspects in *Gentiana lutea* L.

Additional information of **Figure 1**. Scales correspond to 2cm in **A**, **B**, and **C**; in **D**, 150 µm; and **E** and **F**, 50 µm.

A and B - general appearance of rhizomes; **C** - general appearance of rhizome in cross section, **D** - detail of a portion of the rhizome in cross section: cambium (ca), collenchyma (co), vessel elements (ev), cortical parenchyma (pc), periderm (pe), medullary parenchyma (pm), xylem parenchyma (px), **E** - detail showing the region of felema and phellogen with parenchymal and collenchymatic portion: collenchyma (co) lipid droplets (gl), cortical parenchyma (cp), periderm (pe) bark (su); **F** detail of xylem showing xylem vessels. cambium (ca), vessel elements (ev), xylem parenchyma (px);

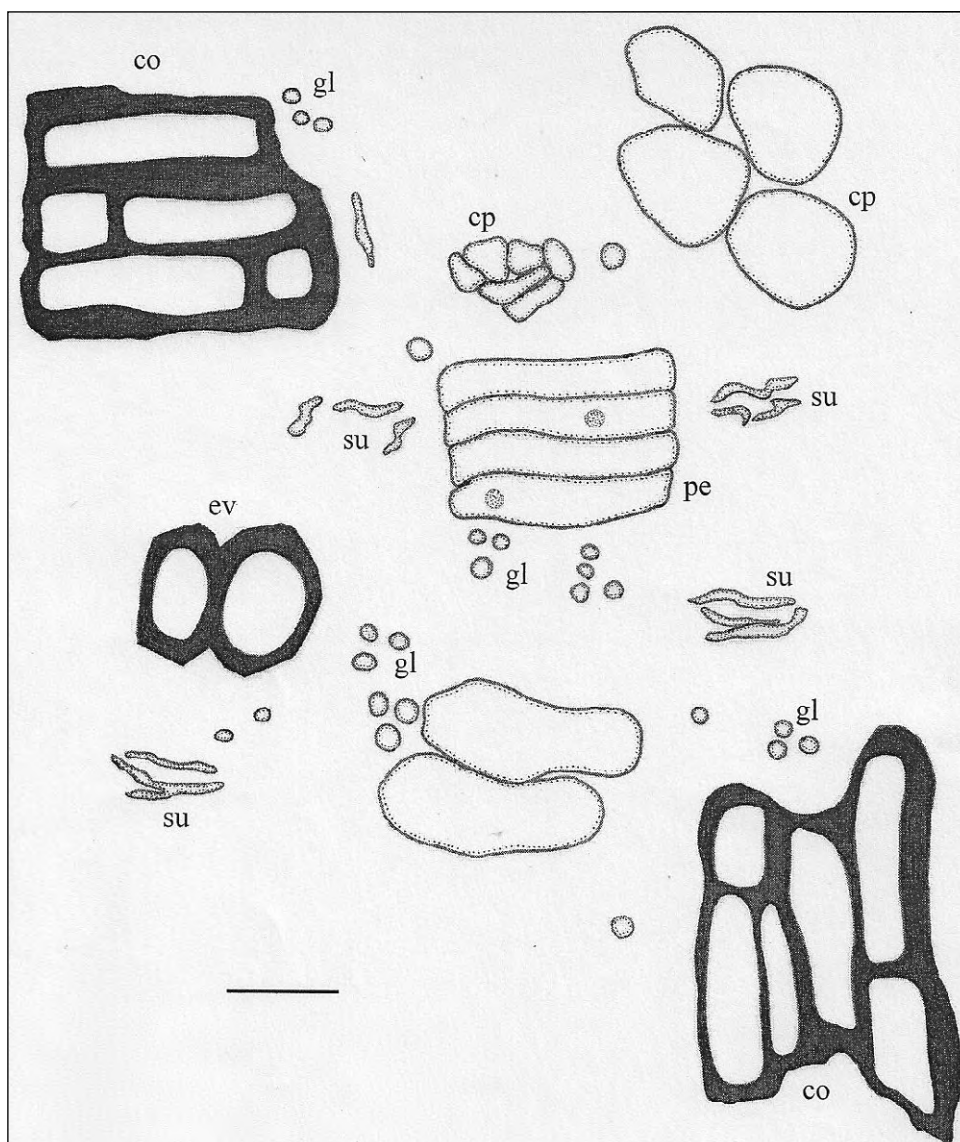


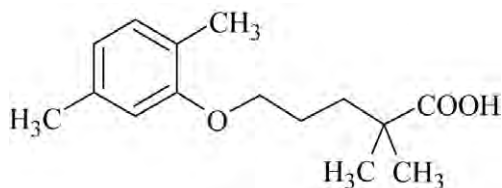
Figure 2 - Macroscopic and microscopic aspects in *Gentiana lutea* L.

Additional information of **Figure 2**. Scales correspond to 50 µm.

General appearance of the powdered drug: collenchyma (co); parenchyma cells (cp); vessel elements (ev); lipid droplets (gl); portions of periderm (pe); portions of bark (su).

GEMFIBROZIL

Gemfibrozilum



$C_{15}H_{22}O_3$; 250.33

gemfibrozil; 04421

5 - (2,5-dimethylphenoxy) -2,2-dimethylpentanoic acid
[25812-30-0]

It contains at least 98.0% and at most 102.0% of $C_{15}H_{22}O_3$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. Crystalline, white or almost white, waxy powder.

Solubility. Practically insoluble in water; freely soluble in methanol.

Physical and chemical constants.

Melting range (5.2.2): 58 °C to 61 °C.

IDENTIFICATION

The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the gemfibrozil SQR spectrum similarly prepared.

PURITY TESTS

Related substances. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 276 nm; 250 mm chromatograph column and 4 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at room temperature; flow of *Mobile phase* of 1.0 mL/min.

Mobile phase: to transfer 10 mL of glacial acetic acid and 750 mL of methanol to 1000 mL volumetric flask. To top up with water and make it homogeneous.

Solution (1): to dissolve accurately weighed amounts of gemfibrozil SQR, 5 - [2,5-dimethyl-4-(1-propenyl) phenoxy] -2,2-dimethylpentanoic acid SQR (Impurity A) and 2,5-dimethylphenol in *Mobile phase*, to obtain respectively about 0.2 mg/mL, 0.05 mg/mL, and 0.05 mg/mL concentrations.

Solution (2): to accurately transfer about 10 mg of gemfibrozil SQR and 10 mg of Impurity A SQR to a 100 mL volumetric flask, dissolve in 50 mL of methanol, top up to the volume with the same solvent, and make it homogeneous. To transfer 5 mL of this solution to 50 mL volumetric flask, top up with *Mobile phase*, and make it homogeneous.

Solution (3): to accurately transfer about 0.1 g of the sample to a 10 mL volumetric flask. To dissolve in *Mobile phase*, top up with the same solvent, and make it homogeneous.

To inject replicates of 100 µL of *Solution (1)*. The relative retention times are about 0.35 to 2,5-dimethylphenol, 1.0 to gemfibrozil, and 2.1 to Impurity A. The relative standard deviation for the replicate areas for the peaks recorded is not greater than 3.0%.

Procedure: to separately inject 100 µL of *Solutions (2) and (3)*, record the chromatograms at least 3 times the retention time of the gemfibrozil peak, and measure the areas under the peaks. To calculate the percentage of Impurity A in the sample according to the equation:

$$1000(C/W)(r_u/r_s)$$

where

C = concentration in mg/mL of Impurity A SQR in *Solution (2)*;

W = mass of sample, in mg, weighed to prepare the *Solution (3)*;

r_u and r_s = area under the peaks related to Impurity A obtained with *Solutions (3) and (2)*, respectively.

At most 0.1% of Impurity A. To calculate the percentage of other impurities in the sample according to the equation:

$$1000(C_G/W)(r_i/r_G)$$

where

C_G = concentration in mg/mL of gemfibrozil SQR in *Solution (2)*;

r_i = area under the peak of each individual impurity obtained in *Solution (3)*;

r_G = area under the gemfibrozil peak obtained in *Solution (2)*; W = mass of sample, in mg, weighed to prepare the *Solution (3)*;

At most 0.1% of any individual impurity. At most 0.5% of total impurities.

Heavy metals (5.3.2.3). To determine in 1 g of the sample by using *Method III*. At most 0.002% (20 ppm).

Water (5.2.20.1). To determine in 1.5 g of the sample. At most 0.25%.

Sulfated ashes (5.2.10). To determine in 2 g of the sample. At most 0.1%.

DOSAGE

To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 276 nm; 300 mm chromatograph column and 3.9 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at room temperature; flow of *Mobile phase* of 0.8 mL/min.

Mobile phase: to transfer 10 mL of glacial acetic acid and 800 mL of methanol to 1000 mL volumetric flask. To top up with water and make it homogeneous.

Sample solution: to accurately weigh about 25 g of the sample and transfer to a 25 mL volumetric flask. To dissolve in methanol and top up with the same solvent. To make it homogeneous. To transfer 5 mL of this solution to 25 mL volumetric flask and top up with *Mobile phase*. To make it homogeneous.

Standard solution: to accurately weigh about 25 mg of gemfibrozil SQR and transfer to a 25 mL volumetric flask. To dissolve in methanol and top up with the same solvent. To make it homogeneous. To transfer 5 mL of this solution to 25 mL volumetric flask and top up with *Mobile phase*. To make it homogeneous.

Resolution solution: to prepare 0.2 mg/mL gemfibrozil solution and 0.05 mg/mL of 2,5-dimethylphenol in *Mobile phase*.

To inject 10 µL of *Resolution solution*. The resolution between gemfibrozil and 2,5-dimethylphenol is not less than 8.0. To inject replicates of 10 µL of *Standard solution*. The relative standard deviation for the replicate areas for the peaks recorded is not greater than 2.0%.

Procedure: to separately inject 10 µL of *Sample and standard solutions*, record the chromatograms, and measure the area under the peaks. To calculate the content of $C_{15}H_{22}O_3$ from the sample, from the responses obtained for the *Sample and standard solutions*.

PACKAGING AND STORAGE

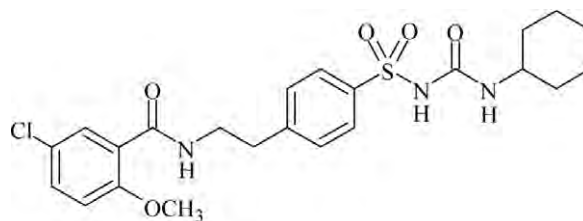
In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antilipemic.

GLIBENCLAMIDE
Glibenclamidum

$C_{23}H_{28}ClN_3O_5S$; 494.00

glibenclamide; 04451

5-Chloro-*N*-[2 - [4 - [[[cyclohexylamino) carbonyl] amino] sulfonyl] phenyl] ethyl]-2-methoxybenzamide [10238-21-8]

It contains at least 98.0% and at most 102.0% of $C_{23}H_{28}ClN_3O_5S$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or almost white, crystalline powder; odorless or almost odorless.

Solubility. Practically insoluble in water; soluble in dimethylformamide; slightly soluble in methylene chloride; sparingly soluble in chloroform, methanol, and ethanol; practically insoluble in ethyl ether.

Physical and chemical constants.

Melting range (5.2.2): 169 °C to 174 °C.

IDENTIFICATION

A. To proceed as described in *Infrared absorption spectrophotometry* (5.2.14). The Infrared absorption spectrum of the sample, dispersed in potassium bromide has absorption maximum at the same wavelength, with the same relative intensities to those observed in the glibenclamide SQR spectrum similarly prepared.

B. To dissolve about 50 mg of the sample in methanol, by using ultrasonic bath, if necessary, and top up to 50 mL with the same solvent. To transfer 10 mL to a 100 mL volumetric flask, add 1 mL of *M* hydrochloric acid, and top up with methanol. The ultraviolet absorption spectrum (5.2.14), ranging from 230 nm to 350 nm, of the resulting solution, exhibits maxima at 300 nm and 275 nm. The absorbance at 300 nm is 0.61 - 0.65 and at 275 nm is 0.27 - 0.32.

C. The principal spot in the chromatogram of *Solution (1)* in the *Related substances* corresponds in position to that obtained with *Solution (3)*.

D. To heat to boiling about 50 mg of sample with 1 mL of 6 *M* sodium hydroxide

The litmus paper changes from red to blue when moistened by the vapors, due to evaporation of water, which have irritating odor that is typical of amines.

E. To mix 0.2 g of the sample with 0.25 g of anhydrous sodium carbonate and 0.25 g of anhydrous potassium carbonate. To incinerate the mixture for 10 minutes, cool down, add 10 mL of hot water to the residue, stir for 1 minute, and filter it. Filtrate responds to the reactions of chloride and sulfate ion (**5.3.1.1**).

PURITY TESTS

Appearance of solution. The 1% solution (w/v) of the sample in ethanol, prepared by heating, is clear (**5.2.25**) and colorless (**5.2.12**).

Related substances. To proceed as described in *Thin-layer chromatography* (**5.2.17.1**) by using GF₂₅₄ silica gel as support and a mixture of chloroform, cyclohexane, ethanol, and glacial acetic acid (45:45:5:5) as mobile phase. To separately apply to the plate 10 µL of each of the solutions freshly prepared, as described below.

Solution (1): to dissolve 0.1 g of the sample in a mixture of chloroform and methanol (1:1) and top up to 5 mL with the same solvent.

Solution (2): to dissolve 20 mg of the sample in a mixture of chloroform and methanol (1:1) and top up to 100 mL with the same solvent. To dilute 2 mL to 10 mL with the same solvent.

Solution (3): to dissolve 0.2 g of glibenclamide SQR in 10 mL of methanol and heat it under reflux for 10 minutes.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). Any secondary spot in the chromatogram obtained with *Solution (1)* is not more intense than that obtained with *Solution (2)* (0.2%). The test is valid only if the chromatogram obtained with *Solution (3)* shows two principal spots clearly separated.

Heavy metals (5.3.2.3). To use *Method III*. At most 0.002% (20 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 105 °C for 6 hours. At most 1.0%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.5%.

DOSAGE

To employ one of the methods described below.

A. To accurately weigh about 0.5 g of the sample and dissolve in 100 mL of hot ethanol, previously neutralized with 0.1 M SV sodium hydroxide. To titrate with 0.1 M SV sodium hydroxide by using phenolphthalein SI as an indicator.

Each mL of 0.1 M SV sodium hydroxide is equal to 49.40 mg of C₂₃H₂₈ClN₃O₅S.

B. To proceed as described in *High performance liquid chromatography* (**5.2.17.4**), by using chromatograph equipped with an ultraviolet detector at 230 nm; 150 mm column and 4.6 mm inner diameter, packed with silica chemically bonded to the octylsilane group (5 µm), flow of *Mobile phase* of 1.5 mL/min.

Phosphate buffer pH 7.0: to dissolve 1.36 g of monobasic potassium phosphate in 900 mL of water, adjust the pH to 3.0 ± 0.1 with phosphoric acid SR, and dilute to 1000 mL with water.

Mobile phase: mixture of *Phosphate buffer pH 3.0* and acetonitrile (45:55).

Sample solution: to exactly transfer about 22 mg of sample to a 100 mL volumetric flask, add 70 mL of methanol, and put it in ultrasound for 20 minutes. To top up with the same solvent and make it homogeneous. To successively dilute with phosphate buffer pH 7.3 to obtain 5.5 µg/mL concentration.

Standard solution: to exactly transfer about 22 mg of glibenclamide SQR to a 100 mL volumetric flask, add 70 mL of methanol, and put it in ultrasound for 20 minutes. To top up with the same solvent and make it homogeneous. To successively dilute with phosphate buffer pH 7.3 to obtain 5.5 µg/mL concentration.

Procedure: to separately inject 10 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of C₂₃H₂₈ClN₃O₅S from the sample, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light, in cool and dry place.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Oral hypoglycemic.

GLIBENCLAMIDE TABLETS

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_{23}H_{28}ClN_3O_5S$.

IDENTIFICATION

A. To weigh and pulverize the tablets. To mix in mortar an amount of powder equivalent to 20 mg of glibenclamide with 20 mL of a mixture of methylene chloride and acetone (2:1).

To filter, evaporate the filtrate to dryness at room temperature, and dry it in an oven at 105 °C for 2 hours. The infrared absorption spectrum (5.2.14) of the residue, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the glibenclamide SQR spectrum similarly prepared.

B. To weigh and pulverize the tablets. To transfer amount of powder equivalent to 20 mg of glibenclamide to a 200 mL volumetric flask. To add 4 mL of 0.5 M hydrochloric acid and stir it. To add 100 mL of methanol, put it in ultrasound for 15 minutes, and mechanically stir for 15 minutes. To top up to volume with methanol and make it homogeneous. To filter it. The ultraviolet absorption spectrum (5.2.14), ranging from 230 nm to 350 nm, of the resulting solution, exhibits maxima at 275 nm and 300 nm.

C. The principal spot in the chromatogram of *Solution (1)* in the *Related substances* corresponds in position, color, and intensity to that obtained with *Solution (3)*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). Within 15 minutes.

Uniformity of dosage units (5.1.6). It complies with the test.

Procedure for content uniformity. To transfer each tablet to 25 mL volumetric flask To add 2.5 mL of water and wait for total disintegration of the tablet. To add 15 mL of methanol and put it in ultrasound for 20 minutes. To top up to volume with methanol and make it homogeneous. To filter it. To proceed as described in *Dosage*.

DISSOLUTION TEST (5.1.5)

Note: before testing, the dissolution medium must be heated at 41 °C and deaerated, by using vacuum filtration system by vigorously stirring. To keep stirring under vacuum for about 5 minutes after filtration in the system. To filter the aliquots of the dissolution medium by using 0.45 µm membrane compatible with the dissolution medium.

Dissolution medium: phosphate buffer pH 7.3, 900 mL

Equipment: blades, 75 rpm

Time: 60 minutes

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 230 nm; 150 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm); flow of *Mobile phase* of 1.5 mL/min.

Phosphate buffer pH 3.0: to dissolve 1.36 g of monobasic potassium phosphate in 900 mL of water, adjust the pH to 3.0 ± 0.1 with phosphoric acid, and dilute to 1000 mL with water.

Mobile phase: mixture of *Phosphate buffer pH 3.0* and acetonitrile (45:55).

Sample solution: after the test, remove aliquot from dissolution medium and filter.

Standard solution: to exactly transfer about 22 mg of glibenclamide SQR to a 100 mL volumetric flask, add 70 mL of methanol, and put it in ultrasound for 20 minutes. To top up with the same solvent and make it homogeneous. To successively dilute with phosphate buffer pH 7.3 to obtain 5.5 µg/mL concentration.

Procedure: to separately inject 10 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of $C_{23}H_{28}ClN_3O_5S$ dissolved in the medium from the responses obtained from *Sample and standard solutions*.

Tolerance: not less than 70% (Q) of the amount stated of $C_{23}H_{28}ClN_3O_5S$ dissolves within 60 minutes.

PURITY TESTS

Related substances. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF₂₅₄ silica gel as support and a mixture of chloroform, cyclohexane, ethanol, and glacial acetic acid (45:45:5:5) as mobile phase. To separately apply to the plate 10 µL of each of the solutions freshly prepared, as described below.

Solution (1): to weigh and pulverize the tablets. To stir in a mortar an amount of powder amount equivalent to 40 mg of glibenclamide with 20 mL of a mixture of methylene chloride and acetone (2:1) and filter it. To evaporate the filtrate to dryness at 40 °C, maximum, under vacuum. To dissolve the residue in 4 mL of mixture of chloroform and methanol (1:1).

Solution (2): 0.24 mg/mL glibenclamide SQR solution in a mixture of chloroform and methanol (1:1).

Solution (3): 10 mg/mL glibenclamide SQR solution in a mixture of chloroform and methanol (1:1).

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (2)* (2.4%).

DOSAGE

To proceed as described in *High performance liquid chromatography (5.2.17.4)*.

To use chromatograph equipped with an ultraviolet detector at 300 nm; 150 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octylsilane group (5 µm); flow of *Mobile phase* of 1.5 mL/min.

Phosphate buffer pH 3.0: to dissolve 1.36 g of monobasic potassium phosphate in 900 mL of water, adjust the pH to 3.0 ± 0.1 with phosphoric acid, and dilute to 1000 mL with water.

Mobile phase: mixture of *Phosphate buffer pH 3.0* and acetonitrile (47:53).

Sample solution: to weigh and pulverize 20 tablets. to exactly transfer amount of powder equivalent to about 5 mg of glibenclamide to a 25 mL volumetric flask, add 15 mL of a mixture of methanol and water (10:1), and put it in ultrasound for 20 minutes. To top up with the same solvent, make it homogeneous, and filter it.

Standard solution: to exactly transfer about 20 mg of glibenclamide SQR to a 100 mL volumetric flask, add 70 mL of a mixture of methanol and water (10:1), and put it in ultrasound for 20 minutes. To top up with the same solvent and make it homogeneous.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of $C_{23}H_{28}ClN_3O_5S$ in the tablets, from the responses obtained for the *Sample solution* and *Standard solution*.

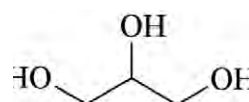
PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

GLYCEROL Glycerolum



$C_3H_8O_3$; 92.09
glycerol; 04469
1,2,3-propanetriol
[56-81-5]

It contains at least 98.0% and at most 101.0% of $C_3H_8O_3$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. Syrupy liquid, colorless or almost colorless, clear, hygroscopic.

Solubility. Miscible with water and ethanol; practically insoluble in benzene, chloroform, petroleum ether, fatty oils, and essential oils.

Physical and chemical constants. Relative density (5.2.5): 1.25 to 1.26.

IDENTIFICATION

To mix 1 mL of the sample and 0.5 mL of nitric acid. To add 0.5 mL of 10.6% potassium dichromate (w/v). A blue ring is developed in the contact surface develops which, during 10 minutes, does not spread in the bottom layer.

PURITY TESTS

Appearance of solution. To dilute 25 g of the sample to 50 mL with carbon dioxide-free water. Solution is clear (5.2.25). To dilute with water 10 mL of resulting solution to 50 mL. The solution is colorless (5.2.12).

Chlorinated compounds. To add 5 g of sample and 15 mL of morpholine to a condenser-adapted round bottom flask. To gently heat under reflux for 3 hours. To wash the condenser with 10 mL of water. To collect the washing in the flask. To transfer to Nessler tube. To acidify with nitric acid SR, add 0.5 mL of 0.5 M silver nitrate, and dilute to 50 mL with water. To stir. To prepare a standard into Nessler tube, by using 15 mL of morpholine, 10 mL of water and 0.2 mL of 0.02 M hydrochloric acid. To proceed as described to prepare sample from "To acidify..." Any turbidity developed in the sample preparation is not more intense than that obtained with standard preparation. At most 0.003% (30 ppm).

Acrolein, glucose, and ammonium compounds. To mix 5 mL of sample and 5 mL of 10% potassium hydroxide (w/v). To heat at 60 °C for 5 minutes. No ammonia vapors are released. No yellow color develops.

Other reducing substances. To mix 5 mL of sample with 5 mL of 10% ammonium hydroxide (w/v) and heat it at 60 °C

for 5 minutes. To rapidly add 0.5 mL of 0.1 *M* silver nitrate, by keeping the pipette tip above the pipe, thus the solution falls directly over the solution without touching the walls of the tube. To stir and keep it at dark place for 5 minutes. No darkening occurs.

Fatty acids and esters. To mix 50 g of sample with 100 mL of hot water freshly boiled. To add 1 mL of phenolphthalein SI and neutralize with 0.1 *M* sulfuric acid. To add 15 mL of 0.2 *M* sodium hydroxide. To heat it under reflux for 5 minutes, cool down, and titrate with 0.1 *M* SV sulfuric acid. To perform sample blank correction by using 140 mL of water freshly boiled. The difference between titrations is not greater than 1.6 mL.

Sucrose. To add 6 mL of 0.5 *M* sulfuric acid to 4 mL of sample. To heat it for 1 minute, cool down, and neutralize with sodium hydroxide SR by using litmus paper.

To add 5 mL of alkaline cupric tartrate SR and heat to boiling for 1 minute. No orange-red precipitate is formed.

Arsenic (5.3.2.5). To proceed as described in *Visual method*. At most 0.00015% (1.5 ppm).

Chlorides. To add 0.25 mL of nitric acid SR and 0.5 mL of 0.1 *M* silver nitrate to 10 mL of 10% solution of the sample (w/v). No turbidity occurs.

Heavy metals (5.3.2.3). To mix 4 g of the sample with 2 mL of 0.1 *M* hydrochloric acid and dilute with water to 25 mL. At most 0.0005% (5 ppm).

Sulfates. To add 3 drops of hydrochloric acid SR and 5 drops of barium chloride SR to 10 mL of 10% solution of the sample (w/v). No turbidity occurs.

Water (5.2.20.1). To determine in 1 g of the sample. At most 2.0%.

Sulfated ashes (5.2.10). To determine in 5 g of the sample. At most 0.01%.

DOSAGE

To exactly weigh about 0.1 g of the sample, transfer to 250 mL erlenmeyer flask, and dissolve in 45 mL of water. To add 25 mL of a mixture of 0.1 *M* sulfuric acid and 2.14% sodium periodate (w/v) (1:20) and let it rest for 15 minutes protected from light. To add 5 mL of 50% ethylene glycol (w/v) and let it rest for 20 minutes. To protect it from light. To titrate with 0.1 *M* SV sodium hydroxide by using 0.5 mL of phenolphthalein SI. To perform sample blank correction and adjust it as required. Each mL of 0.1 *M* SV sodium hydroxide is equal to 9.210 mg of $C_3H_8O_3$.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

CATEGORY

Humectant, solvent.

GLYCEROL SUPPOSITORIES

It contains at least 75.0 % and at most 90 % of the amount stated of $C_3H_8O_3$. It contains sodium stearate.

IDENTIFICATION

A. To dissolve 12 suppositories in 125 mL of water, by heating it. To cool down, add 1.5 mL of hydrochloric acid, and transfer the mixture to a 250 mL separatory funnel.

To extract with 75 mL of hexane, discard the aqueous layer, and collect the organic layer to a beaker. To evaporate to dryness in water bath. The infrared absorption spectrum (5.2.14) of the residue, dispersed in mineral oil, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the stearic acid SQR spectrum similarly prepared.

B. To dissolve 1 g of sodium borate decahydrate in 100 mL of water, add 25 drops of phenolphthalein SI, and make it homogeneous. To add 2 drops of a previously melted suppository to a test tube containing 0.5 mL of this solution. Intense pink color is completely discolored. When the solution is heated, pink color fades in.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

PURITY TESTS

Water (5.2.20.1). At most 15.0%.

DOSAGE

To weigh number of suppositories equivalent 0.25 g of glycerin, dissolve in water, top up to 250 mL, and filter it. To transfer 5 mL of this solution to erlenmeyer flask, add 50 mL of a reagent prepared by mixing 40 mL of 5% sulfuric acid (v/v) and 60 mL of 0.1% potassium periodate (w/v) acidified with 3-5 drops of sulfuric acid. To heat the solution in water bath for 15 minutes, cool down at room temperature, and add 1 g of potassium iodide. To let it rest for 5 minutes. To titrate with 0.02 *M* SV sodium thiosulfate by using starch SI as an indicator. To perform sample blank correction and adjust it as required. Each mL of 0.02 *M* SV sodium thiosulfate is equal to 0.4604 mg of $C_3H_8O_3$.

PACKAGING AND STORAGE

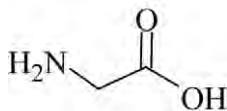
In airtight, opaque containers.

LABELLING

To comply with the legislation in force.

GLYCINE

Glycinum



$C_2H_5NO_2$; 75,07
glycine; 04472
Glycine
[56-40-6]

It contains at least 98.5% and at most 101.5% of $C_2H_5NO_2$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white, odorless powder with sweet taste.

Solubility. Freely soluble in water, sparingly soluble in ethanol, very slightly soluble in ethyl ether.

Physical and chemical constants.

Melting range (5.2.2): 232 °C to 236 °C with decomposition. IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample desiccated at 105 °C, for 2 hours, dispersed in mineral oil, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the glycine SQR spectrum similarly prepared.

B. To prepare 2 mL of a 10% solution (w/v) in water and add 1 mL of ferric chloride SR. An intense red color is observed, which fades out by adding hydrochloric acid in excess and fades in by adding concentrated ammonia solution in excess.

C. To prepare 5 mL of a 0.1% solution (w/v) in water and add 1 mL of cupric sulfate SR. An intense blue color is observed.

D. To prepare 5 mL of a 10% solution (w/v) in water and add 5 drops of hydrochloric acid SR and 5 drops of 50% sodium nitrite (w/v). A strong release of colorless gas is observed.

PURITY TESTS

Appearance of solution. To boil 10 mL of a 10% (w/v) for 1 minute and let it rest for 2 hours. The solution is clear (5.2.25) and colorless (5.2.12).

Chlorides (5.3.2.1). To proceed as described in *Limit test for chlorides*. To determine in 5 g of the sample. At most 0.007% (70 ppm).

Heavy metals (5.3.2.3). To use the *Method I*. To prepare 12 mL of a 10% solution (w/v) of the sample in water and proceed according to *Limit test for heavy metals*. To use the *Lead standard solution (1 ppm Pb)*. At most 0.001% (10 ppm).

Sulfates (5.3.2.1). To dissolve 4 g of the sample in 40 mL of water and proceed as described in *Limit test for sulfates*, by using 0.5 mL of standard solution of 0.005 M sulfuric acid. At most 0.0065% (65 ppm).

Readily carbonisable substances. To dissolve 0.5 g of glycine in 5 mL of sulfuric acid. The solution should be colorless.

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 105 °C for 2 hours. At most 0.2%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To accurately weigh about 0.15 g of sample, dissolve in 100 mL of glacial acetic acid, and gently heat to facilitate solubilization. To add 2 drops of methylrosanilinium chloride SI and titrate with 0.1 M SV perchloric acid to obtain greenish-blue color. To perform sample blank correction and adjust it as required. Each mL of 0.1 M SV perchloric acid is equal to 7.507 mg of $C_2H_5NO_2$.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

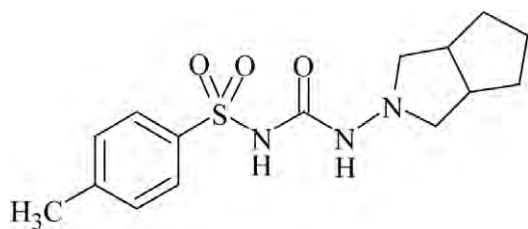
To comply with the legislation in force.

THERAPEUTIC CLASS

Nonessential amino acid.

GLICLAZIDE

Gliclazidum



$C_{15}H_{21}N_3O_3S$; 323.41
gliclazide; 04474

N - [[1-(Hexahydro-cyclopenta [c] pyrrol-2 (1*H*)-yl) amino] carbonyl]-4-methyl-benzenesulfonamide
[21187-98-4]

It contains at least 98.0% and at most 101.0% of $C_{15}H_{21}N_3O_3S$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white or almost white powder.

Solubility. Practically insoluble in water, freely soluble in methylene chloride, slightly soluble in acetone, and sparingly soluble in ethanol.

IDENTIFICATION

The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the gliclazide SQR spectrum similarly prepared.

PURITY TESTS

Related substances. To proceed as described in *High performance liquid chromatography* (5.2.17.4). To immediately prepare the solutions before utilization. To use chromatograph equipped with an ultraviolet detector at 235 nm; 250 mm chromatograph column and 4 mm inner diameter, packed with silica chemically bonded to the octylsilane group (5 μ m), kept at room temperature; flow of *Mobile phase* of 0.9 mL/min.

Mobile phase: mixture of triethanolamine, trifluoroacetic acid, acetonitrile, and water (0.1:0.1:45:55).

Solution (1): to accurately dissolve about 50 mg of the sample in 23 mL of acetonitrile and dilute to 50 mL with water.

Solution (2): to dilute 1 mL of *Solution (1)* to 100 mL of acetonitrile and water (45:55). To dilute 10 mL of the resulting solution to 100 mL with the same diluent.

Solution (3): To accurately dissolve about 5 mg of sample and 15 mg of 1 - (hexahydro cyclopenta [c] pyrrol-2 (1*H*) -

yl) -3 - [(2-methylphenyl) sulfonyl] urea SQR in 23 mL of acetonitrile and dilute to 50 mL with water. To dilute 5 mL of the resulting solution to 100 mL with mixture of acetonitrile and water (45:55).

Solution (4): To accurately dissolve about 10 mg of 1 - (hexahydro cyclopenta [c] pyrrol-2 (1*H*) - yl) -3 - [(2-methylphenyl) sulfonyl] urea SQR in 45 mL of acetonitrile and dilute to 100 mL with water. To dilute 1 mL of the resulting solution to 100 mL with mixture of acetonitrile and water (45:55).

To inject 20 μ L of *Solution (3)*. The resolution between 1 - (hexahydro cyclopenta [c] pyrrol-2 (1*H*) - yl) -3 - [(2-methylphenyl) sulfonyl] urea and gliclazide peaks is greater than 1.8.

Procedure: to separately inject 20 μ L of *Solution (1)*, *Solution (2)*, and *Solution (4)*. Then, run the chromatograms of *Solution (1)* twice the retention of gliclazide, record the chromatograms, and measure the areas under the peaks. In the chromatogram obtained with *Solution (1)*, the area of all peaks corresponding to 1 - (hexahydro cyclopenta [c] pyrrol-2 (1*H*) - yl) -3 - [(2-methylphenyl) sulfonyl] urea is not greater than the area under the peak obtained with *Solution (4)* (0.1%). The sum of all the peaks obtained with *Solution (1)*, except the main peak's one and the 1 - (hexahydro cyclopenta [c] pyrrol-2 (1*H*) - yl) -3 - [(2-methylphenyl) sulfonyl] urea peak, is not greater than the area under the main peak obtained with *Solution (2)* (0.1%). The sum of all the peaks obtained with *Solution (1)* is not greater than twice the area under the main peak obtained with *Solution (2)* (0.2%). To disregard the peaks with an area less than 0.2 times the area under the main peak obtained with *Solution (2)* (0.02%).

Heavy metals (5.3.2.3). To use *Method IV*. At most 0.001% (10 ppm). To prepare a lead standard solution (10 ppm Pb).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 100-105 $^{\circ}$ C for 2 hours. At most 0.25 %.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1 %.

DOSAGE

To accurately weigh about 0.25 g of the sample previously desiccated and dissolve in 50 mL of glacial acetic acid. To titrate with 0.1 M SV perchloric acid, to potentiometrically determine the endpoint. Each mL of 0.1 M SV perchloric acid is equal to 32.341 mg of $C_{15}H_{21}N_3O_3S$.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

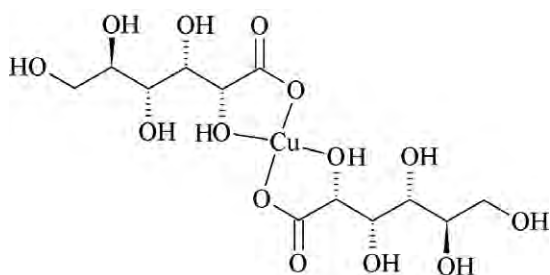
To comply with the legislation in force.

THERAPEUTIC CLASS

Oral antidiabetic.

GLUCONATE, COPPER

Cupri gluconas



$C_{12}H_{22}CuO_{14}$; 453.84
copper gluconate; 04479
Bis (D-gluconate- KO^1 , KO^2)- copper
[527-09-3]

It contains at least 98.0% and at most 102.0% of $C_{12}H_{22}CuO_{14}$.

DESCRIPTION

Physical characteristics. Blue-greenish, fine powder.

Solubility. Freely soluble in water; insoluble in ethanol and benzene.

Physical and chemical constants. *Melting range* (5.2.2): 155 °C to 157 °C.

IDENTIFICATION

A. It responds to the reactions of copper ion (5.3.1.1).

B. It responds to the reactions of calcium ion (5.3.1.1).

PURITY TESTS

Reducing substances. To weigh 1 g of sample, dissolve in 10 mL of water, and add 25 mL of alkaline cupric citrate SR. To cover the vial, gently boil for 5 minutes, and quickly cool down to room temperature. To add 25 mL of 0.6 M acetic acid, 10 mL of 0.1 M SV iodine, and 10 mL of 3 M hydrochloric acid. To titrate with 0.1 M SV sodium thiosulfate, add 3 mL of starch SI near the endpoint. To perform sample blank correction and note the required difference in volumes. Each mL of the difference in volume of 0.1 M SV sodium thiosulfate is equivalent to 2.7 mg of reducing substances (expressed as dextrose). At most 1 %.

Chlorides (5.3.2.1). To dissolve 0.5 g of the sample in 40 mL of boiling water and proceed as described in *Limit test for chlorides*. At most 0.07% (700 ppm).

Sulfates (5.3.2.1). To dissolve 2.4 g of the sample in 40 mL of boiling water and proceed as described in *Limit test for sulfates*. At most 0.05% (500 ppm).

Arsenic (5.3.2.5). To weigh 1 g of the sample and proceed as described in *Method I of Limit test for arsenic*. At most 0.0003% (3 ppm).

Lead. To proceed as described in *Method I of Atomic absorption spectrometry (5.2.13.1)*. To use flame spectrophotometer with graphite furnace, hollow lead cathode lamp, and select the emission line at 283.3 nm. To use the following temperature list, by using the argon flow rate of 3 liters per minute, unless otherwise indicated: 70 °C for 10 seconds, 90 °C for 60 seconds, 120 °C for 15 seconds, 250 °C for 5 seconds (without gas flow), 250 °C for 10 seconds, 250 °C for 2 seconds (without gas flow), and 2000 °C for 3.2 seconds. To determine the absorbance for the last temperature.

Standard solution: to transfer 10 mL of lead SRA to a 100 mL volumetric flask. To add 40 mL of water and 5 mL of nitric acid. To top up with water and mix it. To transfer 0.4 mL of this solution to another volumetric flask. To add 50 mL of water and 1 mL of nitric acid. To top up with water and mix it. This solution contains 0.04 µg/mL of lead.

Sample solution: to accurately transfer about 4 mg of copper gluconate to a 100 mL volumetric flask. To add 50 mL of water and 5 mL of nitric acid. To put it in ultrasonic bath to dissolve the substance. To top up with water and mix it. To transfer 4 mL of this solution to another 100 mL volumetric flask. To add 50 mL of water and 1 mL of nitric acid. To top up with water and mix it.

Blank solution: to transfer 1.2 mL of nitric acid to a 100 mL volumetric flask. To top up with water and mix it.

To prepare analytical solutions from the *Standard solution*, *Sample solution*, and *Blank solution* according to the following proportions by volume: 10:0:10; 10:4:6; 10:7:3, and 10:10:0. These solutions contain respectively 0; 0.008; 0.014, and 0.020 µg/mL of lead. to separately inject 20 µL of Blank solution and each analytical solution. To transfer the results of absorbance and the concentrations corresponding to a graph and calculate the concentration of the *Sample solution*.

DOSAGE

To accurately weigh about 1.5 g of the sample and dissolve in 100 mL of water. To add 2 mL of glacial acetic acid and 5 g of potassium iodide. To titrate with 0.1 M SV sodium thiosulfate to obtain light yellow color. To add 2 g of ammonium thiocyanate. To mix and add 3 mL of starch SI. To proceed the titration until the color changes. Each mL of 0.1 M SV sodium thiosulfate is equal to 45.384 mg of $C_{12}H_{22}O_{14}Cu$.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

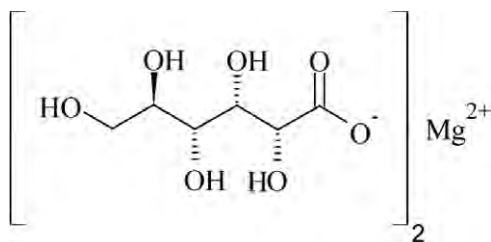
To comply with the legislation in force.

THERAPEUTIC CLASS

Food supplement.

GLUCONATE, MAGNESIUM

Magnesii gluconas



$C_{12}H_{22}MgO_{14}$; 414.60

$C_{12}H_{22}MgO_{14} \cdot 2H_2O$; 450.63

magnesium gluconate; 04480

Magnesium salt of D-gluconic acid (1:2)

[3632-91-5] *Magnesium salt of D-gluconic acid hydrate*
(1:2)

[59625-89-7]

It contains at least 98.0% and at most 102.0% of $C_{12}H_{22}MgO_{14}$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White powder.

Solubility. Soluble in water; slightly soluble in ethanol; insoluble in ethyl ether.

IDENTIFICATION

A. It responds to the reactions of magnesium ion (5.3.1.1).

B. It responds to the reactions of calcium ion (5.3.1.1).

PURITY TESTS

pH (5.2.19). 6.0 to 7.8. To determine in 5 % solution (w/v).

Reducing substances. To weigh 1 g of sample, dissolve in 20 mL of hot water, cool down, and add 25 mL of alkaline cupric citrate SR. To cover the vial, gently boil for 5 minutes, and quickly cool down to room temperature. To add 25 mL of 2 M acetic acid, 10 mL of 0.1 M SV iodine, and 10 mL of 3 M hydrochloric acid. To titrate with 0.1 M SV sodium thiosulfate and add 3 mL of starch SI near the endpoint. To perform sample blank correction and note the required difference in volumes. Each mL of the difference in volume of sodium thiosulfate solution is equivalent to 2.7 mg of reducing substances (expressed as dextrose). At most 1 %.

Volatile organic impurities. To proceed as described in *Gas chromatography* (5.2.17.5). To use chromatograph equipped with flame ionization detector, by using mixture of nitrogen, synthetic air, and hydrogen (1:1:10) as the auxiliary gas to flame detector; 30 m long capillary column and 0.53 mm inner diameter, filled with stationary linked phase of phenyl polysiloxane and methyl polysiloxane (5:95), with a film thickness of 5 μ m; column temperature from 35 °C to 260 °C (35 °C kept for 5 minutes, then rises to 175 °C at 8 °

C per minute, and rises to 260 °C at 35 °C and kept at this temperature for at least 16 minutes); injector temperature at 70 °C and the detector temperature at 260 °C; use helium as carrier gas; flow of carrier gas of 1 mL/minute.

Sample solution: To accurately dissolve about 1 g of sample in 50 mL of water, free of organic compounds.

Standard solution: to prepare a solution in water free of organic compounds containing, in each mL, 10 μ g of methylene chloride, 1 μ g of chloroform, 2 μ g of benzene, 2 μ g of dioxane, and 2 μ g of trichlorethylene to separately inject 1 μ L of *Sample solution* and *Standard solution* into the gas chromatograph. To obtain the chromatograms and measure the area under the peaks. Based on the retention time, identify any peak in the chromatogram of the sample solution. To compare the chromatograms of the Sample solution and Standard solution to establish the presence and identification of peaks in the chromatogram. Limit: Benzene (2 ppm), chloroform (50 ppm), dioxane (100 ppm), methylene chloride (500 ppm), and trichlorethylene (80 ppm). It complies with the test.

Arsenic (5.3.2.5). To use *Method III*. To dissolve 1.0 g of sample in 35 mL of water. To proceed according to *Limit test for arsenic*. At most 0.0003% (3 ppm).

Chlorides (5.3.2.1). To weigh 0.7 g of the sample and proceed as described in *Limit test for chlorides*. At most 0.05% (500 ppm).

Sulfates (5.3.2.1). To dissolve 2.4 g of the sample in 40 mL of boiling water and proceed as described in *Limit test for sulfates*. At most 0.05% (500 ppm).

Heavy metals (5.3.2.3). To use *Method I*. To dissolve 1.0 g of sample in 10 mL of water, add 6 mL of 3.0 M hydrochloric acid, and top up to 25 mL with water. To proceed as described in *Limit test for heavy metals*. At most 0.002% (20 ppm).

Water (5.2.20.1). To use *Indirect method*. At most 12.0 %.

DOSAGE

To accurately weigh about 800 mg of the sample and dissolve in 20 mL of water. To add 5 mL of ammonium chloride SR and 0.1 mL of eriochrome black T SI. To titrate with 0.05 M SV disodium edetate until color becomes blue.

Each mL of 0.05 M disodium edetate is equal to 20.730 mg of $C_{12}H_{22}MgO_{14}$.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

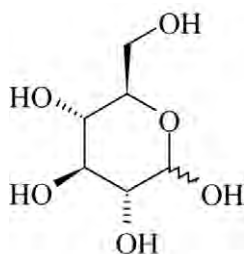
LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Food supplement.

GLUCOSE
Glucosum

 $C_6H_{12}O_6$; 180.16 $C_6H_{12}O_6 \cdot H_2O$; 198.17

glucose; 04485

glucose monohydrate, 04486

D-Glucose

[50-99-7]

D-Glucose hydrate (1:1)

[77938-63-7]

It contains at least 99.0% and at most 101.5% of $C_6H_{12}O_6$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White, crystalline powder, or colorless crystals; odorless; sweet taste.

Solubility. Freely soluble in water, and slightly soluble in ethanol.

Physical and chemical constants.

Specific rotation (5.2.8): +52.5° to +53.5°, in relation to the desiccated substance. To determine in 10% solution (w/v) in 0.012 M ammonium hydroxide.

IDENTIFICATION

A. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using G silica gel as support and a mixture of *n*-propyl alcohol, ethyl acetate, and water (v/v) (70:20:10) as mobile phase. To prepare the following solutions.

Solution (1): To dissolve 0.1 g of the sample in water and top up the volume to 10 mL.

Solution (2): To dissolve 0.1 g of glucose SQR in water and top up the volume to 10 mL.

To separately apply 2 µL of *Solution (1)* and *Solution (2)* to the chromatographic plate. To develop the chromatogram by allowing the solvent front rises 17 cm above the line of application, remove the plate from the tank, and air dry.

To nebulize with a 0.2% sodium periodate solution (w/v). To dry the plate in air for 15 minutes and nebulize with of 2% 4,4-methylene-bis-*N,N*-dimethylaniline solution (w/v) in a mixture of 20 volumes of glacial acetic acid and 80 volumes of acetone. The principal spot obtained in the chromatogram with *Solution (1)* corresponds in position, color, and intensity to that obtained in the chromatogram with *Solution (2)*.

B. To dissolve 0.1 g of the sample in 10 mL of water. To add 3 mL of alkaline cupric tartrate SR and heat. Red precipitate is produced.

PURITY TESTS

Appearance of solution. To dissolve 12.5 g of the sample in water and top up the volume to 25 mL. The solution obtained is not more intensely colored (5.2.12) in relation to the solution prepared by mixing 1 mL of cobaltous chloride SR, 3 mL of ferric chloride SR, and 2 mL of cupric sulfate SR in sufficient amount of water to 10 mL, then diluting 1.5 mL of this solution with water to 25 mL. To compare over a white background in Nessler tubes.

Acidity. To dissolve 5 g of the sample in 50 mL of carbon dioxide-free water, add phenolphthalein SI, and titrate with 0.02 M SV sodium hydroxide to obtain pink color. At most 0.3 mL of titrant is spent for neutralization.

Dextrins and low soluble sugars. To dissolve 1 g of the pulverized sample in 30 mL of 90% ethanol (v/v) and, by stirring, heat it into a reflux flask. After cooling, solution remains clear.

Soluble starch and sulphites. To dissolve 1 g of sample in 10 mL of water and add a drop of 0.1 M SV iodine. solution becomes yellow and not blue color is developed.

Arsenic (5.3.2.5). To determine in 1 g of the sample. At most 0.0001% (1 ppm).

Chlorides (5.3.2.1). To determine in 2 g of the sample. At most 0.018% (180 ppm).

Sulfates (5.3.2.2). To determine in 2 g of the sample. To use 1 mL of 0.005 M sulfuric for the *Standard preparation*. At most 0.025% (250 ppm).

Heavy metals (5.3.2.3). To conduct test in a solution dissolved in 4 g in water and top up the volume to 25 mL. At most 0.0005% (5 ppm).

Water (5.2.20.1). To determine in 0.5 g of the sample. At most 1.0% to anhydrous glucose and 7.0% - 9.5% to glucose monohydrate.

BIOLOGICAL SAFETY TEST

Note: Sample complies with the following additional testing when the substance is for parenteral preparations

without any appropriate treatment for removing bacterial endotoxin.

Pyrogens (5.5.2.1). It complies with the test. To inject 10 mL/kg by using 50 mg/mL glucose solution in water for injection.

DOSAGE

To accurately weigh about 0.1 g of the sample and dissolve in 50 mL of water, in an erlenmeyer flask with ground lid. To add 25 mL of 0.05 M SV iodine and 10 mL of 5% sodium carbonate solution (w/v). To make it homogeneous and let it rest for 20 minutes, protected from light. To add 15 mL of diluted hydrochloric acid and titrate the iodine in excess with 0.1 M SV sodium thiosulfate, by using starch SI as indicator. To perform sample blank correction and adjust it as required. Each mL of 0.05 M SV iodine is equal to 9.008 mg of $C_6H_{12}O_6$ and 9.909 mg of $C_6H_{12}O_6 \cdot H_2O$.

PACKAGING AND STORAGE

In tightly closed containers, protected from light, at room temperature.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Sweetener, energetic, excipient.

GLUCOSE INJECTABLE SOLUTION

It contains at least 95.0% and at most 105.0% of the amount stated of $C_6H_{12}O_6$. The injectable solution of glucose is a sterile, colorless anhydrous glucose or glucose monohydrate in water for injection. It contains no antimicrobial agents.

IDENTIFICATION

A. To heat a portion of injectable solution with alkaline cupric tartrate SR. Red precipitate is formed.

B. The solution obtained in *Dosage* is dextral (5.2.8).

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

pH (5.2.19). 3.2 to 6.5. To determine in solution containing the equivalent to 5% of $C_6H_{12}O_6$ (w/v). To dilute the sample with water for injection, if necessary. To add 0.3 mL of saturated potassium chloride solution per 100 mL of solution.

PURITY TESTS

5-hydroxymethylfurfural and related substances. To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. To dilute volume of injectable solution containing the equivalent to 1 g of $C_6H_{12}O_6$ to 250 mL with water. The absorbance at 284 nm is not greater than 0.25.

Particle contamination (5.1.7). To use *Method I, Sub-visible particles (5.1.7.1)*. It complies with the *test A or B*, as per the volume of containers.

Heavy metals (5.3.2.3). To transfer volume of injection solution equivalent to 4 g of glucose to a suitable container and adjust the volume to 25 mL by evaporation or adding water as needed. At most 0.0005% (5 ppm).

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

Bacterial endotoxins (5.5.2.2). At most 0.25 UE/ mL of injectable solution. To dilute with water for injection, if necessary, to 5% $C_6H_{12}O_6$ (w/v)

DOSAGE

To proceed as described in *Determination of rotation and specific rotation (5.2.8)*. To accurately transfer volume of injectable solution containing between 2 g and 5 g of $C_6H_{12}O_6$ to 100 mL volumetric flask, add 0.2 mL of 5 M ammonia, and top up with water. To make it homogeneous and let it rest for 30 minutes. To determine the optical rotation in 2 dm tube. The rotation angle multiplied by 0.9477 is the mass of $C_6H_{12}O_6$ in the volume used.

PACKAGING AND STORAGE

In tightly closed containers; single-dose; plastic or type I or II glass, preferably.

LABELLING

To comply with the legislation in force.

GUARANA Paullinae semen

Paullinia cupana Kunth - SAPINDACEAE

The vegetable drug consists of seeds without aril and integument (husk), containing at least 5% of methylxanthines calculated as caffeine ($C_8H_{10}N_4O_2$; 194.19) and at least 4% of tannins.

CHARACTERISTICS

Organoleptic characteristics. The drug is odorless, bitter taste, and slightly astringent.

MACROSCOPIC DESCRIPTION

The seed is globose when alone in the fruit or subspherical to ellipsoid, slightly compressed on the lateral when 2 or 3 unevenly convex on both sides generally having a short apical protuberance. In general, it has 0.6 cm - 0.8 cm diameter, covered with an integument, called husk, which must be discarded. The seed without integument is exalbuminous and has two large fleshy cotyledons, thick and firm, unequal, plano-convex, dark brown color. The scar of the aril remains in the cotyledons, but blackened. The embryo is undeveloped and has a lower short stem-radicle axis.

MICROSCOPIC DESCRIPTION

The cotyledons consist of uniseriate epidermis composed of tangentially elongated cells and cotyledon parenchyma with rounded or rounded-polyhedral cells from 40 µm to 80 µm diameter. It contains simple and compound starch grains, variable shapes, globose, polygonal, oval or elliptical, from 10 µm to 25 µm diameter. The hilum is central and sometimes branched. Most of the grains is deformed and agglutinated due to torrefaction.

MICROSCOPIC DESCRIPTION OF POWDER

The powder meets all the requirements for the species, unless the macroscopic characters. Characteristics: light brown to reddish-brown color, portions of cotyledon parenchymal cells, generally isodiametric, yellowish, with or without masses of agglutinated starch grains, masses of agglutinated starch grains, isolated starch grains with central hilum. Fragments of integument, when present to the limit, formed by palisade cells of very thick walls and little pits, sinuous in front view; grouped or isolated stone cells.

MACROSCOPIC DESCRIPTION OF IMPURITIES

The integument, when present as an impurity, called husk, presents shiny coat, glabrous, reddish-brown or brown-black, with a broad hilum provided with fleshy aril, membranous, whitish, which covers the seed up to a maximum of its middle portion. During collection and dissection, the aril is removed, and a opaque cream-brown scar appears as dome, which takes up 1/3 of the longitudinal axis of the seed. During dissection, the integument becomes brittle and easily separates from the cotyledons.

MICROSCOPIC DESCRIPTION OF IMPURITIES

The integument, if present as an impurity, presents in cross section an epidermis composed of large cells arranged in a palisade, thick-walled, with a few pits. These have sinuous walls in front view. Below the epidermis there are several layers of irregularly thickened parenchyma cells with brown appearance. There are numerous stone cells whose walls have clear pits.

IDENTIFICATION

A. Characterization of the presence of tannins. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel, with 250 µm thickness as support and a mixture of ethyl acetate, formic acid, and water (90:5:5) as mobile phase. To separately apply to the plate 10 µL of *Solution (1)* and 5 µL of *Solution (2)* freshly prepared, as described below.

Solution (1): to weigh 1 g of the pulverized drug and transfer to a round bottom flask. To add 20 mL of water and heat under reflux for 15 minutes. To filter through cotton and concentrate 4 mL of the filtrate to dryness in a water bath. To resuspend the residue in 1 mL of methanol.

Solution (2): 1 mg/mL catechin solution in methanol.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity of fluorescence to that obtained with *Solution (2)*. To nebulize the plate with vanillin sulfuric SR. The catechin spot (R_f about 0.72) is fleeting red.

B. Characterization of the presence of methylxanthines. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel as support and a mixture of chloroform, ethanol, and formic acid (90:8:2) as mobile phase. To separately apply to the plate 10 µL of *Solution (1)* and 5 µL of *Solution (2)* freshly prepared, as described below.

Solution (1): to weigh 1 g of the pulverized drug and transfer to erlenmeyer flask with lid. To add 3 mL of 25% ammonium hydroxide (v/v) and 40 mL of methylene chloride. To stir for 15 minutes in a magnetic stirrer. To filter through cotton and concentrate 5 mL of the filtrate to dryness in a water bath. To resuspend the residue in 1 mL of methanol.

Solution (2): 1 mg/mL caffeine SQR solution in methanol.

Solution (3): 1 mg/mL theophylline SQR solution in methanol.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). The chromatogram obtained with *Solution (1)* shows 2 principal spots, which correspond in position, color, and intensity of fluorescence to those obtained with *Solutions (2) and (3)*. Then, nebulize the plate with iodine SR. The theophylline spot (R_f about 0.50) is fleeting purplish and caffeine spot (R_f about 0.70) is reddish-brown.

C. To weigh 3 g of the pulverized drug and transfer to a round bottom flask. To add 60 mL of water and heat under reflux for 15 minutes. To cool down and filter it. To add 2 drops of diluted hydrochloric acid to 2 mL of resulting extract and drip gelatin SR. Clear precipitate is produced.

D. To add 10 mL of water and 4 drops of methanolic ferric chloride to 2 mL of extract obtained in method **C.**, *Identification*. Dark gray color is developed.

E. To add 0.5 mL of 1% vanillin (w/v) and 1 mL of hydrochloric acid to 2 mL of the extract obtained in method **C.** *Identification*. Red color is developed.

PURITY TESTS

Foreign matter (5.4.2.2). At most 3%, including husk.

Water (5.4.2.3). At most 9.5%.

Total ashes (5.4.2.4). At most 3%.

DOSAGE

Total tannins

Note: To protect samples from light during extraction and dilution. To use carbon dioxide-free water in all operations.

To proceed as described in *Visible absorption spectrophotometry (5.2.14)*. To prepare the solutions as described below.

Stock solution: to accurately weigh 0.75 g of crushed drug, transfer to erlenmeyer flask, and add 150 mL of water. To heat to boil and keep it in a water bath at 80 °C - 90 °C for 30 minutes. To cool down in running water, transfer the mixture to a 250 mL volumetric flask, and top up with water.

To decant the sediment and filter by using a filter paper. To discard the first 50 mL of the filtrate.

Sample solution for total polyphenols: to transfer 5 mL of *Stock solution* to 25 mL volumetric flask and top up with water. To mix 5 mL of this solution with 2 mL of phosphotungstic acid SR and dilute to 50 mL with sodium carbonate SR. To measure the absorbance of solution (A_1) at 691 nm (**5.2.14**) exactly 3 minutes after adding the last reagent, by using water as a blank.

Sample solution for polyphenols not adsorbed by hide powder: to add 0.2 g of hide powder SQR to 20 mL of *Stock solution* and mechanically stir for 60 minutes. To filter it. To dilute 5 mL of this solution to 25 mL with water. To mix 5 mL of the previous solution with 2 mL of phosphotungstic acid SR and dilute to 50 mL with sodium carbonate SR. To measure the absorbance of solution (A_2) at 691 nm (**5.2.14**) exactly 3 minutes after adding the last reagent, by using water as a blank.

Reference solution: to dissolve 50 mg of pyrogallol in water and dilute to 100 mL. To dilute 5 mL of this solution to 100 mL with water. To mix 5 mL of this solution with 2

mL of phosphotungstic acid SR and dilute to 50 mL with sodium carbonate SR. To measure the absorbance of solution (A_3) at 691 nm (**5.2.14**) exactly 3 minutes after adding the last reagent and within 15 minutes from dissolution of pyrogallol, by using water as a blank.

To calculate the tannin content, by using the following equation:

$$TT = \frac{13,12 \times (A_1 - A_2)}{A_3 \times m}$$

where

TT = Total tannins

A_1 = absorbance of Sample solution for total polyphenols

A_2 = absorbance of *Sample solution for polyphenols not adsorbed by hide powder*

A_3 = absorbance of *Standard solution*

m = mass of drug by considering the determination of water.

Methylxanthines

To proceed as described in *Visible absorption spectrophotometry (5.2.14)*. To prepare the solutions as described below.

Sample solution: To accurately weigh about 0.25 g of the pulverized drug and extract with 20 mL of 2.5% sulfuric acid (v/v) by mechanically stirring for 15 minutes, 4 times. To filter portions to a 100 mL volumetric flask. To top up the volume with the same solvent. To transfer an aliquot of 10 mL of this solution to a 100 mL volumetric flask and top up with 2.5% sulfuric acid (v/v).

Solutions for analytical curve: To prepare the caffeine analytical curve by accurately dissolving 50 mg of caffeine SQR in 100 mL of 2.5% sulfuric acid (v/v), to obtain 0.05% stock solution (w/v).

To prepare reference solutions by separately transferring aliquots of 1 mL; 2 mL; 3 mL; 4 mL, and 5 mL of stock solution to 100 mL volumetric flasks. To top up to volume with 2.5% sulfuric acid (v/v), to respectively obtain 4 µg/mL, 10 µg/mL, 15 µg/mL, 20 µg/mL, and 25 µg/mL solutions.

To measure the absorbance of *Sample solution* and *Solutions for analytical curve* at 271 nm (**5.2.14**),

by using 2.5% sulfuric acid solution (v/v) to adjust zero. To calculate the caffeine content (methylxanthines) from the sample, from the line equation obtained with Solutions for caffeine analytical curve.

PACKAGING AND STORAGE

In tightly closed containers, protected from light and heat.

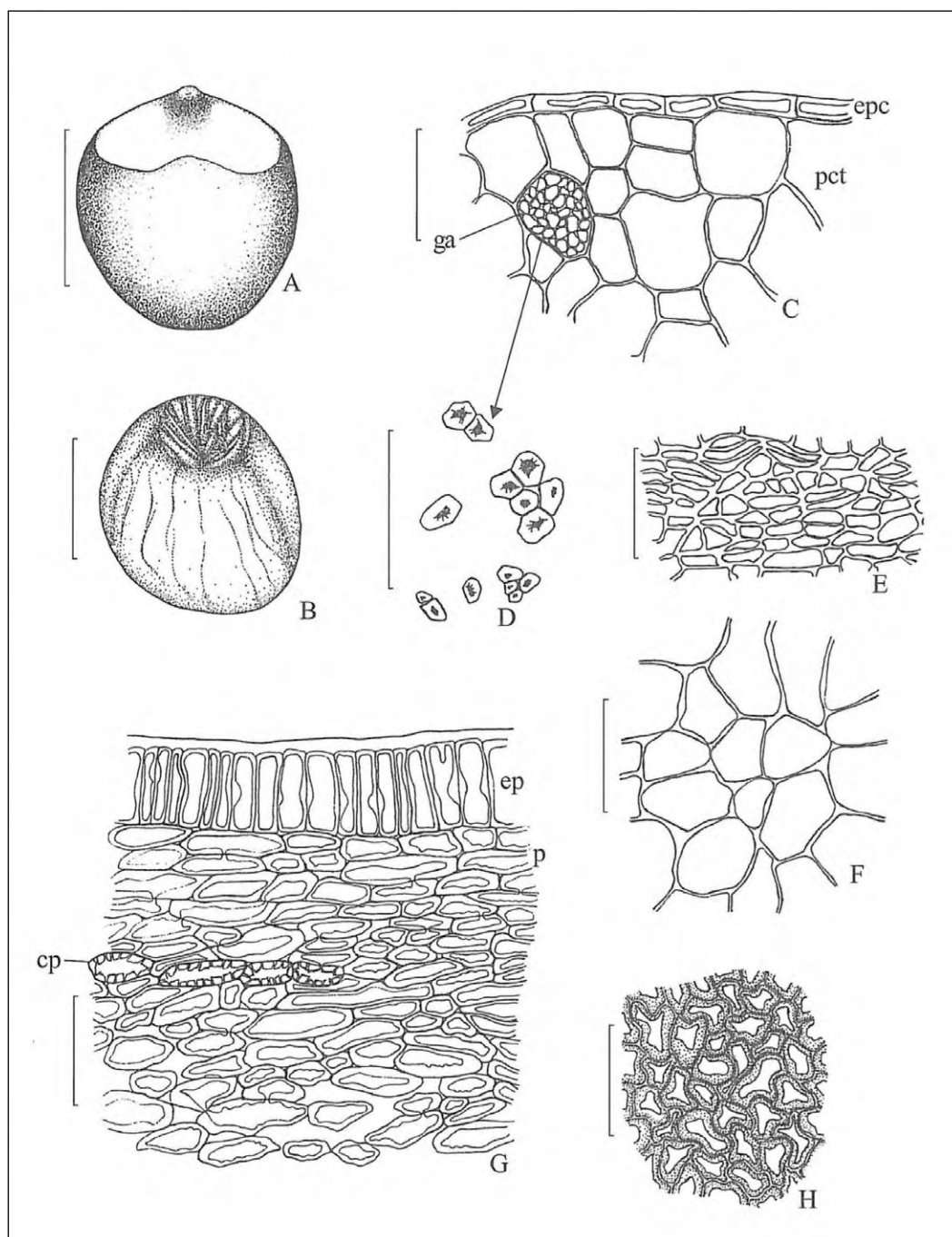


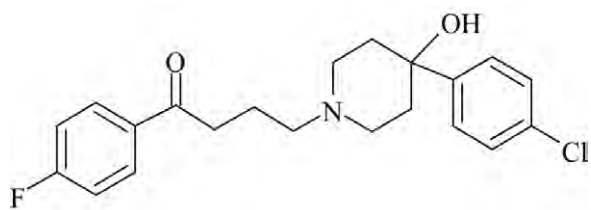
Figure 1 - Macroscopic and microscopic aspects in *Paullinia cupana* Kunth

Additional information of **Figure 1**. Scales correspond to: A and B (4cm), C to H (100 μ m).

A — general appearance of the seed. **B** — general appearance of the cotyledons. **C** — cross section of outer portion of a cotyledon; cotyledon epidermis (epc); cell containing starch grains (ga); cotyledon parenchyma (pct). **D** — detail of starch grains. **E** — epidermal cells of cotyledon in front view. **F** — parenchyma cells of cotyledons. **G** — detail of cross section of the seed integument; stone cells (cp); integument epidermis (ep); parenchyma (p). **H** — epidermal cells of integument in front view.

HALOPERIDOL

Haloperidolum



$C_{21}H_{23}ClFNO_2$; 375.86

haloperidol; 04589

4-[4-(4-Chlorophenyl)-4-hydroxy-1-piperidinyl]-1-(4-fluorophenyl)-1-butanone
[52-86-8]

It contains at least 98.0% and at most 102.0% of $C_{21}H_{23}ClFNO_2$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or almost white powder, amorphous or microcrystalline.

Solubility. Insoluble in water; freely soluble in acetone, benzene, chloroform, and methanol; sparingly soluble in methylene chloride and ethanol. Freely soluble in diluted mineral acids.

Physical and chemical constants.

Melting range (5.2.2): 148 °C to 151 °C. To determine in desiccated sample, in oven, at 105 °C for 1 hour.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, desiccated at 105 °C and dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the haloperidol SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14), ranging from 230 nm to 350 nm, of 0.0002% solution (w/v) in a mixture of 0.1 M hydrochloric acid and isopropyl alcohol (1:9), exhibits maxima at 245 nm. The absorbance at 245 nm does not differ more than 3.0% of reading related to similar solution of haloperidol SQR.

C. The principal spot in the chromatogram of *Solution (2)* in the *Related substances* corresponds in position, color, and intensity to that obtained with *Solution (3)*.

D. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method **B.** corresponds to that of main peak of *Standard solution*.

E. To dissolve 10 mg of the sample in 5 mL of ethanol and add 0.5 mL of 1,3-dinitrobenzene SR and 0.5 mL of 2 M ethanolic potassium hydroxide SR.

Violet color is developed, then becoming brownish-red after 20 minutes.

PURITY TESTS

Appearance of solution. To dissolve 0.2 g of the sample in 20 mL of 1% lactic acid (v/v). To put it in ultrasound, if necessary, to obtain total dissolution. The solution is clear (5.2.25) and is not darker than *Standard solution color SC F* (5.2.12) diluted to 2.5% (v/v) in water.

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel as support and a mixture of chloroform, glacial acetic acid, and methanol (80:10:10) as mobile phase. To separately apply to the plate 10 µL of each of the solutions freshly prepared, as described below.

Solution (1): 10 mg/mL solution of the sample in methylene chloride.

Solution (2): to dilute 1 mL of *Solution (1)* to 10 mL with methylene chloride.

Solution (3): 1 mg/mL haloperidol SQR solution in methylene chloride.

Solution (4): to dilute 0.5 mL of *Solution (2)* to 10 mL with methylene chloride.

Procedure: To develop the chromatogram. To remove the plate, and allow it to dry in a current of air for 15 minutes. To examine under ultraviolet light (254 nm). Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (4)* (0.5%).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 105 °C to obtain constant weight. At most 0.5 %.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1 %.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Titrations in non-aqueous medium* (5.3.3.5). To accurately dissolve about 0.3 g of the sample in 30 mL of glacial acetic acid. To add 5 drops of 1-naphtholbenzein SI and titrate with 0.1 M SV perchloric acid, to change from yellow-orange to green. To perform sample blank correction and adjust it as required. Each mL of 0.1 M SV perchloric acid is equal to 37.586 mg of $C_{21}H_{23}ClFNO_2$.

B. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 254 nm; 250 mm chromatograph column and 4.0 mm inner diameter, packed

with silica chemically bonded to the octadecylsilane group (5 μm), kept at 30 °C; flow of *Mobile phase* of 1 mL/min.

Mobile phase: mixture of methanol, 0.05 M monobasic potassium phosphate, tetrahydrofuran, and triethylamine (50:47:3:0.3). To adjust pH to 3.5 ± 0.1 with phosphoric acid SR.

Sample solution: to dissolve accurately weighed amount of the sample in *Mobile phase*, to obtain a 10 $\mu\text{g/mL}$ solution.

Standard solution: to dissolve accurately weighed amount of haloperidol SQR in *Mobile phase*, to obtain a 10 $\mu\text{g/mL}$ solution.

To inject replicates of 20 μL of the *Standard solution*. The tailing factor should not be greater than 2.0. The relative standard deviation for the replicate areas for the peaks recorded is not greater than 2.0%.

Procedure: to separately inject 20 μL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $\text{C}_{21}\text{H}_{23}\text{ClFNO}_2$ from the sample, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antipsychotic.

HALOPERIDOL TABLETS

It contains at least 90.0 % and at most 110.0 % of the amount stated of $\text{C}_{21}\text{H}_{23}\text{ClFNO}_2$.

IDENTIFICATION

A. To weigh and pulverize the tablets. To transfer amount of powder equivalent to 10 mg of haloperidol to a separatory funnel, add 10 mL of water and 1 mL sodium hydroxide *M*. To extract with 10 mL of water-saturated chloroform. To filter and evaporate to dryness. The infrared absorption spectrum (5.2.14) of the residue, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the haloperidol SQR spectrum similarly prepared.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

Procedure for content uniformity. To transfer each tablet to 50 mL volumetric flask. To add 30 mL of hot methanol and put it in ultrasound for 15 minutes. To mechanically stir for 15 minutes, top up with methanol, and filter it. To dilute, if necessary, in methanol, to obtain 0.002% concentration (w/v). To prepare haloperidol SQR solution at the same concentration by using the same solvent. To measure the absorbances of the resulting solutions at 245 nm (5.2.14), by using methanol to adjust zero. To calculate the amount of $\text{C}_{21}\text{H}_{23}\text{ClFNO}_2$ in the tablets, from the reading obtained.

DISSOLUTION TEST (5.1.5)

Dissolution medium: simulated gastric fluid (no enzyme), 900 mL

Equipment: baskets, 100 rpm

Time: 60 minutes

Procedure: To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 254 nm; 250 mm chromatograph column and 4.0 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μm), kept at 30 °C; flow of *Mobile phase* of 1 mL/min.

Mobile phase: mixture of methanol and 0.05 M monobasic potassium phosphate (60:40). To adjust the pH of the mixture to 4.0 ± 0.1 with phosphoric acid or diluted sodium hydroxide.

Sample solution: after testing, remove aliquot from the dissolution medium, filter, and dilute, if necessary, with dissolution medium, to obtain about 1.11 $\mu\text{g/mL}$ concentration.

Standard solution: to accurately transfer about 27.75 mg of haloperidol SQR to a 250 mL volumetric flask. To dissolve in 5 mL of methanol, top up with dissolution medium, and make it homogeneous. To successively dilute this solution with dissolution medium, to obtain about 1.11 $\mu\text{g/mL}$ concentration.

To inject replicates of 100 μL of the *Standard solution*. The tailing factor is not greater than 2.0. The relative standard deviation should not be greater than 3.0 %.

Procedure: to separately inject 100 µL of *Sample and standard solutions*, record the chromatograms, and measure the areas under the peaks.

To calculate the amount of $C_{21}H_{23}ClFNO_2$ dissolved in the medium from the responses obtained from *Sample and standard solutions*.

Tolerance: not less than 80% (Q) of the amount stated of $C_{21}H_{23}ClFNO_2$ dissolves within 60 minutes.

PURITY TESTS

Related substances. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using G silica gel as support and a mixture of chloroform, glacial acetic acid, and methanol (80:10:10) as mobile phase. To separately apply to the plate 10 µL of each of the solutions freshly prepared, as described below.

Solution (1): to weigh and pulverize the tablets. To stir amount of powder equivalent to 10 mg of haloperidol with 10 mL of chloroform. To filter and evaporate the residue to dryness. To dissolve the residue in 1 mL of chloroform.

Solution (2): to dilute 0.25 mL of *Solution (1)* to 25 mL with chloroform.

Solution (3): to dilute 0,25 mL of *Solution (2)* to 50 mL with chloroform.

To develop the chromatogram. To remove the plate, allow to air dry, and nebulize with potassium iodobismuthate SR. Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (2)* (1%) and only one is more intense than that obtained with *Solution (3)* (0.5 %).

DOSAGE

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 254 nm; 250 mm chromatograph column and 4.0 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at 30 °C; flow of *Mobile phase* of 1 mL/min.

Mobile phase: mixture of methanol and 0.05 M monobasic potassium phosphate (60:40). To adjust the pH of the mixture to 4.0 + 0.1 with phosphoric acid or diluted sodium hydroxide.

Sample solution: to weigh and pulverize 20 tablets. To transfer amount of sample equal to 5 g of haloperidol to a 50 mL volumetric flask. To add 30 mL of *Mobile phase* and put it in ultrasound for 30 minutes. To mechanically stir for 30 minutes. To top up to volume with *Mobile phase*, make it homogeneous, and filter it. To transfer 5 mL to 50 mL volumetric flask and top up with *Mobile phase*.

Standard solution: to accurately transfer about 25 mg of haloperidol SQR to a 250 mL volumetric flask. To dissolve in 5 mL of methanol, top up with *Mobile phase*, and make it homogeneous. To successively dilute this solution with *Mobile phase*, to obtain about 10 µg/mL concentration.

To inject replicates of 20 µL of the *Standard solution*. The tailing factor is not greater than 2.0. The relative standard deviation of the areas should not be greater than 3.0 %.

Procedure: to separately inject 20 µL of *Sample and standard solutions*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of $C_{21}H_{23}ClFNO_2$ in the tablets, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

HALOPERIDOL INJECTABLE SOLUTION

It contains at least 90.0% and at most 110.0% of amount stated of $C_{21}H_{23}ClFNO_2$. The injectable solution can contain lactic acid and suitable preservatives.

IDENTIFICATION

The ultraviolet absorption spectrum (5.2.14) ranging from 200 nm to 400 nm, of sample solution obtained in *Dosage*, exhibits maxima at 245 nm, similar to those observed in the standard solution spectrum.

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test. **pH (5.2.19).** 3.0 to 3.8.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

Bacterial endotoxins (5.5.2.2). At most 71.4 UE/ mg of haloperidol.

DOSAGE

To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. To quantitatively transfer volume of sample volume equivalent to 10 mg of haloperidol to a separatory funnel and add 20 mL of 5% hydrochloric acid (v/v). To extract with 4 portions (25 mL each) of ethyl ether. To gather the ethyl phases and add 3 portions (5 mL each) of dilute hydrochloric acid (1 to 20). To add portions of hydrochloric acid to the aqueous phase. To transfer it to a 50 mL volumetric flask, top up with 5% hydrochloric

acid (v/v), and stir. To transfer 5 mL of this solution to 50 mL volumetric flask and top up with methanol. To prepare haloperidol SQR solution at the same concentration by using the same solvents.

To measure the absorbances of the resulting solutions at 245 nm, by using 5 mL of 5% hydrochloric acid (v/v) in 50 mL of methanol to adjust zero. To calculate the amount of $C_{21}H_{23}ClFO_2$ in the injectable solution, from the readings obtained.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

HALOPERIDOL ORAL SOLUTION

It contains at least 90.0% and at most 110.0% of amount stated of $C_{21}H_{23}ClFNO_2$. The oral solution can contain lactic acid and suitable preservatives.

IDENTIFICATION

A. To transfer volume of oral solution equivalent to 10 mg of haloperidol to a separatory funnel. To add 1 mL of *M* sodium hydroxide, make it homogeneous, and extract with a portion (10 mL) of chloroform. To discard the aqueous phase. To evaporate the extract to dryness. The residue responds to test A., *Identification*, in the monograph *Haloperidol*.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method **B.** corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test. **pH (5.1.19).** 2.5 to 3.5.

PURITY TESTS

Related substances. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using G silica gel as support and a mixture of methylene chloride, methanol, and 13.5 *M* ammonia (92:8:1) as mobile phase. To separately apply to the plate 50 µL of each of the solutions freshly prepared, as described below.

Solution (1): to dilute oral solution, if necessary, with methanol to obtain 1 mg/mL haloperidol solution.

Solution (2): to dilute 1 mL of *Solution (1)* to 100 mL with methanol.

Solution (3): to dilute 1 mL of *Solution (1)* to 200 mL with methanol.

To develop the chromatogram. To remove the plate, and let it drying. To nebulize with diluted potassium iodobismuthate SR. Any secondary spot obtained in the chromatogram with *Solution (1)*,

other than the principal spot, is not more intense than that obtained with *Solution (2)* (1%) and only one is more intense than that obtained with *Solution (3)* (0.5 %).

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. To quantitatively transfer volume of sample equivalent to 10 mg of haloperidol to a separatory funnel and add 20 mL of diluted hydrochloric acid (1 to 20). To extract with 4 portions (25 mL each) of ethyl ether. To gather the ethyl phases and add 3 portions (5 mL each) of diluted hydrochloric acid (1 to 20). To add portions of hydrochloric acid to the aqueous phase. To transfer it to a 50 mL volumetric flask, top up with diluted hydrochloric acid (1 to 20), and stir. To transfer 5 mL of this solution to 50 mL volumetric flask and top up with methanol. To prepare haloperidol SQR solution at the same concentration by using the same solvents. To measure the absorbances of the resulting solutions at 245 nm, by using 5 mL of diluted hydrochloric acid (1 to 20) in 50 mL of methanol to adjust zero. To calculate the amount of $C_{21}H_{23}ClFNO_2$ in the injectable solution, from the readings obtained.

B. To proceed as described in method **B.** *Dosage*, in the monograph *Haloperidol*. To prepare *Sample solution* and *Resolution solution* as described below.

Sample solution: to transfer volume of oral solution equivalent to 10 mg of haloperidol to 100 mL volumetric flask and top up with *Mobile phase*. To transfer 5 mL to 50 mL volumetric flask and top up with *Mobile phase*.

Resolution solution: To prepare solution in *Mobile phase* containing about 10 mg of haloperidol SQR, 3 mg of methylparaben, and 3 mg of propylparaben per milliliter.

To separately inject replicates of 20 µL of *Standard solution* and *Resolution solution* and record the chromatograms. To measure the area under the haloperidol peak. The tailing factor should not be greater than 2.0. The relative standard deviation for replicate areas of the peaks recorded for haloperidol should not be greater than 2.0 %. The resolution

between the haloperidol, methylparaben, and propylparaben peaks is greater than 2.

Procedure: to separately inject 20 µL of *Sample and standard solutions*, record the chromatograms, and measure the area under the peaks.

To calculate the amount of $C_{21}H_{23}ClFNO_2$ in the oral solution, from the responses from the *Standard solution* and *Sample solution*.

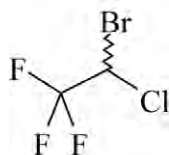
PACKAGING AND STORAGE

In tightly closed containers, protected from light, at room temperature.

LABELLING

To comply with the legislation in force.

HALOTHANE Halothanum



$C_2HBrClF_3$; 197.38
halothane; 04596
2-Bromo-2-chloro-1,1,1-trifluoroethane
[151-67-7]

It contains at least 0.008% and at most 0.012% of thymol, by weight, as a stabilizer.

DESCRIPTION

Physical characteristics. Dense liquid, colorless, mobile, non-flammable, typical odor resembling chloroform, sweet taste, and produces a burning sensation.

Solubility. Slightly soluble in water; miscible with ethanol, chloroform, ethyl ether, and fixed oils.

IDENTIFICATION

A. To add 5 mL of sulfuric acid to 5 mL of the sample. The acid forms a layer over the sample (difference of chloroform and trichlorethylene).

B. To add a clean sodium fragment, about 8 mm diameter to 0.3 mL of sample in a borosilicate glass test tube (12 x 75 mm), and let it rest for several minutes. To hold the tube upright and gently heat with a microburner to melt the metal and the reaction begins. Then, remove the source of heat and cool down the tube. To cautiously add 2 mL of water and wait for total reaction. To filter the solution and

add 0.5 mL of glacial acetic acid to the filtrate. To add 2 drops of this solution to a mixture of 0.1 mL of alizarin SI freshly prepared and 0.1 mL of zirconyl nitrate SR. Red color turns yellow color.

PURITY TESTS

Acidity or alkalinity. To stir 20 mL of sample in 20 mL of carbon dioxide-free water for 3 minutes and let the layers separate. The aqueous layer requires at most 0.1 mL of 0.01 M sodium hydroxide, or at most 0.6 mL of 0.01 M hydrochloric acid to neutralize, by using bromocresol purple SI as an indicator.

Chloride and bromide. To stir 25 mL of sample in 25 mL of water for 5 minutes and wait for total separation of liquids. To remove the aqueous layer and add a drop of nitric acid and five drops of silver nitrate SR to 10 mL of such layer. No opalescence should be produced.

Thymol.

Thymol standard solution: to prepare 0.1 mg/mL thymol solution in 0.25 M sodium hydroxide.

Buffer solution: to use borate buffer pH 8.0.

Chloroimide solution: to dissolve 100 g of 2,6-dibromoquinone-4-chloroimide in 25 mL of absolute ethanol. Solution must be freshly prepared.

Standard thymol curve: to respectively pipette 1 mL, 3 mL, and 5 mL of *Standard thymol solution* into three 100 mL volumetric flasks and add, if necessary, 0.25 M sodium hydroxide, to complete 5 mL. To add 5 mL of 0.25 M sodium hydroxide to a forth flask, to prepare the blank. To add 10 mL of *Buffer solution* to each flask, gently stir, and add 1 mL of *Chloroimide solution*. To put it to rest for exactly 15 minutes, add 3 mL of 0.25 M sodium hydroxide, and top up with water.

By using a suitable spectrophotometer, measure the absorbances of solutions containing thymol and 590 nm blank. To make a graph for the readings and plot the best curve.

Procedure: to add 2 mL of the sample, accurately measured, into a 100 mL volumetric flask containing 5 mL of 0.25 M sodium hydroxide and gently stir it. To evaporate the halothane under a stream of nitrogen and add 10 mL of *Buffer solution* and 1 mL of *Chloroimide solution*. To gently stir and put it to rest for exactly 15 minutes, add 3 mL of 0.25 M sodium hydroxide, and top up with water. To read the absorbance of the resulting solution and, by using *Standard thymol curve* as reference, calculate the percentage of thymol in relation to the weight of halothane used.

Evaporation residue. To evaporate to dryness, in a water bath, 50 mL of sample in a tared capsule. To desiccate the residue in an oven at 105 °C for 2 hours. The weight of the residue should not exceed 1 mg.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

In accordance with legislation in force.

THERAPEUTIC CLASS

General anesthetic (inhalation)

HAMAMELIS, TINCTURE

Hamamelidis tinctura

Hamamelis virginiana L. - HAMAMELIDACEAE

The tincture is obtained from the leaves containing at least 0.6% of total tannins expressed as pyrogallol ($C_6H_6O_3$, 126.1) (w/w).

PREPARATION

The hamamelis tincture is obtained from 1 portion of vegetable drug in 10 portions of 65% ethanol (v/v) by proper procedure.

CHARACTERISTICS

Organoleptic characteristics. Yellowish-brown liquid with astringent taste.

IDENTIFICATION

To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using F_{254} silica gel, with 250 μ m thickness, as support and a mixture of ethyl acetate, toluene, formic acid, and water (60:20:15:15) as mobile phase. To separately apply to the plate, as band, 20 μ L of *Solution* (1) and 10 μ L of *Solution* (2) and *Solution* (3) freshly prepared, as described below.

Solution (1): to reduce in a water bath 5 mL of hamamelis tincture to obtain dry residue. To take up the residue in 10.0 mL of water. To extract the resulting aqueous phase with 3 portions (10 mL each) of ethyl acetate in a 125 mL separatory funnel. To let it rest at -18 °C for 15 minutes, to obtain complete separation of the phases. To collect the organic phases and wash with 20 mL of water.

Solution (2): to weigh about 1 mg of gallic acid and dissolve in 1.0 mL of methanol.

Solution (3): to weigh about 1 mg of catechin and dissolve in 2.0 mL of methanol.

To develop the chromatogram. To remove the plate and dry in fume hood. Then, nebulize the plate with 1% ferric chloride (w/v) in methanol. A yellowish spot and two more intense lower blue-gray spots obtained with *Solution* (1),

in the upper third of the chromatogram, correspond in position to those obtained with *Solution* (2) and *Solution* (3). To observe two yellowish-brown spots in the central third of the chromatogram and one in the lower third, with R_f about 0.35, corresponding to hamamelitannin.

PURITY TESTS

Relative density (5.2.5). 0.902 to 0.914.

Determination of alcohol (5.3.3.8). 58% (v/v) to 62% (v/v).

Dry residue (5.4.3.2.3). At least 1.2%.

DOSAGE

Total tannins

To prepare the solutions as described below. To perform all the extraction and dilution operations protected from the light.

Stock solution: To accurately weigh about 1.5 g of hamamelis tincture in a 250 mL volumetric flask and top up to volume with distilled water. To filter the mixture through filter paper. To discard the first 50 mL of the filtrate.

Sample solution for total polyphenols: to volumetrically transfer 5 mL of *Stock solution* to a 25 mL volumetric flask and top up with distilled water. To volumetrically transfer 2 mL of this solution, 1 mL of phosphotungstic molybdate reagent, and 10 mL of distilled water to a 25 mL volumetric flask and top up with a 29% sodium carbonate solution (w/v). To determine the absorbance at 760 nm (A_1) after 30 minutes by using distilled water as compensation liquid.

Sample solution for polyphenols not adsorbed by hide powder: to add 0.100 g of hide powder SQR to 10 mL of *Stock solution* and mechanically stir in 125 mL erlenmeyer flask for 60 minutes. To use filter paper. To dilute 5 mL of the filtrate in 25 mL volumetric flask with distilled water. To volumetrically transfer 2 mL of this solution, 1 mL of phosphotungstic molybdate reagent, and 10 mL of distilled water to a 25 mL volumetric flask and top up with a 29% sodium carbonate solution (w/v). To determine the absorbance at 760 nm (A_2) after 30 minutes by using distilled water as compensation liquid.

Standard solution: just before using it, dissolve 50 mg of pyrogallol in a 100 mL volumetric flask with distilled water. To volumetrically transfer 5 mL of this solution to a 100 mL volumetric flask and top up with distilled water. To volumetrically transfer 2 mL of this solution, 1 mL of phosphotungstic molybdate reagent, and 10 mL of distilled water to a 25 mL volumetric flask and top up with a 29% sodium carbonate solution (w/v). To determine the absorbance at 760 nm (A_3) after 30 minutes by using distilled water as compensation liquid.

To calculate the tannin content (in percentage) expressed as pyrogallol, according to the equation:

$$TT = \frac{62,5 \times (A_1 - A_2) \times m_2}{A_3 \times m_1}$$

where

A_1 = absorbance of Sample solution for total polyphenols;

A_2 = absorbance of Sample solution for polyphenols not adsorbed by hide powder;

A_3 = absorbance of Standard solution;

m_1 = mass of the sample used in the test, in grams;

m_2 = mass of pyrogallol, in grams.

PACKAGING AND STORAGE

In tightly closed container, protected from light and heat.

HEPARIN CALCIUM Heparinum calcicum

Heparin calcium is a preparation containing the calcium salt of a mixture of sulfated glycosaminoglycans of variable weight in mammalian tissues. It is usually obtained from bovine lungs or porcine intestinal mucosa. It consists of polymers with D-glucosamine (*N*-sulfated or *N*-acetylated) units and uronic acid (L-iduronic or D-glucuronic acid) that combined alternate with glycosidic linkages. It has the property of prolonging the blood clotting primarily by forming complex of some components of the mixture with specific plasma proteins, thus enhancing the inactivation of thrombin (factor IIa). Other proteases involved in the coagulation process, such as activated factor X (factor Xa) are also inhibited. The ratio of anti-factor Xa activity by the potency of anti-factor IIa should be between 0.9 and 1.1. The potency of heparin calcium should not be less than 180 IU/mg in relation to the desiccated substance. The animals from which heparin is derived must fulfill the health requirements for the species, and the production process must ensure the removal or inactivation of infectious agents.

IDENTIFICATION

A. It complies with the requirements of *Dosage*, depending on the methods *Determination of potency* or *Potency anti-factor IIa*.

B. To employ the nuclear magnetic resonance spectroscopy technique. To prepare the solutions as described below.

Sample solution: not less than 20 mg/mL of sample in 99.9% deuterium oxide (w/v) with 0.02% (trimethylsilyl) propionic sodium.

Standard solution: not less than 20 mg/mL of heparin calcium SQR in 99.9% deuterium oxide (w/v) with 0.02% (trimethylsilyl) propionic sodium.

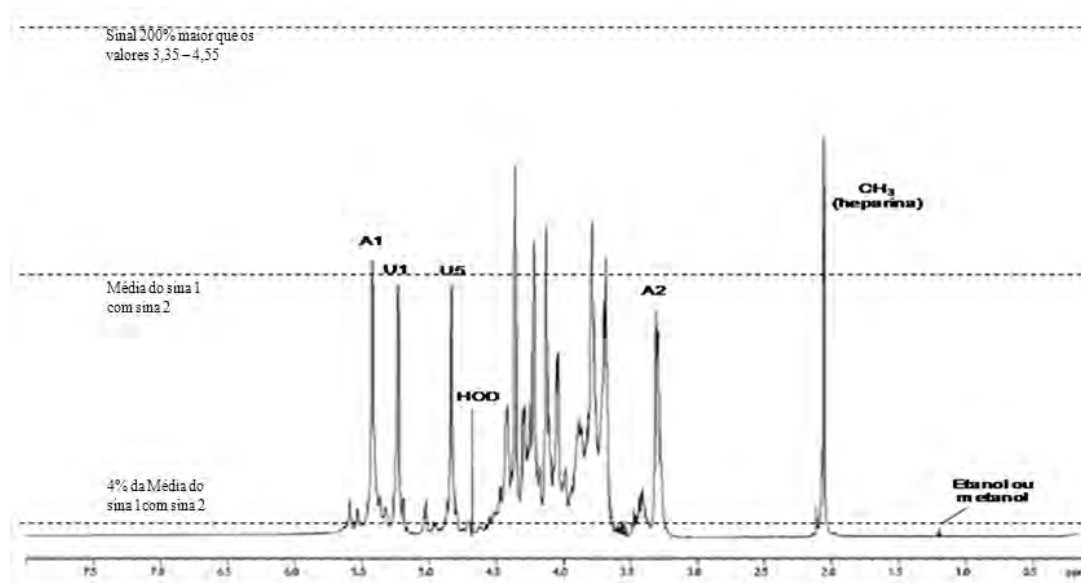
Procedure: in the analysis of samples, use a nuclear magnetic resonance spectrometer (at least 500 MHz operating pulse) (Fourier Transform) to acquire ^1H under free-induction decay, by using 16 scans at 90° pulse. The test shall be conducted at constant temperature (25°C). The spectral window should be at least 10 to -2 ppm. For all the samples, methyl from (trimethylsilyl) propionic compound must be referenced at 0.00 ppm. The chemical shifts of 4 typical regions of heparin (porcine) are H1 of glucosamine *N*-acetylated/glucosamine *N*-sulfated (signal 1) at 5.40 ppm, H1 of iduronic acid 2-sulfated (signal 2) at 5.21 ppm, H2 of glucosamine *N*-sulfated at 3.28 ppm, and glucosamine methyl *N*-acetylated at 2.05 ppm. The ppm values observed for each signal should not vary ± 0.03 ppm.

Acceptance criteria are based on the average height signals 1 and 2. Any signal identified in the following fields: 0.10 - 2.00, 2.10 - 3.20, and 5.70 - 8.00 ppm should not exceed 4% of the average height of both signals mentioned above. Likewise, no signs 200% higher than 3.35 - 4.55 ppm must be found.

Impurities: oversulfated chondroitin sulfate. The chemical shift in the region *N*-acetyl of oversulfated chondroitin sulfate must be observed at 2.16 ± 0.03 ppm.

Dermatan sulfate The chemical shift in the region *N*-acetyl of oversulfated chondroitin sulfate must be observed at 2.10 ± 0.03 ppm.

h



C. To employ ion-exchange liquid chromatography technique. The ion-exchange chromatography is a test for determination of purity of heparin preparations, especially for the detection and separation of dermatan sulfate, chondroitin sulfate, and oversulfated chondroitin sulfate. To use chromatograph equipped with an ultraviolet detector at 202 nm; 50 mm column and 2 mm inner diameter, packed with anion exchange resin (13 mm); 250 mm long column, and 2 mm internal diameter, packed with anion exchange resin (9 mm), kept at 40 °C; flow of *Mobile phase* of 0.22 mL/min

Reference standards: Test solution (a) and Reference solution (a) relative retention of reference heparin (retention time = about 26 min); dermatan and chondroitin sulfate = about 0.9; oversulfated chondroitin sulfate = 1.3, in relation to heparin.

Note: reference solutions should be stabilized for 24 hours at room temperature.

Suitability system: Reference solution; relationship peak/valley: at least 1.3, where H_p = height above the base line of the peak due to dermatan and chondroitin sulfate; H_v = height above the base line the lowest point of the curve that separates the top of this peak due to heparin.

The main peak in the chromatogram obtained with *Test solution (a)* should be similar in form and retention time of the main peak in the chromatogram obtained with *Reference solution (a)*.

To prepare Test solutions as described below.

Test solution (a): to dissolve about 50 mg of the substance, accurately weighed, to be examined in 5 mL of water for chromatography (deionized water having a resistivity not less than 0.18 Mohms). To mix it with a vortex to obtain total dissolution.

Test solution (b): to dissolve about 0.1 g of the substance, accurately weighed, to be examined in 1 mL of water for chromatography. To mix it with a vortex to obtain total dissolution. To mix 0.5 mL of solution and 0.25 mL of *M* hydrochloric acid, then add 50 µL of 250 mg/mL sodium nitrite solution. To gently mix and let it rest at room temperature for 40 min before adding 0.2 mL of *M* sodium hydroxide to stop the reaction.

Reference solution (a): to dissolve 250 mg of heparin SQR in water by chromatography and dilute to 2 mL with the same solvent. To mix it with a vortex to obtain total dissolution.

Reference solution (b): to add 1.2 mL of *Reference solution (a)* and 0.3 mL of dermatan sulfate and oversulfated chondroitin sulfate. To mix it with a vortex, to make it homogeneous.

Reference solution (c): to add 0.1 mL of *Reference solution (b)* and 0.9 mL of water for chromatography. To mix it with a vortex, to make it homogeneous.

Reference solution (d): to add 0.4 mL of *Reference solution (a)* to 0.1 mL of water for chromatography and mix it with a vortex. To add 0.25 mL of *M* hydrochloric acid, then add 50 µL of 250 mg/mL sodium nitrite solution. To gently mix and let it rest at room temperature for 40 min before adding 0.2 mL of *M* sodium hydroxide to stop the reaction.

Reference solution (e): To add 0.5 mL of *Reference solution (b)*, add 250 µL of *M* hydrochloric acid, then add 50 µL of 250 mg/mL sodium nitrite solution. To gently mix and let it rest at room temperature for 40 min before adding 0.2 mL of *M* sodium hydroxide to stop the reaction.

Mobile phase A: to dissolve 0.4 g of monobasic sodium phosphate in 1000 mL of water for chromatography and adjust the pH to 3.0 with diluted phosphoric acid solution.

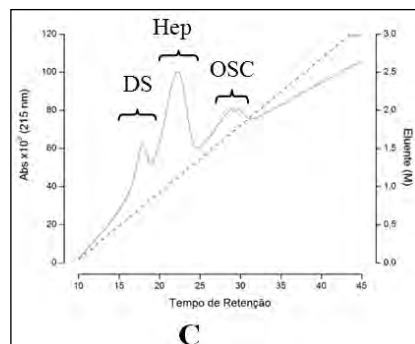
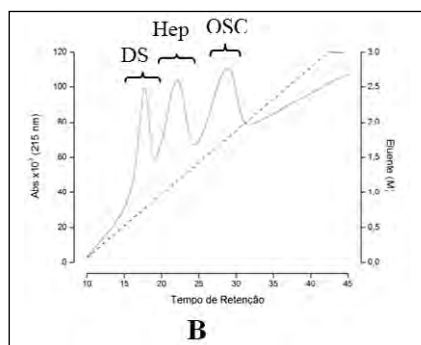
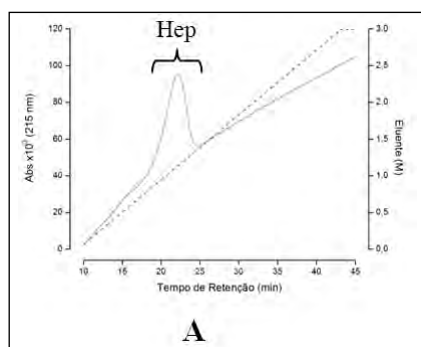
Mobile phase B: to dissolve 0.4 g of monobasic sodium phosphate in 1000 mL of water for chromatography, add 140 g of sodium perchlorate, adjust the pH to 3.0 with diluted phosphoric acid, filter, and degas it.

Mobile phase gradient: to adopt the gradient system described in the following table:

Time (minutes)	Mobile phase A (v/v)	Mobile phase B (v/v)	Elution
0 - 10	75	25	isocratic
10 - 35	75 → 0	25 → 100	linear gradient
35 - 40	0	100	isocratic

Procedure: to inject 20 µL of *Test solution (b)* and *Reference solutions (d)* and *(e)*. Heparin relative retention (retention time = about 26 minutes): dermatan sulfate and chondroitin sulfate = about 0.9; oversulfated chondroitin sulfate = 1.3. Balance: at least 15 min. The resolution is at least 3.0 between the peaks corresponding to dermatan sulfate plus chondroitin sulfate and oversulfated chondroitin sulfate in the chromatogram obtained with reference. The sum of the areas of dermatan sulfate and chondroitin sulfate is not greater than the area under the corresponding peak in the chromatogram obtained with *Reference solution (e)* 2.0%. No other peaks may exist, except the peak corresponding to dermatan sulfate plus chondroitin sulfate and heparin, that is, there should be no impurities.

Suitability of the system: the chromatogram obtained with *Reference solution (d)* shows no peak at the heparin retention time. Example:



A. chromatogram of SQR heparin solution (Hep).

B. chromatogram of standard solution of the mixtures (DS - dermatan Sulfate - 12%; Hep - Heparin - 44%, and OSC - oversulfated chondroitin sulfate - 54%).

C. chromatogram of a sample failed by the presence of oversulfated chondroitin sulfate (OSC).

D. It responds to the reactions of calcium ion (5.3.1.1).

CHARACTERISTICS

Physical characteristics. White or almost white powder, slightly hygroscopic.

Solubility. Soluble in water.

PURITY TESTS

pH (5.2.19). 5.0 to 8.0. To determine in 1% aqueous solution (w/v).

Proteins. To add 5 drops of 20% trichloroacetic acid (w/v) in 1 mL of the 1% aqueous solution (w/v) of the sample. No turbidity or precipitate should occur.

Heavy metals (5.3.2.3). To use the *Method I*. At most 0.003%.

Nitrogen (5.3.3.2). To use the *Method I, macrodetermination*. It contains at least 1.3% and at most 2.5% of nitrogen, in relation to the desiccated substance.

Calcium (5.3.2.7). At least 9.5% and at most 11.5% calcium, calculated with respect to the desiccated substance, determined in 0.2 g by *Complexometric titration (5.3.3.4)*.

Loss on drying (5.2.9). To determine in an oven, under vacuum, at 60 °C for 3 hours. At most 5 %.

Sulfated ashes (5.4.2.6). At least 28% and at most 41 %.

Nucleotide impurities: To dissolve 40 mg in 10 mL of water. The absorbance measured at 260 nm and the result should not be greater than 0.2.

BIOLOGICAL SAFETY TEST

Bacterial endotoxins (5.5.2.2). At most 0.03 UE/ IU of heparin.

DOSAGE

Determination of potency.

The anticoagulant activity of heparin is determined *in vitro* by comparing its capacity under certain conditions to delay the coagulation of reference ovine plasma or reference human plasma, citrated and recalcified with the same capacity of a reference heparin preparation calibrated in international units.

International Unit is the activity contained in an amount specified in the International Standard, which consists of a amount of lyophilized heparin calcium obtained from porcine intestinal mucosa. The equivalence in international units of International Reference Standard is indicated by World Health Organization.

The heparin calcium reference standard is calibrated in international units, as compared to an International Standard by means of the following test.

To conduct the test by using device to mechanically record the change of flow of agitation, by taking care to not agitate the solution during the initial phase of coagulation (coagulometer).

Procedure: The volumes described in the text are given as examples and can be adapted to the device used, since the relationship between the different volumes is observed. To dilute the heparin calcium reference standard in a 0.9% sodium chloride solution (w/v), to provide a precisely known number of International Units per milliliter and prepare a sample solution similar to the preparation to be examined, which should have the same activity. To use a 0.9% sodium chloride solution (w/v) to prepare a series of dilutions in geometric progression, in such a way that the clotting time obtained with the lowest concentration is not less than 1.5 times the time of blank recalcification. After obtaining the highest concentration, a curve in log dose-response should be satisfactory. To place 12 tubes in a water bath with cold water, by labeling them in duplicate: A1, A2, and A3 for dilutions of preparation to be examined and P1, P2, and P3 for dilutions of reference preparation. To add 1 mL of thawed plasma substrate and 1 mL of an appropriate dilution of preparation to be examined or reference preparation to each tube. After each addition, mix it, but no bubbles should be formed. To handle the tubes in the following order: P1, P2, P3, A1, A2, A3. To individually transfer the tube to a water bath at 37 ° C for balancing at 37 ° C for about 15 minutes and add 1 mL of a dilution of cephalin to each tube to which was added an appropriate activator, such as kaolin, so that a suitable recalcification time obtained in blank should not be greater than 60 seconds. When kaolin is used, it must be prepared immediately before using it, a mixture of equal volumes of cephalin

and 0.4% kaolin suspension (w/v) protected from light in a 0.9% sodium chloride solution (w/v). Exactly 2 minutes after adding 1 mL of 3.7 g/mL calcium chloride solution and record the clotting time and interval, in seconds, between the last addition and the start of coagulation determined by the technique selected.

To similarly determine the blank recalcification time at the beginning and end of the process, by using 1 mL of 0.9% sodium chloride solution (w/v) in place of a dilution of heparin, both values obtained in the blank should not significantly distinguish. To convert the clotting time in logarithms, by using the average value for the tubes in duplicate. To repeat the procedure with fresh dilutions and perform the incubation as follows: A1, A2, A3, P1, P2, P3. To calculate the result by employing the customary statistical methods. To conduct at least 3 independent tests. To prepare new reference solutions for each test and preparation to be examined and use another recently thawed plasma portion. To conduct test for heparin. The estimated potency should be at least 90% and at most 111% of the stated potency. The confidence limits of estimated potency should be not less than 80% and not greater than 125% of the stated potency ($P = 0.95$).

Potency of anti-factor IIa.

Buffer pH 8.4: to dissolve 6.1 g of tromethamine, 10.2 g of sodium chloride, 2.8 g of disodium edetate and, if necessary, between 0 and 10 g of polyethylene glycol 6000 and/or 2 g of bovine albumin in 800 mL of water. To adjust it with hydrochloric acid to pH 8.4 and dilute to 1000 mL with water.

Note: 2 g of human albumin can be replaced by 2 g of bovine serum albumin.

Antithrombin solution: to reconstitute a vial of antithrombin in water, to obtain a 5 IU/ mL antithrombin solution. To dilute with *Buffer pH 8*. to obtain a 0.125 IU/mL antithrombin solution.

Human thrombin solution: to reconstitute the human thrombin (Factor IIa) in water, to obtain 20 IU/mL of thrombin, and dilute with *Buffer pH 8.4* to obtain 5 IU/mL thrombin solution.

Note: thrombin should have a specific activity of at least 750 IU/mg.

Chromogenic substrate solution: to prepare a solution of a suitable thrombin substrate for chromogenic amidolytic test with water, to obtain 1.25 mM concentration.

Stop solution: to prepare 20% acetic acid solution (w/v) in water.

Standard solution: to reconstitute the entire content of a vial of heparin calcium SQR in water and dilute with *Buffer pH 8.4* to obtain at least 4 dilutions between the concentration range of 0.005 and 0.03 units/ mL of heparin.

Sample solutions: to proceed as described in the solutions, to obtain the concentrations of heparin calcium similar to those obtained for *Standard solutions*.

Duplicate should be performed for each dilution of *Standard solution* or *Sample solution*.

Numerical labels should be placed depending on the number of repetitions to be tested. For example: if there are 5 blanks to be used B1, B2, B3, B4, and B5; A1, A2, A3, and A4 for each sample in duplicate to be tested and P1, P2, P3, and P4 for each standard solutions in duplicate to be tested. To distribute the blank spaces on the series so that they accurately represent the behavior of the reagents during the experiments.

Note: *Tubes should be handled as follows:* B1, P1, P2, P3, P4, B2, A1, A2, A3, A4, B3, A1, A2, A3, A4, B4, P1, P2, P3, P4, B5.

To note that after adding reagent, the incubated solution should be mixed without bubbles. To add twice the volume (100 - 200 μ L) of Antithrombin solution to each tube containing a volume (50 - 100 μ L), *Buffer pH 8.4* or appropriate dilution of standard and sample solutions. To stir, but avoid formation of bubbles, and incubate it at 37 °C for at least 1 minute. To add 25-50 μ L of *Human thrombin solution* to each tube and incubate for at least 1 minute. To add 50-100 μ L of *Chromogenic substrate solution*. To note that all reagents, standard solutions, and sample solutions must be pre-heated at 37 °C shortly before utilization.

Two different types of measurements can be recorded:

“Endpoint” measurement: To stop the reaction after at least 1 minute with 5-10 μ L of *Stop solution*. To measure the absorbance of each solution at 405 nm by using a suitable spectrophotometer. A relative standard deviation of the blank readings must be less than 10%.

Kinetic Measurement: Following the change in absorbance for each solution for 1 minute at 405 nm by using a spectrophotometer. To calculate the variation in absorbance/ minute (Δ OD/minute). Blanks for kinetic measurement is also expressed as Δ OD/minute and should give higher values that are performed in the absence of heparin. A relative standard deviation of the blank readings must be less than 10%.

Statistical models for analyzing the relation between slope of the straight lines or on parallelism can be used depending on the best model that describes the correlation between concentration and response.

Test on parallelism: For each series, to calculate the regression of absorbance or change in absorbance/minute versus the log concentrations of the sample solutions and standard solutions and calculate the potency of reference heparin calcium in Units/mL, by using statistical methods for parallel line tests. To express the potency of heparin calcium IU/mg on dry basis.

Relationship between slope of the straight lines: For each series, to calculate the regression of log absorbance or log change in absorbance/minute versus concentrations of the sample solutions and standard solutions and calculate the potency of reference heparin calcium in Units/mL, by using statistical methods for slopes.

To express the potency of heparin calcium IU/mg on dry basis.

Acceptance Criteria: The potency of heparin calcium, calculated on a dry basis, is not less than 180 units of heparin per mg.

Anti-factor Xa activity.

Buffer pH 8.4: to dissolve 10.24 g of sodium chloride, 6.6 g of tromethamine, and 2.8 g of disodium edetate in water. If necessary, adjust the pH to 8.4 with diluted hydrochloric acid or sodium hydroxide solution.

Antithrombin solution: to reconstitute the content of a vial containing antithrombin with water (or as recommended by the manufacturer). To dilute with *Buffer pH 8.4*, to obtain 1 UI/mL antithrombin solution.

Bovine factor Xa solution: to reconstitute the content of a vial containing bovine factor Xa with water (or as recommended by the manufacturer). To dilute the solution obtained in *Buffer pH 8.4*, to obtain a solution with absorbance values between 0.65 and 1.25 measured at 405 nm, when tested as described below, but using 30 μ L of *Buffer pH 8.4* instead of 30 μ L of *Standard solution* or *Sample solution*.

The factor Xa solution containing 3 catalytic nano units per mL, but can vary depending on the manufacturer of factor Xa or substrate used.

Chromogenic substrate solution: to prepare a chromogenic solution suitable for amidolytic test specific for factor Xa in water to obtain about 1 mM concentration.

Stop solution: to prepare 20% acetic acid solution (w/v) in water.

Sample solution: to dissolve exact amount of heparin calcium sample in *Buffer pH 8.4* and dilute with it to obtain solutions containing activities roughly equal to the *Standard Solution*.

Standard solution: to use official standard of heparin. Other preparation may be used, whose potency has been calibrated against the official standard. To reconstitute the content of the vial of official standard of heparin in water and gently mix to obtain total dissolution. To prepare dilutions in *Buffer pH 8.4*, to obtain 5-7 solutions containing known activities of 0.375, 0.3125, 0.25, 0.188, 0.125, 0.0625, and 0.0313 in units of heparin per mL.

Procedure: the amounts described can be adapted to perform the test in tubes or microplates, by keeping the ratio between the volumes. To conduct the test with each *Standard solution* and *Sample solution* in duplicate. To transfer 120 µL of *Buffer pH 8.4* to each series of plastic tubes in a water bath at 37 °C. To separately transfer 30 µL of different dilutions of standard solutions or sample solutions to the tubes. To add 150 µL to each tube, mix it, and incubate it for 2 minutes.

To add 300 µL of *Chromogenic substrate solution* preheated at 37 °C for 15 minutes. To add 150 µL of *Stop Solution* to each tube and mix it. To prepare the blank to zero the spectrophotometer, by inversely adding the reagents, starting with *Stop Solution* and ending with the addition of 150 µL of *Buffer pH 8.4*, and excluding the standard solutions or sample solutions. To record the absorbance measured at 405 nm against the blank.

To construct a graph of absorbance values for the standard solutions and sample solutions against heparin concentrations in units. To construct separate lines of best fit, by using linear regression analysis for the least squares to the standard solutions and sample solutions, as well as determining the slope of each regression line. To calculate the potency of heparin calcium by using the equation below.

$$P \times \left(\frac{AS}{SP} \right)$$

where

P = potency of Heparin calcium reference standard;
SA and *SP* = inclinations of lines from the *Sample solutions* and *Standard solutions*, respectively.

$$\left(\frac{\text{anti-factor Xa activity}}{\text{anti-factor IIa potency}} \right)$$

To express the potency of the anti-factor Xa for the sample solution as a percentage of heparin concentration determined in the Test. To calculate the ratio of anti-factor Xa versus potency of factor IIa according to equation. The ratio of anti-factor Xa and potency of anti-factor IIa should be at least 0.9 and at most 1.1.

PACKAGING AND STORAGE

In accordance with legislation in force.

LABELLING

In accordance with legislation in force.

THERAPEUTIC CLASS

Anticoagulant.

HEPARIN SODIUM Heparinum natricum

Heparin calcium is a preparation containing sodium salt of a mixture of sulfated glycosaminoglycans, variable weight, in mammalian tissues. It is usually obtained from bovine lungs or porcine intestinal mucosa. It consists of polymers with D-glucosamine (*N*-sulfated or *N*-acetylated) units and uronic acid (L-iduronic or D-glucuronic acid) that combined alternate with glycosidic linkages. It has the property of prolonging the blood clotting primarily by forming

complex of some components of the mixture with specific plasma proteins, thus enhancing the inactivation of thrombin (factor IIa). Other proteases involved in the coagulation process, such as activated factor X (factor Xa), are also inhibited. The ratio of anti-factor Xa activity by the potency of anti-factor IIa should be between 0.9 and 1.1. The potency of heparin sodium should not be less than 180 IU/mg in relation to the desiccated substance. The animals from which heparin is derived must fulfill the health requirements for the species, and the production process must ensure the removal or inactivation of infectious agents.

IDENTIFICATION

A. It complies with the requirements of *Dosage*, depending on the methods *Determination of potency* or *Potency anti-factor IIa*.

B. To employ the nuclear magnetic resonance spectroscopy technique. To prepare the solutions as described below.

Standard solution: not less than 20 mg/ml of Heparin sodium SQR in 99.9% deuterium oxide (w/v) with 0.02% (trimethylsilyl) propionic sodium acid.

Sample solution: not less than 20 mg/mL of the sample in 99.9% deuterium oxide (w/v) with 0.02% (trimethylsilyl) propionic sodium acid.

Procedure: in the analysis of samples: to use a nuclear magnetic resonance spectrometer (at least 500 MHz operating pulse) (Fourier Transform) to acquire ¹H under free-induction decay, by using 16 scans at 90° pulse. The test shall be conducted at constant temperature (25 °C). The spectral window should be at least 10 to -2 ppm. For all the samples, methyl from (trimethylsilyl) propionic compound must be referenced at 0.00 ppm. The chemical shifts of 4 typical regions of heparin (porcine) are H1 of glucosamine *N*-acetylated/glucosamine *N*-sulfated (signal 1) at 5.40 ppm, H1 of iduronic acid 2-sulfated (signal 2) at 5.21 ppm, H2 of glucosamine *N*-sulfated at 3.28 ppm, and glucosamine methyl *N*-acetylated at 2.05 ppm. The ppm values observed for each signal should not vary ± 0.03 ppm.

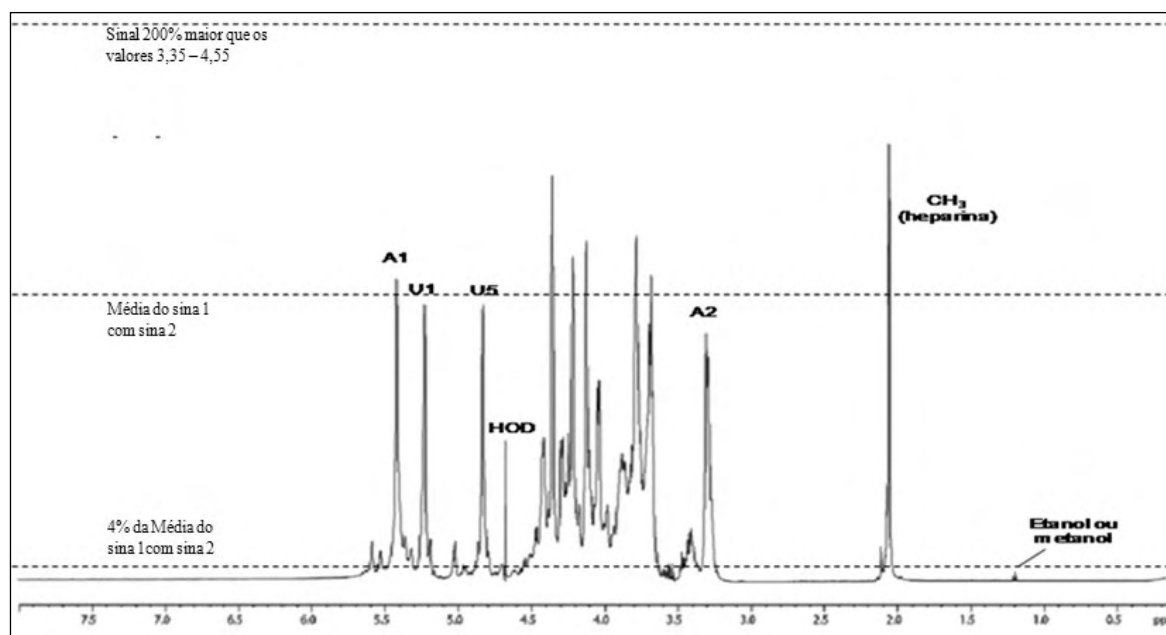
Acceptance criteria are based on the average height signals 1 and 2. Any signal identified in the following fields: 0.10 - 2.00, 2.10 - 3.20, and 5.70 - 8.00 ppm should not exceed 4% of the average height of both signals men-

tioned above. Likewise, no signs 200% higher than 3.35 - 4.55 ppm must be found.

Impurities: oversulfated chondroitin sulfate. The chemical shift in the region N-acetyl of oversulfated chondroitin sul-

fate must be observed at 2.16 ± 0.03 ppm.

Dermatan sulfate: The chemical shift in the region N-acetyl of oversulfated chondroitin sulfate must be observed at 2.10 ± 0.03 ppm.



C. To employ ion-exchange liquid chromatography technique. The ion-exchange chromatography is a test for determination of purity of heparin preparations, especially for the detection and separation of dermatan sulfate, chondroitin sulfate, and oversulfated chondroitin sulfate. To use chromatograph equipped with an ultraviolet detector at 202 nm; 50 mm column and 2 mm inner diameter, packed with anion exchange resin (13 mm); 250 mm long column, and 2 mm internal diameter, packed with anion exchange resin (9 mm), kept at 40 °C; flow of *Mobile phase* of 0.22 mL/min

Reference standards: *Test solution (a)* and *Reference solution*, relative retention of reference heparin (retention time = about 26 min); dermatan and chondroitin sulfate = about 0.9; oversulfated chondroitin sulfate = 1.3, in relation to heparin.

Note: reference solutions should be stabilized for 24h hours at room temperature.

Suitability system: *Reference solution:* relationship peak/valley: at least 1.3, where H_p = height above the base line of the peak due to dermatan plus chondroitin sulfate; H_v = height above the base line the lowest point of the curve that separates the top of this peak due to heparin.

The main peak in the chromatogram obtained with *Test solution (a)* should be similar in form and retention time of the main peak in the chromatogram obtained with *Reference solution (a)*.

To prepare Test solutions as described below.

Test solution (a): to dissolve about 50 mg of the substance, accurately weighed, to be examined in 5.0 mL of water for

chromatography (deionized water having a resistivity not less than 0.18 Mohms). To mix it with a vortex to obtain total dissolution.

Test solution (b): to dissolve about 0.1 g of the substance, accurately weighed, to be examined in 1.0 mL of water for chromatography. To mix it with a vortex to obtain total dissolution. To mix 500 µL of solution and 250 µL of *M* hydrochloric acid, then add 50 µL of 250 mg/mL sodium nitrite solution. To gently mix and let it rest at room temperature for 40 min before adding 200 µL of *M* sodium hydroxide to stop the reaction.

Reference solution (a): to dissolve 250 mg of heparin SQR in water by chromatography and dilute to 2.0 mL with the same solvent. To mix it with a vortex to obtain total dissolution.

Reference solution (b): to add 1200 µL of *Reference solution (a)* and 300 µL of dermatan sulfate and oversulfated chondroitin sulfate. To mix it with a vortex, to make it homogeneous.

Reference solution (c): to add 100 µL of *Reference solution (b)* and 900 µL of water for chromatography. To mix it with a vortex, to make it homogeneous.

Reference solution (d): to add 400 µL of *Reference solution (a)* to 100 µL of water for chromatography and mix it with a vortex. To mix 250 µL of *M* hydrochloric acid, then add 50 µL of 250 mg/mL sodium nitrite solution. To gently mix and let it rest at room temperature for 40 min before adding 200 µL of *M* sodium hydroxide to stop the reaction.

Reference solution (e): To add 250 μL of *M* hydrochloric acid to 500 μL of *Reference solution (b)*, then add 50 μL of 250 mg/mL sodium nitrite solution. To gently mix and let it rest at room temperature for 40 min before adding 200 μL of *M* sodium hydroxide to stop the reaction.

Mobile phase A: to dissolve 0.40 g of sodium dihydrogen phosphate in 1000 mL of water for chromatography and adjust the pH to 3.0 with diluted phosphoric acid solution.

Mobile phase B: to dissolve 0.40 g of sodium dihydrogen phosphate in 1000 mL of water for chromatography, add 140 g of sodium perchlorate, adjust the pH to 3.0 with diluted phosphoric acid, filter, and degas it.

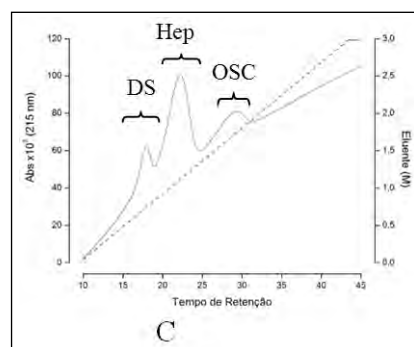
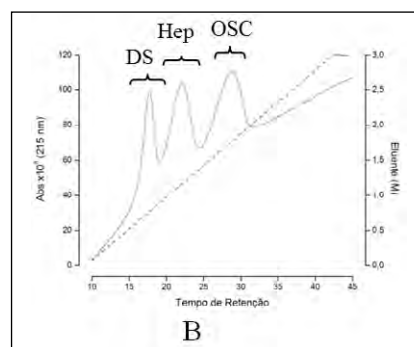
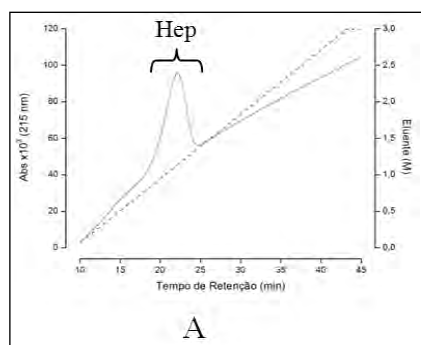
Mobile phase gradient: to adopt the gradient system described in the following table:

Time (minutes)	Mobile phase A%	Mobile phase B%
	(v/v)	(v/v)
0 - 10	75	25
10 - 35	75 $\rightarrow$ 0	25 $\rightarrow$ 100
35 - 40	0	100

Procedure: to inject 20 μL of *Test solution (b)* and *Reference solutions (d)* and *(e)*. Heparin relative retention (retention time = about 26 minutes): dermatan sulfate and chondroitin sulfate = about 0.9 of oversulfated chondroitin sulfate = 1.3. Balance: at least 15 min. The resolution is at least 3.0 between the peaks corresponding to dermatan sulfate plus chondroitin sulfate and oversulfated chondroitin sulfate in the chromatogram obtained with reference. The sum of the areas of dermatan sulfate and chondroitin sulfate is not greater than the area under the corresponding peak in the chromatogram obtained with *Reference solution (e)* 2.0%. No other peaks may exist, except the peak corresponding to dermatan sulfate plus chondroitin sulfate and heparin, that is, there should be no impurities.

Suitability of the system: the chromatogram obtained with *Reference solution (d)* shows no peak at the heparin retention time.

Example:



A - Chromatogram of Hep solution - Reference Heparin.
B - chromatogram of standard solution of the mixtures (DS - dermatan Sulfate - 12%; Hep - Heparin - 44%, and OSC - oversulfated chondroitin sulfate - 54%).
C - chromatogram of a sample failed by the presence of oversulfated chondroitin sulfate (OSC).

D. It responds to the reactions of sodium ion (5.3.1.1).

CHARACTERISTICS

Physical characteristics. White or almost white powder, slightly hygroscopic.

Solubility. Soluble in water.

PURITY TESTS

pH (5.2.19). 5.0 to 8.0. To determine in 1% aqueous solution (w/v).

Proteins. To add 5 drops of 20% trichloroacetic acid (w/v) in 1 mL of the 1% aqueous solution (w/v) of the sample. No turbidity or precipitate should occur.

Heavy metals. To use *Method I*. At most 0.003%.

Nitrogen (5.3.3.2). To use the *Method I, macrodetermination*. It contains at least 1.3% and at most 2.5% of nitrogen, in relation to the desiccated substance.

Sodium (5.2.13.1). 9.5% to 12.5% of sodium determined as described in *Atomic absorption spectrophotometry*.

Loss on drying (5.2.9). To determine in an oven, under vacuum, at 60 °C for 3 hours. At most 5 %.

Sulfated ashes (5.4.2.6). At least 28% and at most 41 %.

Nucleotide impurities: To dissolve 40 mg in 10 mL of water. The absorbance measured at 260 nm and the result should not be greater than 0.2.

BIOLOGICAL SAFETY TEST

Bacterial endotoxins (5.5.2.2). At most 0.03 UE/ IU of heparin.

DOSAGE

Determination of potency.

The anticoagulant activity of heparin is determined in vitro by comparing its capacity under certain conditions to delay the coagulation of reference ovine plasma or reference human plasma, citrated and recalcified with the same capacity of a reference heparin preparation calibrated in international units.

International Unit is the activity contained in an amount specified in the International Standard, which consists of a amount of lyophilized heparin sodium obtained from porcine intestinal mucosa. The equivalence in international units of International Reference Standard is indicated by World Health Organization.

The heparin sodium reference standard is calibrated in international units, as compared to an International Standard by means of the following test.

To conduct the test by using device to mechanically record the change of flow of agitation, by taking care to not agitate the solution during the initial phase of coagulation (coagulometer).

Procedure: The volumes described in the text are given as examples and can be adapted to the device used, since the relationship between the different volumes is observed. To dilute the heparin sodium reference standard in a 9 g/L sodium chloride solution, to provide a precisely known number of International Units per milliliter and prepare a sample solution similar to the preparation to be examined, which should have the same activity. To use a 9 g/L sodium chloride solution (w/v) to prepare a series of dilutions in geometric progression, in such a way that the clotting time obtained with the lowest concentration is not less than 1.5 times the time of blank recalcification. After obtaining the highest concentration, a curve in log dose-response should be satisfactory. To place 12 tubes in a water bath with cold water, by labeling them in duplicate: A1, A2, and A3 for dilutions of preparation to be examined and P1, P2, and P3 for dilutions of reference preparation. To add 1.0 mL of thawed plasma substrate R1 and 1.0 mL of an appropriate dilution of preparation to be examined or reference preparation to each tube. After each addition, mix it, but no bubbles should be formed. To handle the tubes in the following order: P1, P2, P3, A1, A2, A3. To individually transfer the tube to a water bath at 37 ° C for balancing at 37 ° C for about 15 minutes and add 1 mL of a dilution of cephalin to each tube to which was added an appropriate activator, such as kaolin, so that a suitable

recalcification time obtained in blank should not be greater than 60 seconds. When kaolin is used, it must be prepared immediately before using it, a mixture of equal volumes of cephalin and 4 g/L kaolin suspension protected from light in a 9 g/L sodium chloride solution (w/v). Exactly 2 minutes after adding 1 mL of 3.7 g/mL calcium chloride solution and record the clotting time and interval, in seconds, between the last addition and the start of coagulation determined by the technique selected.

To similarly determine the blank recalcification time at the beginning and end of the process, by using 1 mL of 9 g/L sodium chloride solution in place of a dilution of heparin, both values obtained in the blank should not significantly distinguish. To convert the clotting time in logarithms, by using the average value for the tubes in duplicate. To repeat the procedure with fresh dilutions and perform the incubation as follows: A1, A2, A3, P1, P2, P3. To calculate the result by employing the customary statistical methods. To conduct at least 3 independent tests. To prepare new reference solutions for each test and preparation to be examined and use another recently thawed plasma portion. To conduct test for heparin. The estimated potency should be at least 90% and at most 111% of the stated potency. The confidence limits of estimated potency should be not less than 80% and not greater than 125% of the stated potency ($P = 0.95$).

Potency of anti-factor IIa.

Buffer pH 8.4: to dissolve 6.10 g of tris (hydroxymethyl) aminomethane, 10.20 g of sodium chloride, 2.80 g of sodium edetate and, if necessary, between 0 and 10.00 g of polyethylene glycol 6000 and/or 2.00 g of bovine albumin in 800 mL of water. To adjust it with hydrochloric acid to pH 8.4 and dilute to 1000 mL with water.

Note: 2.00 g of human albumin can be replaced by 2.00 g of bovine serum albumin. To adjust it with hydrochloric acid to pH 8.4 and dilute to 1000 mL with water.

Antithrombin solution: to reconstitute a vial of antithrombin in water, to obtain a 5 IU/ mL antithrombin solution. To dilute this Buffer pH 8.4 to obtain a 0.125 IU/mL antithrombin solution.

Human thrombin solution: to reconstitute the human thrombin (Factor IIa) in water, to obtain 20 IU/mL of thrombin, and dilute with Buffer pH 8.4 to obtain 5 IU/mL thrombin solution.

Note: thrombin should have a specific activity of at least 750 IU/mg.

Chromogenic substrate solution: to prepare a solution of a suitable thrombin substrate for chromogenic amidolytic test with water, to obtain 1.25 mM concentration.

Stop solution: 20% (v/v) of acetic acid.

Standard solution: To reconstitute the entire content of a vial of heparin sodium SR in water and dilute with Buffer

pH 8.4 to obtain at least 4 dilutions between the concentration range of 0.005 and 0.03 units/ mL of heparin.

Sample solutions: To proceed as described in the solutions, to obtain the concentrations of heparin sodium similar to those obtained for Standard solutions.

To conduct the test with each Standard solution and Sample solution in duplicate, in relation to each dilution. Numerical labels should be placed depending on the number of repetitions to be tested. For example: if there are 5 blanks to be used B1, B2, B3, B4, and B5; A1, A2, A3, and A4 for each sample in duplicate to be tested and P1, P2, P3, and P4 for each standard solutions in duplicate to be tested. To distribute the blank spaces on the series so that they accurately represent the behavior of the reagents during the experiments.

Note: Tubes should be handled as follows: B1, P1, P2, P3, P4, B2, A1, A2, A3, A4, B3, A1, A2, A3, A4, B4, P1, P2, P3, P4, B5.

To note that after adding reagent, the incubated solution should be mixed without bubbles. To add twice the volume (100 - 200 μ L) of Antithrombin solution to each tube containing a volume (50 - 100 μ L), Buffer pH 8.4 or appropriate dilution of standard or sample solutions. To stir, but avoid formation of bubbles, and incubate it at 37 °C for at least 1 minute. To add 25-50 μ L of Human thrombin solution to each tube and incubate for at least 1 minute. To add 50-100 μ L of Chromogenic substrate solution. To note that all reagents, standard solutions, and sample solutions must be pre-heated at 37 °C shortly before utilization.

Two different types of measurements can be recorded:

“Endpoint” measurement: To stop the reaction after at least 1 minute with 5-10 μ L of Stop solution. To measure the absorbance of each solution at 405 nm by using a suitable spectrophotometer. A relative standard deviation of the blank readings must be less than 10%.

Kinetic Measurement: Following the change in absorbance for each solution for 1 minute at 405 nm by using a spectrophotometer. To calculate the variation in absorbance/ minute (Δ OD/minute). Blanks for kinetic measurement is also expressed as Δ OD/minute and should give higher values that are performed in the absence of heparin. A relative standard deviation of the blank readings must be less than 10%.

Statistical models for analyzing the relation between slope of the straight lines or on parallelism can be used depending on the best model that describes the correlation between concentration and response.

Test on parallelism: For each series, to calculate the regression of absorbance or change in absorbance/minute versus the log concentrations of the sample solutions and standard solutions and calculate the potency of reference heparin sodium in Units/mL, by using statistical methods for parallel line tests. To express the potency of heparin sodium IU/mg on dry basis.

Relationship between slope of the straight lines: For each series, to calculate the regression of log absorbance or log change in absorbance/minute versus concentrations of the sample solutions and standard solutions and calculate the potency of reference heparin sodium in Units/mL,

by using statistical methods for slopes. To express the potency of heparin sodium IU/mg on dry basis.

Acceptance Criteria: The potency of heparin sodium, calculated on a dry basis, is not less than 180 units of heparin per mg.

Anti-factor Xa activity.

Buffer tris (hydroxymethyl) aminomethane and EDTA pH 8.4: To dissolve 10.24 g of sodium chloride, 6.6 g of tris (hydroxymethyl) aminomethane and 2.8 g of disodium EDTA in water. If necessary, adjust the pH to 8.4 with diluted hydrochloric acid or sodium hydroxide solution.

Antithrombin SR solution: to reconstitute the content of a vial containing antithrombin with water (or as recommended by the manufacturer). To dilute with Buffer tris (hydroxymethyl) aminomethane and EDTA pH 8.4, to obtain 1 UI/mL antithrombin solution.

Bovine factor Xa solution: to reconstitute the content of a vial containing bovine factor Xa with water (or as recommended by the manufacturer). To dilute the solution obtained in Buffer pH 8.4, to obtain a solution with absorbance values between 0.65 and 1.25 measured at 405 nm, when tested as described below, but using 30 μ L of Buffer pH 8.4 instead of 30 μ L of *Standard solution* or *Sample solution*.

The factor Xa solution containing 3 catalytic nano units per mL, but can vary depending on the manufacturer of factor Xa or substrate used.

Chromogenic substrate solution: To prepare a chromogenic solution suitable for amidolytic test (see Specifications for reagents in reagents, indicators, and Solutions) specific for factor Xa in water, to obtain about 1 mM concentration.

Stop solution: To prepare 20% acetic acid solution (w/v) in water.

Sample solution: to dissolve exact amount of heparin sodium sample in Buffer pH 8.4 and dilute with it to obtain solutions containing activities roughly equal to the Standard Solution.

Standard solution: to use official standard of heparin. Other preparation may be used, whose potency has been calibrated against the official standard. To reconstitute the content of the vial of official standard of heparin in water and gently mix to obtain total dissolution. To prepare dilutions in Buffer pH 8.4, to obtain 5-7 solutions containing known activities of 0.375, 0.3125, 0.25, 0.188, 0.125, 0.0625, and 0.0313 in units of heparin per mL.

Procedure: the amounts described can be adapted to perform the test in tubes or microplates, by keeping the ratio between the volumes. To conduct the test with each Standard solution and Sample solution in duplicate. To transfer 120 µL buffer 8.4 for each series of plastic tubes in a water bath at 37 °C.

To separately transfer 30 µL of different dilutions of standard solutions or sample solutions to the tubes. To add 150 µL to each tube, mix, and incubate for 2 minutes. To add 300 µL of Chromogenic substrate solution preheated at 37 °C for 15 minutes. To add 150 µL of Stop Solution to each tube and mix it. To prepare the blank to zero the spectrophotometer, by inversely adding the reagents, starting with Stop Solution and ending with the addition of 150 µL of Buffer pH 8.4, and excluding the standard solutions or sample solutions. To record the absorbance measured at 405 nm against the blank.

To construct a graph of absorbance values for the standard solutions and sample solutions against heparin concentrations in units. To construct separate lines of best fit, by using linear regression analysis for the least squares to the standard solutions and sample solutions, as well as determining the slope of each regression line. To calculate the potency of heparin sodium by using the equation below.

$$P \times \left(\frac{AS}{SP} \right)$$

where

P = potency of *Heparin calcium reference standard*;

SA and *SP* = inclinations of lines from the *Sample solutions* and *Standard solutions*, respectively.

$$\left(\frac{\text{anti-factor Xa activity}}{\text{anti-factor IIa potency}} \right)$$

To express the potency of the anti-factor Xa for the sample solution as a percentage of heparin concentration determined in the Test. To calculate the ratio of anti-factor Xa versus potency of factor IIa according to equation. The ratio of anti-factor Xa and potency of anti-factor IIa should be at least 0.9 and at most 1.1.

PACKAGING AND STORAGE

In accordance with legislation in force.

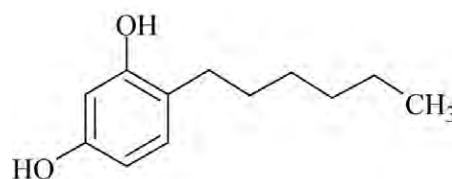
LABELLING

In accordance with legislation in force.

THERAPEUTIC CLASS

Anticoagulant.

HEXYLRESORCINOL Hexylresorcinolum



$C_{12}H_{18}O_2$; 194.27
hexylresorcinol; 04636
4-hexyl-1,3-benzenediol
[136-77-6]

It contains at least 98.0% and at most 101.0% of $C_{12}H_{18}O_2$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or slightly yellowish acicular crystals or plates or clusters of acicular masses or crystalline powder, pronounced styptic odor and flavor, no sensation in tongue.

Solubility. Very slightly soluble in water; freely soluble in benzene, chloroform, ethanol, ethyl ether, glycerol, and fixed oils; practically insoluble in petroleum ether.

Physical and chemical constants.

Melting range (5.2.2): 62 °C to 67 °C.

IDENTIFICATION

A. To add 2 drops of ferric chloride SR to 10 mL of 1% hexylresorcinol solution (w/v) in ethanol. Green color is produced.

B. To add 1 mL of nitric acid SR to 1 mL of hexylresorcinol saturated solution. Slightly red color is produced.

C. To add 1 mL of 0.1 M bromine to 1 mL of hexylresorcinol saturated solution. Yellow flaky precipitate is produced, which is dissolved by adding 2 mL of ammonia SR, thus producing yellow solution.

PURITY TESTS

Acidity. To dissolve 0.25 g of the sample in 500 mL of distilled water. At most 1 mL of 0.02 M sodium hydroxide is spent to neutralize, by using methyl red SI as an indicator.

Resorcinol and other phenols. To stir about 1 g of sample with 50 mL of water for a few minutes. To filter it. To add 3 drops of ferric chloride SR to the filtrate. No red or blue color should be developed.

anti-factor Xa activity **anti-factor IIa potency**

DOSAGE

To accurately dissolve about 70 - 100 mg of sample, previously desiccated over silica gel for 4 hours, in 10 mL of methanol, in a 250 mL iodine bottle. To add 30 mL of 0.1 *M* SV bromine and then quickly add 5 mL of hydrochloric acid by immediately closing it. To cool down the at room temperature with cold running water. To vigorously stir for 5 minutes and let it rest for 5 minutes. To add 6 mL of potassium iodide SR around the stopper and carefully loosen it. Then, close tightly and gently stir it. To add 1 mL of chloroform and titrate the released iodine with 0.05 *M* SV sodium thiosulfate, add 3 mL of starch SI when the endpoint gets closer. To perform sample blank correction. Each mL of 0.1 *M* SV bromine is equal to 4.857 mg of $C_{22}H_{24}N_2O_8$.

PACKAGING AND STORAGE

In airtight closed containers, protected from light.

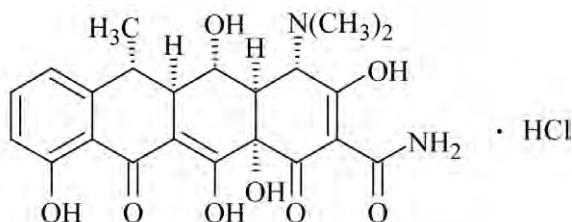
LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Anthelmintic (nematodes and trematodes).

HYCLATE, DOXYCYCLINE Doxycyclini hyclas



$C_{22}H_{24}N_2O_8$; 444.43

$C_{22}H_{24}N_2O_8 \cdot HCl$. $1/2C_2H_6O \cdot 1/2H_2O$; 512.94

doxycycline; 03217

doxycycline hyclate; 03222

(4S, 4aR, 5S, 5aR, 6R, 12aS) -4 - (Dimethylamino)

- 1,4,4a,5,5a,6,11,12 a-octahydro-3,5,10,12,12a-

Pentahydroxy-6-methyl-1,11-dioxo-2-

naphthacenecarboxamide

[564-25-0]

(4S, 4aR, 5S, 5aR, 6R, 12aS) -4 - (dimethylamino)

- 1,4,4a, 5,5a,6,11,12a-octahydro-3,5,10,12,12a-

pentahydroxy-6-methyl-1,11-dioxo-2-naphthacene

carboxamide ethanol hydrate (2:2:1:1)

[24390-14-5]

It contains the equivalent to at least 800 µg and at most 920 µg of doxycycline ($C_{22}H_{24}N_2O_8$) per milligram.

DESCRIPTION

Physical characteristics. Crystalline powder, yellow, hygroscopic, and slightly alcoholic odor, bitter taste.

Solubility. Freely soluble in water and methanol; slightly soluble in ethanol. Soluble in alkalis.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the doxycycline hyclate SQR spectrum similarly prepared.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

C. To weigh 2 mg of the sample and add 5 mL of sulfuric acid. Yellow color is produced.

D. It responds to the reactions of chloride ion (5.3.1.1).

PURITY TESTS

pH (5.2.19). 2.0 to 3.0. To determine in 1% aqueous solution (w/v).

Specific rotation (5.2.8): -105° to -120°. to dissolve 0.25 g of the sample in a mixture of *M* hydrochloric acid and methanol (1:99) and dilute to 25 mL with the same solvent. To perform reading within 5 minutes after preparation.

Absorption of light. to dissolve 25 mg in a mixture of *M* hydrochloric acid and methanol (1:99) and dilute to 25 mL with the same mixture of solvents. To dilute 1 mL of this solution to 100 mL with a mixture of *M* hydrochloric acid and methanol (1:99). To measure 1 hour after preparing the solution. The absorbance of the anhydrous solution (ethanol-free) measured at 349 nm is between 0.300 and 0.335.

Impurities that absorb light. To dissolve 0,10 g of the sample in a mixture of *M* hydrochloric acid and methanol (1:99) and dilute to 10 mL with the same mixture of solvents. To measure 1 hour after preparing the solution. The absorbance of the anhydrous solution (ethanol-free) measured at 490 nm is at most 0.7.

Related substances. To proceed as described in *Dosage*. To prepare the solutions as described below.

Solution (1): to use the *Sample solution* prepared as described in *Dosage*.

Solution (2): to dissolve accurately weighed amount of methacycline hydrochloride SQR with *Diluent* and quantitatively dilute to obtain a 1.2 mg/mL solution.

Solution (3): to prepare as described for *Standard solution* in *Dosage*.

Solution (4): to transfer 2 mL of *Solution (3)* and 2 mL of *Solution (2)* to 100 mL volumetric flask, dilute with *Diluent* to top up the volume, and make it homogeneous. This solution contains about 0.024 mg of doxycycline hyclate SQR and methacycline hydrochloride SQR per mL.

Solution (5): to prepare as described for *Resolution solution* in *Dosage*.

To inject replicates of 20 µL of *Resolution solution* as described in *Dosage*. The relative retention times are about 0.4 to 4-epidoxycycline (the main degradation product), 0.6 to methacycline, and 1.0 to doxycycline. The resolution between 4-epidoxycycline and doxycycline peaks should not be less than 3.0. The tailing factor should not be greater than 2.0. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2.0 %.

Procedure: to separately inject 20 µL of *Solution (4)* and *Solution (1)*, record the chromatograms, in relation to the time, corresponding to 1.7 times the retention time of doxycycline peak, and measure the areas under the peaks. To calculate the percentage of methacycline, according to the equation.

$$10000 (C_M / W)(r_U / r_M)$$

where

C_S = methacycline hydrochloride SQR concentration, in mg/mL, in *Solution (4)*,

W = weight of doxycycline hyclate, in mg, in *Solution (1)*;

r_u = response of methacycline peak in the chromatogram of *Solution (1)*,

r_M = response of methacycline peak in the chromatogram of *Solution (4)*,

No more than 2% of methacycline is found. To calculate the percentages of other related substances in the sample according to the equation:

$$10000 (C_s / W)(r_i / r_s)$$

where

C_s = doxycycline hyclate SQR concentration, in mg/mL, in *Solution (4)*;

W = weight of doxycycline hyclate, in mg, in *Solution (1)*;

r_i = response of each related substance peak in the chromatogram in *Solution (1)*;

r_s = response of methacycline peak in the chromatogram of *Solution (4)*;

No more than 0.5% of any impurity is eluted before finding methacycline; no more than 2% of 6-epidoxycycline is found; and no more than 0.5% of any impurity eluted after the doxycycline main peak is found.

Water (5.2.20.1). 1.4% to 2.8%.

Heavy metals (5.3.2.3). To proceed as described in *Methods of reaction with thioacetamide, Method IV*. At most 0.005% (50 ppm).

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.4 %.

BIOLOGICAL SAFETY TEST

When the label states that the substance is sterile, the sample complies with Bacterial endotoxins and Sterility tests. Should the substance be indicated to sterilization for sterile preparations, the sample complies with Bacterial endotoxins tests.

Sterility (5.5.3.2.1). It complies with the test. To use the *Membrane filtration method*.

Bacterial endotoxins (5.5.2.2). At most 1.14 UE/ mg of doxycycline.

DOSAGE

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 270 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with spherical styrene divinylbenzene copolymer (5 µm), kept at 60 °C ± 1 °C; flow of *Mobile phase* of 1.0 mL/min.

Mobile phase: to dissolve 2.72 g of monobasic potassium phosphate, 0.74 g of sodium hydroxide, 0.5 g of tetrabutylammonium sulfate, and 0.4 g of disodium edetate in 850 mL of water in a 1000 mL volumetric flask. To add 60 g of tert-butyl alcohol by using water, top up with water, and adjust pH to 8.0 ± 0.1 with *M* sodium hydroxide. To filter and deaerate the solution before using it. The decrease in the proportion of tert-butyl alcohol results in prolongation of the retention time of doxycycline and facilitates doxycycline to be separated from its related substances.

Diluent: 0.01 *M* hydrochloric acid.

Sample solution: to accurately transfer about 120 mg of the sample to a 100 mL volumetric flask. To dissolve and top up with *Diluent*. To make it homogenous and filter it.

Standard solution: to accurately transfer about 12 mg of doxycycline hyclate SQR to a 10 mL volumetric flask. To add 6 mL of *Diluent*, stir for 5 minutes or until dissolve it, top up with *Diluent*, and make it homogeneous.

Resolution solution: to prepare 6 mg/mL doxycycline hyclate SQR solution by using the *Diluent*. To transfer 5 mL to 25 mL volumetric flask, heat in a steam bath for 60 minutes, and evaporate to dryness on hot plate, by taking care to not incinerate the residue. To dissolve the residue and top up with *Diluent*. To make it homogenous and filter it. This solution contains a mixture of 4-epidoxycycline, 4-epidoxycycline, and doxycycline.

When stored in the refrigerator, this solution can be used within 14 days.

To inject replicates of 20 µL of the *Resolution solution*. The relative retention times are about 0.4 to 4-epidoxycycline (the main degradation product), 0.7 to 6-epidoxycycline, and 1.0 to doxycycline. The resolution between 4-epidoxycycline and doxycycline peaks should not be less than 3.0. The tailing factor for doxycycline peak should not be greater than 2.0. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2.0 %.

Procedure: to separately inject 20 µL of *Standard and sample solutions*, record the chromatograms, in relation to the time, corresponding to 1.7 times the retention time of doxycycline, and measure the areas under the peaks. To calculate the doxycycline content ($C_{22}H_{24}N_2O_8$) in the sample, per milligram, from the standard content and responses obtained for the *Sample and Standard solutions*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force. Label must indicate whether the product is sterile or must be sterilized during the process, if substance is intended for parenteral preparations.

THERAPEUTIC CLASS

Antibacterial. Parasiticide (plague, trachoma, and malaria).

GOLDEN SEAL Hydrastidis radix

Hydrastis canadensis L. - RANUNCULACEAE

The vegetable drug consists of dried, fragmented rhizomes and roots, containing at least 2.5% hydrastine ($C_{21}H_{21}NO_6$, 383.4), dry basis, and at least 3.0% berberine ($C_{20}H_{18}NO_4$, 336.4), dry basis.

CHARACTERISTICS

Organoleptic characteristics. The drug has a faint odor and strongly bitter taste.

MACROSCOPIC DESCRIPTION

The rhizome horizontally or obliquely grows and sustains several small branches, in addition to adventitious roots. The rhizome is cylindrical, tortuous, often expanded, longitudinally corrugated, from 1 cm to 6 cm long and from 0.2 cm to 1.0 cm diameter; it is externally yellowish-brown or gray-brown and internally is light yellow in the center to greenish-yellow near the edge.

Externally, it is marked by numerous scars, somewhat circular, from the fall of the stems, and other smaller from the fall of shoots and roots. The roots, from the ventral and lateral surfaces, are numerous, filiform, 0.1 cm diameter and 3.5 cm long, bent, twisted, fragile, easily separable and detachable, color similar to that of rhizome.

MICROSCOPIC DESCRIPTION

The rhizome shows, from the periphery to the center, the following tissues: yellowish-brown fragments of bark, composed of polygonal cells in front view, thin and lignified walls; fragments, in cross section, often with irregular brown granular mass, which can darker the bark cells on the outer portion. Cortical parenchyma with about 25 layers of cells, thin-walled, polygonal to rounded, in cross section, and elongated in the longitudinal section, containing starch grains and yellow mass. The starch grains are mostly simple and may also have 2-4 components. The cells in the outer region of the parenchyma have thickened walls with appearance of those of a collenchyma. There is a circle of 12-20 collateral vascular bundles, separated by wide rows of orange-yellow to yellow-greenish parenchyma cells. The xylem consists of small vessel elements, helical, pits, and crosslinked (more rare), with perforation plate in oblique terminal walls. The central portion is occupied by a large medullary parenchyma. The cross-section of the root shows epidermis composed of a single layer of cells, yellowish-brown, with outer suberized walls. Those in front view are more irregular and elongated than those of the rhizome; some of them give rise to trichomes. The cortical parenchyma, with thickened-wall cells, contains starch. The endoderm has cells with slightly lignified walls; in the young roots, in tangential section, the cells are elongated, thin-walled, and markedly sinuous. The vascular system has 2-6 edges of xylem, by alternating with the phloem. The medulla consists of a small central area of unclear parenchyma cells.

MICROSCOPIC DESCRIPTION OF POWDER

The powder meets all the requirements for the species, unless the macroscopic characters. Characteristics: yellowish to greenish-yellow; abundant spherical starch grains, isolated or grouped in 2-4 components, fragments of parenchyma containing starch grains; few fragments of yellowish-brown bark, composed of polygonal cells in front view; in sectional view, it shows irregular masses of dark brown granular substance on the outer portion of the bark; fragments of vascular tissue containing vessel elements with areolar pits, some of them with helical thickening, infrequent xylem fibers from 200 µm to 300 µm long, thin walls and simple pores; occasional fragments of endoderm; numerous spherical to ovoid masses, with orange-brown granular substance, dispersed throughout the powder. The powder does not contain crystals of calcium oxalate or sclerified cells (sclereids).

IDENTIFICATION

A. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel, with 250 µm thickness as support and a mixture of n-propyl alcohol, formic acid, and water (90:1:9) as mobile phase. To separately apply to the plate, as band, 15 µl - 20 µl of *Sample solution* and 3 µl - 5 µl of *Reference solution* prepared as described below.

Solution (1): to extract 0.5 g of the pulverized drug with 15 mL of 60% ethanol (v/v) under magnetic stirring, for 15 minutes. To filter it. To repeat twice the operation and collect the extracts. To concentrate under vacuum to 5 mL. To adjust the residue to 10 mL.

Solution (2): freshly prepared solution of hydrastine hydrochloride and 0.1% berberine hydrochloride (w/v) in 60% ethanol (v/v).

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (365 nm). To develop with potassium iodide and bismuth subnitrate SR and examine it again. The bright yellow fluorescence spot obtained with *Solution (1)*, at the bottom of chromatoplate, corresponds in position to that obtained with *Solution (2)* corresponding to berberine. A second spot, which is dark blue fluorescent, obtained with *Solution (1)*, corresponds in position to that obtained with *Solution (2)*, corresponding to hydrastine. A blue dark-blue fluorescence spot and a bright light blue spot can also be observed. After development with potassium iodide and bismuth subnitrate SR, yellowish spots are observed.

B. To add 5 mL of methylene chloride to 1 g of pulverized drug and macerate it for 1 hour, by occasionally stirring it. To filter it. To evaporate the filtrate. To add 1 mL of sulfuric acid and a crystal of ammonium molybdate to the residue, which will become intense blue, thus characterizing the presence of hydrastine.

PURITY TESTS

Foreign matter (5.4.2.2). At most 2%.

Water (5.4.2.3). At most 10%.

Total ashes (5.4.2.4). At most 8%.

DOSAGE

To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 235 nm; pre-column packed with silica chemically bonded to the octadecylsilane group and 75 mm analytical column and

4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (3.5 µm), flow of *Mobile phase* of 0.30 mL/min.

Mobile phase: mixture of 0.05 M monobasic potassium phosphate and acetonitrile (73:27).

Sample solution: To add 50 mL of an alcoholic solution of 1% ammonia to (v/v) to a 100 mL round bottom flask containing 1000 g of the pulverized sample. To heat to boiling under reflux for 30 minutes. To cool down to room temperature and filter through cotton wool, by collecting the filtrate into an erlenmeyer flask. To collect the cotton wool to the residue in round bottom flask and repeat twice the extraction with 30 mL of an alcoholic solution of 1% ammonia (v/v), by heating under reflux for 10 minutes and filtering through cotton to the same erlenmeyer flask used previously. By using filter paper, filter the filtrates in the erlenmeyer flask into a 250 mL round bottom flask, wash the flask and filter paper with 20 mL of an alcoholic solution of 1% ammonia (v/v). To evaporate the filtrate to dryness at 55°C, in water bath, under reduced pressure. To dissolve the residue in 50 mL of *Mobile phase*. To dilute 1 mL of the resulting solution to 50 mL, by using the *Mobile phase*. To filter through a 0.45 µm membrane and inject into chromatograph.

Standard solution A: to dissolve 10 mg of hydrastine hydrochloride and 10 mg of berberine chloride in methanol and top up to 25 mL with the same solvent.

Standard solution B: to dilute 1 mL of *Standard solution A* to 25 mL by using methanol as solvent.

Solutions for analytical curve: to dilute 2.0 mL of *Standard solution A* to 25 mL volumetric flask and top up with *Mobile phase*. To dilute to 10 mL, with *Mobile phase*, aliquots of 1 mL, 2 mL, 3 mL, 5 mL, and 7 mL, thus obtaining 3.2 µg/mL, 6.4 µg/mL, 9.6 µg/mL, 16.0 µg/mL, and 22.4 µg/mL solutions for hydrastine hydrochloride and berberine chloride. To filter the solutions through a 0.45 µm membrane and inject into chromatograph.

To inject 10 µL of *Standard solution B*. The retention time of hydrastine is less than that of berberine. The resolution between the hydrastine and berberine peaks is at least 1.5.

Procedure: to separately inject 10 µL of *Standard solution A*, *Standard solution B*, and *Sample solution*, record the chromatograms, and measure the areas under the hydrastine and berberine peaks. The relative retention time is about 8.2 minutes to hydrastine and 10.8 minutes to berberine. To calculate the hydrastine and berberine content in the sample, from the line equation obtained from the analytical curves of hydrastine hydrochloride and berberine chloride. The result is expressed as the mean of the determinations in grams for hydrastine and berberine, individually, per 100 g of drug, by taking into account the water determination.

PACKAGING AND STORAGE

In tightly closed container, protected from light and heat.

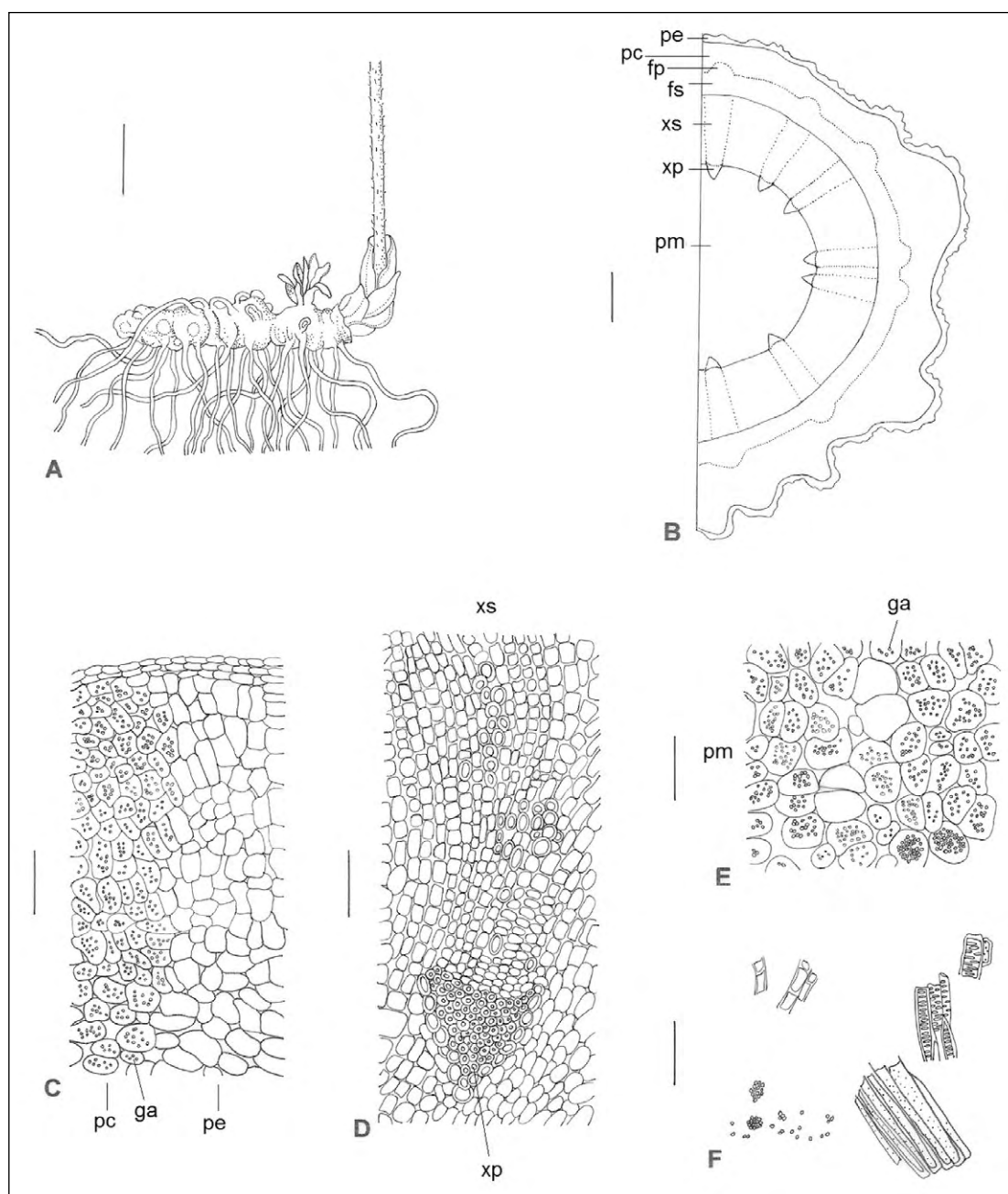


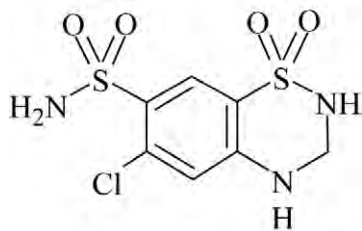
Figure 1 - Macroscopic and microscopic aspects of *Hydrastis canadensis* L.

Additional information of **Figure 1**. Scales correspond to 100 mm in **A**; in **B-F**, 100 μ m.

A - general appearance of rhizome. **B** - scheme of the rhizome in cross section: primary phloem (fp), region of secondary phloem (fs), cortical parenchyma (cp), periderm (pe), medullary parenchyma (pm), primary xylem (xp), secondary xylem (xs). **C** - detail of periderm (pe) and cortical parenchyma (pc) in cross section, numerous starch grains (ga) are represented only in cells to the left. **D** - detail in cross sectional of the following regions, from top to bottom: secondary xylem (xs) showing multiseriate parenchyma beams, fibers, and vessel elements arranged in radial series; primary xylem (xp) with fiber, metaxylem and protoxylem covered with medullary parenchyma; the abundant starch grains present in the medullary parenchyma and parenchyma beams not were expressed. **E** - detail of cross section of medullary parenchyma (pm) presenting numerous starch grains (ga) in most of cells. **F** - general appearance of the powdered rhizome with fragments of bark (above left), vessels (above, right), fiber (below right), starch grains, isolated or aggregated.

HYDROCHLOROTHIAZIDE

Hydrochlorothiazidum



$C_7H_8ClN_3O_4S_2$; 297.74
hydrochlorothiazide; 04652
1,1-Dioxide 6-chloro-3,4-dihydro-2H-1,2,4-
benzothiadiazine-7-sulphonamide
[58-93-5]

It contains at least 98.0% and at most 102.0% of $C_7H_8ClN_3O_4S_2$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white or almost white, and odorless powder.

Solubility. Very slightly soluble in water, soluble in acetone, sparingly soluble in ethanol. Soluble in dilute alkalis.

Physical and chemical constants.

Melting range (5.2.2): 266 °C to 270 °C, with decomposition. IDENTIFICATION

The identification test **A.** may be omitted if the tests **B.** and **C.** are conducted.

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the hydrochlorothiazide SQR spectrum similarly prepared.

B. To determine the absorbance (5.2.14) of the following solutions:

Solution (1): to dissolve 50 mg of the sample in 10 mL of 0.1 M sodium hydroxide solution and top up to 100 mL with water. To dilute 10 mL of this solution to 100 mL with 0.01 M sodium hydroxide solution. The ultraviolet absorption spectrum (5.2.14), ranging from 200 nm to 400 nm, exhibits a maximum at 323 nm. The absorbance at 323 nm is between 0.45 and 0.475.

Solution (2): to dilute 2 mL of *Solution (1)* to 100 mL with 0.01 M sodium hydroxide. The ultraviolet absorption spectrum (5.2.14), ranging from 200 nm to 400 nm, exhibits a maximum at 273 nm. The absorbance at 273 nm is between 0.0505 and 0.053.

C. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

PURITY TESTS

Acidity or alkalinity. To stir 0.5 g of the sample with 25 mL of water for 2 minutes and filter it. To add 0.2 mL of 0.01 M sodium hydroxide and 5 drops of methyl red SI to 10 mL of this solution. Solution turns yellow. At most 0.4 mL of 0.01 M hydrochloric acid are spent to turn pink color.

Chlorides (5.3.2.1). To use *Method II*. To dissolve 1 g of the sample in 25 mL of acetone and top up the volume to 30 mL with water. Fifteen milliliters of this solution should meet the *Limit test for chlorides*. To use 10 mL of standard chloride solution (5 ppm Cl) plus 5 mL of acetone solution in water (85:15) as *Standard preparation*.

Heavy metals (5.3.2.3). At most 0.001 %.

To use the *Method III*.

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 105 °C for 1 hour. At most 0.1 %.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1 %.

DOSAGE

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 254 nm; 250 mm column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm); kept at room temperature; flow of *Mobile phase* of 2.0 mL/min.

Mobile phase: mixture 0.1 M potassium phosphate solution and acetonitrile (9:1). To degas it, adjust the pH to 3.0, and filter.

Sample solution: To accurately transfer about 30 mg of the sample to a 200 mL volumetric flask, dissolve in volume of acetonitrile not exceeding 10% of the capacity of the flask, add *Mobile phase* to top up, and mix it.

Standard solution: to dissolve accurately weighed amount of hydrochlorothiazide SQR in *Mobile phase* to obtain a 0.15 mg/mL solution. Volume of acetonitrile not exceeding 10% of the total volume of the solution can be used to dissolve the standard.

Resolution solution: to dissolve accurately weighed amount of hydrochlorothiazide SQR and chlorothiazide in *Mobile phase* to obtain about 0.15 mg/mL solution.

To inject replicates of 20 µL of the *Standard solution*. The relative standard deviation under the peaks recorded should not be greater than 1.5%.

The relative retention times are about 0.8 to chlorothiazide and 1 to hydrochlorothiazide. The resolution between chlorothiazide and hydrochlorothiazide should be not less than

2. *Procedure*: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of $C_7H_8ClN_3O_4S_2$ from the responses from the *Standard solution* and *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, at 15 °C - 25 °C.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Diuretic.

HYDROCHLOROTHIAZIDE TABLETS

It contains at least 93.0 % and at most 107.0 % of the amount stated of $C_7H_8ClN_3O_4S_2$.

IDENTIFICATION

A. To weigh and pulverize the tablets. To stir amount of powder equivalent to 10 mg of hydrochlorothiazide with 20 mL of acetone. To filter and evaporate the filtrate to dryness. The residue responds to test **A.**, *Identification*, in the monograph *Hydrochlorothiazide*.

B. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF_{254} silica gel as support and a mixture of ethyl acetate and isopropyl alcohol (v/v) (85:15) as mobile phase. To separately apply to the plate 10 µL of each of the solutions freshly prepared, as described below.

Solution (1): to weigh and pulverize the tablets. To stir amount of powder equivalent to 10 mg of hydrochlorothiazide with 10 mL of acetone. To filter it.

Solution (2): 1 mg/mL hydrochlorothiazide SQR solution in acetone.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). The principal spot obtained with *Solution (1)* corresponds in position and intensity to that obtained with *Solution (2)*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 900 mL

Equipment: baskets, 100 rpm

Time: 30 minutes

Procedure: after testing, remove aliquot from the dissolution medium, filter, and dilute, if necessary, with 0.1 M hydrochloric acid, to obtain suitable concentration. To measure the absorbances at 272 nm (5.2.14), by using the same solvent to adjust zero. To calculate the amount of $C_7H_8ClN_3O_4S_2$ dissolved in the medium, by comparing the readings obtained with that of 0.001% hydrochlorothiazide SQR solution (w/v) prepared in the same solvent.

Tolerance: not less than 60% (Q) of the amount stated of $C_7H_8ClN_3O_4S_2$ dissolves within 30 minutes.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

A. To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To weigh and pulverize 20 tablets. To stir for 20 minutes amount of powder equivalent to 30 mg of hydrochlorothiazide with 50 mL of 0.1 M sodium hydroxide. To dilute to 100 mL with the same solvent. To make it homogenous and filter it. To dilute with water to the 0.0015% concentration (w/v). To prepare a standard solution under similar conditions, by using the same solvents. To measure the absorbances of the solutions at 273 nm by using water to adjust zero. To calculate the amount of $C_7H_8ClN_3O_4S_2$ in the tablets, from the readings obtained. Alternatively, make the calculations by taking into account that $A(1\%, 1\text{ cm}) = 520$ at 273 nm.

B. To proceed as described in *Dosage*, in the monograph *Hydrochlorothiazide*. To prepare the Sample solution as described below.

Sample solution: to weigh and pulverize 20 tablets. To transfer amount of powder equivalent to 30 mg of hydrochlorothiazide to a 200 mL volumetric flask, add 20 mL of *Mobile phase*, and put it in ultrasound for 5 minutes. To add 20 mL of acetonitrile and put it in ultrasound for 5 minutes. To add 50 mL of *Mobile phase* and mechanically stir for 10 minutes.

To top up to volume with *Mobile phase*, make it homogeneous, and filter it, by discarding the first 10 mL of filtrate.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of $C_{21}H_{30}ClN_3O_4S_2$ in the tablets, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

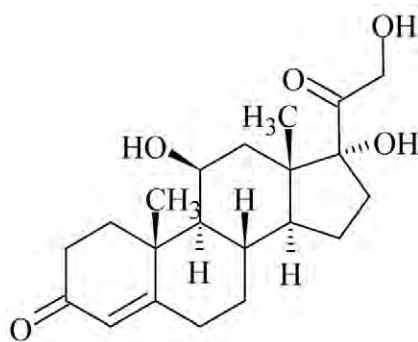
In tightly closed containers.

LABELLING

To comply with the legislation in force.

HYDROCORTISONE

Hydrocortisonum



$C_{21}H_{30}O_5$; 362.46
hydrocortisone; 04664
(11β)-11,17,21-Trihydroxy-pregn-4-ene-3,20-dione
[50-23-7]

It contains at least 97.0% and at most 102.0% of $C_{21}H_{30}O_5$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white or almost white, and odorless powder.

Physical and chemical constants.

Melting range (5.2.2): 214 - 215 °C with decomposition.

Specific rotation (5.2.8): +150° to +156°, in relation to the desiccated substance. To determine in 1% solution (w/v) in dioxane.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the desiccated sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the hydrocortisone SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14), from 200 nm to 400 nm, 0.0002% solution (w/v) in ethanol has absorption maximum similar to those observed in the spectrum of a similar solution of hydrocortisone SQR.

PURITY TESTS

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using 60 silica gel as support and a mixture of chloroform and ethanol (85:15) as mobile phase. To separately apply to the plate 10 µl of each of the solutions as described below.

Solution (1): to dissolve about 20 mg of sample in 10 mL of a mixture of chloroform and methanol (9:1).

Solution (2): to dissolve 1 mL of the *Solution (1)* to 50 mL volumetric flask and top up with a mixture of chloroform and methanol (9:1).

To develop the chromatogram. To remove the plate, allow to air dry, and examine it under ultraviolet light (254 nm). No secondary spot obtained with *Solution (1)* is more intense than the principal spot obtained with *Solution (2)*.

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 105 °C for 3 hours. At most 1.0 %.

Sulfated ashes (5.2.10). To determine in 100 mg of the sample. At most 0.1 %.

DOSAGE

A. To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To accurately transfer 20 mg of the sample to a 100 mL volumetric flask, top up with ethanol, and stir. To transfer 5 mL of the solution to 100 mL volumetric flask and top up with ethanol. To prepare hydrocortisone SQR solution at the same concentration by using the same solvents. To measure the absorbances of the resulting solutions at 242 nm, by using ethanol to adjust zero. To calculate the amount of $C_{21}H_{30}O_5$ from the readings obtained.

B. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 254 nm; 150 mm column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm); flow of *Mobile phase* of 1.0 mL/min.

Mobile phase: mixture of water, acetonitrile, and methanol (50:25:25).

Diluent: mixture of methanol and water (1:1).

Internal standard solution: to prepare about 1 mg/mL propylparaben solution in methanol.

Stock standard solution: to accurately weigh about 25 mg of hydrocortisone SQR and transfer to a 25 mL volumetric

flask. To dissolve in methanol, top up to volume, and make it homogeneous, to obtain about 1 mg/mL solution.

Standard solution: to transfer 2.0 mL of the *Stock standard solution* and 2.0 mL of the *Internal standard solution* to a 50 mL volumetric flask. To top up with *Diluent* and make it homogeneous.

Sample solution: to accurately weigh about 50 mg of the sample and transfer to a 50 mL volumetric flask. To dissolve and dilute with methanol to top up volume and make it homogeneous. To transfer 2.0 mL of the solution and 2.0 mL of *Internal standard solution* to a 50 mL volumetric flask. To top up with *Diluent* and make it homogeneous.

To inject replicates of 10 µL of the *Standard solution*. The column efficiency is greater than 3000 theoretical plates to hydrocortisone. The relative retention times are about 1.8 to propylparaben and 1.0 to hydrocortisone. The tailing factor should not be greater than 1.2. The resolution between hydrocortisone and propylparaben should be greater than 9.0. The relative standard deviation for replicate areas of the peaks recorded should not be greater than 2.0 %.

Procedure: to separately inject 10 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the area under the peaks. To calculate the amount of $C_{21}H_{30}O_5$ from the responses from the *Standard solution* and *Sample solution* by using the equation below.

$$1250.C (A_A/A_P)$$

where

C is the concentration in mg/mL of the *Standard solution*; A_A/A_P are the area ratios of hydrocortisone and propylparaben obtained from the *Standard solution* and *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

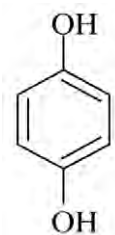
LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Anti-inflammatory

HYDROQUINONE



$C_6H_6O_2$; 110.11
hydroquinone; 09457
1,4-Benzenediol
[123-31-9]

It contains at least 99.0% and at most 100.5% of $C_6H_6O_2$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White, crystalline crystals that get darker upon exposure to air.

Solubility. Soluble in water, freely soluble in ethanol and ethyl ether, practically soluble in chloroform; practically insoluble in benzene.

Physical and chemical constants.

Melting range (5.2.2): 170 °C to 171 °C.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the hydroquinone SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14), from 200 nm to 400 nm, of 0.0025% solution (w/v) of the sample, in methanol, exhibits absorption maxima similar to those observed in the spectrum of hydroquinone SQR solution at the same concentration.

PURITY TESTS

Water (5.2.20.1). To determine in 3 g of the sample. At most 0.5 %.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.5 %.

DOSAGE

To accurately weigh about 0.25 g of the sample, dissolve in a mixture of 100 mL of water and 0.05 M sulfuric acid (w/v), and add 3 mL of diphenylamine SR. To titrate with 0.05 M SV ceric sulfate to obtain red color.

Each mL of 0.05 M SV ceric sulfate is equal to 5.506 mg of $C_6H_6O_2$.

PACKAGING AND STORAGE

In containers protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Depigmenting agent.

HYDROXOCOBALAMIN INJECTABLE SOLUTION

It contains at least 95% and at most 1151 % of the amount stated of $C_{62}H_{89}CoN_{13}O_{15}P$.

IDENTIFICATION

To dilute 3 mL of injectable solution to 100 mL, by using *buffer pH 4.0*, as described below. The ultraviolet and visible absorption spectra (5.2.14) exhibit maxima at 352 ± 1 nm and 528 ± 2 nm. The ratio between the absorbance values measured at 352 nm and 528 nm is between 2.7 and 3.3.

Buffer pH 4.0: to dissolve 2.61 g of sodium acetate and 20.5 g of sodium chloride in 5.25 mL of glacial acetic acid and water sufficient to top up 1500 mL of solution. To adjust the pH, if necessary.

CHARACTERISTICS

pH (5.2.19). 3.5 to 5.0.

Determination of volume (5.1.2). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

BIOLOGICAL SAFETY TEST

Bacterial endotoxins (5.5.2.2). At most 0.4 EU/ μ g of hydroxocobalamin.

Sterility (5.5.3.2.1). It complies with the test. To use Membrane filtration method.

DOSAGE

To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*.

Borate buffer pH 9.3: to dissolve 28.3 g of sodium tetraborate and 402 mg of boric acid in water sufficient to complete 1500 mL of solution, and make it homogeneous.

To adjust the pH, if necessary.

Sample solution: transfer an accurately measured volume of injectable solution, equivalent to about 5mg of hydroxocobalamin to a 50 mL volumetric flask containing 25mL of *Borate buffer pH 9.3*. To add 5 mL of potassium cyanide solution (1:10000). To let it rest for 30 minutes at room temperature and dilute with *Borate buffer pH 9.3* to indicated volume, and make it homogeneous. To transfer 15 mL of this solution to a second 50 mL volumetric flask, dilute with *Borate buffer pH 9.3* to the indicated volume, and make it homogeneous.

Standard solution: to dissolve in *Borate buffer pH 9.3* an accurately weighed amount of cyanocobalamin SQR and quantitatively dilute step by step, if necessary, to obtain about 30 μ g/mL solution.

Procedure: to simultaneously determine the absorbances of solutions in a 1 cm cuvette at (about 361 nm wavelength) of maximum absorption with a suitable spectrophotometer, by using *Borate buffer pH 9.3* as a blank. To calculate in mg the amount of $C_{62}H_{89}CoN_{13}O_{15}P$ per mL of injectable solution, according to the equation:

$$\left(\frac{1346,38}{1355,39} \right) \times \left(\frac{0,1667 \times c}{V} \right) \times \left(\frac{Au}{As} \right)$$

where

1346.38 and 1355.39 = molecular weights of hydroxocobalamin and cyanocobalamin, respectively;

C = concentration in μ g/mL of cyanocobalamin SQR solution to prepare *Standard solution*;

V = volume in mL of injectable solution taken;

Au and *As* = absorbances of *Sample solution* and *Standard solution*, respectively.

PACKAGING AND STORAGE

In type I glass containers, single dose or multiple dose, protected from light.

LABELLING

To comply with the legislation in force.

HYDROXIDE, ALUMINUM

Aluminii hydroxidum

Al(OH)₃; 78.00
Al₂O₃; 101.96
aluminum hydroxide; 04694
Aluminum hydroxide
[21645-51-2]

It contains at least 47.0% and at most 60.0% of Al₂O₃.

DESCRIPTION

Physical characteristics. White or almost white, amorphous powder.

Solubility. It is practically insoluble in water. Soluble in alkalis and acid solutions.

IDENTIFICATION

A. The solution obtained in *Appearance of solution* responds to the reactions of aluminum ion (5.3.1.1).

B. To dissolve 15 mg of the sample in 2 mL of water and 0.5 mL of 2 M hydrochloric acid. To filter it. To add 0.5 mL of thioacetamide SR. No precipitate is produced. To add dropwise 2 M sodium hydroxide. Gelatinous white precipitate is formed, which dissolves in 2 M sodium hydroxide in excess. To gradually add ammonium chloride SR. Gelatinous white precipitate is produced.

PURITY TESTS

Appearance of solution. To dissolve 2.5 g of the sample in 15 mL of hydrochloric acid SR, heat in a water bath, and top up to 100 mL with water. The solution obtained is not more opalescent than the *Reference suspension II* (5.2.25) and is not more colored than the *Reference color solution* (5.2.12) prepared as described below.

Reference color solution: to prepare mixture of *Ferric chloride base solution*, *Cobaltous chloride base solution*, and *Cupric sulfate base solution* (96:2:2) (5.2.12). To transfer 1.5 mL of the resulting solution to a 100 mL volumetric flask and top up with 1% hydrochloric acid (w/v).

Alkalinity. To accurately stir exactly about 1 g of the sample in 20 mL of carbon dioxide-free water for 1 minute. To filter it. To add 0.1 mL of phenolphthalein SI to 10 mL of filtrate. Should pink color be developed, at most 0.3 mL of 0.1 M hydrochloric acid is spent to neutralize 10 mL of the filtrate.

Neutralizing capacity. To stir 0.5 g of the sample in 100 mL of water kept at 37 °C throughout the test time.

To add 100 mL of 0.1 M hydrochloric acid at 37 °C and continuously stir it. The pH of the solution after 10 minutes, 15 minutes, and 20 minutes, is not less than 1.8, 2.3, and 3.0, respectively. It is not greater than 4.5 at any time. To add 10 mL of 0.5 M hydrochloric acid at 37 °C and continuously stir it

for 1 hour. To add 0.1 M sodium hydroxide at 37 °C to obtain pH 3.5. At most 35 mL of 0.1 M sodium hydroxide are spent.

Chlorides (5.3.2.1). To proceed as described in *Limit test for chlorides*, except that the *Standard preparation* and *Sample preparation* should not be diluted to 50 mL. At most 1.0% (10000 ppm).

Sample preparation: to dissolve, by heating, 0.106 g of the sample in 10 mL of 2 M nitric acid and top up the volume to 100 mL with water. To transfer 10 mL of the resulting solution to suitable tube and dilute to 25 mL with water.

Standard preparation: To transfer 0.3 mL of 0.01 M hydrochloric acid to suitable tube and dilute to 25 mL with water.

Sulfates (5.3.2.2). To dilute 4 mL of resulting solution obtained in *Appearance of solution* to 100 mL with water. To use 15 mL of this solution. To use 0.3 mL of 0.005 M sulfuric for the *Standard preparation*. At most 1.0% (10000 ppm).

Arsenic (5.3.2.5). To use *Method III*. To use 10 mL of the solution obtained in *Appearance of solution*. At most 0.0004% (4 ppm).

Heavy metals (5.3.2.3). To dissolve, by heating, 0.33 g of the sample in 10 mL of 3 M hydrochloric acid, filter, if necessary, and dilute to 25 mL with water. At most 0.006% (60 ppm).

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). Total aerobic bacteria: At most 1000 UFC/g.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test for enterobacteriaceae and *Escherichia coli*.

DOSAGE

To proceed as described in *Complexometric titrations* (5.3.3.4) for *Aluminum*. To accurately dissolve about 0.8 g of sample in 10 mL of hydrochloric acid SR by heating it in a water bath. To cool down and dilute to 50 mL with water. To add ammonia SR to 10 mL of this solution, to initiate the formation of precipitate. To add sufficient amount of hydrochloric acid SR to dissolve the precipitate and dilute to 20 mL with water. Each mL of 0.1 M SV disodium edetate is equal to 5.098 mg of Al₂O₃.

PACKAGING AND STORAGE

In tightly closed containers, at temperature below 30 °C.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antacid.

HYDROXIDE, ALUMINUM CHEWABLE TABLETS

It contains at least 90.0% and at most 110.0% of the amount stated of $\text{Al}(\text{OH})_3$. Chewable tablets may contain sweeteners.

IDENTIFICATION

A. To weigh and pulverize the tablets. To dissolve the amount of powder equivalent to 15 mg of aluminum hydroxide in 2 mL of water. It responds to the reactions of aluminum ion (5.3.1.1).

B. To weigh and pulverize the tablets. To use amount of powder equivalent to about 15 mg of aluminum hydroxide, add 2 mL water and 0.5 mL 2 M hydrochloric acid, and filter it. To add 0.5 mL of thioacetamide SR. No precipitate is produced. To add dropwise 2 M sodium hydroxide. Gelatinous white precipitate is formed, which dissolves in 2 M sodium hydroxide in excess. To gradually add ammonium chloride SR. Gelatinous white precipitate is produced.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

ACID NEUTRALIZING CAPACITY

To transfer an amount equivalent to a tablet to 250 mL beaker. To add 70 mL of water and mix it for 1 minute, by using magnetic stirrer. To transfer 25 mL of 0.05 M hydrochloric acid and stir for 15 minutes. To immediately titrate the acid in excess within 5 minutes, by using 0.05 M sodium hydroxide, to obtain a stable pH 3.5 (10 - 15 seconds).

To conduct the test at $(37 \pm 3)^\circ\text{C}$. At least 5 mEq of acid is consumed, and at least 55.0% of the expected mEq value from the amount stated of aluminum hydroxide is obtained. Each mg of $\text{Al}(\text{OH})_3$ can neutralize 0.0385 mEq.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To weigh and pulverize 20 tablets. To weigh an amount of powder equivalent to 1.2 g of $\text{Al}(\text{OH})_3$, add 15 mL of concentrated hydrochloric acid, and heat to dissolve it. To dilute with

water to about 100 mL, stir, quantitatively filter to a 500 mL volumetric flask, by washing with water. To top up the volume with water and stir. To transfer 20 mL of this solution to a 250 mL erlenmeyer flask, add 25 mL of 0.05 M SV disodium edetate and 20 mL of acetic acid-ammonium acetate buffer and heat to boiling for 5 minutes. To cool down, add 50 mL of ethanol and 2 mL of 0.025% dithizone (w/v). To titrate the solution with 0.05 M SV zinc sulphate to obtain pinkish color. To perform sample blank correction, by using 20 mL of water instead of 20 mL of sample solution. Each mL of 0.05 M SV disodium edetate is equal to 3.900 mg of $\text{Al}(\text{OH})_3$.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

HYDROXIDE, ALUMINUM, GEL

Aluminum hydroxide gel is an amorphous aluminum hydroxide suspension in which there is partial replacement of carbonate hydroxide. It contains at least 90.0 % and at most 110.0 % of the amount stated of $\text{Al}(\text{OH})_3$.

IDENTIFICATION

A. To add 4 mL of concentrated hydrochloric acid to 1 g of sample. To heat at 60°C for 1 hour, cool down, dilute to 50 mL with water, and filter it, if necessary. To add 0.5 mL of 2 M hydrochloric acid and 0.5 thioacetamide SR to 10 mL of the resulting solution. No precipitate is produced. To slowly add 5 mL of 2 M sodium hydroxide and let it rest for 1 hour.

Gelatinous white precipitate is produced, which is dissolved by adding 5 mL of 2 M sodium hydroxide. To slowly add 5 mL of ammonium chloride SR and let sit rest for 30 minutes. Gelatinous white precipitate is produced again.

B. To add 4 mL of concentrated hydrochloric acid to 1 g of sample. To heat at 60°C for 1 hour, cool down, dilute to 50 mL with water, and filter it, if necessary. The resulting solution responds to the reactions of aluminum ion (5.3.1.1).

CHARACTERISTICS

pH (5.2.19). 5.5 to 8.0.

PURITY TESTS

Arsenic (5.3.2.5). To dissolve accurately weighed amount of sample equivalent to 0.3 g of $\text{Al}(\text{OH})_3$, in 20 mL of 10 M sulfuric acid. To proceed as described in *Spectrophotometric method, Method I*. At most 0.001% (10 ppm).

Chlorides. To transfer accurately weighed amount of sample equivalent to 0.6 g of $\text{Al}(\text{OH})_3$ to a porcelain capsule. To add 0.1 mL of potassium chromate SR and 25 mL of water.

To stir. To drip 0.1 *M* silver nitrate to obtain persistent pink color. At most 8 mL of 0.1 *M* silver nitrate are spent (4.7%).

Heavy metals (5.3.2.3). To dissolve 2.3 g of the sample in 20 mL of *M* hydrochloric acid and 10 mL of water. To add 0.5 mL of nitric acid and boil it for 30 seconds. To cool down, add 2 g of ammonium chloride and 2 g of ammonium thiocyanate, and extract with 2 portions (10 mL each) of a mixture of isoamyl alcohol and ethyl ether (1:1). To add 2 g of citric acid to the aqueous phase and dilute to 40 mL with water. To use 18 mL of the resulting solution and proceed as described in *Method I*. At most 0.002% (20 ppm).

Sulfates (5.3.2.2). To dissolve accurately weighed amount of sample equivalent to 0.15 g of $\text{Al}(\text{OH})_3$ in 5 mL of 3 *M* hydrochloric acid, by heating it, if necessary. To filter and proceed as described in *Limit test for sulfates*. At most 0.8% (8000 ppm).

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

To cool down, transfer to a 500 mL volumetric flask, and top up with water. To transfer 20 mL of the solution to 250 mL erlenmeyer flask and add, by constantly stirring, 25 mL of 0.05 *M* SV disodium edetate and 20 mL of acetic acid-ammonium acetate buffer. To heat for 5 minutes. To cool down, add 50 mL of ethanol and 2 mL of 0.025% dithizone (w/v) in ethanol. To titrate with 0.05 *M* SV zinc sulfate to violet-greenish becomes pink. To perform sample blank correction, by using 20 mL of water, and adjust it as required. Each mL of 0.05 *M* SV disodium edetate is equal to 3.900 mg of $\text{Al}(\text{OH})_3$.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

HYDROXIDE, CALCIUM

Calcii hydroxidum
 $\text{Ca}(\text{OH})_2$; 74.09
 calcium hydroxide; 04696
 Calcium hydroxide
 [1305-62-0]

It contains at least 95.0% and at most 100.5% of $\text{Ca}(\text{OH})_2$.

DESCRIPTION

Physical characteristics. Fine, white or almost white powder.

Solubility. Practically insoluble in water; soluble in glycerol. Soluble in acids, with heat-releasing.

IDENTIFICATION

A. To transfer 0.8 g of the sample to glass mortar, add 10 mL of water, 0.5 mL of phenolphthalein SI, and make it homogeneous. The red color develops. To add 17.5 mL of *M* hydrochloric acid. Suspension becomes colorless, with no effervescence. To grind the mixture for 1 minute. Red color fades in again. To add 6 mL of *M* hydrochloric acid and grind it. Solution becomes colorless.

B. To dissolve 0.1 g of the sample in hydrochloric acid SR and dilute to 10 mL with water. Solution responds to the reactions of calcium ion (5.3.1.1).

DOSAGE

To transfer accurately weighed amount of sample equivalent to 1.5 g of $\text{Al}(\text{OH})_3$ to the beaker and add 15 mL of concentrated hydrochloric acid, by heating, to obtain total solubilization.

PURITY TESTS

Alkalinity and alkaline earth metals. To dissolve 1 g of the sample in the mixture of 10 mL of hydrochloric acid and 40 mL of water. To heat to boiling and add 50 mL of oxalic acid MR. To neutralize with ammonia and top up the volume to 200 mL with water.

To let it rest for 1 hour and filter through suitable filter. To carefully add 0.5 mL of sulfuric acid to 100 mL of the filtrate. To evaporate to dryness and incinerate it. At most 20 mg of residues (4.0% of sulfates).

Carbonates. To add 5 mL of *M* SV hydrochloric acid to solution obtained in *Dosage*. To titrate with *M* SV hydrochloric acid, by using 0.5 mL of methyl orange SI as an indicator. Each mL of *M* SV hydrochloric acid is equal to 50.05 mg of calcium carbonate. At most 5% of CaCO_3 .

Arsenic (5.3.2.5). To dissolve 0.75 g of the sample in 15 mL of hydrochloric acid. To proceed as described in *Spectrophotometric method, Method I*. The addition of 20 mL of 3.5 *M* sulfuric acid specified in the procedure may be omitted. At most 0.0004% (4 ppm).

Chlorides (5.3.2.1). To dissolve 1.07 g of the sample in 7 mL of nitric acid. To dilute to 40 mL with water and proceed as described in *Limit test for chlorides*. At most 0.033% (330 ppm).

Sulfates (5.3.2.1). To dissolve 0.3 g of the sample in 10 mL of hydrochloric acid SR and proceed as described in *Limit test for sulfates*. At most 0.4% (4000 ppm).

Heavy metals (5.3.2.3). To dissolve 1 g of the sample in 10 mL of 3 *M* hydrochloric acid and evaporate to dryness in a water bath. To dissolve the residue in 20 mL of water.

To filter it. To proceed as described in *Method I*. At most 0.002% (20 ppm).

Substances insoluble in acid. To dissolve 2 g of the sample in 30 mL of hydrochloric acid. To heat to boiling and filter. To wash the residue with hot water and incinerate it. The weight of the residue is not greater than 10 mg. At most 0.5 %.

DOSAGE

To accurately transfer about 1.5 g of the sample to a glass mortar. To add 20 mL - 30 mL of water and 0.5 mL of phenolphthalein SI. To titrate with *M* SV hydrochloric acid, by grinding the substance until red color fades in. Each mL of *M* SV hydrochloric acid is equal to 37.045 mg of $\text{Ca}(\text{OH})_2$.

PACKAGING AND STORAGE

In nonmetallic containers, tightly closed.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Astringent.

HYDROXIDE, MAGNESIUM Magnesii hydroxidum

$\text{Mg}(\text{OH})_2$; 58.32
magnesium hydroxide; 04697
Magnesium hydroxide
[1309-42-8]

It contains at least 95.0% and at most 100.5% of $\text{Mg}(\text{OH})_2$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or almost white, fine, amorphous, odorless powder.

Solubility. It is practically insoluble in water. Soluble in diluted acid solutions.

IDENTIFICATION

A. To prepare an aqueous suspension of the sample and add phenolphthalein SI. The pinkish color develops.

B. To dissolve about 15 mg of the sample in 2 mL of nitric acid SR and neutralize with 8.5% sodium hydroxide solution (w/v). The solution responds to the reactions of magnesium ion (**5.3.1.1**).

PURITY TESTS

Appearance of solution. To dissolve 5 g of sample in a mixture of 50 mL of acetic acid SR and 50 mL of water. Mild effervescence is developed. To boil for 2 minutes and dilute to 100 mL with 2 *M* acetic acid. To filter in the porcelain or silica filtering crucible previously calcined and tared, with adequate porosity to obtain a clear filtrate. The solution is not intensely darker than the *Reference color solution* (**5.2.12**).

Reference color solution: to prepare a mixture of 3 parts of *Cobaltous chloride base solution*, 2.4 parts of *Cupric sulfate base solution*, 3 parts of *Ferric chloride base solution*, and 1.6 parts of 1% hydrochloric acid (w/v). To dilute 3.75 volumes of this solution with 6.25 volumes of 1% hydrochloric acid (w/v) at the moment of using it.

Soluble substances. To mix 2 g of the sample in 100 mL of water and boil for 5 minutes. By using a sintered glass funnel, filter the hot mixture, cool down, and dilute to 100 mL with water. To evaporate to dryness 50 mL of the resulting solution at 100 °C - 105 °C. The weight of the residue is not greater than 20 mg. At most 2.0 %.

Substances insoluble in acetic acid. The weight of the residue obtained in *Appearance of solution*, after washing with 2 *M* acetic acid, dessication, and incineration at 600 °C, is not greater than 5 mg. At most 0.1 %.

Arsenic (5.3.2.5). To determine in 5 mL of the solution obtained in *Appearance of solution*. To proceed as described in *Visual method*. At most 0.0004% (4 ppm).

Calcium (5.3.2.7). To dilute 1.3 mL of resulting solution obtained in *Appearance of solution* to 150 mL with water. To determine in 15 mL of this solution. At most 1.5 %.

Chlorides (5.3.2.1). To determine in 7 mL of the solution obtained in *Appearance of solution*. To use 1 mL of 0.01 *M* hydrochloric acid for the *Standard preparation*. At most 0.1% (1000 ppm).

Iron (5.3.2.4). To dissolve 0.143 g of the sample in 5 mL of 2 *M* hydrochloric acid and dilute to 10 mL with distilled water. To use 1 mL of this solution and proceed as described in *Method I*. To use 10 mL of *Iron standard solution* (1 ppm Fe). At most 0.07% (700 ppm).

Sulfates (5.3.2.2). To determine in 2 mL of the solution obtained in *Appearance of solution*. To use 1 mL of 0.005 *M* sulfuric acid for the *Standard preparation*. At most 0.5% (5000 ppm)

Heavy metals (5.3.2.3). To dissolve 1 g of the sample in 15 mL of 3 *M* hydrochloric acid and evaporate to dryness in a water bath. About to end the evaporation, continuously stir the residue to disintegrate it, to obtain a fine dry powder. To dissolve the residue in 20 mL of water and filter it. To add 2 mL of *M* acetic acid to the filtrate and dilute with water to 25 mL. At most 0.002% (20 ppm).

Loss on drying (5.2.9). To determine in an oven at 105 °C for 2 hours. At most 2.0 %.

Sulfated ashes (5.2.10). To determine in 0.5 g of the sample. To gradually heat up to 900 °C and calcine to obtain constant weight. From 29.0% to 32.5%.

DOSAGE

To dissolve 0.1 g of the sample in 2 mL of 2 M hydrochloric acid and proceed as described in *Complexometric titrations (5.3.3.4)* for magnesium. Each mL of 0.1 M SV disodium edetate is equal to 5.832 mg of $\text{Mg}(\text{OH})_2$.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antacid.

HYDROXIDE, POTASSIUM Kalli hydroxidum

KOH; 56.11
potassium hydroxide; 04698
Potassium hydroxide
[1310-58-3]

It contains at least 85.0% and at most 100.5% of KOH.

DESCRIPTION

Physical and chemical characteristics. White or slightly yellowish particles, crystalline, provided as spheres, shavings, rods, or irregular pieces. Hygroscopic and deliquescent; it quickly absorbs atmospheric carbon dioxide. Melting point (5.2.2): it melts around 360 °C.

Solubility. Very soluble in water, and freely soluble in ethanol.

IDENTIFICATION

A. To dissolve 0.1 g of the sample in 10 mL of water. To dilute 1 mL to 100 mL with the same solvent. The pH (5.2.19) of the resulting solution is greater than 10.5.

B. The 1% aqueous solution (w/v) of the sample responds to the reactions of potassium ion (5.3.1.1).

PURITY TESTS

Appearance of solution. To dissolve 5 g of the sample in carbon dioxide-free water and dilute to 50 mL with the

same solvent. The solution is clear (5.2.25) and colorless (5.2.12).

Chlorides (5.3.2.1). To accurately weigh about 8.75 g of the sample and transfer to a 25 mL volumetric flask. To dissolve in 10 mL of water. To slowly add 2 mL of nitric acid, cool down, and top up with nitric acid SR. To use 20 mL of this solution and proceed as described in *Limit test for chlorides*. At most 0.005% (50 ppm).

Carbonates. To proceed as described in *Dosage*. Each mL of M hydrochloric acid required to change color of bromophenol blue SI solution is equal to 69.103 mg of K_2CO_3 . At most 2%.

Sulfates (5.3.2.1). To accurately weigh about 15 g of the sample and transfer to a 50 mL volumetric flask. To dissolve in 15 mL of water. To slowly add 12 mL of hydrochloric acid, cool down, and dilute to 50 mL with diluted hydrochloric acid. To use 30 mL of the resulting solution and 1 mL of 0.005 M standard hydrochloric acid solution. To proceed as described in *Limit test for sulfates*. At most 0.005% (50 ppm).

Sodium. To proceed as described in *Atomic spectrometry (5.2.13.1.1)*, direct calibration method. To dissolve 1 g of the sample in 50 mL of water, add 5 mL of sulfuric acid, and top up to 100 mL with water. To dilute 1 mL of this solution to 10 mL with water. To prepare reference solution by dissolving, in water, 0.5084 g of sodium chloride previously desiccated at 105 °C for 3 hours, and dilute to 1000 mL with the same solvent (0.2 mg/mL of sodium). To dilute with water to prepare standard solutions as required. To make reading at 589 nm by using hollow sodium cathode lamp and air-acetylene flame as source of radiation. At most 1.0% (10000 ppm).

Iron (5.3.2.4). To use *Method III*. To dissolve 10 g of the sample in 15 mL of water. To carefully add 12 mL of hydrochloric acid, cool down, dilute with 7.3% hydrochloric acid (w/v), and top up to 50 mL with the same solvent. To use 5 mL of this solution. At most 0.001% (10 ppm).

Aluminum (5.3.2.10). It is required for potassium hydroxide to prepare hemodialysis solutions. To dissolve 20 g of the sample in 100 mL of water and add 10 mL of acetate buffer pH 6.0. To proceed as described in *Limit test for aluminum*. To use as a reference solution a mixture of 2 mL of *Standard aluminum solution (2 ppm Al)*, 10 mL of acetate buffer pH 6.0, and 98 mL of water. To prepare the blank, use a mixture of 10 mL of acetate buffer solution pH 6.0 and 100 mL of water. At most 0.00002% (0.2 ppm).

Heavy metals (5.3.2.3). To use *Method I*. To dilute 10 mL of the solution obtained in the test for *Iron* to 20 mL with water. To use the *Standard lead solution (1 ppm Pb)* as reference. At most 0.001% (10 ppm).

DOSAGE

To accurately weigh about 2 g of the sample and dissolve in 25 mL of carbon dioxide-free water. To add 25 mL of 0.025 *M* barium chloride, freshly prepared, and 0.3 mL of phenolphthalein SI. By stirring, slowly add 25 mL of *M* SV hydrochloric acid. To continue the titration with *M* SV hydrochloric acid to indicator changes from pink to colorless. To add 0.3 mL of bromophenol blue SI and continue the titration with *M* SV hydrochloric acid to indicator changes from violet-blue to yellow. Each mL of *M* SV hydrochloric acid used in the second part of the titration is equal to 69.103 mg of K_2CO_3 . Each mL of *M* SV hydrochloric acid used to combine titrations is equal to 56.110 mg of total alkalinity, calculated as KOH.

PACKAGING AND STORAGE

In nonmetallic containers, tightly closed.

LABELLING

To comply with the legislation in force.

CATEGORY

Alkalizing agent.

HYPOCHLORITE, SODIUM DILUTED SOLUTION

Aqueous solution of sodium hypochlorite. It contains at least 2.0% (w/v) and at most 3.0% (w/v) of NaClO equivalent to at least 1.9% (w/v) and at most 2.9% (w/v) of active chlorine.

IDENTIFICATION

A. To mix 5 mL of sample and 1 mL of 15% potassium iodide (w/v). Orange color rapidly develops, but it fades out soon. To add 5 drops of 2 *M* hydrochloric acid and drops of starch SR. Solution becomes blue.

B. To mix 1 mL of the sample with 50 mg of phenolphthalein. Liquid becomes red.

C. To add 5 drops of 2 *M* hydrochloric acid to 5 mL of sample. Greenish color becomes intense and chlorine gas bubbles are produced.

D. To immerse litmus paper red in 1 mL of sample. Paper turns blue, then it fades out.

E. The acidified solution obtained in test **C**. *Identification* responds to the flame test for sodium ions (**5.3.1.1**).

CHARACTERISTICS

Description. Clear liquid, pale yellow to greenish, chlorine odor. It is susceptible to light and gradually deteriorates.

Determination of volume (5.1.2). It complies with the test. **pH (5.2.19).** Greater than 11.0.

PURITY TESTS

Calcium. To transfer 10 g of sample to 150 mL beaker, add 10 mL of water, 5 mL of hydrochloric acid, and 1 g of potassium iodide. To heat for 5 minutes, cool down, and add 2 mL of 30% hydrogen peroxide (v/v). To evaporate to dryness, cool down, add 2 mL of hydrochloric acid and 2 mL of 30% hydrogen peroxide (v/v). To wash the inner walls of the beaker with a few milliliters of water and filter it, if necessary. To add 3 mL of ammonium hydroxide and 5 mL of 3.5% ammonium oxalate (w/v) to the filtrate. To quantitatively transfer to Nessler tube, top up to 25 mL, and make it homogeneous. No turbidity produced within 15 minutes exceeds that of the standard preparation obtained by using 10 mL of standard calcium solution (10 ppm Ca) to the same treatment as the sample. At most 0.001% (10 ppm).

DOSAGE

To accurately transfer 3 mL of sample volume containing 60 mg - 90 mg of NaClO or 57 mg - 87 mg of active chlorine to a 250 mL erlenmeyer flask with lid. To add about 50 mL of water, 1 g of potassium iodide, and 10 mL of 6 *M* acetic acid. To cover, stir, and let it for 15 minutes, protected from light. To wash the sides of the flask with a few milliliters of water and titrate the iodine with 0.1 *M* SV sodium thiosulfate. To add 1 mL of starch SR when the color of the solution turns greenish-yellow. To continue the titration to blue color fades out. Each mL of 0.1 *M* SV sodium thiosulfate is equal to 3.723 mg of NaClO and 3.545 of active chlorine.

PACKAGING AND STORAGE

In tightly closed, opaque containers; preferably at temperatures below 25 °C.

LABELLING

To comply with the legislation in force.

PEPPER MINT *Menthae piperitae folium*

Mentha x piperita L. - LAMIACEAE; 09914

The vegetable drug consists of dried, whole, broken, cut, or pulverized leaves of the species and its varieties, containing at least 1.2% of volatile oil in whole leaves and at least 0.9% of volatile oil in scratched leaves.

CHARACTERISTICS

Organoleptic characteristics. The drug has a strong odor, aromatic, penetrating, similar to menthol and spicy aromatic flavor with pleasant feeling of freshness.

MACROSCOPIC DESCRIPTION

Whole, membranous, rough, brittle, cross-opposite, petiole, green to brownish-green, when dry, leaves, with numerous glandular trichomes on the lower face of the blade, visible in sixfold increase or against the light, as bright spots, yellow, bright, and tector trichomes distributed on the ribs; camptodrome-brochidodromous venation, thick midrib and pronounced on both sides, secondary ribs at about 45°, depression on the adaxial face and prominent on the lower face. Ovate to ovate-lanceolate blade, acute apex, irregularly rounded and asymmetrical base, irregularly serrate edge, with sharp teeth, measuring from 3.0 cm to 9.0 cm long and from 1.0 cm to 5.0 cm wide. Petiole, measuring from 0.5 cm to 1.0 cm long, green; when dried becomes brown, concave on the adaxial face, convex on the lower face, with lateral ribs and trichomes similar to that of the blade.

MICROSCOPIC DESCRIPTION

Leaf blade of dorsiventral symmetry, hypo-amphistomatic with diacytic stomata. In front view, the cuticle is smooth and epidermal cells have anticlinal walls with wavy contour in the region between the ribs and straight walls on the ribs. There are five types of trichomes on both faces: (1) multicellular tector trichomes, long, thin, acute, uniseriate, with 2-14 cells, the longest basal cell and apical with obtuse apex; some of these trichomes with increased number of cells have a crown of basal cells; thick cuticle and distinctly striated; (2) multicellular tector trichomes, with 2-6 cells, biserial at base, with thickened and striated cuticle; (3) glandular trichome with unicellular pedicel, short and unicellular head, rounded, with thin cuticle; (4) glandular trichome with unicellular, bicellular, or tricellular pedicel, short and elliptical unicellular head, with thin cuticle; (5) peltate glandular trichome, short pedicel, composed of 1-2 cells in the basal portion and multicellular head with 8 cells radially arranged, usually with dilated and brownish cuticle. In cross section, the cuticle is thin and the epidermis is uniseriate, with tangentially flattened cells, rich in oil drops; stomata are pronounced; type 1 tector trichomes in greater numbers on the lower face and on the midrib, and type 2 are rare; glandular trichomes, types 3 and 4, are distributed throughout the blade; peltate glandular trichomes, type 5, are in depression in the epidermis and more frequent on the lower face of intercostal region; unistratified palisade parenchyma with compact and short cell; tri-stratified (or more) spongy parenchyma, filling about 60% of the section; abundant oil droplets; no crystals of calcium oxalate. The midrib, in cross section, has a thick cuticle on the lower face, the epidermal cells are polygonal, oval, small, with thick outer periclinal wall; the collenchyma is angular, unistratified or more layers along the adaxial face, followed in this face by a chlorenchyma with up to

five layers of polygonal cells and the parenchyma. The latter, together with the lower face, is composed of up to ten layers of isodiametric cells with large intercellular spaces. The vascular system is composed of one or more collateral bundles, open or not, showing well developed phloem with fiber calottes facing the lower face or with some isolated fibers externally located. The petiole, in cross section, has a thick and smooth cuticle, unistratified epidermis of polyhedral cells, pronounced stomata with higher frequency of trichomes, type 1; the cortex shows angular collenchyma with up to 8 layers in the ribs and unistratified on the lower face, chlorenchyma more compressed in the ribs; cortical parenchyma formed by oval cells, large volume, with larger intercellular spaces along the lower face; endoderm rich in starch grains; vascular system with three or more collateral bundles, the central one is wide open, with expressive phloem, with or without fibers. Oil drops in chlorenchyma, cortical parenchyma, and endoderm.

Adulteration: Mentha crispa L. has glandular trichome with 12-cell head and tector trichomes with thin walls of 1-6 cells.

MICROSCOPIC DESCRIPTION OF POWDER

The powder meets all the requirements for the species, unless the macroscopic characters. To perform microscopic examination by using chloral hydrate solution. Characteristics: broken or cut leaves, often wrinkled, green-brown; fragments of blade with epidermal cells with sinuous walls and various diacytic stomata, especially on the lower face; trichomes as described, mainly those of type 1; fragments of heterogeneous asymmetric mesophyll as described; fibers; tracheary elements with helical thickening; yellow crystals of menthol under the cuticle of peltate trichomes can be seen; no crystals of calcium oxalate.

MACROSCOPIC DESCRIPTION OF IMPURITIES

The stems, branches, flowers, fruits, and seeds of the species itself, if considered an impurity, are characterized by: quadrangular stem with ribs well defined up to the fourth node, branched, it is vinous in most varieties when adult, light green when young, and whitish at basal nodes; trichomes are not visible to the naked eye; flowers gathered into spindly inflorescences; glabrous calyx, with five teeth, pinkish-violet or white corolla, with four lobes, the superior one is extended; four stamens, didynam, included in the corolla, supero ovary, 4-lobed, gynobasic stylet; rare and sterile seeds.

MICROSCOPIC DESCRIPTION OF IMPURITY FOR THE STEM

The stems of the species itself, if present as an impurity, present, in the secondary structure and cross section, thick and striated cuticle, uniseriate epidermis of polygonal cells, with or without idioblasts of crystalline sand, with or without cells containing anthocyanin; trichome and stomata are rare; angular collenchyma, consisting of one to many layers in the ribs; chlorenchyma with up to ten layers, with

isolated sclereids and idioblasts of crystalline sand; endodermis with clear Casparian strip without starch grains; phloem with or without fibers, isolated or small groups; clear cambium zone up to four layers; xylem totally sclerified or not; oil droplets in all tissues, except cambium and xylem, developed medullary parenchyma. When in primary structure, the epidermal cells are filled with anthocyanin; trichomes as described for the leaf, collenchyma comprised of a layer in the intercostal regions and up to nine layers in the ribs; chlorenchyma full of chloroplast and starch grains; clear endoderm and rich in grain starch; vascular system with collateral bundles well developed in the ribs; clear fascicular and interfascicular cambium; medullary parenchyma with large isodiametric cells.

IDENTIFICATION

To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF₂₅₄ silica gel with 250 µm thick as stationary phase and a mixture of toluene and ethyl acetate (95:5) as mobile phase. To separately apply to the plate, as band, 20 µl of *Solution (1)* and 10 µl of *Solution (2)* freshly prepared, as described below.

Solution (1): to stir 0.2 g of drug freshly pulverized with 2 mL of methylene chloride. To filter it. To evaporate to dryness (40 °C) and dissolve the residue in 0.1 mL of toluene.

Solution (2): to dilute 50 mg of menthol SQR, 20 µL of 1,8-cineole, 10 mg of thymol, and 10 µL of menthyl acetate in toluene, and top up to 10 mL with the same solvent.

To develop the chromatogram. To remove the plate, and let it drying. To observe under ultraviolet light (254 nm). The chromatogram obtained with *Solution (1)*, on top of the chromatoplate, shows 4 principal spots corresponding

in position, color, and intensity of fluorescence with those obtained with *Solution (2)*. Then nebulize the plate with anisaldehyde SR and put it in an oven at 100 °C - 105 °C for 5 - 10 minutes. The spot corresponding to menthyl acetate (Rf about 0.81) is blue-violet; the spot corresponding to thymol (Rf about 0.65) is pink; the spot corresponding to 1,8-cineole (Rf about 0.60) is blue to violet-brown; and the spot corresponding to menthol (Rf about 0.55) is violet to blue.

PURITY TESTS

Foreign matter (5.4.2.2). At most 10% of quadrangular stems, glabrous or tector trichomes; limited fragments of stem recognized by fibers, in addition to numerous vessel elements; fragments of flowers as described.

Water (5.4.2.3). At most 12.0%.

Total ashes (5.4.2.4). At most 15.0%.

DOSAGE

Volatile oil

To proceed as described in *Determination of volatile oils in vegetable drugs (5.4.2.7)*. To use 500 mL flask containing 200 mL of water as distillation liquid. To add 0.5 mL of xylene through the opening k. To use dried scratched plant. To immediately proceed to determine the volatile oil from 20 g of the drug. To distill for 4 hours.

PACKAGING AND STORAGE

In tightly closed glass container, protected from light and heat.

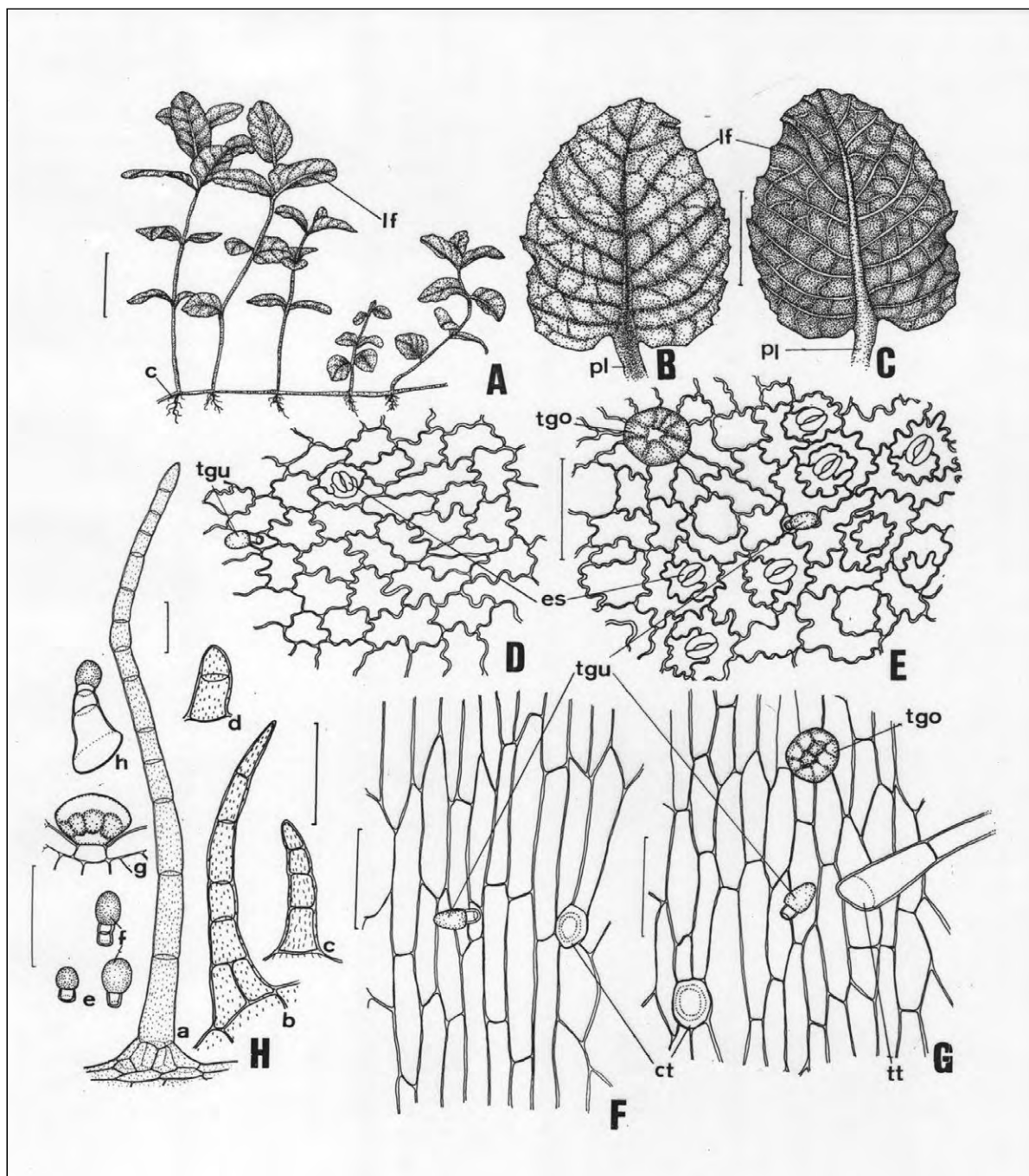


Figure 1 — Macroscopic and microscopic aspects in *Mentha piperita* L.

Additional information of **Figure 1**. Scales correspond to 2.5cm in **A**; 1 cm, in **B** and **C**; 100 µm, in **D-H**.

A - general appearance of a branch: stem (c), leaf blade (lf). **B** — view of the adaxial face of leaf: leaf blade (lf); petiole (pl). **C** — view of the lower face of a leaf: leaf blade (lf); petiole (pl). **D** — detail of a portion of the adaxial face of epidermis in the leaf blade, in the intercostal region, in front view: stomata (es), glandular trichomes with unicellular head (tgu). **E** — detail of a portion of the lower face of epidermis in the leaf blade, in the intercostal region, in front view: stomata (es); glandular trichome with 8-cell head (tgo); glandular trichomes with unicellular head (tgu). **F** — detail of a portion of the adaxial face of epidermis in the leaf blade, on the midrib, in front view: scar of tector trichomes (ct); glandular trichomes with unicellular head (tgu). **G** — detail of a portion of the lower face of epidermis in the leaf blade, on the midrib, in front view: scar of tector trichomes (ct); glandular trichome with 8-cell head (tgo); glandular trichomes with unicellular head (tgu); tector trichomes (tt). **H** — trichomes: detail of a multicellular uniseriate tector trichome with basal cell crown, in side view (a); detail of a multicellular uniseriate tector trichome with bisseriate base, in side view (b); detail of a tetracelluar uniseriate tector trichome in side view (c); detail of a bicellular uniseriate tector trichome in side view (d); detail of glandular trichome with rounded head and unicellular pedicel in side view (e); detail of glandular trichomes with unicellular elliptical head, unicellular or bicellular uniseriate pedicel in side view (f); detail of glandular trichome with 8-cell secretory head in side view (g); detail of glandular trichome with unicellular head, tricellular uniseriate pedicel in side view (h).

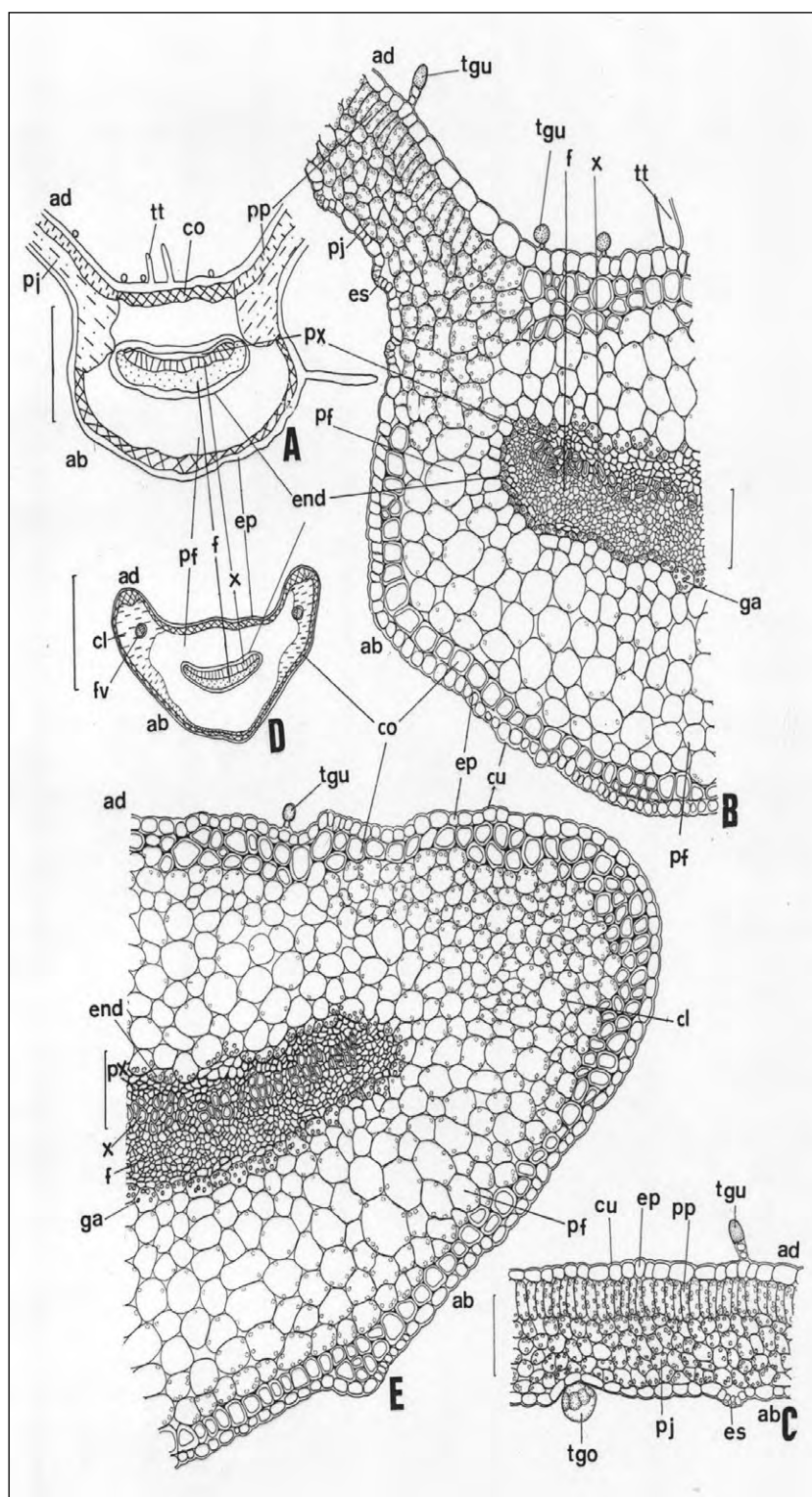


Figure 2 — Microscopic aspects in *Mentha piperita* L.

Additional information of **Figure 2**. Scales correspond to 400 µm in **A**; 100 µm, in **B**, **C**, and **E**; 1000 µm, in **D**.

A - schematic representation of the general appearance of the midrib and the portion of intercostal region, in cross-section: lower face (ab); adaxial face (ad); spongy parenchyma (pj); tector trichomes (tt); collenchyma (co); palisade parenchyma (pp); xylem parenchyma (px); endoderm (end); epidermis (ep); xylem (x), phloem (f); fundamental parenchyma (pf). **B** - detail of region of the midrib and the portion of intercostal region, in cross-section: lower face (ab); adaxial face (ad); palisade parenchyma (pp); glandular trichomes with unicellular head (tgu); spongy parenchyma (pj); phloem (f); xylem (x); tector trichomes (tt); stomata (es), xylem parenchyma (px); fundamental parenchyma (pf); endoderm (end); collenchyma (co); epidermis (ep), cuticle (cu); starch grain (ga). **C** — detail of the leaf blade in the intercostal region, in cross-section: lower face (ab); adaxial face (ad); glandular trichomes with unicellular head (tgu); palisade parenchyma (pp); epidermis (ep), cuticle (cu); stomata (es), spongy parenchyma (pj); glandular trichomes with 8-cell head (tgo). **D** — scheme of general appearance of the petiole in cross-section: lower face (ab); adaxial face (ad); palisade parenchyma (pp); endoderm (end); collenchyma (co); vascular bundle (fv); chlorenchyma (cl); fundamental parenchyma (pf). **E** — detail of the portion of the petiole in cross section: lower face (ab); adaxial face (ad); glandular trichomes with unicellular head (tgu), collenchyma (co); epidermis (ep), cuticle (cu); chlorenchyma (cl); fundamental parenchyma (pf); starch grain (ga); phloem (f); xylem (x), xylem parenchyma (px); endoderm (end).

PEPPER MINT, VOLATILE OIL

Menthae piperitae aetheroleum

Mentha x piperita L. - LAMIACEAE

Volatile oil obtained by water vapor drag of the aerial parts, recently collected, from the plant species. The volatile oil consists of at least 35.0% of menthol.

CHARACTERISTICS

Organoleptic characteristics. Colorless, pale yellow or pale greenish-yellow liquid, with typical odor and flavor, followed by a feeling of freshness.

IDENTIFICATION

To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ with 250 µm thick as support and a mixture of toluene and ethyl acetate (95:5) as mobile phase. To separately apply to the plate, as band, 20 µl of *Solution* (1) and 10 µL of *Solution* (2) freshly prepared, as described below.

Solution (1): to dissolve 0.1 mL of volatile oil in 1 mL of toluene.

Solution (2): to dilute 50 mg of menthol SQR, 20 µL of 1,8-cineole, 10 mg of thymol, and 10 µL of menthyl acetate in toluene, and top up to 10 mL with the same solvent.

To develop the chromatogram. To remove the plate, and let it drying. To observe under ultraviolet light (254 nm). The 4 principal spots obtained with *Solution* (1), on top of the chromatogram, corresponds in position, color, and fading of fluorescence with those obtained with *Solution* (2). Then nebulize the plate with anisaldehyde SR and put it in an oven at 100 °C - 105 °C for 5 - 10 minutes. To immediately examine under visible light. The principal spots corresponding to menthyl acetate (R_f about 0.81, blue-violet), thymol (R_f about 0.65, pinkish), 1,8-cineole (R_f about 0.60, blue to violet-brown), and menthol (R_f about 0.55, blue to violet).

Refractive index (5.2.6): 1.457 to 1.467.

Rotation (5.2.8): -10° to -30°.

Index of acidity (5.2.29.7). At most 1.4. To determine in 5g of volatile oil diluted in 50 mL of a mixture of solvents.

CHROMATOGRAPHIC PROFILE

To proceed as described in *Gas chromatography* (5.2.17.5). To use chromatograph equipped with flame ionization detector, by using mixture of nitrogen, synthetic air, and hydrogen (1:1:10) as the auxiliary gas to flame detector; 30 m long capillary column and 0.25 mm inner diameter, filled with poly (dimethyl/diphenyl siloxane), with a film thickness of 0.25 µm; column temperature from 60 °C to 300 °C, at 3 °C per minute (total: 80 minutes), injector temperature at 220 °C and detector temperature at 250 °C; use helium at 80 kPa as carrier gas; flow of gas of 1 mL/minute.

Sample solution: to dilute the volatile oil in ethyl ether (2:100).

Procedure: to inject 1 µL of *Sample solution* into the gas chromatograph, by using 1:50 flow division. The relative retention index of the oil constituents is calculated in relation to homologous series of hydrocarbons and compared to reference samples. The relative concentration is obtained by normalization (integrating manually or electronically).

To calculate the Relative Retention Index (RRI), according to the equation:

$$IR = 100 \times n + \frac{100 \times (tr_x - tr_z)}{(tr_{z+1} - tr_z)}$$

where

n = number of carbon atoms in the alkane with retention time immediately before the constituent “x” to be characterized;

tr_x = retention time of constituent “x” (intermediate to *tr_z* and *tr_{z+1}*);

tr_z = retention time of the alkane immediately before the constituent “x”;

tr_{z+1} = retention time of the alkane with “n + 1” carbons (immediately after the constituent “x”).

PURITY TESTS

Relative density (5.2.5). 0.900 to 0.916.

To observe the **Figure 1** below.

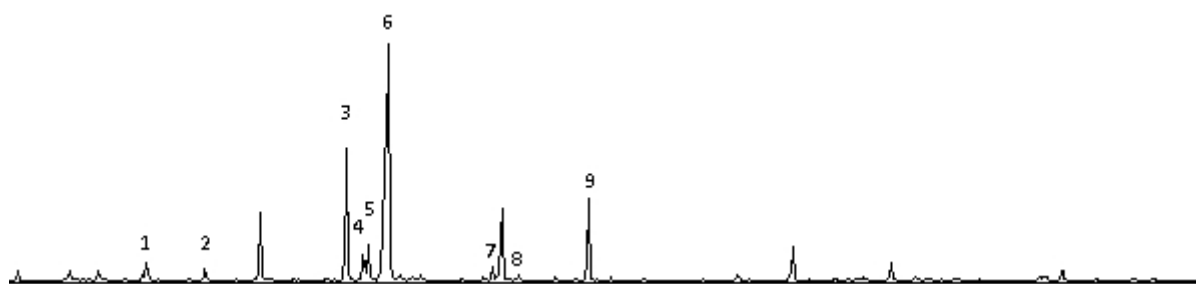


Figure 1 - Chromatogram obtained with volatile oil of *Mentha x piperita*

The percentages of the main constituents are within the following ranges:

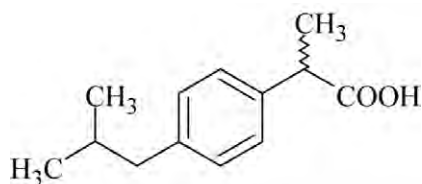
Peak	Retention index	Constituent	Content (%)
1	1023	Limonene	0.5 - 5.0
2	1025	1,8-Cineole	0.5 - 13.0
3	1147	Mentone	6.0 - 30.0
4	1156	Iso-mentone	2.0 - 10.0
5	1160	Neo-menthol	2.0 - 3.5
6	1165	Menthol	35.0 - 79.0
7	1230	Pulegone	At most 2.0
8	1237	Carvone	At most 1.0
9	1290	Menthyl acetate	3.0- 10.0

PACKAGING AND STORAGE

In airtight closed glass containers, protected from light, oxygen, and heat.

IBUPROFEN

Ibuprofenum



$C_{13}H_{18}O_2$; 206.28

ibuprofen; 04766

α -methyl-4-(2-methylpropyl) benzeneacetic acid
[15687-27-1]

It contains at least 97.0% and at most 103.0% of $C_{13}H_{18}O_2$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. Crystalline powder, white or nearly white, typical odor.

Solubility. Practically insoluble in water; freely soluble in ethanol, acetone, methanol, and chloroform; slightly soluble in ethyl acetate. Freely soluble in aqueous alkalis.

Physical and chemical constants.

Melting range (5.2.2): 75 °C to 78 °C.

Specific rotation (5.2.8): +0.05° to -0.05°. To determine in 2.5% solution (w/v) in methanol.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the ibuprofen SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14) ranging from 240 nm to 300 nm, of 0.025% solution (w/v) in 0.1 M SV sodium hydroxide, exhibits maxima and minima only at the same wavelength of a similar ibuprofen SQR solution. The corresponding absorbances calculated in relation to anhydrous substance, in tablets at 264 nm and 273 nm wavelength, do not differ more than 3%.

PURITY TESTS

Appearance of solution. The 10% solution (w/v) in ethanol is clear (5.2.25).

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using G silica gel as support and a mixture of *n*-hexane, ethyl acetate, glacial acetic acid (15:5:1) as mobile phase.

To separately apply to the plate 5 μ L of each of the solutions freshly prepared, as described below.

Solution (1): 100 mg/mL solution of the sample in methylene chloride.

Solution (2): 1 mg/mL solution of the sample in methylene chloride.

To develop the chromatogram. To remove the plate, and let it drying. To nebulize with 1% *p*-dimethylaminobenzaldehyde (w/v) in a mixture of ethanol and hydrochloric acid (10:1), heat it in an oven at 100 °C for 5 min, and nebulize with 5% ferric chloride (w/v). Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (2)* (1%).

Heavy metals (5.3.2.3). To use Method III. At most 0.002% (20 ppm).

Water (5.2.20.1). At most 1%.

Sulfated ashes (5.2.10). To determine in 1 g of the substance. At most 0.5 %.

DOSAGE

To accurately weigh about 0.5 g of the sample and dissolve in 100 mL of ethanol. To add 3 mL of phenolphthalein SI and titrate with 0.1 M SV sodium hydroxide to it turns pink. To perform sample blank correction and adjust it as required. Each mL of 0.1 M SV sodium hydroxide is equal to 20.628 mg of $C_{13}H_{18}O_2$.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Analgesic, anti-inflammatory.

IBUPROFEN TABLETS

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_{13}H_{18}O_2$.

IDENTIFICATION

A. To weigh and pulverize the tablets. To mix amount of powder equivalent to 0.5 g of ibuprofen with 20 mL of acetone, stir, filter, and evaporate the filtrate to dryness in a stream of air, without heating. The infrared absorption spectrum (5.2.14) of the resulting residue, dispersed in

potassium bromide, has absorption maximum only at the same wavelength, with the same

relative intensities to those observed in the ibuprofen SQR spectrum similarly prepared.

B. To recrystallize the residue obtained in test **A.**, *Identification*, with petroleum ether. The melting temperature (**5.2.2**) of the residue is 75 °C.

C. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method **B.** corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: phosphate buffer pH 7.2, 900 mL

Equipment: baskets, 150 rpm

Time: 30 minutes

Procedure: after testing, remove aliquot from the dissolution medium and dilute, if necessary, with phosphate buffer pH 7.2, to obtain suitable concentration. To measure the absorbances at 221 nm (**5.2.14**), by using the same solvent to adjust zero. To calculate the amount of $C_{13}H_{18}O_2$ dissolved in the medium, by comparing the readings obtained with that of 0.002% ibuprofen SQR solution (w/v) prepared in the same solvent.

Tolerance: not less than 60% (Q) of the amount stated of $C_{13}H_{18}O_2$ dissolves within 30 minutes.

PURITY TESTS

Related substances. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using silica gel as support and a mixture of n-hexane, ethyl acetate, and glacial acetic acid (15:5:1) as mobile phase. To apply separately 5 µl of each of the solutions as described below.

Solution (1): to stir amount of powder equivalent to 0.2 g of ibuprofen with 10 mL of chloroform, filter, and set apart the filtrate. To repeat the procedure with 2 portions (10 mL each) of chloroform and gather the chloroform extracts. To evaporate the filtrate to about 1 mL and add sufficient amount of chloroform to 2 mL.

Solution (2): to dilute 1 volume of *Solution (1)* to 100 volumes with chloroform.

To develop the chromatogram. To remove the plate, let it drying, nebulize with 1% *p*-dimethylaminobenzaldehyde (w/v) in a mixture of ethanol and hydrochloric acid (10:1), heat it in an oven at 100 °C for 5 min, and nebulize with 5% ferric chloride (w/v) aqueous solution.

No other secondary spot obtained in the chromatogram with *Solution (1)* is more intense than that obtained with *Solution (2)* (1%).

Water (5.2.20.1). At most 5.0%.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To weigh and pulverize the tablets. To stir amount of powder equivalent to 0.5 mg of ibuprofen with 20 mL of chloroform. To filter through a sintered glass funnel and wash the resulting residue with 50 mL of ethanol previously neutralized with 0.1 M SV sodium hydroxide, by using phenolphthalein SI as an indicator. To titrate with 0.1 M SV sodium hydroxide to it turns pink. Each mL of 0.1 M SV sodium hydroxide is equal to 20.628 mg of $C_{13}H_{18}O_2$.

B. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 264 nm; 150 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm); flow of *Mobile phase* of 1.2 mL/min.

Mobile phase: mixture of phosphoric acid, water, and methanol (3:247:750).

Sample solution: to weigh and pulverize 20 tablets. To transfer amount of powder equivalent to 100 mg of ibuprofen to 100 mL volumetric flask, add 70 mL of *Mobile phase*, stir for 30 minutes, top up to volume with *Mobile phase*, and make it homogeneous. To centrifuge a portion of suspension obtained and use the supernatant.

Standard solution: to dissolve accurately weighed amount of ibuprofen SQR in *Mobile phase* to obtain a 1 mg/mL solution.

To inject replicates of 20 µL of the *Standard solution*. The tailing factor is not greater than 2.0. The relative standard deviation for replicate areas of the peaks recorded is greater than 2.0 %.

Procedure: to separately inject 20 µL of *Sample and standard solutions*, record the chromatograms, and measure the area under the peaks. To calculate the amount of $C_{13}H_{18}O_2$ in the tablets, from the responses obtained for the *Sample and standard solutions*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

ANTI-D HUMAN IMMUNOGLOBULIN Immunoglobulinum Humanum anti-D

Anti-D Human immunoglobulin is a sterile, liquid or lyophilized preparation containing immunoglobulins, especially immunoglobulin G. The preparation is intended to be administered intramuscularly. It is obtained from plasma from D-negative donors containing high titers of specific anti-D immunoglobulins and small amounts of antibodies against other blood groups. Normal human immunoglobulin can be added to it. Anti-D human immunoglobulin meets the requirements set forth in the monograph Normal Human Immunoglobulin, except with regard to the minimum number of donors and the minimum content in total proteins.

Sterility. For liquid preparation, conducting accelerated degradation test conducted by heating at 37 °C for four weeks in the final product, the loss of anti-D activity is not more than 20% of the initial value.

To limit B19 viral load in plasma pools used by manufacturers of anti-D immunoglobulin, the plasma pool is tested for the presence of B19 virus through the use of techniques for nucleic acid amplification properly validated. No more than 10 IU per microliter.

A positive control with 10 IU of B19 virus DNA per microliter and an internal control prepared by adding an appropriate marker in a sample from the plasma pool to be used in the test. The test is invalid if the positive control is not reagent or the result obtained with the internal control indicates the presence of inhibitors.

If added to the preparation human immunoglobulin and or human albumin solution the plasma pool or origin pools must meet the requirements stated above for B19 virus DNA.

DOSAGE

To proceed as for *Determination of anti-D human immunoglobulin (5.5.1.15)*. The estimated potency is less than 90% of the stated potency. The confidence limits ($P=0.95$) are not less than 80% and not greater than 120% of the stated potency.

PACKAGING AND STORAGE

See the monograph Normal Human Immunoglobulin..

LABELLING

To comply with the legislation in force. See the monograph *Normal Human Immunoglobulin*. In the label it is indicated the number of International Units per container.

IMMUNOGLOBULIN, HUMAN, ANTI- HEPATITIS A

Immunoglobulinum Humanum Hepatitis A

Anti-hepatitis A human immunoglobulin is a sterile, liquid or lyophilized preparation containing immunoglobulins, especially immunoglobulin G. The preparation is intended to be administered intramuscularly. It is obtained from plasma from selected donors containing antibodies against hepatitis A. Normal human immunoglobulin can be added to it.

Anti-hepatitis A human immunoglobulin meets the requirements set forth in the monograph Normal Human Immunoglobulin, except with regard to the minimum number of donors and the minimum content in total proteins.

DOSAGE

The activity of anti-Hepatitis A human immunoglobulin is evaluated by comparing to the activity of a reference preparation measured in International Units, by means of a test with appropriate sensitivity and specificity (To proceed as described in *Immunochemical methods* (5.6)). The international unit corresponds to the activity of a certain amount of international reference preparation of anti-hepatitis A human immunoglobulin. The correspondence between the International Unit and the preparation of international reference is indicated by the World Health Organization

The activity indicated is not less than 600 IU per milliliter and not lower than the activity indicated. The confidence limits ($P=0.95$) of the determined activity are not less than 80% and not greater than 120%.

PACKAGING AND STORAGE

See the monograph Normal Human Immunoglobulin.

LABELLING

To comply with the legislation in force. See the monograph Normal Human Immunoglobulin. In the label it is indicated the number of International Units per milliliter.

IMMUNOGLOBULIN, HUMAN, ANTI-HEPATITIS B

Immunoglobulinum Humanum Hepatitis B

Anti-hepatitis B human immunoglobulin is a sterile, liquid or lyophilized preparation containing immunoglobulins, especially immunoglobulin G. The preparation is intended to be administered intramuscularly. It is obtained from plasma from selected donors containing antibodies against hepatitis B. Normal human immunoglobulin can be added to it. Anti-hepatitis B human immunoglobulin meets the requirements set forth in the monograph Normal Human Immunoglobulin, except with regard to the minimum number of donors and the minimum content in total proteins.

DOSAGE

The activity of anti-Hepatitis B human immunoglobulin is evaluated by comparing to the activity of a reference preparation measured in International Units, by means of a test with appropriate sensitivity and specificity (To proceed as described in *Immunochemical methods* (5.6)). The international unit corresponds to the activity of a certain amount of international reference preparation of anti-hepatitis B human immunoglobulin. The correspondence between the International Unit and the preparation of international reference is indicated by the World Health Organization

The activity indicated is not less than 100 IU per milliliter and not lower than the activity indicated. The confidence limits ($P=0.95$) of the determined activity are not less than 80% and not greater than 125%.

PACKAGING AND STORAGE

In accordance with the monograph *Vaccines for human use*.

LABELLING

To comply with the legislation in force.

IMMUNOGLOBULIN, HUMAN, ANTI-HEPATITIS B, INTRAVENOUS ADMINISTRATION

Immunoglobulinum Humanum Hepatitis B ad Usum Intravenosum

Human immunoglobulin against hepatitis B for intravenous administration is a sterile, liquid or lyophilized preparation, containing immunoglobulins, especially immunoglobulin G. It is obtained from plasma from

donors carrying specific antibodies against the surface antigen of hepatitis B. It can be added to normal human immunoglobulin administered intravenously. Anti-hepatitis B human immunoglobulin for intravenous administration meets the requirements set forth in the monograph Normal Human Immunoglobulin, except

with regard to the minimum number of donors and the minimum content in total proteins and the limit of osmolality.

DOSAGE

The activity of anti-Hepatitis B human immunoglobulin is evaluated by comparing to the activity of a reference preparation measured in International Units, by means of a immunodosage with appropriate sensitivity and specificity (To proceed as described in *Immunochemical methods* (5.6)). The international unit corresponds to the activity of a certain amount of international reference preparation of anti-hepatitis A human immunoglobulin. The correspondence between the International Unit and the preparation of international reference is indicated by the World Health Organization

The activity indicated is not less than 50 IU per milliliter. The specific activity is not inferior to the activity indicated. The confidence limits ($P=0.95$) of the determined activity are not less than 80% and not greater than 125%.

PACKAGING AND STORAGE

See monograph Normal Human Immunoglobulin for Intravenous Administration.

LABELLING

To comply with the legislation in force. See monograph Normal Human Immunoglobulin for Intravenous Administration. In the label it is indicated the number of International Units per container.

IMMUNOGLOBULIN, HUMAN, ANTI- RABIES

Immunoglobulinum Humanum Rabicum

Anti-rabies human immunoglobulin is a sterile, liquid or lyophilized preparation containing immunoglobulins, especially immunoglobulin G. The preparation is intended to be administered intramuscularly. It is obtained from plasma from donors carrying specific antibodies against rabies. Normal human immunoglobulin can be added to it. Anti-rabies human immunoglobulin meets the requirements set forth in the monograph Normal Human Immunoglobulin, except with regard to the minimum number of donors and the minimum content in total proteins.

DOSAGE

The activity of anti-rabies human immunoglobulin is evaluated by comparing between the dose required to neutralize the infectious power of rabies virus and the dose of a reference preparation measured in International Units needed to ensure the same degree of neutraliza-

tion (*Immunochemical methods (5.6)*). To perform the measurement in sensitive cell cultures and reveal the presence of the virus not neutralized by immunofluorescence. The International Unit corresponds to the specific neutralizing activity for the rabies virus in a given amount of an international reference preparation of anti-rabies human immunoglobulin.

The correspondence between the International Unit and the international reference preparation is indicated by the World Health Organization

To perform measurement in appropriate sensitive cells. To use the cell line BHK 21, multiplied in the culture medium described below, and submit from 18 to 30 passages from ATCC seed lot. To collect the cells after incubation for 2 to 4 days. To treat the cells with trypsin. To prepare a suspension of 500 000 cells per milliliter (suspension of cells). To increase the sensitivity of cells add, if necessary, 10 minutes before the use of this suspension, 10 µg of diethylaminoethyl dextran per milliliter.

To use a fixed strain of virus multiplied in sensitive cells, for example, the CVS strain adapted to culture in cell line BHK 21 (parent virus suspension). To titrate the parent virus suspension as follows:

To prepare serial dilutions of the virus suspension. On plates with cameras for cell cultures (8 cameras per plate), distribute 0.1 mL of each dilution. To add 0.1 mL of culture medium and 0.2 mL of the cell suspension. To incubate at 37 °C in an atmosphere of carbon dioxide for 24 hours. To fix core by immunofluorescence and make the calculations following the procedures described below. To determine the titer of the parent virus suspension and prepare a working dilution of virus corresponding to 100 TCID₅₀ per 0.1 mL.

In each test to check the amount of the virus by performing a control titration: from dilution corresponding to 100 TCID₅₀ per 0.1 mL, perform three successive dilutions of ratio 10. To distribute respectively 0.1 mL of each dilution into four cameras containing 0.1 mL of culture medium and add 0.2 mL of the cell suspension. The test is valid only if the titration is between 30 and 300 TCID₅₀.

To dilute the reference preparation with no additional culture medium to a concentration of 2 IU per milliliter (reference parent dilution and at a temperature below -80 °C). To prepare two appropriate pre-dilutions (1/8 and 1/10) of reference parent dilution so that the dilution of the reference preparation, which reduces by 50% the number of fluorescent fields in the cell culture plate

is among the four dilutions. To add 0.1 mL of medium to each camera except the first one in each of two rows to which it is added, respectively, 0.2 mL of the two pre-dilutions of the parent dilution of reference and then successively transfer 0.1 mL to the other cameras.

To dilute the sample at 1/100 with unsupplemented culture medium (immunoglobulin parent dilution) to minimize errors due to the viscosity of the undiluted preparation. To prepare three appropriate pre-dilutions of the parent dilution of immunoglobulin so that the sample dilution which reduces by 50% the number of fluorescent fields in the cell culture plate is between four dilutions.

To add 0.1 mL of medium to each camera except in the first one in each of three rows to which is added respectively 0.2 mL of the three pre-dilutions of the parent dilution of the immunoglobulin. To prepare a series of dilution of ration 2 successively transferring 0.1 mL for the other cameras.

To all cameras containing dilutions of the reference preparation and sample dilutions, add 0.1 mL of virus suspension corresponding to 100 TCID₅₀ per 0.1 mL (working dilution), stir manually and rest at 37 °C in an atmosphere of carbon dioxide for 90 minutes, add 0.2 mL of the cell suspension stir manually and rest at 37 °C in an atmosphere of carbon dioxide for 24 hours. After 24 hours, to remove the medium and withdrawal the plastic walls.

To wash monocellular cameras with phosphate saline buffered of pH 7.4 and then with a mixture of 20 volumes of water and 80 volumes of acetone and fix for 3 minutes with a mixture of 20 volumes of water and 80 volumes of acetone at -20°C. To spread on blades fluorescent conjugated of anti-rabies serum ready to be used.

Rest for 30 minutes at 37 °C in an atmosphere with a very high humidity. To wash with phosphate saline buffered of pH 7.4 and dry. To examine 20 fields of each camera at a magnification of 250 times with a microscope equipped for fluorescence readout. To record the number of fields containing at least one fluorescent cell. To check the dose of test virus used in the plaque for virus titration and determine the preparation dilution of the reference and sample which reduces by 50% the number of fluorescent fields performing the calculations for the set of two or three dilutions by means of a interactive probability analysis. The test is valid only when the statistical analysis demonstrates a significant inclination of the dose/effect curve and does not reveal deviation of linearity or parallelism.

The activity indicated is at least 150 IU per milliliter.

The specific activity is not inferior or superior to twice the activity indicated. The confidence limits (P= 0.95) of the determined activity are not less than 80% and not greater than 120%.

CULTURE MEDIUM FOR GROWTH OF CELLS BHK 21

The mediums marketed with a slightly different composition from that shown can also be used.

Sodium chloride	6.4 g
Chloride, Potassium	0.40 g
Chloride, Calcium, Anhydrous	0.20 g
Sulfate, Magnesium, Heptahydrate	0.20 g
Sodium phosphate monohydrate	0.124 g
Glucose monohydrate	4.5 g
Ferric nitrate monohydrate	0.10 mg
L-arginine hydrochloride	42.0 mg
L-cystine	24.0 mg
L-histidine	16.0 mg
L-isoleucine	52.0 mg
L-leucine	52.0 mg
L-lysine hydrochloride	74.0 mg
L-phenylalanine	33.0 mg
L-threonine	48.0 mg
L-tryptophan	8.0 mg
L-tyrosine	36.0 mg
L-valine	47.0 mg
L-methionine	15.0 mg
L-glutamine	0.292 g
I-inositol	3.60 mg
Choline chloride	2.0 mg
Folic acid	2.0 mg
Nicotinamide	2.0 mg
Calcium pantothenate	2.0 mg
Pyridoxal hydrochloride	2.0 mg
Thiamine hydrochloride	2.0 mg
Riboflavin	2.0 mg
Phenol Red	15.0 mg
Sodium bicarbonate	2.75 g
Water q.s.p.	1000 mL

To add to the medium the following supplement:

Fetal calf serum	
(heated at 56°C for 30 minutes)	10%
Tryptose phosphate broth	10%
Benzylpenicillin sodium	60 mg/L
Streptomycin	0.1 g/L

PACKAGING AND STORAGE

See the monograph *Normal Human Immunoglobulin*.

LABELLING

See the monograph *Normal Human Immunoglobulin*. In the label it is indicated the number of International Units per container.

IMMUNOGLOBULIN, HUMAN, ANTI-RUBELLA

Immunoglobulinum Humanum Rubellae

Anti-rubella human immunoglobulin is a sterile, liquid or lyophilized preparation containing immunoglobulins, especially immunoglobulin G. The preparation is intended to be administered intramuscularly. It is produced from plasma containing specific antibodies against rubella virus. Normal human immunoglobulin can be added to it. Anti-rubella human immunoglobulin meets the requirements set forth in the monograph Normal Human Immunoglobulin, except with regard to the minimum number of donors and the minimum content in total proteins.

DOSAGE

The activity of anti-rubella human immunoglobulin is evaluated by comparison with the activity of a reference preparation measured in International Units, by means of an appropriate test of inhibition of hemagglutination. The international unit corresponds to the activity of a certain amount of international reference preparation of anti-rubella human serum. The correspondence between the International Unit and the international reference preparation is indicated by the World Health Organization

The indicated activity is not less than 4500 IU per milliliter. The confidence limits ($P=0.95$) of the determined activity are not less than 50% and not greater than 200%.

PACKAGING AND STORAGE

See the monograph Normal Human Immunoglobulin.

LABELLING

To comply with the legislation in force. See the monograph Normal Human Immunoglobulin. In the label it is indicated the number of International Units per milliliter.

IMMUNOGLOBULIN, HUMAN, ANTI-MEASLES

Immunoglobulinum Humanum Morbillicum

Anti-measles human immunoglobulin is a sterile, liquid or lyophilized preparation containing immunoglobulins, especially immunoglobulin G. The preparation is intended to be administered intramuscularly. It is produced from plasma containing specific antibodies against measles virus. Normal human immunoglobulin can be added to it. Anti-measles human immunoglobulin meets the requirements set forth in the monograph Normal Human Immunoglobulin, except with regard to the minimum number of donors and the minimum content in total proteins.

DOSAGE

The activity of the liquid preparation, or lyophilized preparation reconstituted according to the indications on the label is at least 50 IU of neutralizing antibodies of the measles virus per milliliter.

The activity is evaluated by comparing the titration of antibodies in the sample and a reference preparation measured in International Units, using a test dose of measles virus in appropriate cell culture.

The international unit corresponds to the specific neutralizing activity for the measles virus of a certain amount of international reference preparation of anti-rubella human serum.

The correspondence between the International Unit and the international reference preparation is indicated by the World Health Organization

To prepare serial dilutions of the sample and the reference preparation. To mix equal volumes of each dilution and a measles virus suspension containing approximately 100 TCID₅₀ in 0.1 mL. Incubate this mixture in the dark at 37 °C for 2 hours. To use at least six cell cultures for each mixture and inoculate 0.2 mL of the mixture per culture. To incubate at least for 10 days. To examine cultures for the development of the virus.

To determine the activity comparing the dilution which contains the lowest amount of the sample that has neutralized the virus with the reference preparation, which manifests similar activity. To calculate the activity of the sample in international units of neutralizing antibodies of the measles virus per milliliter.

PACKAGING AND STORAGE

See the monograph Normal Human Immunoglobulin.

LABELLING

See the monograph Normal Human Immunoglobulin. In the label it is indicated the number of International Units per container.

IMMUNOGLOBULIN, HUMAN, ANTI-TETANUS

Immunoglobulinum Humanum Tetanicum

The anti-tetanus human immunoglobulin is a sterile, liquid or lyophilized preparation containing immunoglobulins, especially immunoglobulin G. It is produced from plasma containing specific antibodies against the toxin of *Clostridium tetani*. Normal human immunoglobulin can be added to it. Anti-tetanus human immunoglobulin meets the requirements set forth in the monograph Normal Human

Immunoglobulin, except with regard to the minimum number of donors and the minimum content in total proteins.

During production it is necessary to establish a satisfactory relationship between the activity determined by the method immunoassay Determination of human activity against tetanus and the activity determined by the method antitoxic activity in mice in *dosage*.

DOSAGE

Antitoxic activity in mice

To evaluate the activity by determining the dose that ensures the protection of mice against the paralyzing effects of a given dose of tetanus toxin. This dose is compared with a reference preparation of tetanus human immunoglobulin measured in international units required to provide the same protection. The International Unit of antitoxin corresponds to specific neutralizing activity regard to tetanus toxin contained in a certain amount of international standard consisting of lyophilized human immunoglobulin. The correspondence between the International Unit and the International Standard is indicated by the World Health Organization. The tetanus human immunoglobulin is measured in International Units by comparison with the international standard.

Choice of animals. To use mice weighing between 16 and 20 g.

Preparation of test toxin. To prepare the toxin test by a suitable method from the sterile filtrate of a culture of *C. tetani* in liquid medium. The two methods mentioned below are given by way of example, but any other appropriate method may be used.

(1) To a culture filtrate of about nine days, add 1 to 2 volumes of glycerin and maintain the mixture in a liquid state at a temperature slightly lower than 0 °C.

(2) To precipitate the toxin by addition to the culture of ammonium sulfate, dry the precipitate under vacuum in the presence of phosphorus pentoxide, spray it and keep it dry in sealed ampoules under vacuum in the presence of phosphorus pentoxide.

Determination of test toxin dose (dose Lp/10). To prepare a solution of the reference preparation in a suitable liquid so that it contains 0.5 IU of antitoxin per milliliter. If the toxin is stored in a dry state, reconstitute using a suitable liquid. To prepare a series of mixtures of the solution preparation of the sample and reference so that each one contains 2 mL of the solution of the reference preparation and a variable amount of the sample. To complete each mixture with the same final volume of 5 mL using a suitable liquid. Let it rest for 60 minutes protected from light. To use a group of six mice for each mixture. To inject each of them subcutaneously, 0.5 mL of the mixture assigned to its group. To keep the mice under observation for 96 hours. Those who are afflicted by paralysis can be sacrificed. The toxin test

dose corresponds to the amount present in 0.5 mL of the mixture containing the lowest amount of toxin that causes, during the period of observation, paralysis in 6 mice to which it was administered, despite partial neutralization due to reference preparation.

Determination of the activity of the immunoglobulin

To prepare a solution of the reference preparation in a suitable liquid so that it contains 0.5 IU of antitoxin per milliliter. Prepare a solution of the test toxin in a suitable liquid so that it contains 5 doses/ mL. To prepare a series of mixtures of the solution of the test toxin and of the sample so that each one contains 2 mL of the solution of the toxin and a variable amount of the sample. To complete each mixture with the same final volume of 5 mL using a suitable liquid. To prepare a second series of mixtures of the toxin test solution and reference preparation solution so that each one contains 2 mL of solution of the test toxin and a variable amount of the reference preparation. In this second series, the average dilution of the reference preparation corresponds to the mixture containing 1 IU of antitoxin (2 mL of solution of the reference preparation). To complete each mixture with the same final volume of 5 mL using a suitable liquid. Let it rest for 60 minutes protected from light. To use a group of six mice for each mixture. To inject each of them subcutaneously, 0.5 mL of the mixture assigned to its group. To keep the mice under observation for 96 hours. Those who are afflicted by paralysis can be sacrificed. The mixture containing the maximum amount of immunoglobulin that does not protect any mice from paralysis corresponds to 1 IU. This amount is used to calculate the activity of the immunoglobulin in international units per milliliter.

The test is valid only if all the mice inoculated with the mixture containing up to 2 mL of the solution of the reference preparation are affected by paralysis and all mice inoculated with mixtures containing larger amounts of this solution did not show symptoms of paralysis.

Determination of the activity of tetanus human immunoglobulin

The activity of anti-tetanus human immunoglobulin is evaluated by comparison of antibody titer of the sample and a reference preparation, measured in International Units, with the aid of a test immunoassay with appropriate sensitivity and specificity (*Immunochemical methods*). The tetanus human immunoglobulin is measured in International Units by comparison with the international standard. The indicated activity is not less than 100 IU of tetanus antitoxin per milliliter. The specific activity is not inferior to the activity indicated. The confidence limits ($P=0.95$) of the determined activity are not less than 80% and not greater than 125%.

PACKAGING AND STORAGE

See the monograph Normal Human Immunoglobulin.

LABELLING

To comply with the legislation in force. See the monograph Normal Human Immunoglobulin. The label must indicate the number of international units in the vial.

IMMUNOGLOBULIN, HUMAN, ANTI-VARICELLA

Immunoglobulinum Humanum Varicellae

Anti-varicella human immunoglobulin is a sterile, liquid or lyophilized preparation containing immunoglobulins, especially immunoglobulin G. The preparation is intended to be administered intramuscularly. It is produced from plasma containing specific antibodies against varicella virus. Normal human immunoglobulin can be added to it. Anti-varicella human immunoglobulin meets the requirements set forth in the monograph Normal Human Immunoglobulin, except with regard to the minimum number of donors and the minimum content in total proteins and, in authorized cases, to the test of antibodies against the surface antigen of hepatitis B.

DOSAGE

The activity of anti-varicella human immunoglobulin is evaluated by comparing to the activity of a reference preparation measured in International Units, by means of a test with appropriate sensitivity and specificity (To proceed as described in *Immunochemical methods* (5.6)). The international unit corresponds to the activity of a certain amount of international reference preparation of anti-varicella human immunoglobulin. The correspondence between the International Unit and the international reference preparation is indicated by the World Health Organization

The indicated activity is not less than 100 IU per milliliter and not lower than the indicated activity.

The confidence limits ($P=0.95$) of the determined activity are not less than 80% and not greater than 125%.

PACKAGING AND STORAGE

See the monograph Normal Human Immunoglobulin.

LABELLING

To comply with the legislation in force. See the monograph Normal Human Immunoglobulin. In the label it is indicated the number of International Units per milliliter.

IMMUNOGLOBULIN, HUMAN, ANTI-VARICELLA, INTRAVENOUS ADMINISTRATION

Immunoglobulinum Humanum Varicella ad Usum Intravenosum

The anti-varicella human immunoglobulin for intravenous use is a sterile, liquid or lyophilized preparation containing immunoglobulins, especially immunoglobulin G. It is produced from plasma containing specific antibodies against human herpesviruses 3 (varicella-zoster 1). Normal human immunoglobulin can be added for intravenous administration. Anti-varicella human immunoglobulin for intravenous administration meets the requirements set forth in the monograph Normal Human Immunoglobulin, except with regard to the minimum number of donors and the minimum content in total proteins and the limit of osmolality.

DOSAGE

The activity of anti-varicella human immunoglobulin is evaluated by comparing to the activity of a reference preparation measured in International Units, by means of immunoassay with appropriate sensitivity and specificity (To proceed as described in *Immunochemical methods* (5.6)). The international unit corresponds to the activity of a certain amount of international reference preparation of anti-varicella human immunoglobulin. The correspondence between the International Unit and the international reference preparation is indicated by the World Health Organization

The indicated activity is not less than 25 IU per milliliter. The specific activity is not inferior to the activity indicated. The confidence limits ($P=0.95$) of the determined activity are not less than 80% and not greater than 125%.

PACKAGING AND STORAGE

See monograph Normal Human Immunoglobulin for Intravenous Administration.

LABELLING

To comply with the legislation in force. See monograph Normal Human Immunoglobulin for Intravenous Administration. In the label it is indicated the number of International Units per milliliter.

NORMAL HUMAN IMMUNOGLOBULIN Immunoglobulinum Humanum Normale

Normal human immunoglobulin is a sterile, lyophilized or liquid preparation, containing mainly IgG. Other proteins may also be present. Normal human immunoglobulin contains IgG antibodies can be administered intramuscularly or subcutaneously. Normal human immunoglobulin is obtained from human plasma, which meets the requirements

of the monograph of Human Plasma for Fractionation. Antibiotics should not be added in the preparation.

The method of preparation should include one or more steps demonstrating to remove or inactivate known infectious agents. If substances are used for viral inactivation, must demonstrate that there is no residue in the final preparation showing adverse events in patients treated with immunoglobulin. It is necessary to demonstrate, through appropriate testing in animals and evaluation during clinical trials, that the product is well tolerated when administered intramuscularly or subcutaneously. Normal human immunoglobulin is prepared from pooled plasma of at least 1000 donors, by a known method which provides a sterile final product and with a protein concentration of 160 g/l, containing antibodies to at least two agents (of which one viral and bacterial) available in Reference Preparation, or International Standard. The concentration of each antibody should be at least ten times higher than in the original pooled plasma.

If normal human immunoglobulin is prepared for subcutaneous administration, the production method should be suitable for a yield consistent of the product that meets the test of F_c function of immunoglobulin. Normal human immunoglobulin is prepared as a stabilized solution, for example, in a solution of sodium chloride at 0.9% (w/v); in glycine solution 2.25% (w/v), or if the preparation is lyophilized, in glycine solution of 6% (w/v). Multidose preparations should contain an antimicrobial agent. Multidose preparations should contain an antimicrobial agent. In the final product the amount of antimicrobial agents or stabilizers used must not present health adverse effects. The substance should be filtered through a bacterial-retaining filter (sterilizing filtration). The preparation may subsequently be lyophilized and the vials sealed under vacuum or an inert gas.

The stability of the preparation must be established through appropriate testing during the development of the stability study.

IDENTIFICATION

A. To subject the sample to precipitation tests, by using a suitable range of specific sera of different species of domestic animals. It is recommended that testing be performed in specific sera of plasma proteins of each domestic animal, commonly used for the preparation of biological products. The normal human immunoglobulin contains human proteins and it gives negative results to specific sera with plasma protein from other species.

B. To perform in the sample a test of immunoelectrophoresis as per proper technique. To use a normal human antiserum, comparing with normal human serum with the sample diluted in order to contain 10 g/L of proteins. The main component of the sample corresponds to the IgG component of normal human serum and there may be other plasma proteins in small amounts. If the human albumin has

been added as a stabilizer it may be regarded as an important compound.

CHARACTERISTICS

Appearance. The liquid preparation is clear or yellow or slightly brown during storage, and may be slightly turbid or a small amount of particle formation. The lyophilized preparation is a powder or solid friable mass, white or slightly yellowish. To prepare lyophilized reconstitution should be according to LABELLING immediately before the identification and other tests, except for solubility and water.

pH (5.2.19). From 5.0 to 7.2. To dilute the preparation to be examined in a solution of sodium chloride at 0.9% (w/v) at a protein concentration of 1% (w/v).

Osmolality (05.02.28). The osmolality of the sample is at least 240 mosmol/kg.

Solubility. For lyophilized preparation add the volume of the diluent according to the label. The preparation dissolves completely within 20 minutes at a temperature from 20 °C to 25 °C.

Protein composition. To proceed as described in *Electrophoresis (5.2.22)*, use the zone electrophoresis technique. To use suitable strips of cellulose acetate gel or agarose as the support medium, and barbital buffer pH 8.6 as the electrolyte solution. If the cellulose acetate is the support material, use the method described below. If it is the agarose gel, and because it is usually part of the automatic system, use the manufacturer's instruction manual.

Solution of the sample: to dilute the sample with solution of sodium chloride at 0.9% (w/v) to a concentration of 50 g/L proteins.

Reference solution: to reconstitute a reference standard for human immunoglobulin electrophoresis and dilute with sodium chloride at 0.9% (w/v) up to a concentration of 5% (w/v) in proteins.

System Suitability: in the electrophoretogram obtained with the reference solution, cellulose acetate or agarose gel, the proportion of proteins in the main band is in accordance with the limits set in the instructions accompanying the reference preparation.

Procedure: apply to the strip 2.5 µL of *Sample solution* or 0.25 µL per milliliter if used a narrower strip. For other strips, apply the same way the same amount of the reference solution. To apply a suitable electric field so that the band of human serum albumin, applied to the control strip, migrates at least 30 mm. Blush strip with starch black 10B SR for 5 minutes. Discolor with a mixture of glacial acetic acid and methanol (10:90) so that the bottom is free from coloration. To develop transparency of the strips with a mixture of glacial acetic acid and methanol (19:81). To measure the absorbance of the band in linear response in-

struments and wavelength of 600 nm. Calculate the result as the average of three measurements for each band.

The mobility of the protein is not greater than 10% of the main protein band.

Distribution of molecular size. To proceed as described in *high efficiency liquid chromatography (5.2.17.4)*. To use chromatograph equipped with an ultraviolet detector at 280 nm, column 600 mm length and 7.5 mm internal diameter and 300 mm length and 7.8 mm internal diameter packed with hydrophilic silica gel (for an adequate degree for fractionation of globular proteins with relative molecular mass between 10 000 and 500 000), mobile phase flow of 0.5 mL/min.

Mobile phase: to dissolve 4.873 g of dibasic dihydrate sodium phosphate, 1.741 g monobasic monohydrate sodium phosphate, 11,688 g of sodium chloride and 50 mg of sodium azide in 1000 mL of water.

Sample solution: to dilute the *Sample solution* with sodium chloride at 0.9% (w/v) up to concentration suitable to chromatographic system used. The range of concentration of 4 g/L to 12 g/L, and injection from 50 µg to 600 µg of protein is usually adequate.

Standard solution: dilute human immunoglobuline standard with solution of sodium chloride at 0.9% (w/v) to a concentration in proteins equal to the *Sample solution*.

In the chromatogram obtained with the *Standard solution*, the main peak corresponds to IgG monomers and there is a peak corresponding to dimmer with relative retention of main peak of 0.85. To identify peaks in the chromatogram obtained with *Sample solution* compared to the chromatogram of the *Standard solution*, no peak at the retention time less than that corresponding to the dimmer polymers and aggregates. The preparation to be examined complies with the test if the chromatogram obtained with *Sample solution* meets the following items:

- the relative retention time, compared to the corresponding peak of the chromatogram obtained with the *Standard solution*, is 1 ± 0.02 for the monomer and dimmer,
- **area under the peak:** the sum of the area under the peaks of the monomer and dimmer is not less than 85% of the total area of the chromatogram and the total area under the peaks of polymers and aggregates is not more than 10% of the total area of the chromatogram. This requirement does not apply to preparations to which albumin is added as a stabilizer, in the case of preparations stabilized with albumin, a molecular size distribution test is performed during manufacturing before adding the stabilizer.

PHYSICAL AND CHEMICAL TESTS

Water. Determined by an appropriate method, such as the semi-micro method (5.2.20.3), Loss on drying (5.2.9) or

Infrared absorption spectrophotometry (5.2.14). At most 2.0%.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

Pyrogens (5.5.2.1). It complies with the test. To inject into each rabbit, per kilogram of body weight, volume corresponding to at least 1 mL of immunoglobulin.

DOSAGE ANTI-D ANTIBODY

If the normal immunoglobulin is for subcutaneous use, it must comply with the determination of anti-D human immunoglobulin (5.5.1.15) in normal immunoglobulin for intravenous administration.

Antibody for the surface antigen of Hepatitis B

To determine by a suitable *Immunochemical method* (5.6). Is not less than 0.5 IU/g of immunoglobulin.

Antibody for Hepatitis A Virus

If the intention is to use for the prophylaxis of hepatitis A, it must meet the following additional requirements. To determine the content of antibodies in comparison with preparation of a reference standard measured in IU by using a suitable, specific and sensitive *Immunochemical method* (5.6).

The International Unit is the amount of activity of the international standard of anti-hepatitis A immunoglobulin. The equivalent in international standard unit is declared by the World Health Organization

The reference standard of human immunoglobulin against Hepatitis A is measured in international units compared to the international standard. The stated potency is not less than 100 IU/mL. The estimated potency is less than the stated potency. The confidence interval ($P = 0.95$) of the estimated potency is not less than 80% and not greater than 125%.

Anti-A and anti-B hemagglutinins

To perform the test if the normal human immunoglobulin is for subcutaneous preparation. To proceed as described in *Determination of hemagglutinin titration Anti-A and Anti-B* (5.5.1.9). To dilute the preparation to be examined at a concentration of 30 g/L of immunoglobulin prior to the preparation of serial dilutions to be used in the test. The agglutination is less than 1:64 dilution.

Total protein

To proceed as described in *Determination of nitrogen by the Kjeldahl method* (5.3.3.2). To dilute the sample with a 0.9% sodium chloride solution (w/v) to obtain a solution containing about 15 mg of protein in 2 mL. To add 2 mL

of this solution, 2 mL of 7.5% sodium molybdate solution (w/v), and 2 mL of a mixture of nitrogen-free sulfuric acid and water (1:30) to a round bottom centrifuge tube. To stir and centrifuge for 5 minutes. The supernatant liquid must be decanted, thus allowing the tube to be dry on a filter paper. Calculate the protein content by multiplying the nitrogen content by 6.25. The protein content is not less than 100 g/L and not more than 180 g/L. It contains at least 90.0 % and at most 110 % of the amount stated in the label.

PACKAGING AND STORAGE

To keep the liquid preparation in colorless glass container, protected from light and temperature indicated on the label. Save lyophilized preparation in colorless glass container under reduced pressure or under inert gas, in the dark and at a temperature not exceeding 25 °C.

LABELLING

To comply with the legislation in force. The label must state:

- for the liquid preparation, the volume of the preparation and protein content should be expressed in g/L,
- for lyophilized preparation, the amount of protein in the vial;
- the route of administration;
- for lyophilized preparation, the name or composition and volume of diluent for reconstitution to be added;
- where applicable, that the preparation is suitable for use in the prophylaxis of hepatitis A infection;
- where applicable, the activity of anti-hepatitis A immunoglobulin in IU/mL;
- in multidose preparations, the name and concentration of the antimicrobial agent.

IMMUNOGLOBULIN, HUMAN, NORMAL, INTRAVENOUS ADMINISTRATION

Immunoglobulinum Humanum Normale ad Usus Intravenosum

Normal human immunoglobulin is a sterile, liquid or lyophilized preparation containing immunoglobulins, especially immunoglobulin G (IgG). Other proteins may also be present. Contains IgG antibodies from normal individuals. This monograph does not apply to preparations produced by a process which purports to obtain a preparation containing fragments or chemically modified. Normal human immunoglobulin for intravenous administration is obtained from plasma that meets the requirements of the monograph of Human Plasma for Fractionation. No antimicrobial is added to the plasma used.

The method of preparation comprises one or more stages that showed that inactivate or eliminate infectious agents known. It has been shown that residues in the final product of the substances may be used in processes to inactivate

viruses do not have any undesirable effect in patients treated with the immunoglobulin. The safety of the preparation ready for administration intravenously has been shown by suitable tests on animals and by a study during clinical tests. Normal human immunoglobulin for intravenous administration is prepared from plasma collected from at least one thousand donors, using a method whereby it is known to be possible to obtain a preparation which:

- will not transmit infection;
- in the immunoglobulin concentration of 5% (w/v) contains at least two antibodies (one bacterial and another viral) for which there is an international standard or a reference preparation, the concentration of such antibodies is at least three times greater than the initial raw material; has a distribution defined in subclasses of immunoglobulin G and meets the test of Determination of Fc function of the immunoglobulin (5.5.1.16).

Normal human immunoglobulin for intravenous administration is prepared either in the form of a stabilized solution or lyophilized. A stabilizer can be added. In both cases, the preparation is subjected to a sterilizing filtration. No antimicrobial preservative is added during the fractionation of plasma and final plasma pool. The stability of the final product is demonstrated by tests performed during the development studies.

IDENTIFICATION

A. To subject the sample to precipitation tests, by using a suitable range of specific sera of different species

of domestic animals. It is recommended that testing be performed in specific sera of plasma proteins of each domestic animal, commonly used for the preparation of biological products. The normal human immunoglobulin contains human proteins and it gives negative results to specific sera with plasma protein from other species.

B. To subject the sample to a immunoelectrophoresis test according to the appropriate technique described in *Immunochemical methods* (5.6). To use a normal human antiserum, compare with normal human serum sample diluted in order to contain 10 g/L of protein. The main component of the sample corresponds to the IgG component of normal human serum and there may be other plasma proteins in small amounts. If the human albumin has been added as a stabilizer it may be regarded as an important compound.

CHARACTERISTICS

Appearance. The liquid preparation is clear or slightly opalescent, colorless or pale yellow. The lyophilized preparation is a powder or solid friable mass, white or slightly yellowish. In the case of a lyophilized preparation, its reconstitution is carried out according to the indications on the label immediately before performing the identification and testing, except for solubility and water content.

pH (5.2.19). The pH of the solution is between 4.0 and 7.4. To dilute the sample with solution of sodium chloride at 0.9% (w/v) to a concentration of 1% (w/v) in proteins.

Osmolality (05.02.28). The osmolality of the sample is at least 240 mosmol/kg.

Solubility. In the case of a lyophilized sample, add the volume of the diluent indicated on the label. At a temperature of 20 °C to 25 °C, the sample dissolves completely in 30 minutes.

Composition in proteins. To proceed as described in *Electrophoresis* (5.2.22), use the zone electrophoresis technique. To use strips of cellulose acetate gel suitable as a support and barbital buffer pH 8.6 as the electrolyte solution.

Sample solution: to dilute the sample with solution of sodium chloride at 0.9% (w/v) to a concentration of 3% (w/v) in immunoglobulin.

Standard solution: to reconstitute a reference standard for human immunoglobulin electrophoresis and dilute with sodium chloride at 0.9% (w/v) up to a concentration of 3% (w/v) in proteins.

To apply on a strip 4 µL of the *Sample solution* in 10 mm spot or apply 0.4 µL per millimeter when using a narrower strip. In another strip apply under the same conditions, the same volume of the *Standard solution*. To apply an electric field so that the appropriate normal human serum albumin band in a standard electrophoretogram migrates at least 30 mm. To handle strips with starch black 10B SR for 5 minutes with a mixture of 10 volumes of glacial acetic acid with 90 volumes of methanol for the time strictly necessary to achieve the discoloration of the frame. To provoke the transparency frame with a mixture of 19 volumes of glacial acetic acid with 81 volumes of methanol. To determine the absorbance of the bands at 600 nm with the aid of an apparatus that in this wavelength gives linear response in the range of measurement. To perform three determinations on each strip and calculate average of readings on each strip. In electrophoretogram of the sample, at most 5% of the protein may have different mobility from the main band. This limit does not apply if albumin was added to the preparation as a stabilizer, in the case of preparations stabilized with albumin, there will be a test in protein composition during production before adding the stabilizer. The test is only valid if the electrophoretogram obtained with the *Standard solution*, the proportion of proteins contained in the main band is between the limits indicated in the literature accompanying the preparation of the reference standard.

Distribution of molecular size. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 280 nm; 300 mm chromatograph column and 7.8 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group Flow of Mobile phase of 0.5 mL/min.

Mobile phase: dissolve 4.873 g of dibasic dihydrate sodium phosphate, 1.741 g monobasic monohydrate sodium phosphate, 11,688 g of sodium chloride and 50 mg of sodium azide in 1000 mL of water.

Sample solution: to dilute the *Sample solution* with sodium chloride at 0.9% (w/v) up to concentration suitable to chromatographic system used. Generally, it is convenient a concentration between 4 g/L and 12 g/L and injection of 500 µg to 600 µg of protein.

Standard solution: to dilute human immunoglobuline standard with solution of sodium chloride at 0.9% (w/v) to a concentration in proteins equal to the *Sample solution*.

To inject the *Sample solution* and the *Standard solution*. In the chromatogram obtained with the *Standard solution*, the main peak corresponds to IgG monomers and there is a peak corresponding to dimer with relative retention of main peak of 0.85. To identify peaks in the chromatogram obtained with *Sample solution* compared to the chromatogram of the *Standard solution*, no peak at the retention time less than that corresponding to the dimer polymers and aggregates. The sample meets the test if in the chromatogram obtained with *Sample solution* the retention time, compared to the corresponding peak in the chromatogram obtained with the *Standard solution*, is 1 ± 0.02 for the monomer and dimer, and the sum of the monomer and the dimer represents, at least, 90.0% of the total area of the chromatogram and aggregates and polymers represent at most 3.0% of the total area. This requirement does not apply to preparations with added albumin as a stabilizer, in case of stabilized preparations of albumin, perform a test of molecular size distribution during manufacturing before adding the stabilizer.

PHYSICAL AND CHEMICAL TESTS

Water. To determine by using one of the following methods: Determination of water by semi-micro method (5.2.20.3), Loss on drying (5.2.9) or Infrared absorption spectrophotometry (5.2.14). At most 2.0%.

Activator of prekallikrein. To proceed as described in *Determination of the activator of prekallikrein* (5.5.1.11). At most, 35 IU/mL, calculated in relation to a dilution of the sample containing 30 g/L of immunoglobulin.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

Pyrogens (5.5.2.1). It complies with the test. To inject into each rabbit, per kilogram of body weight, volume corresponding to at least 1 mL of immunoglobulin.

DOSAGE

Antibody against the surface antigen of Hepatitis B

The content of the sample in antibodies against the surface antigen of hepatitis-B determined by an *Immunochemical method* (5.6) suitable, is not less than 0.5 IU/g of immunoglobulin.

Anticomplementarity activity

To proceed as described in *Determination of anticomplementary activity of immunoglobulin* (5.5.1.13). The proportion of the consumed complement is at most 50.0% (1 CH50 per milligram of immunoglobulin).

Anti-A and anti-B hemagglutinins

To proceed as described in *Determination of anti-A and anti-B hemagglutinin titrations* (5.5.1.9). To perform tests of anti-A and anti-B Hemagglutinins. If the sample contains a amount of immunoglobulins greater than 30 g/L diluted to this concentration before preparing dilutions for the test. The dilutions to 1/64 have no signs of agglutination.

Immunoglobulin A

As determined by appropriate *Immunochemical methods* (5.6) content of immunoglobulin A is not greater than that indicated in the label content.

Total protein

To dilute the sample with solution of sodium chloride at 0.9% (w/v) to a concentration of about 15 mg of proteins in 2 mL. To put 2.0 mL of this solution into a round bottom centrifuge tube. To add 2 mL of solution of sodium molybdate 7.5% (w/v) and 2 mL of a mixture of 1 volume of nitrogen-free sulfuric acid with 30 volumes of water. To stir, centrifuge for 5 minutes. The supernatant liquid must be decanted, thus allowing the tube to be dry on a filter paper. To determine the nitrogen content by Determination of nitrogen by the Kjeldahl method (5.3.3.2) and calculate the amount of proteins by multiplying amount of nitrogen by 6.0. The content of proteins is not less than 30 g/L and contains at least 90.0% and at most 100.0% of the amount given on the label.

PACKAGING AND STORAGE

To keep the liquid preparation in colorless glass container, protected from light and temperature indicated on the label. Save lyophilized preparation in colorless glass container under reduced pressure or under inert gas, in the dark and at a temperature not exceeding 25 °C.

LABELLING

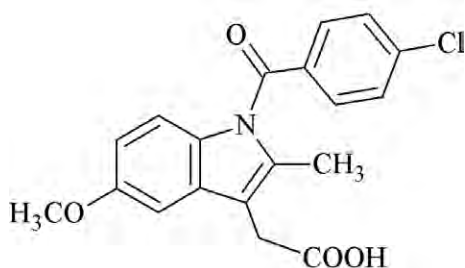
The label indicates the following:

- in the case of a liquid, the volume of the preparation in the container and protein content in grams per liter;
- in case of a lyophilized product, the amount of protein in the vial;
- the amount of immunoglobulin in the vial;

- the route of administration;
- storage conditions;
- the expiry date;
- in the case of the lyophilized product, the name or the composition and volume of diluent;
- the distribution of subclasses of immunoglobulin G in the preparation,
- where appropriate, the amount of albumin added as a stabilizer;
- the maximum level of immunoglobulin A.

INDOMETHACIN

Indometacinum



$C_{19}H_{16}ClNO_4$; 357,79

indomethacin; 04889

1 - (4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indole-3-acetic acid
[53-86-1]

It contains at least 98.0% and at most 101.0% of $C_{19}H_{16}ClNO_4$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white to yellow powder. It shows polymorphism.

Solubility. Practically insoluble in water, sparingly soluble in ethanol, ethyl ether, and chloroform.

Physical and chemical constants. *Melting range* (5.2.2): 158 °C to 162 °C.

IDENTIFICATION

The tests **B.**, **C.**, and **D.** may be omitted if the test **A.** is conducted.

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the indomethacin SQR spectrum similarly prepared. To separately dissolve the substances in methanol, if the spectra are not identical, and evaporate them to dryness. To obtain new spectra from these residues.

B. The ultraviolet absorption spectrum (5.2.14), ranging from 300 nm to 400 nm, of 0.0025% solution (w/v) in a mixture of methanol and hydrochloric acid (9:1), exhibits

maxima at 318 nm. The absorbance at 318 nm is between 0.425 and 0.475.

C. To add 2 mL of a mixture of 25% hydroxylamine hydrochloride (w/v), freshly prepared, with sodium hydroxide SR (1:3) to 0.1 mL of 1% solution of the sample (w/v) in ethanol. To add 2 mL of 20% hydrochloric acid (w/v) and 1 mL of 1.3% ferric chloride (w/v), and make it homogeneous. Pink-violet color develops.

D. To add 0.5 mL of *p*-dimethylaminobenzaldehyde SR to 0.5 mL of 1% solution of the sample (w/v) in ethanol. To solubilize the formed precipitate by stirring. To heat it in water bath. Bluish-green color is produced. To heat for 5 minutes and cool down in an ice bath for 2 minutes. Precipitate is formed, and color turns grayish-green. To add 3 mL of ethanol. Solution becomes clear and pink-violet.

PURITY TESTS

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1), by using HF_{254} silica gel suspension in 4.68% monobasic sodium phosphate (w/v), to prepare the chromatoplate support and a mixture of petroleum ether and ethyl ether (30:70) as mobile phase. To separately apply to the plate 10 µL of each of the solutions freshly prepared, as described below.

Solution (1): 20 mg/mL solution of the sample in methanol. To immediately prepare before using it.

Solution (2): to dilute 1 mL of *Solution (1)* to 200 mL in methanol, to obtain a 0.1 mg/mL solution.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (2)* (0.5%).

Heavy metals (5.3.2.3). To use 2 g of sample and proceed as described in *Method IV*. To prepare the standard solution by using 4 mL of *Standard lead solution* (10 ppm Pb). At most 0.002% (20 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample in oven at 105 °C, to obtain constant weight. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.2%.

DOSAGE

To employ one of the methods described below.

A. To accurately dissolve about 0.3 g of the sample in 75 mL of acetone. To bubble carbon dioxide-free nitrogen for 15 minutes. To add 0.1 mL of phenolphthalein SI and titrate with 0.1 *M* SV sodium hydroxide, by keeping a constant flow of nitrogen. To titrate with 0.1 *M* SV sodium hydroxide to obtain pinkish color. To perform sample blank

correction and adjust it as required. Each mL of 0.1 M SV sodium hydroxide is equal to 35.779 mg of $C_{19}H_{16}ClNO_4$.

B. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 254 nm; 300 mm column and 4.0 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μ m), flow of *Mobile phase* of 1.0 mL/min.

Mobile phase: 0.01 M monobasic sodium phosphate solution and 0.01 M dibasic sodium phosphate prepared by using a mixture of acetonitrile and water (1:1) as solvent.

Sample solution: to accurately transfer about 0.1 g of the sample to a 100 mL volumetric flask. To dissolve in *Mobile phase* and top up with the same solvent. To make it homogeneous. To transfer 10 mL of this solution to 100 mL volumetric flask and top up with *Mobile phase*. To make it homogeneous.

Standard solution: 0.1 mg/mL indomethacin SQR solution in *Mobile phase*.

The column efficiency is greater than 500 theoretical plates/column. The relative standard deviation for the replicate areas for the peaks recorded is not greater than 1.0%.

Procedure: to separately inject 20 μ L of *Standard solution* and *Sample solution*, record the chromatograms, and measure the areas under the peaks.

To calculate the content of $C_{19}H_{16}ClNO_4$ from the sample, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Analgesic, anti-inflammatory.

INDOMETHACIN CAPSULES

It contains at least 90.0% and at most 110.0 % of the amount stated of $C_{19}H_{16}ClNO_4$.

IDENTIFICATION

A. To weigh the capsules, remove the content, and weigh them again. To make the content of the capsules homogeneous. To mix an amount of powder equivalent to 50 mg of indomethacin with 10 mL of acetone for 2 minutes

and filter. To transfer 5 mL of the filtrate to an erlenmeyer flask with lid, add 20 mL of water, and stir for 2 minutes to obtain crystalline precipitate. To filter and collect the crystals. To dry the crystals at room temperature and desiccate in a vacuum oven at 100 °C for 2 hours. The infrared absorption spectrum (5.2.14) of the crystals, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the indomethacin SQR spectrum similarly prepared.

B. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using silica gel as support and a mixture of chloroform and methanol (4:1) as mobile phase. To separately apply to the plate 2 μ L of each of the solutions freshly prepared, as described below.

Solution (1): to weigh the capsules, remove the content, and weigh them again. To make the content of the capsules homogeneous. To mix an amount of powder equivalent to 25 mg of indomethacin with 25 mL of methanol, thus obtaining 1 mg/mL solution. To filter it.

Solution (2): 1 mg/mL indomethacin SQR solution in methanol.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). The principal spot obtained with *Solution (1)* corresponds in position and intensity to that obtained with *Solution (2)*.

C. The ultraviolet absorption spectrum (5.2.14), ranging from 300 nm to 350 nm, of sample solution obtained in *Dosage* exhibits absorption maximum at 320 nm, similar to those observed in the standard solution spectrum.

D. To weigh the capsules, remove the content, and weigh them again. To make the content of the capsules homogeneous. To mix an amount of powder equivalent to 25 mg of indomethacin with 2 mL of water and add 2 mL of 2 M sodium hydroxide. Light yellow color develops, which quickly fades out.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

Procedure for content uniformity. To transfer the content of each capsule to a 100 mL volumetric flask. To add 10 mL of water and let it rest for 10 minutes by occasionally stirring. To add 75 mL of methanol, mechanically stir for 10 minutes, top up with methanol, make it homogeneous, and filter it. To successively dilute in a mixture of methanol and phosphate buffer pH 7.2 (1:1), to obtain 0.0025% concentration (w/v). To proceed as described in *Dosage*.

DISSOLUTION TEST (5.1.5)

Dissolution medium: mixture of phosphate buffer and water (1:4), 750 mL

Equipment: baskets, 100 rpm

Time: 20 minutes

Procedure: after testing, remove aliquot from the dissolution medium, filter, and dilute, if necessary, in a mixture of phosphate buffer pH 7.2 and water (1:4), to obtain suitable concentration. To measure the absorbances at 318 nm (5.2.14), by using the same solvent to adjust zero. To calculate the amount of $C_{19}H_{16}ClNO_4$ dissolved in the medium, by comparing the readings obtained with that of 0.0025% indomethacin SQR solution (w/v) prepared in the same solvent.

Tolerance: not less than 80% (Q) of the amount stated of $C_{19}H_{16}ClNO_4$ dissolves within 20 minutes.

PURITY TESTS

Related substances. To proceed as described in *Related substances*, in the monograph *Indomethacin*. To prepare *Solutions (1)* and *(2)* as described below.

Solution (1): to weigh the capsules, remove the content, and weigh them again. To make the content of the capsules homogeneous. To mix an amount of powder equivalent to 0.1 g of indomethacin with 5 mL of chloroform, thus obtaining 20 mg/mL solution.

Solution (2): to transfer 1 mL of *Solution (1)* to 200 mL volumetric flask and top up with chloroform, thus obtaining 0.1 mg/mL solution.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (2)* (0.5%).

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. To weigh 20 capsules, remove the content, and weigh them again. To make the content of the capsules homogeneous. To accurately transfer an amount of powder equivalent to about 50 mg of indomethacin to a 100 mL volumetric flask, add 10 mL of water, and let it rest for 10 minutes, by occasionally stirring. To add 75

mL of methanol, make it homogeneous, top up with methanol, and filter it. To transfer 5 mL of the filtrate to a 100 mL volumetric flask and top up with a mixture of phosphate buffer pH 7.2 and methanol (1:1), to obtain 0.0025% solution (w/v). To prepare a standard solution at the same concentration by using the same solvent. To measure the absorbance of the resulting solutions at 320 nm, by using a mixture of phosphate buffer pH 7.2 and methanol (1:1) to adjust zero. To calculate the amount of $C_{19}H_{16}ClNO_4$ in the capsules, from the readings obtained. Alternatively, make the calculations by taking into account that $A(1\%, 1\text{ cm}) = 193$, at 320 nm, in a mixture of phosphate buffer pH 7.2 and methanol (1:1).

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

INDOMETHACIN SUPPOSITORIES

It contains at least 90.0% and at most 110.0 % of the amount stated of $C_{19}H_{16}ClNO_4$.

IDENTIFICATION

A. To weigh the suppositories, grind or cut them into small pieces, and mix to obtain homogenous mass. To dissolve an amount of powder equivalent to 0.1 g of indomethacin in 50 mL of hot water and filter it.

To wash the residue with hot water, and allow to air dry. To dissolve the residue in 5 mL of chloroform and evaporate to dryness. The infrared absorption spectrum (5.2.14) of the residue, dispersed in potassium bromide, has absorption maxima only at the same wavelength, with the same relative intensities to those observed in the indomethacin SQR spectrum similarly prepared.

A. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using silica gel as support and a mixture of chloroform and glacial acetic acid (19:1) as mobile phase. To separately apply to the plate 10 μ L of each of the solutions freshly prepared, as described below.

Solution (1): to weigh the suppositories, grind or cut them into small pieces, and mix to obtain homogenous mass. To transfer an amount equivalent to 25 mg of indomethacin to a 125 mL separatory funnel, add 15 mL of water and 50 mL of ethyl ether, and stir to dissolve it. To transfer the ether layer to a 200 mL volumetric flask and extract the aqueous layer with 2 portions (50 mL each) of ethyl ether. To combine the ether extracts and top up with ethyl ether.

Solution (2): 0.125 mg/mL indomethacin SQR solution in a mixture of methanol and ethyl ether (1:100). To previously dissolve in methanol.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). The principal spot obtained with *Solution (1)* corresponds in position and intensity to that obtained with *Solution (2)*.

B. To weigh the suppositories, grind or cut them into small pieces, and mix to obtain homogenous mass. To stir an amount equivalent to 25 mg of indomethacin with 5 mL of water, to produce a white suspension. To add 2 mL of 2 *M* sodium hydroxide. Light yellow color develops, which quickly fades out.

CHARACTERISTICS

Disintegration test (5.1.4.2). To perform the test for 90 minutes in phosphate buffer pH 6.8, by using 3 accurately weighed suppositories. After testing, remove each suppository, dry on filter paper, and weigh them. Not less than 75% of each suppository is dissolved.

Uniformity of dosage units (5.1.6). It complies with the test.

Procedure for content uniformity. To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. To transfer each suppository to 100 mL volumetric flask containing 80 mL of a mixture of methanol and glacial acetic acid (199:1), mechanically stir to obtain total dissolution of suppository, and top up with the same solvent. To make it homogenous and filter it. To successively dilute in a mixture of methanol and glacial acetic acid (199:1), to obtain 0.0025% concentration (w/v). To prepare a standard solution at the same concentration by using the same solvent. To measure the absorbances of the resulting solutions at 320 nm, by using a mixture of methanol and glacial acetic acid (199:1) to adjust zero.

To calculate the amount of $C_{19}H_{16}ClNO_4$ in the suppositories, from the readings obtained.

DOSAGE

To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. To weigh 10 suppositories, grind or cut them into small pieces, and mix to obtain homogenous mass. To accurately weigh an amount equivalent to about 0.1 g of indomethacin, transfer to a 50 mL volumetric flask by using 40 mL of methanol, and stir to obtain total dispersion. To top up with methanol and filter it. To transfer 2 mL of the filtrate to a 100 mL volumetric flask and top up with a mixture of phosphate buffer pH 7.2 and methanol (1:1). To prepare a standard solution at the same concentration, by using the same solvents. To measure the absorbances of the resulting solutions at 318 nm, by using a mixture of phosphate buffer pH 7.2 and methanol (1:1) to adjust zero. To calculate the amount of $C_{19}H_{16}ClNO_4$ in the suppositories, from the readings obtained. Alternatively, make the calculations by taking into account that $A(1\%, 1\text{ cm}) = 193$, at 318 nm, in a mixture of phosphate buffer pH 7.2 and methanol (1:1).

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

IODIDE, POTASSIUM Kalii iodidum

KI; 166.00
potassium iodide; 04965
Potassium iodide
[7681-11-0]

It contains at least 99.0% and at most 100.5% of KI, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Colorless crystals, or white powder.

Solubility. Very soluble in water, freely soluble in glycerol, soluble in ethanol.

IDENTIFICATION

A. The 10% solution (w/v) in carbon dioxide-free water responds to the reactions of iodide ion (5.3.1.1).

B. The 10% solution (w/v) in carbon dioxide-free water responds to the reactions of potassium ion (5.3.1.1).

PURITY TESTS

Appearance of solution. The solution used in Test A., *Identification*, is clear (5.2.25) and colorless (5.2.12).

Alkalinity. To add 0.1 mL of bromothymol blue SI to 12.5 mL of solution used in the test A. *Identification*, and titrate with 0.01 *M* hydrochloric acid to obtain yellow color. At most 0.5 mL of 0.01 *M* hydrochloric acid

Iodides. To add 0.25 mL of iodide-free starch SI and 0.2 mL of *M* sulfuric acid to 10 mL of the solution used in the test A. *Identification*. To let it rest protected from light for 2 minutes. No blue color develops.

Thiosulfate. To add 0.1 mL of iodine starch SI and 0.1 mL of 0.005 *M* iodine to 10 mL of the solution used in the test A. *Identification*. Blue color develops.

Iron (5.3.2.4). To dilute 5 mL of the solution obtained in Test A. *Identification* to 10 mL with water. To proceed as described in *Limit test for iron, Method I*. At most 0.002% (20 ppm).

Heavy metals (5.3.2.3). To use *Method I*. To use 20 mL of solution obtained in the test A. *Identification*. At most 0.001% (10 ppm).

Sulfates (5.3.2.2). To dissolve 8 g of sample in 15 mL of water. To proceed as described in *Limit test for sulfates*. At most 0.015% (150 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 105 °C for 3 hours. At most 1%.

DOSAGE

To accurately weigh about 1.5 g of the sample, dissolve in water, and top up to 100 mL with the same solvent. To add 40 mL of concentrated hydrochloric acid to 20 mL of this solution and titrate with 0.05 M SV potassium iodate, to change color from brown to yellow. To add 5 mL of chloroform. To keep titrating, by vigorously stirring to the chloroform layer discolors. Each mL of 0.05 M SV potassium iodate is equal to 16.600 mg of KI.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Anti-thyroid medication

IODIDE, SODIUM Natrii iodidum

NaI; 149.89
sodium iodide; 04969
Sodium iodide
[7681-82-5]

It contains at least 99.0% and at most 101.5% of NaI, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White, crystalline powder, or colorless crystals; hygroscopic.

Solubility. Very soluble in water, freely soluble in ethanol.

IDENTIFICATION

A. To dissolve 10 g of the sample in carbon dioxide-free water and top up the volume to 100 mL with the same solvent. Resulting solution responds to the reactions of iodide ion (5.3.1.1).

B. It responds to the reactions of sodium ion (5.3.1.1).

PURITY TESTS

Appearance of solution. To dissolve 10 g of the sample in carbon dioxide-free water and top up the volume to 100 mL with the same solvent. The solution is clear (5.2.25) and colorless (5.2.12).

Alkalinity. To add 0.1 mL of bromothymol blue SI to 12.5 mL of the solution obtained in *Appearance of solution*. At most 0.7 mL of 0.01 M hydrochloric acid are spent to change the indicator.

Barium. A 20% solution (w/v) of the sample acidified with hydrochloric acid should not blur by adding 1% potassium sulfate (w/v).

Iodides. To add 0.25 mL of iodide-free starch SI and 0.2 mL of M sulfuric acid to 10 mL of the solution obtained in the *Appearance of solution*. To let it rest protected from light for 2 minutes. No blue color develops.

Nitrate, nitrite, and ammonia. To add 5 mL of M sodium hydroxide and about 0.2 g of metallic aluminum to a solution of 1 g of the sample in 5 mL of water in a 40 mL test tube. To place a wad of cotton in the upper part of the tube and put a piece of litmus paper red mouth on the top of the test tube. To heat in water bath for 15 minutes. No blue color is observed on the paper.

Potassium. A solution of 1 g of the sample in 2 mL of water should not precipitate with 1 mL of sodium bitartrate SR.

Thiosulfate. To add 0.1 mL of iodine starch SI and 0.1 mL of 0.005 M iodine to 10 mL of the solution obtained in *Appearance of solution*. Blue color develops.

Iron (5.3.2.4). To use *Method I*. To use 5 mL of the solution obtained in *Appearance of solution* and proceed as described in *Limit test for iron*. To use 1 mL of the *Standard iron solution* (1 ppm Fe). At most 0.002% (20 ppm).

Heavy metals (5.3.2.3). To use the *Method I*. To weigh 2 g of sample, solubilize in 2 mL of water and proceed according to *Limit test for heavy metals*. At most 0.001% (10 ppm).

Sulfates (5.3.2.2). To use 10 mL of resulting solution obtained in *Appearance of solution* and dilute to 15 mL with water. To proceed as described in *Limit test for sulfates*. At most 0.015% (150 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 105 °C for 3 hours. At most 3.0%.

DOSAGE

To accurately weigh about 0.3 g of the sample previously desiccated and solubilize in 10 mL of water. To add 15 mL of hydrochloric acid and titrate with 0.1 M SV potassium iodate, to change color from red to yellow. To add 5 mL of chloroform and keep titrating, by vigorously stirring to the

chloroform layer discolors. Each mL of 0.1 *M* SV potassium iodate is equal to 29.978 mg of NaI.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Expectorant and anti-hyperthyroidism.

IODINE Iodum

I_2 ; 253.81
iodine; 04983
Iodine
[7553-56-2]

It contains at least 99.5% and at most 100.5% of I.

DESCRIPTION

Physical characteristics. Fine crystals, violet, and metallic luster.

Solubility. Very slightly soluble in water, soluble in ethanol, sparingly soluble in glycerin. Very soluble in concentrated iodide solutions.

IDENTIFICATION

A. To heat a small portion of the sample in a test tube. Violet vapors are released, which condense on the walls of the tube as bluish crystals.

B. To add starch SR solution to a saturated solution of the sample. Blue color is produced. To heat it to discolor. By cooling down, blue color fades out.

PURITY TESTS

Cyanide. To vigorously stir 1 g of sample with 30 mL of water and filter it. To add 10 drops of 0.1 *M* sodium thiosulfate, a crystal of ferrous sulfate, and a drop of ferric chloride and SR to 5 mL of the filtrate and boil it. To cool down. To acidify with hydrochloric acid. No blue color develops.

Bromide and chloride. To crush 3 g of the sample and mix with 20 mL of water. To filter and wash the filter with water. To dilute to 30 mL with the same solvent. To add 1 g of zinc powder. Upon discolor of solution, filter and wash the filter with water and top up to 40 mL with the same solvent. To add 3 mL of ammonia and 6 mL of 0.1 *M* silver nitrate solution to 10 mL of the solution. To filter again, wash the

filter with water, and top up to 20 mL with the same solvent. To treat 10 mL of solution with 1.5 mL of nitric acid. After a minute, the opalescence presented by preparation should not be more intense than that of a standard preparation simultaneously prepared with a mixture of 10.75 mL of water, 0.25 mL of 0.01 *M* hydrochloric acid, 0.2 mL of 20% nitric acid (w/v), and 0.3 mL of 0.1 *M* silver nitrate. At most 0.025% (250 ppm).

Sulfate. To use water to dilute 3 mL of the resulting filtrate in *Cyanide* to 5 mL, add a drop of hydrochloric acid and 5 drops of barium chloride SR. No turbidity is observed.

Evaporation residue. To accurately transfer about 5 g of the sample to a porcelain capsule, heat it in a water bath to all the iodine be sublimated, and dry it in an oven at 105 °C for 1 hour. At most 0.05%.

DOSAGE

To accurately transfer about 0.2 g of iodine to an erlenmeyer flask containing 1 g of potassium iodide and 2 mL of water. To add 1 mL of diluted acetic acid. After dissolution, add 50 mL of water.

To titrate with 0.1 *M* SV sodium thiosulfate at a temperature below 15 °C to change from dark yellow to pale yellow. To add a few drops of starch SI and keep titrating to blue color fades out. Each mL of 0.1 *M* SV sodium thiosulfate is equal to 12.691 mg of I.

PACKAGING AND STORAGE

In tightly closed containers, protected from light and heat.

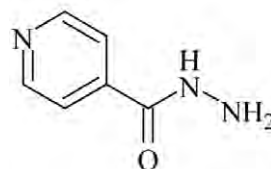
LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Anti-infective, anti-hyperthyroidism.

ISONIAZID Isoniazidum



$C_6H_7N_3O$; 137.14
isoniazid; 05092
4-Pyridinecarboxylic acid hydrazide
[54-85-3]

It contains at least 98.0% and at most 102.0% of $C_6H_7N_3O$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white or colorless powder.

Solubility. Freely soluble in water; slightly soluble in ethanol; sparingly soluble in chloroform; practically insoluble in ethyl ether and benzene.

Physical and chemical constants.

Melting range (5.2.2): 170 °C to 174 °C.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the isoniazid SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14) ranging from 200 nm to 350 nm, of sample solution obtained in *Dosage* method B., exhibits maxima at 212 nm and 265 nm similar to those observed in the standard solution spectrum.

C. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method C. corresponds to that of main peak of *Standard solution*.

PURITY TESTS

pH (5.2.19). 6.0 to 8.0. To determine in 5% aqueous solution (w/v).

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel with 5 µm thick as support and a mixture of water, acetone, methanol, and ethyl acetate (5:20:10:75) as mobile phase. To separately apply to the plate 5 µL of each of the solutions freshly prepared, as described below.

Solution (1): to dissolve 1 g of the sample in a mixture of water and acetone (1:1) and top up to 10 mL with the same solvent.

Solution (2): to dissolve 50 mg of hydrazine sulfate in 50 mL of water and top up to 100 mL with acetone. To transfer 10 mL of this solution to 100 mL volumetric flask, add 0.2 mL of *Solution (1)*, and top up with a mixture of water and acetone (1:1).

To develop the chromatogram. To remove the plate, and let it air drying. To examine under ultraviolet light (254 nm). Any secondary spot obtained in the chromatogram with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (2)* (0.2%). To nebulize the plates with *p*-dimethylaminobenzaldehyde SR1. An additional spot corresponding to hydrazine appears in the chromatogram. Any spot related to hydrazine obtained in the chromatogram with *Solution (1)* is not more intense than that obtained in the chromatogram with *Solution (2)* (0.05%).

Heavy metals (5.3.2.3). To use *Method III*. At most 0.001% (10 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 105 °C for 4 hours. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To employ one of the methods described below.

A. To accurately weigh about 250 mg of the sample. To transfer 100 mL volumetric flask and top up with water. To volumetrically transfer 20 mL of this solution to a 250 mL erlenmeyer flask. To add 100 mL of distilled water, 20 mL of hydrochloric acid SR, 0.2 g of potassium bromide, and 0.05 mL of methyl red SI. To titrate with 0.0167 M SV potassium bromate to red color fades out.

Each mL of 0.0167 M SV potassium bromate is equal to 3.429 mg of isoniazid (C₆H₇N₃O).

B. To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To accurately weigh about 25 mg of the sample and dissolve in 0.01 M hydrochloric acid. To put in ultrasound, if necessary, and top up to 250 mL with the same solvent. To dilute in 0.01 M hydrochloric acid to obtain 0.001% concentration (w/v). To prepare a standard solution at the same concentration by using the same solvent. To measure the absorbances of the solutions at 265 nm, by using hydrochloric acid to adjust zero. To calculate the content of C₆H₇N₃O in the sample from the readings obtained.

C. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 254 nm; 250 mm column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at room temperature; flow of *Mobile phase* of 1.5 mL/minute.

Phosphate buffer pH 6.9: to prepare 0.1 M monobasic potassium phosphate solution and adjust the pH to 6.9 with concentrated sodium hydroxide SR solution. To add 5 drops of triethanolamine per liter of buffer and make it homogeneous.

Mobile phase: mixture of *Phosphate buffer pH 6.9* and methanol (95:5).

Sample solution: to accurately transfer 32 mg of sample to a 100 mL volumetric flask by using 40 mL of *Mobile phase*, and put it in ultrasound for 10 minutes. To top up with the same solvent, to obtain 0.32 mg/mL solution.

Standard solution: to dissolve accurately weighed amount of isoniazid SQR in *Mobile phase* to obtain a 0.32 mg/mL solution.

To inject replicates of 20 µL of the *Standard solution*. The column efficiency is greater than 1800 theoretical plates. The retention factor should be greater than 2.35. The tailing factor should not be greater than 2.0. The relative standard deviation for replicate areas of the peaks recorded is not greater than 1.0 %.

Procedure: to separately inject 20 µL of *Standard solution* and *Sample solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_6H_7N_3O$ from the sample, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Tuberculostatic.

ISONIAZID TABLETS

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_6H_7N_3O$.

IDENTIFICATION

A. The ultraviolet absorption spectrum (5.2.14) ranging from 200 nm to 350 nm, of sample solution obtained in *Dosage* method B., exhibits absorption maxima at 212 nm and 265 nm similar to those observed in the standard solution spectrum.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method C. corresponds to that of main peak of *Standard solution*.

C. To weigh and pulverize the tablets. To transfer an amount of powder equivalent to 1 mg of isoniazid to erlenmeyer flask, add 50 mL ethanol, and stir. To add 0.1 g sodium tetraborate and 5 mL of 5% 1-chloro-2,4-dinitrobenzene (w/v) in ethanol to 5 mL of the resulting solution. To evaporate to dryness in a water bath and heat for extra 10 minutes. To add 10 mL of methanol to the residue and make it homogeneous. Reddish-purple color is developed.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.01 M hydrochloric acid, 900 mL

Equipment: baskets, 100 rpm

Time: 45 min.

Procedure: after testing, remove aliquot from the dissolution medium, filter, and dilute in 0.01 M hydrochloric acid, to obtain suitable concentration. To measure the absorbances of the solutions at 265 nm (5.2.14), by using the same solvent to adjust zero. To calculate the amount of $C_6H_7N_3O$ dissolved in the medium, by comparing the readings obtained with that of 0.001% isoniazid SQR solution (w/v) prepared in the same solvent.

Tolerance: not less than 80% (Q) of the amount stated of $C_6H_7N_3O$ dissolves within 45 minutes.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To weigh and pulverize 20 tablets. To transfer an amount of powder equal to 0.4 g of isoniazid in water, transfer to a 250 mL volumetric flask, top up with water, and stir. To filter it. To transfer 50 mL of the resulting solution to an erlenmeyer flask. To add 50 mL of water, 20 mL of hydrochloric acid SR, and 0.2 g of potassium bromide and titrate with 0.0167 M SV potassium bromate solution, by potentiometrically determining the endpoint. Each mL of 0.0167 M SV potassium bromate is equal to 3.429 mg of $C_6H_7N_3O$.

B. To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. To weigh and pulverize 20 tablets. To transfer an amount of powder equivalent to 0.1 g of isoniazid to a 100 mL volumetric flask and add 50 mL of 0.01 M hydrochloric acid. To put it in ultrasound for 15 minutes, mechanically stir for 15 minutes, and top up to volume with the same solvent. To make it homogenous and filter it. To successively dilute to obtain 0.001% concentration (w/v), by using the same solvent. To prepare standard solution under the same conditions. To measure the absorbances of the solutions at 265 nm, by using 0.01 M hydrochloric acid to adjust zero. To calculate the amount of $C_6H_7N_3O$ in the tablets, from the readings obtained.

C. To proceed as described in *Dosage*, method **C.**, in the monograph *Isoniazid*. To prepare the *Sample solution* as described below.

Sample solution: To weigh and pulverize 20 tablets. To transfer an amount of powder equivalent to 32 mg of isoniazid to a 100 mL volumetric flask, add 40 mL of *Mobile phase*, and put it in ultrasound for 10 minutes. To cool down at room temperature, top up with *Mobile phase*, and centrifuge for 5 minutes, to obtain a 0.32 mg/mL solution.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of isoniazid ($C_6H_7N_3O$) in the tablets, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers.

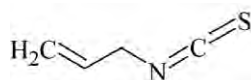
LABELLING

To comply with the legislation in force.

PACKAGING AND STORAGE

In tightly closed containers.

ISOTHIOCYANATE, ALLYL



C_4H_5NS ; 99.15
3-isothiocyanato-1-propene; 09889
[57-06-7]

It contains at least 93.0% and at most 105.0% of C_4H_5NS .

DESCRIPTION

Physical characteristics. Viscous liquid, ranging from colorless to slightly yellow. It is strongly tearing agent, with irritating odor. To wear eye protection when handling it.

Physical and chemical constants.

Relative density (5.2.5): 1.013 to 1.020.

Distillation range (5.2.3): 148 °C to 154 °C.

Refractive index (5.2.6): 1.527 to 1.531, determined at 20 °C.

IDENTIFICATION

The infrared absorption spectrum (5.2.14) of the sample dispersed in sodium chloride has absorption bands at 700

cm^{-1} , 950 cm^{-1} , 980 cm^{-1} , 1300 cm^{-1} , 1340 cm^{-1} , 1350 cm^{-1} , 1410 cm^{-1} , 1420 cm^{-1} , 1650 cm^{-1} , 2100 cm^{-1} , and 2200 cm^{-1} .

PURITY TESTS

Phenols. To dilute 1 mL of the sample in 5 mL of ethanol and add a drop of ferric chloride SR. No blue color.

DOSAGE

To accurately transfer about 4 mL of the sample to a 100 mL volumetric flask and top up the volume with ethanol. To transfer 5 mL of this solution to a distillation flask with 50 mL of 0.1 M SV silver nitrate and 5 mL of 10% ammonia solution (v/v). To connect the flask with a reflux condenser, heat in a water bath for 1 hour, and wait to cool down at room temperature. To disconnect the flask from reflux condenser, transfer the contents to a 100 mL volumetric flask, and top up with water. To filter the solution, by discarding 10 mL from the initial volume of the filtrate.

To add 5 mL of nitric acid and 2 mL of ferric ammonium sulfate SR to every 50 mL of filtrate and titrate the silver nitrate in excess with 0.1 M SV ammonium thiocyanate. To perform sample blank correction, by using 5 mL of ethanol instead of sample solution. Each mL of 0.1 M silver nitrate is equivalent to 4.958 mg of C_4H_5NS .

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antisecretory

ISOTRETINOIN CAPSULES

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_{20}H_{28}O_2$.

IDENTIFICATION

The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak in the chromatogram of *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 353 nm; 250 mm column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (10 µm), kept at room temperature; flow of *Mobile phase* of 1.4 mL/minute.

Mobile phase: mixture of methanol and water (77:23) with 0.5% glacial acetic acid (v/v).

Sample solution: to weigh 20 capsules, remove the content, and weigh them again. To make the content of the capsules homogeneous. To transfer an amount of powder equivalent to 20 mg of ISOTRETINOIN to a 100 mL amber volumetric flask. To add 80 mL of methanol and mechanically stir for 15 minutes. To top up with the same solvent, make it homogeneous, and filter it. To transfer 5 mL of the filtrate

to 25 mL amber volumetric flask and top up with methanol, thus obtaining 40 µg/mL solution.

Standard solution: to transfer about 20 mg of isotretinoin SQR, accurately weighed, to a 50 mL amber volumetric flask. To dissolve in methanol and top up with the same solvent. To transfer 5 mL of previous solution to 50 mL amber volumetric flask and top up with methanol.

Procedure: to separately inject 20 µL of *Sample and standard solutions*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of isotretinoin (C₂₀H₂₈O₂) in the capsules from the responses obtained from *Sample and standard solutions*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

JABORANDI TINCTURE

Jaborandi tinctura

The tincture is prepared from the 10.0% dried leaves of *Pilocarpus microphallus* Stapf - RUTACEAE (w/v), by maceration or percolation, with 65.0% ethanol (v/v) as the extraction liquid. It contains at least 0.06% of total alkaloid expressed as pilocarpine ($C_{11}H_{16}N_2O_2$; M 208.26).

CHARACTERISTICS

Organoleptic characteristics. The tincture is brown-yellow-greenish, pleasant aromatic odor, and bitter taste.

IDENTIFICATION

A. To evaporate 50 mL of jaborandi tincture, treat the residue with 10 mL of water and 5 drops of hydrochloric acid. To filter and wash the filtrate with ethyl ether. To alkalize with 6 M ammonium hydroxide and stir twice with 5 mL of chloroform. To collect the chloroform fractions with 5 mL of distilled water and add a drop of nitric acid. To stir and observe the phases. To add a small crystal of potassium dichromate, 2 mL of chloroform, and 1 mL of 3% hydrogen peroxide (w/v) to the acid solution. Chloroform is blue-purple or blue-anil and indicates the presence of imidazole or glyoxalic nucleus.

B. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel as stationary phase and a mixture of methylene chloride, methanol, and ammonium hydroxide (85:14:1) as mobile phase. To separately apply to the plate, as band, 40 µL of *Solution* (1) and 2 µL of *Solution* (2).

Solution (1): jaborandi tincture.

Solution (2): to dissolve 10 mg of pilocarpine hydrochloride SQR in methanol and top up to 2 mL with the same solvent.

To develop the chromatogram in the path length of 15 cm. To remove the chromatoplate, dry it in an oven at 100 °C - 105 °C for 10 minutes, and wait to cool down. The principal spot obtained with *Solution* (1) corresponds in position, color, and intensity to that obtained with *Solution* (2).

To nebulize with aqueous acetic potassium iodobismuthate SR followed by a sodium nitrite SR solution. The spot in

the chromatogram corresponding to pilocarpine is reddish-brown.

PURITY TESTS

Ethanol (5.3.3.8.1). $65 \pm 5\%$ (v/v). To proceed as described in *Method by distillation, Liquids with more than 30% of alcohol*.

Dry residue (5.4.3.2.2). At least 0.8%.

DOSAGE

To perform vacuum evaporation to 100 g of jaborandi tincture, at low temperature, to reduce to about 20 g. To totally transfer the residue to a separatory funnel, by using a few milliliters of methylene chloride. To add 10 mL of 6 M ammonium hydroxide. To successively extract fractions (20 mL each) of methylene chloride to completely extract the alkaloids, that is, when a few drops of aqueous phase have no turbidity by adding a drop of mercury potassium iodide SR solution. To combine the organic layers and then extract several times by using 0.05 M sulfuric acid. To slowly alkalize by using 6 M ammonium hydroxide to obtain pH 9 and then extract with methylene chloride to completely remove the alkaloids. To wash the organic solution with 20 mL of water. To evaporate the organic portion to about 5 mL. To dissolve the residue in 20 mL of 0.02 M SV hydrochloric acid and dry the remaining methylene chloride in a water bath at 40 °C. To titrate the hydrochloric acid in excess with 0.02 M SV sodium hydroxide. To se 5 drops of methyl red SI to pink turns yellow. To calculate the percentage (w/w) of total alkaloids expressed as pilocarpine according to the equation:

$$AT = \frac{(V_{\text{ácido}} - n) \times 0,4166}{m}$$

where

AT = total alkaloids in %;

$V_{\text{ácido}}$ = volume of 0.02 M hydrochloric acid in mL;

n = volume of 0.02 M sodium hydroxide in mL;

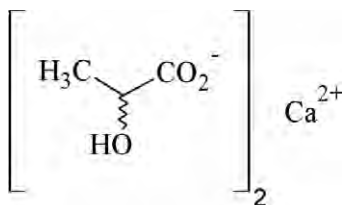
m = mass of the sample in g.

PACKAGING AND STORAGE

In tightly closed amber glass containers, protected from light and heat.

LACTATE, CALCIUM

Calcii lactas



$C_6H_{10}CaO_6$; 21822
 $C_6H_{10}CaO_6 \cdot xH_2O$
 calcium lactate; 00275
 Calcium salt of 2-hydroxypropanoic acid (1:2)
 [814-80-2]

It contains at least 98.0% and at most 101.0% of $C_6H_{10}CaO_6$ in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White granules or powder, practically odorless. The pentahydrate is efflorescent and becomes anhydrous at 120 °C.

Solubility. The pentahydrate is soluble in water and practically insoluble in ethanol.

IDENTIFICATION

A. It responds to the reactions of calcium ion (5.3.1.1).

B. It responds to the reactions of the lactate (5.3.1.1).

PURITY TESTS

Volatile fatty acids. To stir about 0.5 g with 1 mL of sulfuric acid and heat. No release of odor of volatile fatty acids.

Acidity. To titrate 20 mL of a solution of the sample (1:20) with 0.1 M sodium hydroxide, by using phenolphthalein SI as an indicator. The neutralization is achieved by using no more than 0.5 mL of sodium hydroxide (0.45% as lactic acid).

Loss on drying (5.2.9). To evenly distribute 1 - 2 g of the sample over the 3 mm (maximum) layer, in an appropriate weighting filter. To desiccate at 120 °C for 4 hours. Water loss is as follows: pentahydrate, 20% - 30%; trihydrate, 15% - 20%; monohydrate, 5% - 8%; anhydrous, at most 3%.

DOSAGE

To accurately weigh an amount of the sample containing about 0.35 g of lactate anhydrous. To dissolve in 150 mL of water acidulated with 2 mL of diluted hydrochloric acid. To add about 30 mL of 0.05 M SV disodium edetate by stirring (preferably magnetic stirrer).

To add 15 mL of sodium hydroxide SR and 0.3 g of hydroxy naphthol blue indicator, and continue titration to it turns blue. Each mL of 0.05 M SV disodium edetate is equal to 10.91 mg of $C_6H_{10}CaO_6$.

PACKAGING AND STORAGE

In airtight containers.

LABELLING

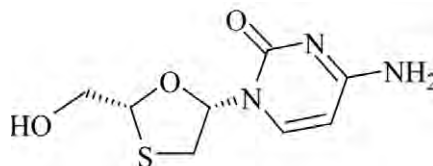
To comply with the legislation in force.

THERAPEUTIC CLASS

Calcium and electrolyte replenishment.

LAMIVUDINE

Lamivudinum



$C_8H_{11}N_3O_3S$; 229.26
 lamivudine; 05152
 4-Amino-1-[(2R, 5S) -2 - (hydroxymethyl)
 -1,3-oxathiolan-5-yl] -2 (1H)-pyrimidone
 [134678-17-4]

It contains at least 98.0% and at most 102.0% of $C_8H_{11}N_3O_3S$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. Crystalline, white to light yellow powder.

Solubility. freely soluble in water, slightly soluble in methanol and ethanol, insoluble in acetone. Freely soluble in 0.1 M sodium hydroxide and 0.1 M hydrochloric acid.

Physical and chemical constants.

Melting range (5.2.2): 176 °C to 178 °C.

Specific rotation (5.2.8): -135° to -146° in relation to the anhydrous substance. To determine in 0.8% solution (w/v) in methanol.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the lamivudine SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14) ranging from 200 nm to 400 nm, of Sample solution obtained in Dosage method A., exhibits absorption maxima at 270 nm similar to those observed in the Standard solution spectrum.

C. The retention time of the main peak in the chromatogram of the Sample solution obtained in the Dosage method B. corresponds to that of main peak of Standard solution.

PURITY TESTS

Related substances. To proceed as described in High performance liquid chromatography (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 270 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with β -cyclodextrin bonded to hydroxypropyl ether (5 μ m), flow of Mobile phase of 1 mL/min.

Mobile phase: mixture of 0.1 M ammonium acetate and methanol (95:5).

Solution (1): to dissolve 15 g of the sample in Mobile phase and top up to 10 mL with the same solvent.

Procedure: to inject 20 μ L of Solution (1), record the chromatograms, and measure the areas under the peaks. The sum of the areas obtained for the secondary peak, except the area under the main peak, is not greater than 1.0% of the total area of the peaks obtained. To disregard the peaks related to solvent.

Water (5.2.20.1). At most 2.0%.

Heavy metals (5.3.2.3). To use the Method I. At most 0.001% (10 ppm).

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.2%.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in Ultraviolet absorption spectrophotometry (5.2.14). To accurately weigh about 50 mg of the sample and dissolve in water. To top up the volume to 100 mL with the same solvent. To successively dilute in water to obtain 0.0015% concentration (w/v). To prepare a standard solution at the same concentration by using the same solvent. To measure the absorbance of the resulting solutions at 270 nm, by using water to adjust zero. To calculate the content of $C_8H_{11}N_3O_3S$ in the sample from the readings obtained.

B. To proceed as described in High performance liquid chromatography (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 277 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octylsilane group (5 μ m), kept at room temperature; flow of Mobile phase of 1 mL/min.

Acetate buffer pH 3.8: to dissolve 1.9 g of ammonium acetate in 900 mL of water, adjust the pH (3.8 ± 0.2) with glacial acetic acid, and top up to 1000 mL.

Mobile phase: mixture of Acetate buffer pH 3.8 and methanol (95:5).

Sample solution: to transfer 20 mg of the sample to a 50 mL volumetric flask. To dissolve in Mobile phase and top up with the same solvent.

Standard solution: to transfer 20 mg of lamivudine to a 50 mL volumetric flask. To dissolve in Mobile phase and top up with the same solvent.

To inject replicates of 10 μ L of the Standard solution. The relative standard deviation for the replicate areas for the peaks recorded is not greater than 2.0%.

Procedure: to separately inject 10 μ L of Sample solution and Standard solution, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_8H_{11}N_3O_3S$ from the sample, from the responses obtained for the Sample solution and Standard solution.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antiretroviral.

LAMIVUDINE TABLETS

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_8H_{11}N_3O_3S$. Tablets may be coated.

IDENTIFICATION

A. To weigh and pulverize the tablets. To transfer an amount of powder equivalent to 0.15 g of lamivudine to a mortar, add 10 mL of methanol, mix, and filter it. To evaporate the filtrate to obtain residue and desiccate it in an oven at 40 °C for 2 hours. The residue responds to test A., Identification, in the monograph Lamivudine.

B. The ultraviolet absorption spectrum (5.2.14) ranging from 200 nm to 400 nm, of Sample solution obtained in Dosage method A., exhibits absorption maxima at 270 nm similar to those observed in the Standard solution spectrum.

C. The retention time of the main peak in the chromatogram of the Sample solution obtained in the Dosage method B. corresponds to that of main peak of Standard solution.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). Within 30 minutes.

Uniformity of dosage units (5.1.6). It complies with the test.

Procedure for content uniformity. To transfer each tablet to a 100 mL volumetric flask containing 70 mL of water and stir to obtain total disintegration of the tablet. To put it in ultrasound for 15 minutes, top up with the same solvent, make it homogeneous, and filter it. To proceed as described in Method A. *Dosage* from "To dilute to obtain concentration..."

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL

Equipment: blades, 50 rpm

Time: 30 minutes

Procedure: after testing, remove aliquot from the dissolution medium, filter, and dilute with water to obtain suitable concentration. To measure the absorbances at 270 nm (5.2.14), by using water to adjust zero. To calculate the amount of $C_8H_{11}N_3O_3S$ dissolved in the medium, by comparing the readings obtained with that of 0.0015% lamivudine SQR solution (w/v) prepared in the same solvent.

Tolerance: not less than 80% (Q) of the amount stated of $C_8H_{11}N_3O_3S$ dissolves within 30 minutes.

PURITY TESTS

Water (5.2.20.1). At most 3.0%.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To weigh and pulverize 20 tablets. To transfer an amount of powder equivalent to 0.15 g of lamivudine to 100 mL volumetric flask, add 70 mL of water, put it in ultrasound for 15 minutes, and top up with

the same solvent. To make it homogenous and filter it. To successively dilute to obtain 0.0015% concentration (w/v), by using water as solvent. To prepare a standard solution at the same concentration by using the same solvent.

To measure the absorbance of the resulting solutions at 270 nm, by using water to adjust zero. To calculate the amount of $C_8H_{11}N_3O_3S$ in the tablets, from the readings obtained.

B. To proceed as described in *High performance liquid chromatography* (5.2.17.4). To proceed as described in method B., *Dosage*, in the monograph *Lamivudine*. To prepare the *Sample solution* as described below.

Sample solution: To weigh and pulverize 20 tablets. To transfer an amount of powder equivalent to 0.2 g of lamivudine to 100 mL volumetric flask and add 50 mL of *Mobile phase*. To mechanically stir for 10 minutes and top up with *Mobile phase*. To make it homogenous and filter it. To transfer 10 mL to 50 mL volumetric flask, top up with *Mobile phase*, and make it homogeneous.

Procedure: to separately inject 10 µL of *Sample and standard solutions*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of $C_8H_{11}N_3O_3S$ in the tablets, from the responses obtained for the *Sample and standard solutions*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

LAMOTRIGINE Lamotriginum



$C_9H_7Cl_2N_5$; 256.09

lamotrigine; 05153

6 - (2,3-dichlorophenyl) -1,2,4-triazine-3,5-diamine
[84057-84-1]

It contains at least 98.0% and at most 102.0% of $C_9H_7NCl_2N_5$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white or almost white powder.

Solubility. Freely soluble in dimethylformamide, slightly soluble in methanol, sparingly soluble in benzene and acetone.

Physical and chemical constants. *Melting range (5.2.2):* 216 °C to 218 °C.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the lamotrigine SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14), from 200 nm to 370 nm, 0.002% solution (w/v) in 0.01 M hydrochloric acid, has maximum at 269 nm, similar to those observed in the spectrum of a similar solution of lamotrigine SQR.

C. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using 60 F₂₅₄ silica gel as support and a mixture of chloroform, methanol, and dimethylformamide (16:3.5:0.5) as mobile phase. To separately apply to the plate 10 µL of each of the solutions freshly prepared, as described below.

Solution (1): 1.0 mg/mL solution of the sample in methanol.

Solution (2): 1.0 mg/mL lamotrigine SQR solution in methanol.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm) or expose the plate to iodine vapor. The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

D. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

PURITY TESTS

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 60°C under reduced pressure for 2 hours. At most 0.5%.

DOSAGE

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 279 nm; 150 mm column and 4.6 mm inner diameter, packed with silica chemically

bonded to the octadecylsilane group (5 µm), kept at room temperature; flow of *Mobile phase* of 1.0 mL/minute.

Mobile phase: mixture of 0.3% triethylamine (v/v), adjusted to pH 4.0 with 10% phosphoric acid, and methanol (62:38).

Sample solution: to dissolve accurately weighed amount of the sample in methanol, to obtain a 0.5 mg/mL solution. To transfer 4 mL of this solution to 50 mL volumetric flask and top up with *Mobile phase*, thus obtaining 40 µg/mL solution.

Standard solution: to dissolve an accurately weighed amount of lamotrigine SQR in methanol to obtain a 0.5 mg/mL solution. To transfer 4 mL of this solution to 50 mL volumetric flask and top up with *Mobile phase*, thus obtaining 40 µg/mL.

The column efficiency should be greater than 5000 theoretical plates for lamotrigine peak. The tailing factor for lamotrigine peak is not greater than 2.0. The relative standard deviation for replicate areas of the peaks recorded should not be greater than 2.0 %.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the amount, in mg, of C₉H₇NCl₂N₅ in the sample, from the responses from the *Standard solution* and *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Anticonvulsant.

LAMOTRIGINE TABLETS

It contains at least 90.0 % and at most 110.0 % of the amount stated of C₉H₇Cl₂N₅.

IDENTIFICATION

A. To weigh and pulverize the tablets. To use an amount of powder equivalent to 10 mg of lamotrigine. To proceed as described in *Identification*, test **B.**, in the monograph *Lamotrigine*.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness (5.1.3.1). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

A. To proceed as described in *Dosage*, in the monograph *Lamotrigine*. To prepare the *Sample solution* as described below.

Sample solution: To weigh and pulverize 20 tablets. To transfer an amount of powder equal to 25 mg of lamotrigine to a 50 mL volumetric flask by using 30 mL of methanol. To put it in ultrasound for 15 minutes. To top up with the same solvent, filter, and make it homogeneous. To transfer 4 mL of this solution to 50 mL volumetric flask, top up with *Mobile phase*, and make it homogeneous.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_{19}H_{17}Cl_2N_3$ in the tablets, from the responses obtained for the *Sample solution* and *Standard solution*.

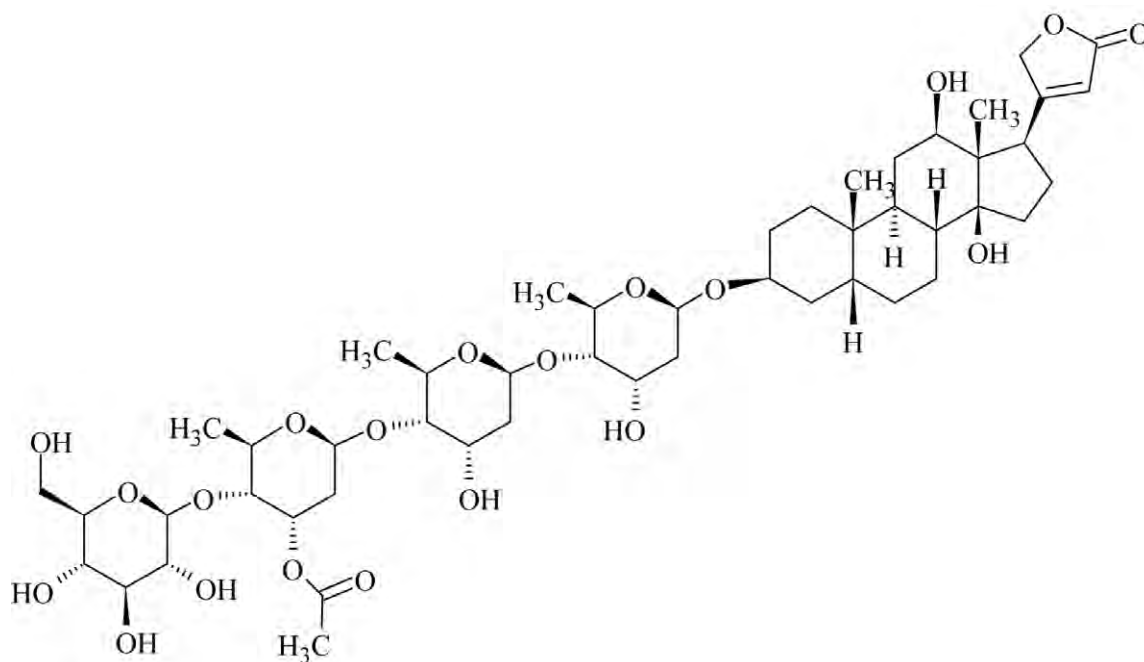
PACKAGING AND STORAGE

In tightly closed containers, at room temperature.

LABELLING

To comply with the legislation in force.

LANATOSIDE C
Lanatosidum C



$C_{49}H_{76}O_{20}$; 985.12

lanatoside C; 05156

(3β,5β,12β)- 3-{[β-D- glucopyranosyl- (1→4)- 3-O-acetyl- 2,6-dideoxy- β-D-ribo- hexopyranosyl- (1→4)- 2,6-dideoxy- β-D- ribo- hexopyranosyl- (1→4)- 2,6-dideoxy- β-D- ribo- hexopyranosyl] oxy}- 12,14-dihydroxycard- 20(22)- enolide [17575-22-3]

It contains at least 97.0% and at most 102.0% of $C_{49}H_{76}O_{20}$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white or yellowish-white, odorless powder; hygroscopic.

Solubility. Practically insoluble in water, soluble in methanol, dioxane, and pyridine anhydrous, insoluble in chloroform and ethyl ether.

Physical and chemical constants.

Specific rotation (5.2.8): +32,0° a +35,5°, in relation to the desiccated substance. To determine in 2% solution (w/v) in methanol.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the lanatoside C SQR spectrum similarly prepared.

B. The principal spot in the chromatogram of *Solution (2)* in the *Related substances* corresponds in position, color, and intensity to that obtained with *Solution (3)*.

C. To dissolve 0.5 g of the sample in 0.2 mL of 60% ethanol (v/v). To add 0.1 mL of 2% 3,5-dinitrobenzoic (w/v) in ethanol and 0.1 mL of 2 M sodium hydroxide. Violet color develops.

D. To dissolve 5 mg of the sample in 5 mL of glacial acetic acid and add 0.05 ferric SR. To carefully add 2 mL of sulfuric acid, but with no stirring. Let it rest. A non-reddish brown ring develops at the interface, and a yellowish-green color, which turns blue-green, diffuses from the ring.

PURITY TESTS

Appearance of solution. The 2% solution (w/v) in methanol is clear (5.2.25) and colorless (5.2.12).

Related substances. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using G silica gel as support and a mixture of toluene, ethanol, methylene chloride, and water (60:30:20:1) as mobile phase. To separately apply to the plate 5 µL of each of the solutions freshly prepared, as described below.

Solution (1): 20 mg/mL solution of the sample in methanol.

Solution (2): 2 mg/mL solution of the sample in methanol.

Solution (3): 2 mg/mL lanatoside C SQR solution in methanol.

Solution (4): 0.3 mg/mL lanatoside C SQR solution in methanol.

Solution (5): 0.2 mg/mL lanatoside C SQR solution in methanol.

Solution (6): 0.1 mg/mL lanatoside C SQR solution in methanol.

To develop the chromatogram. To remove the plate, and let it drying. To nebulize with 5% sulfuric acid (v/v) in ethanol.

In the chromatogram obtained with *Solution (1)* no secondary spot is more intense than the principal spot in the chromatogram obtained with *Solution (4)* (1.5%); no more than

3 secondary spots are more intense than the principal spot in the chromatogram obtained with *Solution (6)* (0.5%); and no more than one of these spots is more intense than the principal spot obtained with *Solution (5)* (1.0%).

Loss on drying (5.2.9). To determine in 0.5 g of the sample, over phosphorus pentoxide in, in an oven at 105 °C, under reduced pressure, to obtain constant weight. At most 7.5%.

Sulfated ashes (5.2.10). To determine in 0.1 g of the sample. At most 0.5%.

DOSAGE

To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. To accurately weigh about 50 mg of the sample and dissolve in ethanol. To dilute to 50 mL with the same solvent. To successively dilute in ethanol to obtain 0.005% concentration (w/v). To prepare a standard solution at the same concentration by using the same solvent. To add 3 mL of alkaline sodium picrate SR to 5 mL of each diluted solution and let it rest in a water bath at 19 °C - 21 °C for 40 minutes, protected from light. To measure the absorbances of the solutions at 484 nm, by using a mixture of 5 mL of ethanol and 3 mL of alkaline sodium picrate SR to adjust zero. To calculate the content of C₄₉H₇₆O₂₀ in the sample from the readings obtained.

PACKAGING AND STORAGE

In airtight closed glass containers, protected from light, stored at temperature below 10 °C.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Cardiotonic glycoside.

BITTER ORANGE

Aurantii amari exocarpium

Citrus aurantium L. subsp. *aurantium* - *RUTACEAE*

The vegetal drug consists of dried exocarp portions corresponding to the mature fruit flavedo, free of most of the flesh which is the white spongy tissue corresponding to the albedo. Contains at least 2.0% of volatile oil.

SCIENTIFIC SYNONYMY

Citrus aurantium L. subsp. *amara* (L.) Engler

CHARACTERISTICS

Organoleptic characteristics. The drug has a strong odor, aromatic, distinctive and aromatic flavor and very bitter.

MACROSCOPIC DESCRIPTION

The exocarp is in irregular portions of up to 8.0 cm in length and up to 4.0 cm wide. The outer surface, in front view, is yellowish, yellowish-brown to brown-yellow, wavy and coarsely punctuated by numerous glands secreting translucent. The inner surface, in front view, is yellowish-white to whitish-brown, rough and spongy. In side view glands are visible in the form of cavities.

MICROSCOPIC DESCRIPTION

The flavedo is composed of the epidermis and the parenchyma adjacent tissues. The albedo is formed by spongy parenchyma. The flavedo in front view, presents epidermis with small cells with different forms of anticlinal walls straight, containing lipid droplets. The stomata are ciclocytic and situated slightly above the other cells. Secreting glands are visible by transparency. In cross section, the cuticle is thick and smooth. The epidermis consists of small cells, polygonal with dense protoplast containing lipid droplets and chromoplasts. Subepidermally occur four to five layers of other yellow, collenchyma, compact, formed of small cells with dense contents, presenting chromoplasts and lipid droplets. Below these, there are larger parenchyma cells, of thinner walls, with intercellular visible spaces, large amount of lipid droplets and prismatic single crystals of calcium oxalate, of different shapes and sizes. In the first layers of the parenchyma circular to ovoid glands with up to 1.0 mm in diameter, at different stages of development and irregularly arranged occur. The parenchyma located laterally at glands is formed by elongated, compact cells, with large amounts of lipid droplets and crystals. Small collateral vascular bundles are distributed in this tissue. Vessel elements with helical thickening are visible longitudinally. The inner parenchyma is loose and composed of hyaline cells and thin walls of different shapes and sizes, containing single crystals. The parenchyma near the albedo present cells of greater volume, thicker walls and smaller amounts of crystals. Hesperidin crystals are common in all parenchyma. The albedo is composed of spongy parenchyma with brachiform cells with intercellular spaces and few crystals and lipid droplets.

MICROSCOPIC DESCRIPTION OF POWDER

The powder meets all the requirements for the subspecies, less macroscopic characters. Clear brown powder. With the addition of

chloral hydrate are characteristic: epidermis fragments of flavedo with cells as described in front view; epidermis fragments of flavedo with stomata in front view; fragments of flavedo, in cross section, showing epidermis and collenchymatous parenchyma; fragments of parenchyma collenchymatous, in cross section; fragments flavedo parenchyma, with cells containing lipid droplets in cross section; fragments of flavedo parenchyma, in cross section, containing lipid droplets, single crystals of calcium oxalate crystals and hesperidin; fragments of parenchyma flavedo,

in cross section, with portions of vascular bundles, seen in longitudinal view; fragments of parenchyma flavedo, in cross section, containing lipid droplets and hesperidin crystals; flavedo fragments with portions of the secretory glands in cross-section; fragments of parenchyma flavedo, in cross section with vascular bundle portion, seen in longitudinal view; fragments of parenchyma hesperidin crystals, crystal idioblasts of flavedo with single crystals of calcium oxalate in cross section; crystals of calcium oxalate alone; hesperidin crystals isolated in the form needle only observed with addition of lugol; portions tracheal thickening helical elements, in longitudinal view; fragments albedo, in small amounts, or in longitudinal cross section.

IDENTIFICATION

To proceed as described in *Thin layer chromatography* (5.2.17.1) using GF₂₅₄ silica gel with a thickness of 250 µm, as support, and a mixture of water, formic acid and ethyl acetate (10:15:75) as mobile phase. To apply separately to the plate-shaped band 20 µL of *solution (1)* and 10 µL of *solution (2)*, freshly prepared as described below.

Solution (1): to add 1 g of the milled drug (710 µm), 10 mL of methanol. Heat in water bath at approximately 60 °C for 10 minutes, stirring often. Cool and filter.

Solution (2): To dissolve 1 µg of naringin and 10.0 µg of caffeic acid in 1 mL of methanol.

To develop the chromatogram. To remove the plate, allow to air dry. Then nebulize the plate with aminoethanol difenilborato SR (Natural Reagent A) at 1% (w/v) in methanol and observe under ultraviolet light (365 nm). The fluorescent yellow stain obtained in the middle part of the chromatogram with the *Solution (1)* with R_f of approximately 0.6, corresponding in position and color to that obtained with *Solution (2)*, referring to naringin. The fluorescent blue stain obtained in the superior part near the chromatogram with the *Solution (1)* with R_f of approximately 0.9, corresponding in position and color to that obtained with *Solution (2)*, referring to caffeic acid. Among the spots related to naringin and caffeic acid are obtained two clear fluorescent spots with *solution (1)* being the closest to naringin corresponding to hesperidin. Other spots

are observed in the lower half of the chromatogram: yellow fluorescent spot (R_f about 0.58), red fluorescent (R_f about 0.5), and other lower blue spot and fluorescent orange.

PURITY TESTS

Water (5.2.20.2). To determine 20.0 g of powdered sample (355 µm). At least 10%.

Total ash (5.4.2.4). At most 7.0%

DOSAGE

Volatile oils

To proceed as described in *Determination of volatile oils in herbal drugs (5.4.2.7)*. To use 500 mL flask containing 200 mL of distilled water as the liquid. To add 0.5 mL of xylene through the side opening k. To use reduced plant dry powder (710 µm). To proceed immediately to determine the volatile oil from 15 g of powdered drug. Distill for 90 minutes.

PACKAGING AND STORAGE

In tightly closed container, away from light, heat and humidity.

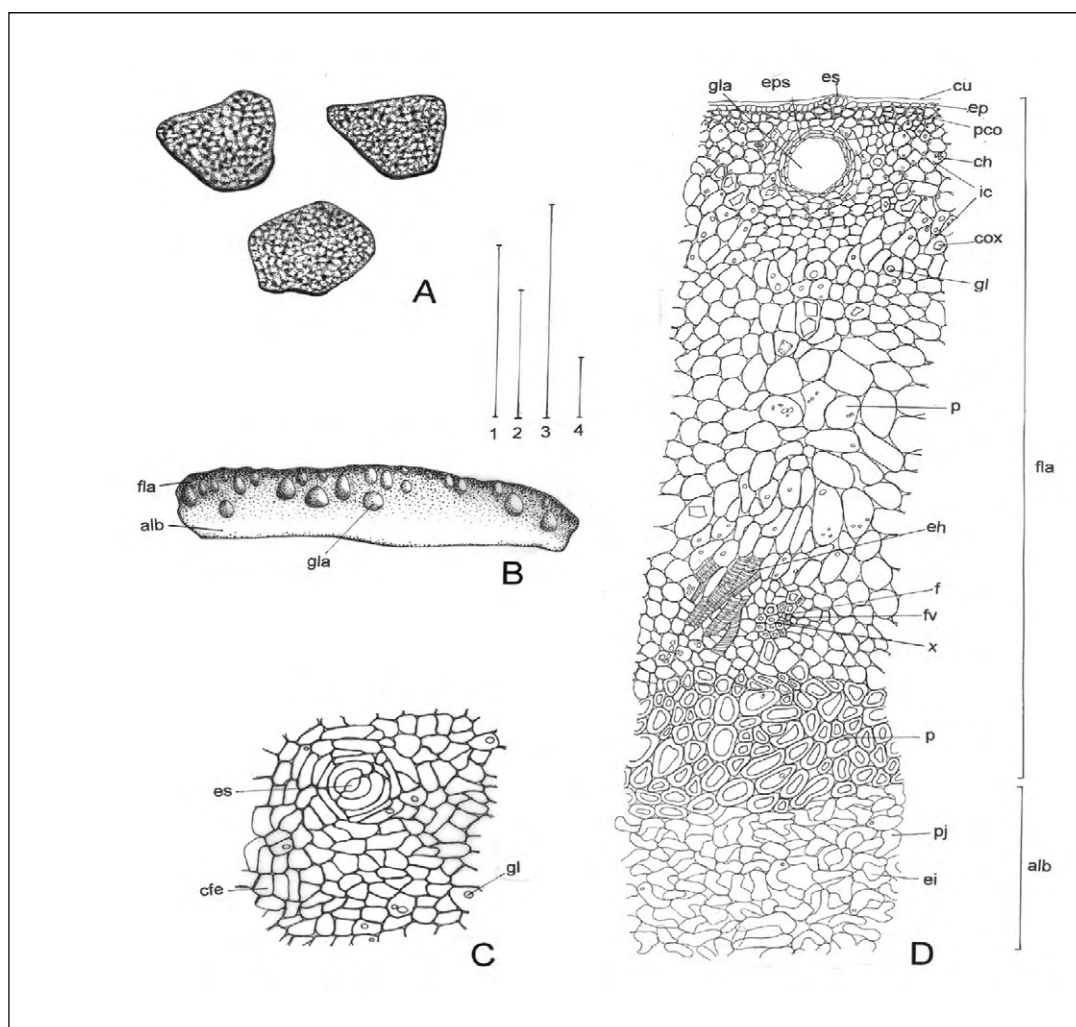


Figure 1 - Macroscopic and microscopic aspects in *Citrus aurantium* L. subsp. *aurantium*

Additional information of Figure 1. The scales correspond in A to 1 cm (rule 1), in B at 0.5 cm (rule 2), in C at 100 µm (rule 3), in D at 100 µm (rule 4).

A - schematic representation of the external surface of the drug in front view. **B** - Schematic representation of the drug, in cross-section: albedo (alb); flavedo (fla); secretory gland (gla). **C** - detail of a portion of the epidermis of flavedo in front view: fundamental cell of the epidermis (cfe); stoma (s), lipid droplet (gl). **D** - detail of portion of the drug, in cross-section: albedo (alb); hesperidin crystal (ch); calcium oxalate crystal (cox); cuticle (Cu); vessel element with helicoidal thickness (h), intercellular space (ei); epidermis (p); secretory epithelium (eps); stoma (s), phloem (f); flavedo (fla), vascular bundle (fv), lipid droplet (gl); secretory gland (gla); crystal idioblast (ic); parenchyma (p); collenchymatous parenchyma (pco); spongy parenchyma (pj); xylem (x).

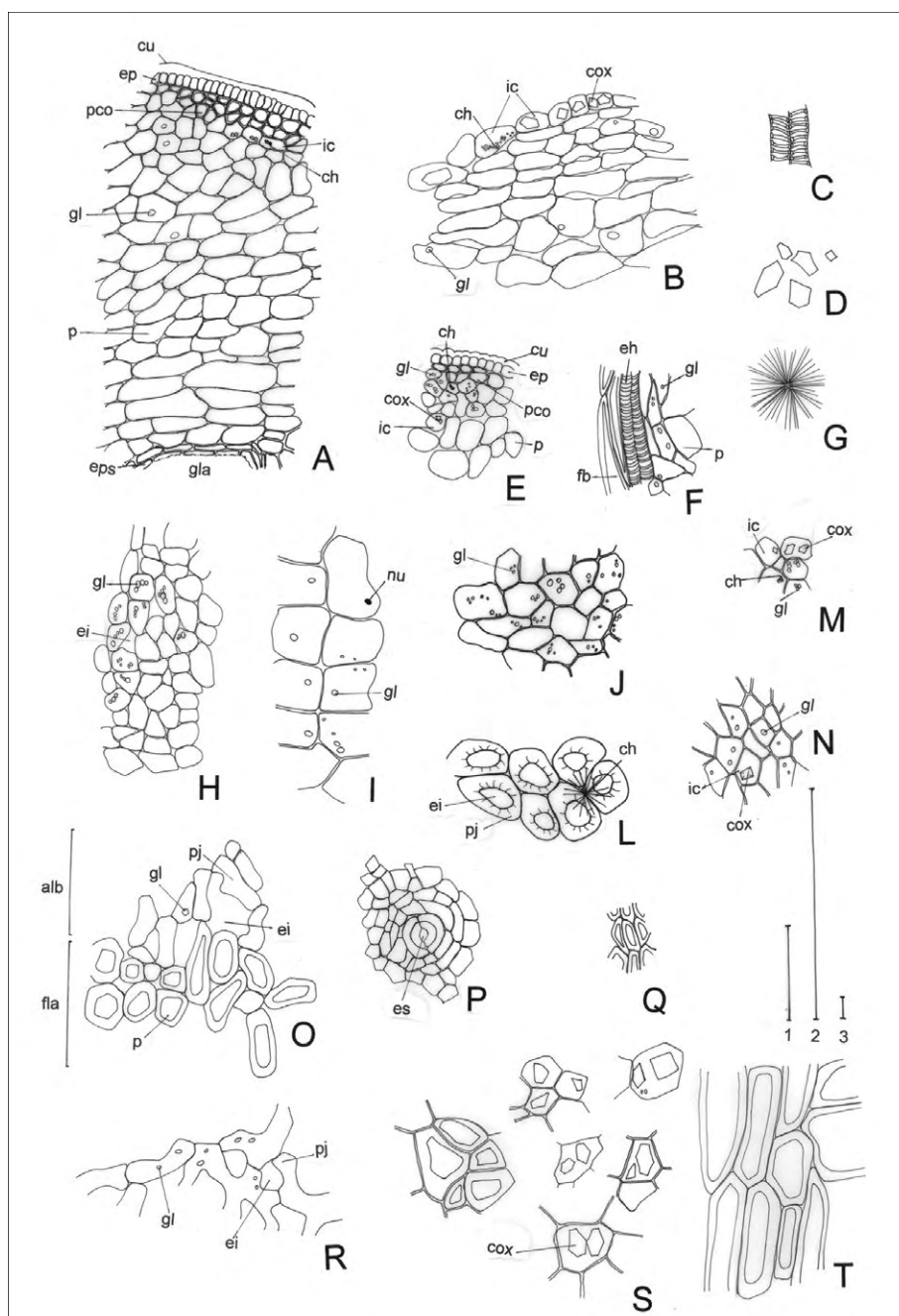


Figure 2 - Macroscopic and microscopic aspects of powder in *Citrus aurantium* L. subsp. *aurantium*

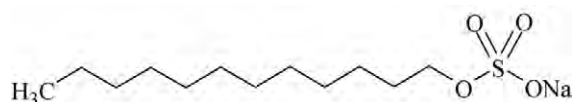
Additional information of Figure 2. Correspond to the ranges A to G, I to O and R to T 100 pm (rule 1), in H and P at 100 pm (rule 2), in Q at 100 pm (rule 3).

A - fragment of flavedo with epidermis, parenchyma and remnants of secretory epithelium in cross-section: hesperidin crystal (ch); cuticle (cu); epidermis (ep); secretory epithelium (eps); lipid droplet (gl); secretory gland (gla); crystal idioblast (ic); parenchyma (p); collenchymatous parenchyma (pco). **B** - fragment of parenchyma of flavedo, in cross-section: hesperidin crystal (ch); crystal of calcium oxalate (cox), lipid droplet (gl); crystal idioblast (ic). **C** - portion of tracheary elements with helical thickening in longitudinal view. **D** - isolated calcium oxalate crystals. **E** - fragment of flavedo, in cross-section: hesperidin crystal (ch); crystal of calcium oxalate (cox), lipid droplet (gl); crystal idioblast (ic). **F** - fragment of parenchyma of flavedo, in cross-section, with a portion of the vascular bundles, seen in side view: vessel element with helical thickening (eh), fiber (fb), lipid droplet (gl); parenchyma (p). **G** - hesperidin crystal, only observed with addition of Lugol. **H** - fragment of parenchyma of flavedo, in cross-section: intercellular space (ei); lipid droplet (gl). **I** - fragment of parenchyma of flavedo, in cross-section: lipid droplet (gl); nucleus (nu). **J** - fragment of the epidermis, in front view: lipid droplet (gl). **L** - fragment of albedo in front view: hesperidin crystal (ch); intercellular space (ei); spongy parenchyma (pj). **M** - fragment of parenchyma of flavedo, in cross-section: hesperidin crystal (ch);

crystal of calcium oxalate (cox), lipid droplet (gl); crystal idioblast (ic). **N** - fragment of parenchyma of flavedo, in cross-section: crystal of calcium oxalate (cox), lipid droplet (gl); crystal idioblast (ic). **O** - fragment of parenchyma of flavedo, in cross-section: albedo (alb), intercellular space (ei); flavedo (fla), lipid droplet (gl); parenchyma (p); spongy parenchyma (pj). **P** - fragment of epidermis of flavedo with stomata in front view: stomata (es). **Q** - fragment of collenchymatous parenchyma in cross-section. **R** - fragment of the albedo, in cross-section: intercellular space (ei); lipid droplet (gl); spongy parenchyma (pj). **S** - crystal idioblasts of flavedo, in cross-section: calcium oxalate crystal (cox). **T** - fragment of flavedo, with parenchymal cells of thickened-wall in cross-section.

LAURYL SULFATE, SODIUM

Natrii laurilsulfas



$C_{12}H_{25}NaO_4S$; 288.38
sodium lauryl sulfate; 05178
sulfuric acid monododecyl ester sodium salt (1:1)
[151-21-3]

Sodium lauryl sulfate is a mixture of sodium alkyl sulfates mainly consisting of sulfuric acid monododecyl ester sodium salt (1:1) - sodium lauryl sulfate. It contains at least 85.0% of sodium alkyl sulfates, expressed as $C_{12}H_{25}NaO_4S$, in relation to the desiccated substance. The total content of sodium chloride and sodium sulfate is at most 8.0%.

DESCRIPTION

Physical characteristics. White or slightly yellowish powder or crystal. Mild typical odor.

Solubility. Freely soluble in water, to form an opalescent solution or mixture, sparingly soluble in ethanol.

IDENTIFICATION

A. To dissolve 0.1 g of the sample in 10 mL of water and stir. Abundant foam is produced.

B. To mix 0.1 mL of the solution obtained in test **A**. *Identification*, with 0.1 mL of 0.1% methylthioninium chloride (w/v) and 2 mL of diluted sulfuric acid. To add 2 mL of methylene chloride and stir. An intense blue color develops in the methylene chloride layer.

C. To mix about 10 mg of sample with 10 mL of ethanol. To heat to boiling in a water bath, by frequently stirring. To immediately filter and evaporate the ethanol. To dissolve the residue in 8 mL of water, add 3 mL of hydrochloric acid SR, evaporate the solution to half volume, and wait to cool down. To separate the solidified fatty alcohols by filtering them. To add 1 mL of 6.1% barium chloride (w/v) to the filtrate. White crystalline precipitate is formed.

D. The 10% solution (w/v) of the sample responds to the reactions of sodium ion (**5.3.1.1**).

E. A 10% solution (w/v) of the sample acidified with hydrochloric acid and simmered for 20 minutes responds to the reactions of the sulfate ion (**5.3.1.1**).

PURITY TESTS

Alkalinity. To accurately weigh about 1 g of the sample and dissolve in 100 mL of carbon dioxide-free water. To add 0.1 mL phenol red SI and titrate with 0.1 M hydrochloric acid.

No more than 0.6 mL of 0.1 M hydrochloric acid are spent.

Non-esterified alcohols. To accurately weigh about 10 g of sample, dissolve it in 100 mL of water, add 100 mL of ethanol, and extract the solution three times with 50 mL of n-amyl alcohol each time. If necessary, add sodium chloride to facilitate the separation of the two phases. To collect the organic phases and wash three times with 50 mL of water. To discard the water from organic solution with sodium sulfate anhydrous, filter, and evaporate in water bath to remove all the solvent. To heat the residue at 105 °C for 15 minutes and cool down. The mass of the residue should be at most 4%.

Total alcohols. To accurately weigh about 5 g of sample into a 800 mL Kjeldahl flask. To add 150 mL of water, 50 mL of hydrochloric acid, and some boiling bead. To connect the Kjeldahl flask with a reflux condenser. To carefully heat to avoid excessive foaming and boil for 4 hours. To cool down, wash the condenser with ethyl ether, by collecting the ethyl ether to the vial, and transfer the contents to a separatory funnel. To wash the flask twice with ethyl ether and add the washings to the separatory funnel. To extract the solution with 2 portions (75 mL each) of ethyl ether. In a pre-weighed beaker, collect the combined ether extracts, evaporate in a water bath, and dry the residue at 105 °C for 30 minutes. To cool down and weigh it. The residue amounts the total alcohols. The mass of the residue should be at least 59.0% of the mass of sample used.

Sodium chloride. To accurately weigh about 0.5 g of the sample and dissolve in 50 mL of water. To add dropwise diluted nitric acid (1:20) to the solution becomes neutral to litmus paper. To add 2 mL of potassium chromate SR and titrate with 0.1 M SV silver nitrate. Each mL of 0.1 M SV silver nitrate is equivalent to 5.844 mg of sodium chloride.

Sodium sulfate. To accurately weigh about 1 g of the sample and transfer to a 250 mL beaker. To add 35 mL of water, and heat it to dissolve. To add 2 mL of M nitric acid to the heated solution, mix, and add 50 mL of ethanol. To heat the solution to boiling. To slowly add by stirring 10 mL of 3.31% lead nitrate solution (w/v). To cover the beaker with a watch glass, simmer for 5 minutes, and let it rest.

If the supernatant is cloudy, wait for extra 10 minutes, heat to boiling, let it rest again. When the solution is about to boil, decant the liquid as much as possible through 9 cm quantitative filter paper, black band, rapid filtration, ashless. To wash four times by decantation, by using 50 mL of 50% ethanol (v/v) each time, and boil the mixture. To transfer the filter paper to the original beaker and immediately add 30 mL of water, 20 mL of 0.05 M SV disodium edetate, and 1 mL of ammonium chloride buffer pH 10.7. To heat the precipitate to dissolve. To wait for cooling down. To add 0.2 mL of eriochrome black T SI and titrate with 0.05 M SV zinc sulfate. Each mL of 0.05 M disodium edetate is equal to 7.102 mg of sodium sulfate.

Heavy metals (5.3.2.3). To use *Method III*. To accurately weigh about 1 g of the sample. At most 0.002% (20 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample in oven at 105 °C for 2 hours. At most 3%.

DOSAGE

To accurately weigh about 0.115 g of the sample, dissolve in 20 mL of water, and heat it, if necessary. To transfer to a 100 mL volumetric flask and top up with the same solvent. To remove 20 mL aliquot of this solution and transfer to 125 mL erlenmeyer flask, add 15 mL of chloroform and 10 mL of dimidium bromide-sulfan blue SR. To titrate with 0.004 M SV benzethonium chloride by vigorously stirring, from pink changes to blue-gray in the chloroform layer. Before adding each titrant, check the complete separation of the layers. Each mL of 0.004 M SV benzethonium chloride is equivalent to 1.154 mg of sodium alkyl sulfates, calculated on $C_{12}H_{25}NaO_4S$.

PACKAGING AND STORAGE

In tightly closed containers.

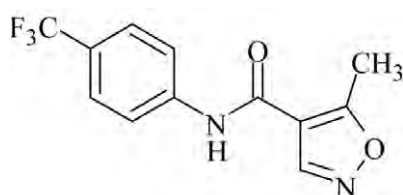
LABELLING

To comply with the legislation in force.

CATEGORY

Anionic surfactant

LEFLUNOMIDE Leflunomidum



$C_{12}H_9F_3N_2O_2$; 270.21

leflunomide; 05192

5-Methyl-N-[4-(trifluoromethyl)phenyl]-4-isoxazole carboxamide
[75706-12-6]

It contains at least 98.0% and at most 102.0% of $C_{12}H_9F_3N_2O_2$ in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White crystalline powder. It shows polymorphism.

Solubility. Practically insoluble in water, soluble in methanol, ethanol, isopropyl alcohol, and ethyl acetate.

Physical and chemical constants. *Melting range (5.2.2):* 165 °C to 167 °C.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the scopolamine leflunomide SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14), from 200 nm to 370 nm, 0.001% solution (w/v) of the sample, in a mixture of acetonitrile and water (50:50), has absorption maximum at 260 nm, similar to those observed in the spectrum of a similar solution of leflunomide SQR.

C. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using 60 F₂₅₄ silica gel as support and a mixture of methylene chloride and ethyl acetate (97:3) as mobile phase. To separately apply to the plate 10 µl of each of the solutions freshly prepared, as described below.

Solution (1): 0.1 mg/mL solution of the sample in ethyl acetate.

Solution (2): 0.1 mg/mL leflunomide SQR solution in ethyl acetate.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm) or expose the plate to iodine vapor.

The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

D. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

PURITY TESTS

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 60°C under reduced pressure for 2 hours. At most 1%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 254 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at 25 °C; flow of *Mobile phase* of 1.0 mL/min.

Mobile phase: mixture of water and acetonitrile (50:50).

Sample solution: to transfer 20 mg of the sample, accurately weighed, to a 100 mL volumetric flask, by using 60 mL of the *Mobile phase*. To stir, if necessary, top up with the same solvent, and make it homogeneous. To transfer 5 mL of this solution to a 25 mL volumetric flask and top up to volume with *Mobile phase* (immediately inject this solution or within 24 hours after preparation, if it is stored under refrigeration).

Standard solution: to dissolve an amount accurately weighed of leflunomide SQR, in *Mobile phase*, to obtain 40 µg/mL solution (immediately inject this solution or within 24 hours after preparation, if it is stored under refrigeration).

To inject replicates of 20 µL of the *Standard solution*. The column efficiency should be greater than 3000 theoretical plates for leflunomide peak. The tailing factor should not be greater than 2. The relative standard deviation for replicate areas of the peaks recorded should not be greater than 2.0 %.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_{12}H_9F_3N_2O_2$ from the sample, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In airtight closed containers, protected from light, under refrigeration.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antirheumatic.

LEFLUNOMIDE TABLETS

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_{12}H_9F_3N_2O_2$. Tablets may be coated.

IDENTIFICATION

A. To weigh and pulverize the tablets. To use an amount of powder equivalent to 10 mg of leflunomide and transfer to a 100 mL volumetric flask, by using 60 mL of a mixture of acetonitrile and water (50:50). To stir for 10 minutes and top up with the same solvent. To make it homogenous and filter it. To transfer 5 mL of this solution to a 50 mL volumetric flask and top up with a mixture of acetonitrile and water (50:50). The infrared absorption spectrum (5.2.14) of this solution, ranging from 200 nm to 370 nm, has maximum at 260 nm similar to that observed in the leflunomide SQR spectrum similarly prepared.

B. To weigh and pulverize the tablets. To use amount of powder equivalent to 5 mg of leflunomide, dissolve in 50 mL of ethyl acetate, make it homogeneous, and filter. To proceed as described in *Identification*, test C., in the monograph *Leflunomide*.

C. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: deaerated water, 1000 mL, for tablets containing 10 or 20 mg.

Equipment: blades, 100 rpm

Time: 30 minutes

Procedure: after testing, remove aliquot from the dissolution medium, immediately filter through 0.45 µm filter, and dilute, if necessary, with *Dissolution medium*, to obtain suitable concentration. To measure the absorbances of the solutions at 260 nm (5.2.14), by using water to adjust zero. To calculate the amount of $C_{12}H_9F_3N_2O_2$ dissolved in the medium, by comparing the readings obtained with the 10 µg leflunomide SQR solution prepared in the same solvent (acetonitrile can be used to dissolve leflunomide in volume not exceeding 2% in the final solution).

Tolerance: not less than 80% (Q) of the amount stated of $C_{12}H_9F_3N_2O_2$ dissolves within 30 minutes.

DOSAGE

To proceed as described in *Dosage*, in the monograph *Leflunomide*. To prepare the *Sample solution* as described below.

Sample solution: to weigh and pulverize 20 tablets. To transfer an amount of powder equal to 10 mg of leflunomide to a 50 mL volumetric flask by using 30 mL of *Mobile phase*. To mechanically stir for 15 minutes, top up with the same solvent, make it homogeneous, and filter it. To transfer 5 mL of this solution to 25 mL volumetric flask, top up with *Mobile phase*, and make it homogeneous.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas of the peaks. To calculate the amount of

$C_{12}H_9F_3N_2O_2$ in the tablets, from the responses obtained for the *Sample solution* and *Standard solution*.

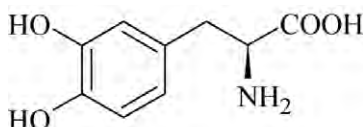
PACKAGING AND STORAGE

In tightly closed containers, protected from light at room temperature.

LABELLING

To comply with the legislation in force.

L-DOPA Levodopum



$C_9H_{11}NO_4$; 197.19
l-dopa; 05249
3-Hydroxy-L-tyrosine
[59-92-7]

It contains at least 98.0% and at most 102.0% of $C_9H_{11}NO_4$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white or almost white powder.

Solubility. Sparingly soluble in water, practically insoluble in ethanol and ethyl ether.

Freely soluble in *M* hydrochloric acid, slightly soluble in 0.1 *M* hydrochloric acid.

Physical and chemical constants.

Rotation (5.2.8): -1.27° to -1.34° , in relation to the desiccated substance. To dissolve 0.2 g of sample and 5 g of methenamine in 10 mL of *M* hydrochloric acid. To dilute to 25 mL with the same acid and make it homogenous. To protect the solution from light (25 °C) for 3 hours.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the l-dopa SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14), from 230 nm to 350 nm, 0.003% solution (w/v) in 0.1 *M* hydrochloric acid, has absorption maximum at 280 nm, similar to

those observed in the spectrum of a similar solution of l-dopa SQR.

C. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage method B*, corresponds to that of main peak of *Standard solution*.

PURITY TESTS

pH (5.2.19). 4.5 to 7.0. To determine in 1% suspension (w/v) in carbon dioxide-free nitrogen after 15 minutes stirring the sample in the solvent.

Absorption of light. The ultraviolet absorption spectrum (5.2.14), ranging from 230 nm to 350 nm, of 0.003% solution (w/v) in 0.1 *M* hydrochloric acid, exhibits maxima at 280 nm. The specific absorptivity, *A* (1%, 1 cm), is from 137 to 147 at 280 nm, in 0.1 *M* hydrochloric acid.

Related substances. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using cellulose plate as support and a mixture of glacial acetic acid, water, and 1-butanol (25:25:50) as mobile phase. To separately apply to the plate 10 µL of each of the solutions freshly prepared, as described below.

Solution (1): to dissolve 0.1 g of the sample in 5 mL of formic acid anhydrous and dilute to 10 mL with methanol. To extemporaneously prepare it.

Solution (2): to transfer 0.5 mL of the *Solution (1)* to 100 mL volumetric flask and top up with methanol.

Solution (3): to dissolve 30 mg of tyrosine in 1 mL of formic acid anhydrous and dilute to 100 mL with methanol. To mix 1 mL of this solution with 1 mL of *Solution (1)*.

To develop the chromatogram. To remove the plate, and allow it to dry in hot air. To nebulize with a mixture of ferric chloride SR and potassium ferricyanide SR (1:1) freshly prepared. To immediately examine it.

Any secondary spot obtained in the chromatogram with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (2)* (0.5%). The test is valid only if the chromatogram obtained with *Solution (3)*, above the principal spot, has a different spot more intense than the spot in the chromatogram obtained with *Solution (2)*.

Heavy metals (5.3.2.3). To use *Method III*. To prepare the standard solution by using 2 mL of *Standard lead solution (10 ppm Pb)*. At most 0.001% (10 ppm).

Loss on drying (5.2.9). To determine in 0.5 g of the sample. To desiccate in an oven at 105 °C for 4 hours. At most 1%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Titration in non-aqueous medium* (5.3.3.5). To accurately weigh about 0.18 g of the sample and dissolve in 5 mL of formic acid anhydrous. To heat it, if necessary. To cool down and add 25 mL of glacial acetic acid and 25 mL of dioxane. To titrate with 0.1 M SV perchloric acid, by using 0.1 mL of methylrosanilinium chloride SI to become green. To perform sample blank correction and adjust it as required. Each mL of 0.1 M SV perchloric acid is equal to 19.719 mg of $C_9H_{11}NO_4$.

B. To proceed as described in *High performance liquid chromatography* (5.2.17.4). To protect the procedure from light and keep the solutions at 10 ° C until injection. To use chromatograph equipped with an ultraviolet detector at 280 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at room temperature; flow of *Mobile phase* of 1.0 mL/min.

Diluent: mixture of trifluoroacetic acid and water (1:1000).

Mobile phase: mixture of *Diluent* and tetrahydrofuran (97:3).

Sample solution: to dissolve accurately weighed amount of the sample in *Diluent*, to obtain a 0.4 mg/mL solution.

Standard solution: to dissolve accurately weighed amount of l-dopa SQR in *Diluent*, to obtain a 0.4 mg/mL solution.

Resolution solution: to dissolve accurately weighed amount of l-dopa SQR and L-tyrosine SQR in *Diluent*, to obtain a 10 µg/mL solution for each substance.

To inject replicates of 20 µL of the *Resolution solution*. The relative retention times are about 1.0 to l-dopa and 1.3 to L-tyrosine. The resolution between l-dopa and L-tyrosine peaks should be greater than 3.0. The tailing factor for l-dopa should not be greater than 2.0.

The relative standard deviation for the replicate areas for the peaks recorded is not greater than 2.0%.

Procedure: to separately inject 20 µL of *Sample and standard solutions*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_9H_{11}NO_4$ from the sample, from the responses obtained for the *Sample and standard solutions*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

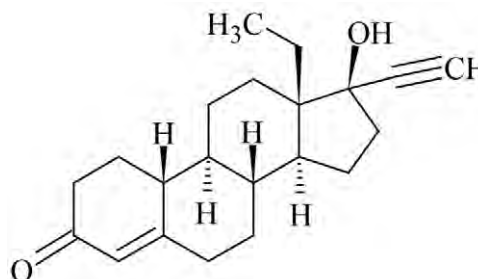
To comply with the legislation in force.

THERAPEUTIC CLASS

Antiparkinsonian.

LEVONORGESTREL

Levonorgestrelum



$C_{21}H_{28}O_2$; 312.45

levonorgestrel; 05279

(17 α)-13-Ethyl-17-hydroxy-18,19-dinorpregn-4-en-20-yn-3-one

[797-63-7]

It contains at least 98.0% and at most 102.0% of $C_{21}H_{28}O_2$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white or almost white powder.

Solubility. Practically insoluble in water, slightly soluble in methylene chloride, sparingly soluble in ethanol.

Physical and chemical constants.

Melting range (5.2.2): 232 °C to 239 °C. The range should not exceed 4 °C.

Specific rotation (5.2.8): -30° to -35°. To determine in 2% solution (w/v) in chloroform.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maxima only at the same wavelength, with the same relative intensities to those observed in the levonorgestrel SQR spectrum similarly prepared.

PURITY TESTS

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using G silica gel as support and a mixture of acetone and chloroform (20:80) as mobile phase. To separately apply to the plate 10 µL of each of the solutions freshly prepared, as described below.

Solution (1): to dissolve 0.5 g of the sample in chloroform and dilute to 25 mL with the same solvent.

Solution (2): to transfer 2.5 mL of *Solution (1)* to 25 mL volumetric flask and top up with chloroform. To transfer 2.5 mL of the previous solution to 50 mL volumetric flask and top up with the same solvent.

Solution (3): to transfer 10 mL of *Solution (2)* to 25 mL volumetric flask and top up with chloroform.

Solution (4): to dissolve 5 mg of levonorgestrel SQR and 5 mg of ethinylestradiol SQR in chloroform and dilute to 50 mL with the same solvent.

To develop the chromatogram. To remove the plate, and let it drying. To nebulize with 10% phosphomolybdic acid (w/v) in *n*-propyl alcohol. To heat the plate at 100 °C - 105 °C for 15 minutes and examine immediately. Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (2)* (0.5%) and not more than two spots is more intense than that obtained with *Solution (3)* (0.2%). The test is valid only if the chromatogram obtained with *Solution (4)* shows two principal spots clearly separated.

Limit for ethinyl group. To proceed as described in *Dosage* method **A**. Each mL of 0.1 M SV sodium hydroxide is equal to 2.503 mg of ethinyl. At least 7.81% and at most 8.18%.

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 105 °C for 5 hours. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

A. To accurately weigh about 0.2 g of the sample and dissolve in 45 mL of tetrahydrofuran. To add 10 mL of 10% silver nitrate (w/v) to water. After a minute, titrate with 0.1 M SV sodium hydroxide, to potentiometrically determine the endpoint. To perform sample blank correction and adjust it as required. Each mL of 0.1 M SV sodium hydroxide is equal to 31.245 mg of $C_{21}H_{28}O_2$.

B. To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To accurately weigh about 100 mg of the sample and dissolve in ethanol. To successively dilute in ethanol to obtain 0.001% concentration (w/v). To prepare a standard solution at the same concentration by using the same solvent. To measure the absorbances of the resulting solutions at 241 nm, by using ethanol to adjust zero. To calculate the content of $C_{21}H_{28}O_2$ in the sample from the readings obtained.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Contraceptive.

LEVONORGESTREL AND ETHINYLESTRADIOL TABLETS

It contains at least 90.0 % and at most 110.0 % of the amount stated of levonorgestrel ($C_{21}H_{28}O_2$) and ethinylestradiol ($C_{20}H_{24}O_2$).

IDENTIFICATION

A. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel as support and a mixture of methanol and chloroform (1:99) as mobile phase. To separately apply to the plate 20 µL of each of the solutions freshly prepared, as described below.

Solution (1): to weigh and pulverize 15 tablets and extract with 30 mL of acetone. To filter and evaporate to dryness. To dissolve the residue in 1 mL of chloroform.

Solution (2): to prepare a 0.75 mg/mL levonorgestrel SQR solution in chloroform.

Solution (3): to prepare a 0.45 mg/mL ethinylestradiol SQR solution in chloroform.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm).

The levonorgestrel and ethinylestradiol spots obtained with *Solution (1)* correspond in position and color to those obtained with *Solutions (2) and (3)*. To nebulize with 2% *p*-toluenesulfonic acid (w/v) in water. To heat at 110 °C for 10 minutes. To examine under ultraviolet light (365 nm). The levonorgestrel and ethinylestradiol spots are blue.

B. The retention times of the main peaks in the chromatogram of the *Sample solution* obtained in the *Dosage* correspond to those of main peaks of *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.0005% polysorbate 80 solution (w/v) in water, 500 mL

Equipment: blades, 75 rpm

Time: 60 minutes

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 274 nm (for determination of levonorgestrel) and spectrofluorometric detector (for determination of ethinylestradiol) with excitation wavelength at 285 nm and emission at 310 nm; 150 mm column and 4.0 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm to 10 µm) kept at room temperature, flow of *Mobile phase* of 1.0 ml/min.

Mobile phase: mixture of acetonitrile and water (60:40).

Sample solution: after testing, remove aliquot from the dissolution medium, filter through polyvinylidene filter, by discarding the first few milliliters. For uncoated tablets, remove aliquots from the dissolution medium at 30 minutes (to replace the volume of each tank) and 60 minutes. For dragees, perform this procedure at 60 minutes only.

Standard solution: to prepare a solution containing ethinylestradiol SQR and norgestrel standard in the *Dissolution medium*, to obtain concentrations near those of levonorgestrel and ethinylestradiol, respectively, of the *Sample solution*.

To inject replicates of 100 µL of the *Standard solution*. The relative retention times are about 0.7 to ethinylestradiol and 1.0 to levonorgestrel.

The relative standard deviation for the replicate areas for the peaks recorded should not be greater than 3.0%.

Procedure: to separately inject 100 µL of *Sample and standard solutions*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of levonorgestrel ($C_{21}H_{28}O_2$) and ethinylestradiol ($C_{20}H_{24}O_2$) dissolved in the medium, from the responses obtained from *Sample and standard solutions*.

Tolerance: uncoated tablets: not less than 80% (Q) of the stated amount of levonorgestrel ($C_{21}H_{28}O_2$) and 75% (Q) of the stated amount of ethinylestradiol ($C_{20}H_{24}O_2$) dissolves within 60 minutes. dragees: not less than 60% (Q) of the stated amount of levonorgestrel ($C_{21}H_{28}O_2$) and 60% (Q) of the stated amount of ethinylestradiol ($C_{20}H_{24}O_2$) dissolves within 60 minutes.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 215 nm; 150 mm column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at room temperature; flow of *Mobile phase* of 1.5 mL/minute.

Mobile phase: mixture of water and acetonitrile (51:49).

Diluent: mixture of water and acetonitrile (40:60).

Sample solution: to weigh and pulverize 20 tablets. To transfer an amount of powder equivalent to 250 mg of levonorgestrel to a centrifuge tube and add 4 mL of *Diluent*. To heat it at 60 °C for 25 minutes, stir, and put it in ultrasound for 25 minutes. To cool down, centrifuge, and use the clear supernatant.

Standard solution: to prepare A levonorgestrel SQR and ethinylestradiol SQR solution in the *Diluent* containing, respectively, 0.625 mg/mL and 0.125 mg/mL. To transfer 5 mL of this solution to 50 mL volumetric flask and top up with *Diluent*, to obtain a levonorgestrel SQR and ethinylestradiol SQR solution containing, respectively, 62,5 mg/mL and 12.5 mg/mL.

Procedure: to separately inject 20 µL of *Sample and standard solutions*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of levonorgestrel ($C_{21}H_{28}O_2$) and ethinylestradiol ($C_{20}H_{24}O_2$) in the tablets from the responses obtained for the *Sample and Standard solutions*.

PACKAGING AND STORAGE

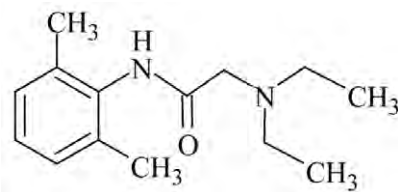
In tightly closed containers.

LABELLING

To comply with the legislation in force.

LIDOCAINE

Lidocainum



$C_{14}H_{22}N_2O$; 234.34

lidocaine; 05313

2 - (diethylamino)-*N*-(2,6-dimethylphenyl) acetamide
[137-58-6]

It contains at least 99.0% and at most 101.0% of $C_{14}H_{22}N_2O$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. Crystalline, white or almost white powder.

Solubility. Practically insoluble in water, freely soluble in methylene chloride, soluble in ethanol. Soluble in diluted hydrochloric acid.

Physical and chemical constants.

Melting range (5.2.2): 66 °C to 70 °C.

IDENTIFICATION

The identification tests **B.**, **C.**, and **D.** may be omitted if the test **A.** is conducted.

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the lidocaine SQR spectrum similarly prepared.

B. By heating, dissolve 0.2 g of the sample in the mixture of 0.5 mL of diluted hydrochloric acid and 10 mL of water. To add 10 mL of 1% picric acid (w/v). The precipitate washed with water and desiccated has melting range (5.2.2) close to 230 °C with decomposition.

C. To add 0.5 mL of fuming nitric acid to about 5 mg of sample. To evaporate to dryness in a water bath, cool down, and dissolve the residue in 5 mL of acetone. To add 0.2 mL of 0.5 *M* ethanolic potassium hydroxide. Green color develops.

D. To dissolve about 0.1 g of sample in 1 mL of ethanol and add 0.5 mL of 10% cobalt nitrate solution (w/v). Bluish-green precipitate is produced.

PURITY TESTS

Appearance of solution. To dissolve 1 g of the sample in 3 mL of diluted hydrochloric acid and dilute to 10 mL with water. The solution is clear (5.2.25) and colorless (5.2.12).

2,6 dimethylaniline. To dissolve 0.25 g of the sample in methanol and dilute to 10 mL with the same solvent. To add 1 mL of 1% *p*-dimethylaminobenzaldehyde solution (w/v) in methanol, freshly prepared, and 2 mL of glacial acetic acid to 2 mL of previous solution. Let it rest for 10 minutes. Any yellow color in the solution examined is not more intense than that of a reference solution simultaneously prepared by using 2 mL of 0.00025% 2,6-dimethylaniline (w/v) in methanol.

Chlorides (5.3.2.1). To dissolve 1.4 g of the sample in a mixture of 3 mL of nitric acid and 12 mL of water and proceed as described in *Limit test for chlorides*. At most 0.0035% (35 ppm).

Sulfates (5.3.2.2). To dissolve 0.5 g of sample in 5 mL of ethanol and dilute to 25 mL in water. At most 0.1% (1000 ppm).

Heavy metals (5.3.2.3). To use *Method III*. To determine in 1 g of the sample. At most 0.002% (20 ppm).

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

Water (5.2.20.1). To determine in 1 g of the sample. At most 1.0 %.

DOSAGE

To proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. To dissolve 0.2 g of the sample vacuum-desiccated over silica gel, for 24 hours, in 50 mL of glacial acetic acid, and stir to obtain total dissolution. To titrate with 0.1 *M* SV perchloric acid, to potentiometrically determine the endpoint. To perform sample blank correction and adjust it as required. Each mL of 0.1 *M* perchloric acid is equal to 23.434 mg of $C_{14}H_{22}N_2O$.

PACKAGING AND STORAGE

In airtight containers.

LABELLING

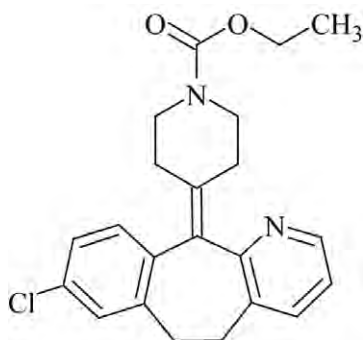
To comply with the legislation in force.

THERAPEUTIC CLASS

Local anesthetic.

LORATADINE

Loratadinum



$C_{22}H_{23}ClN_2O_2$; 382.88

loratadine; 05416

4 - (8-chloro-5,6-dihydro-11H-benzo [5,6] cyclohepta [1,2-b] pyridin-11-ylidene) -1 - piperidinecarboxylic acid ethyl ester
[79794-75-5]

It contains at least 98.5% and at most 102.0% of $C_{22}H_{23}ClN_2O_2$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white or almost white powder. It shows polymorphism.

Solubility. Insoluble in water, freely soluble in acetone, chloroform, methanol, and toluene.

Physical and chemical constants.

Melting range (5.2.2): 132 °C to 137 °C.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the loratadine SQR spectrum similarly prepared. To separately dissolve the substances in acetone, if the spectra are not identical, and evaporate them to dryness. To obtain new spectra from these residues.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method **B.** corresponds to that of main peak of *Standard solution*.

PURITY TESTS

Related substances.

Note: according to the synthesis route, perform the *Test 1* or *Test 2*. The second test is recommended if the 4,8-dichloro-6, 11 - dihydro-5H-benzo [5,6] cyclohepta [1,2-b] pyridin-11-one is a potential related substance.

Test 1. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 254 nm; 150 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octylsilane group (5 µm), kept at 25 °C - 35 °C; flow of *Mobile phase* of 1.0 mL/min.

Mobile phase: mixture of 0.01 M dibasic potassium phosphate anhydrous prepared as described in Method B. **Dosage,** methanol, and acetonitrile (7:6:6). To adjust pH to 7.2 with phosphoric acid.

Diluent: To transfer 400 mL of 0.05 M hydrochloric acid and 80 mL of 0.6 M dibasic potassium phosphate anhydrous prepared as described in Method B. **Dosage** to 100 mL volumetric flask and top up with a mixture of methanol and acetonitrile (1:1). To make it homogeneous.

Solution (1): 0.8 µg/mL loratadine SQR solution in *Diluent*.

Solution (2): to accurately transfer about 40 mg of the sample to a 100 mL volumetric flask. To dissolve, put it in ultrasound for 10 minutes, and top up with the *Diluent*.

The relative retention times are about 0.79 to 4 - (8-chloro-11-fluoro-6,11-dihydro-5H-benzo [5,6] cyclohepta [1,2-b] pyridin-11-yl)-1-piperidinecarboxylate acetate and 1.00 to loratadine. The relative standard deviation for the replicate areas for the peaks recorded is not greater than 4.0%.

Procedure: to separately inject 50 µL of each solution, record the chromatograms, and measure the areas of all peaks of *Solution (2)* and main peak of *Solution (1)*. To calculate the percentage of each impurity in relation to the area under the main peak of *Solution (1)* and the response factors for impurities (the response factor for 4 - (8-chloro-11-fluoro-6,11-dihydro-5H-benzo [5,6] cyclohepta [1,2-b] pyridin-11-yl)-1-piperidinecarboxylate acetate is 0.25). At most 0.2% for 4 - (8-chloro-11-fluoro-6,11-dihydro-5H-benzo [5,6] cyclohepta [1,2-b] pyridin-11-yl)-1-piperidinecarboxylate acetate, 0.1% for individual impurity, and 0.3% for total impurities.

Test 2. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 254 nm; 250 mm column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), flow of *Mobile phase* of 1.2 mL/min.

Eluent A: to dissolve 0.96 g of sodium pentane 1-sulfonate monohydrate in 900 mL of water.

To adjust pH to 3.00 ± 0.05 with 10% phosphoric acid (v/v) and dilute to 1000 mL with water.

Eluent B: to use acetonitrile.

Mobile phase gradient: to adopt the gradient system described in the following table:

Time (min)	Eluent A (%)	Eluent B (%)	Elution
0	75	25	Isocratic
0-20	75→50	25→50	Linear gradient
20-30	50→40	50→60	Linear gradient
30-35	40→30	60→70	Linear gradient
35-45	30	70	Isocratic
45-50	75	25	Isocratic

Solution (1): to dissolve accurately weighed amounts of loratadine SQR, 8-chloro-6,11-dihydro-11-(4-piperidylidene) - 5H-benzo [5,6] cyclohepta [1,2-6] pyridine SQR (compound loratadine related A SQR) and 8-chloro-6,11-dihydro-11-(N-methyl-4-piperiniliden)-5H-benzo [5,6] cyclohepta [1,2-6] pyridine SQR (compound loratadine related B SQR) in methanol, to obtain a 0.1 mg/mL solu-

tion for each compound. To transfer 1 mL of this solution to 10 mL volumetric flask, add 2 mL of *Eluent A*, and top up with methanol.

Solution (2): to accurately weigh about 0.1 g of the sample and transfer to a 10 mL volumetric flask. To add 2 mL of methanol and stir to dissolve it. To add 2 mL of *Eluent A* and top up with methanol.

To inject replicates of 20 µL of *Solution (1)*. The resolution between compound loratadine related A and compound loratadine related B peaks is not less than 1.5. The relative standard deviation of the areas in loratadine peak, in the replicates, is not greater than 10%. The relative retention times and response factors are described in the following table. For unknown impurities, the response factor is 1.00.

Related compound	Relative retention time	Response factor
compound loratadine related A	0.50	1.00
compound loratadine related B	0.53	0.89
8-Chloro-6,11-dihydro-5H-benzo [5,6] cyclohepta [1,2- <i>b</i>] pyridin-11-one	0.70	0.60
8-Chloro-6,11-dihydro-11-[N-methyl-4-piperidinyl]-11-hydroxy-5H-benzo [5,6] cyclohepta [1,2- <i>b</i>] pyridine	0.75	0.46
4,8-Dichloro-6,11-dihydro-5H-benzo [5,6] cyclohepta [1,2- <i>b</i>] pyridin-11-one	1.23	0.92
8-Chloro-6,11-dihydro-11-[N-ethoxycarbonyl-4-piperidinyl]-11-hydroxy-5H-benzo [5,6] cyclohepta [1,2- <i>b</i>] pyridine	1.60	0.42
4,8-Dichloro-6,11-dihydro-11-[N-ethoxycarbonyl-4-piperidylidene]-5H-benzo [5,6] cyclohepta [1,2- <i>b</i>] pyridine	1.83	1.08
Loratadine	1.00	1.00

Procedure: to separately inject 20 µL of each solution and record the chromatograms. At most 0.1% of loratadine related compound A, 0.1% of loratadine related compound B, 0.1% of each individual impurity, and 0.3% of total impurities.

Heavy metals (5.3.2.3). To proceed as described in *Method III*. At most 0.001% (10 ppm).

Loss on drying (5.2.10). To determine in 1 g of the sample. To desiccate in an oven at 100 °C to obtain constant weight. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. To dissolve 0.3 g of the sample in 50 mL of glacial acetic acid. To titrate with 0.1 M SV perchloric acid, to potentiometrically determine the endpoint.

Each mL of 0.1 M SV perchloric acid is equal to 38.288 mg of $C_{22}H_{23}ClN_2O_2$.

B. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 254 nm; 150 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octylsilane group (5 µm), kept at room temperature; flow of *Mobile phase* of 1.0 mL/min.

Mobile phase: mixture of 0.01 M dibasic potassium phosphate anhydrous, methanol, and acetonitrile (7:6:6). To adjust pH to 7.2 with phosphoric acid.

Diluent: to transfer 400 mL of 0.05 M hydrochloric acid and 80 mL of 0.6 M dibasic potassium phosphate anhydrous to 1000 mL volumetric flask and top up with a mixture of methanol and acetonitrile (1:1). To make it homogeneous.

Sample solution: to accurately transfer about 40 mg of the sample to a 100 mL volumetric flask. To dissolve, put it in ultrasound for 10 minutes, and top up with the Diluent. To make it homogeneous.

Standard solution: 0.4 mg/mL loratadine SQR solution in *Diluent*.

Procedure: to separately inject 15 µL of *Standard solution* and *Sample solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_{22}H_{23}ClN_2O_2$ from the sample, from the responses obtained for the *Sample solution* and *Standard solution*. The relative standard deviation for the replicate areas for the peaks recorded is not greater than 2.0%.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antihistamine.

LORATADINE TABLETS

It contains at least 90.0 % and at most 110 % of the amount stated of loratadine ($C_{22}H_{23}ClN_2O_2$).

IDENTIFICATION

A. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using G silica gel as support and a mixture of ethyl ether and diethylamine (40:1) as mobile phase. To separately apply to the plate 5 µL of each of the solutions freshly prepared, as described below.

Solution (1): to transfer an amount of powdered tablets equivalent to about 20 mg of loratadine to a centrifuge tube. To add 5 mL of a mixture of chloroform and methanol (1:1), stir for 30 minutes, and centrifuge it.

Solution (2): 4 mg/mL loratadine SQR solution in a mixture of chloroform and methanol (1:1).

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 900 mL

Equipment: blades, 50 rpm

Time: 60 minutes

Procedure: after testing, remove aliquot from the dissolution medium, filter, and dilute in 0.1 M hydrochloric acid, to obtain suitable concentration. To measure the absorbances of the solutions at 280 nm (5.2.14), by using the same solvent to adjust zero. To calculate the amount of $C_{22}H_{23}ClN_2O_2$ dissolved in the medium, by comparing the readings obtained with that of 0.001% loratadine SQR solution (w/v) prepared in the same solvent.

Tolerance: not less than 80% (Q) of the amount stated of $C_{22}H_{23}ClN_2O_2$ dissolves within 60 minutes.

PURITY TESTS

Related substances. To proceed as described in *Dosage*, method **B**, in the monograph *Loratadine*. To prepare the solutions as described below.

Solution (1): to use the *Sample solution* described in *Dosage*, in this monograph.

Solution (2): to transfer 5 mL of *Standard solution* to 100 mL volumetric flask, top up with *Diluent*, and make it homogeneous. To dilute this solution to obtain 0.8 µg/mL loratadine SQR.

To inject replicates of 50 µL of *Solution (1)*. The relative retention times are 0.79 to 4 - (8-chloro-11-fluoro-6,11-dihydro-5H-benzo [5,6] cyclohepta [1,2-b] pyridin-11-yl) - piperidinecarboxylate acetate and 1.0 to loratadine. The relative standard deviation of the replicates areas for the loratadine peak is not greater than 4.0%.

Procedure: to separately inject 50 µL of *Solutions (1)* and (2), record the chromatograms, and measure the areas under the peaks. At most 0.2% of 4 - (8-chloro-6-fluoro-5,11-dihydro-5H-benzo [5,6] cyclohepta [1,2-b] pyridin-11-yl)-1-piperidinecarboxylate. At most 0.1% of any individual impurity. The sum of all impurities except 4 - (8-chloro-11-fluoro-6, 11 - dihydro-5 H-benzo [5,6] cyclohepta [1,2-b] pyridin-11-yl) -1 - piperidinecarboxylate does not exceed 0.1%.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To proceed as described in *Dosage*, method **B.**, in the monograph *Loratadine*. To prepare the *Sample solution* as described below.

Sample solution: to weigh and pulverize 20 tablets. To transfer an amount of powder equivalent to 100 mg of loratadine to a 250 mL volumetric flask. To transfer 100 mL of 0.05 M hydrochloric acid and stir for 40 minutes. To add 75 mL of a mixture of methanol and acetonitrile (1:1) and make it homogeneous. To add 20 mL of 0.6 M dibasic potassium phosphate anhydrous and make it homogeneous for 5 minutes. To top up with a mixture of methanol and acetonitrile (1:1) and make it homogeneous.

To inject replicates of 15 µL of the *Standard solution*. The retention factor is greater than 3.5. The tailing factor is not greater than 1.7. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2.0 %.

Procedure: to separately inject 15 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of $C_{22}H_{23}ClN_2O_2$ in the tablets, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers at 2 °C - 30° C.

LABELLING

To comply with the legislation in force.

LORATADINE ORAL SOLUTION

It contains at least 94.0 % and at most 105.0 % of the amount stated of loratadine ($C_{22}H_{23}ClN_2O_2$).

IDENTIFICATION

A. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using G silica gel as support and a mixture of ethyl ether and diethylamine (40:1) as mobile phase. To separately apply to the plate 5 µL of each of the solutions freshly prepared, as described below.

Solution (1): to transfer a volume of oral solution equivalent to about 10 mg of loratadine to a centrifuge tube. To add 10 mL of 0.2 M ammonium hydroxide and 2 mL of methylene chloride. To stir for 10 minutes. To centrifuge it. To use the organic phase.

Solution (2): 5 mg/mL loratadine SQR solution in methylene chloride.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm).

The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in *Dosage* corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

pH (5.2.19). 2.5 to 3.1.

PURITY TESTS

Related substances. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 254 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octylsilane group (5 µm), kept at 30 °C - 40 °C; flow of *Mobile phase* of 2.0 mL/min.

Mobile phase: 0.015 M sodium lauryl sulfate solution in a mixture of water and acetonitrile (1:1). To adjust the pH to 2.6 ± 0.1 with phosphoric acid.

Diluent: mixture of *Mobile phase* and water (2:1).

System suitability solution (1): 0.002 mg/mL loratadine SQR solution in *Diluent*.

System suitability solution (2): to transfer 5 mL of *System suitability solution (1)* to 50 mL volumetric flask and top up with *Diluent*.

Resolution solution: to transfer a volume of oral solution containing 20 mg of loratadine to a glass vial with lid. To add 1 mL of a 3% hydrogen peroxide solution (w/v) and make it homogeneous. To cover the vial and heat at 65 °C for 18 - 24 hours. To cool down at room temperature. To transfer 5 mL to 25 mL volumetric flask and top up with *Diluent*.

Sample solution: to transfer volume of oral solution equal to 5 mg of loratadine to a 25 mL volumetric flask and top up with *Diluent*. To make it homogeneous.

To inject replicates of 50 µL of the *Resolution solution*. The relative retention times are about 0.70 to 4 - (8-chloro-5,6-dihydro-4-(hydroxymethyl)-11H-benzo [5,6] cyclohepta [1,2-b] pyridin-11-ylidene) -1 - piperidinecarboxylate, 0.84 to 4 - [8-chloro-5,6 - dihydro-2-(hydroxymethyl)-11H-benzo [5,6] cyclohepta [1,2-b] pyridin-11-ylidene]-1-piperidinecarboxylate, and 1.0 to loratadine. The resolution between loratadine and 4 - [8-chloro-5,6-dihydro-2-(hy-

droxymethyl) - 11*H*-benzo [5,6] cyclohepta [1,2-*b*] pyridin-11-ylidene] - 1 - piperidinecarboxylate acetate peaks is greater less than 3.0. To inject 50 µL of replicates of *System suitability solution* (1). The tailing factor for loratadine peak is 0.7 - 1.1. To inject replicates of 50 µL of *System suitability solution* (2).

The relative standard deviation of the replicates areas for the loratadine peak is not greater than 10.0%.

Procedure: to inject 50 µL of *Sample solution*, record the chromatograms, and measure the areas under the peaks. At most 0.3% of 4 - (8-chloro-5,6-dihydro-4- (hydroxymethyl) -11*H*-benzo [5,6] cyclohepta [1,2-*b*] pyridin-11-ylidene) - 1-piperidinecarboxylate. At most 0.3% of 4 - (8-chloro-5,6-dihydro-2-(hydroxymethyl) - 11 *H*-benzo [5,6] cyclohepta [1,2-*b*] pyridin-11-ylidene) - 1-piperidinecarboxylate. At most 0.2% of any individual impurity, and the sum of all impurities does not exceed 0.5%.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 254 nm; 300 mm chromatograph column and 4 mm inner diameter, packed with silica chemically bonded to the phenyl group (10 µm), kept at 20 °C e 30 °C; flow of *Mobile phase* of 2.0 mL/min.

0.05 M monobasic potassium phosphate: to transfer about 6.8 g of monobasic potassium phosphate to 1000 mL volumetric flask. To dissolve, top up with water, and make it homogeneous. To adjust the pH to 3.0 ± 0.1 with phosphoric acid.

Mobile phase: mixture of 0.05 M monobasic potassium phosphate and acetonitrile (7:3).

Internal standard solution: 0.3 mg/mL butylparaben solution in a mixture of water and acetonitrile (7:3).

Sample solution: to transfer a volume of oral solution equal to 5 mg of loratadine to a 50 mL volumetric flask. To add 5 mL of *Internal standard solution* and top up with a mixture of water and acetonitrile (7:3). To make it homogeneous.

Standard solution: to prepare 1 mg/mL loratadine SQR solution in acetonitrile. To transfer 5 mL of this solution to a 50 mL volumetric flask and 5 mL of *Internal standard solution* and 12 mL of water. To top up with a mixture of water and acetonitrile (7:3) and make it homogeneous.

To inject replicates of 10 µL of the *Standard solution*. The relative retention times are about 0.78 to butylparaben and 1.0 to loratadine. The resolution between butylparaben and loratadine is greater than 1.9. The tailing factor is not greater than 1.6. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2.0 %.

Procedure: to separately inject 10 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the butylparaben and loratadine peaks. To calculate the amount of C₂₂H₂₃ClN₂O₂ in the oral solution, from the responses to relationship butylparaben/loratadine with *Standard solution* and *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

LORATADINE AND PSEUDOEPHEDRINE SULFATE ORAL SOLUTION

It contains at least 94.0 % and at most 105,0% of the amounts stated of loratadine (C₂₂H₂₃ClN₂O₂) and pseudoephedrine ((C₁₀H₁₅NO)₂.H₂SO₄) sulfate.

IDENTIFICATION

The relationship between the retention times of the main peak and internal standard peak, in the chromatogram of the *Sample solution*, obtained in *Dosage*, corresponds to the relationship between the retention times of the main peak and internal standard peak in the chromatogram of the *Standard solution*.

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

pH (5.2.19). 4.5 to 5.5.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 247 nm; 300 mm chromatograph column and 3.9 mm inner diameter, packed with

silica chemically bonded to the octadecylsilane group (10 µm), kept at 20 °C - 30 °C; flow of *Mobile phase* of 2 mL/min.

Solution A: to dissolve 3 g of monobasic ammonium phosphate in a mixture of water, methanol, and phosphoric acid (150:110:1).

Mobile phase: mixture of *Solution A* and acetonitrile (60:40).

Internal standard solution: 0.1 mg/mL butylparaben solution in *Mobile phase*.

Sample solution: to transfer a volume of oral solution equivalent to 5 mg of loratadine to a 50 mL volumetric flask, top up with *Mobile phase*, and make it homogeneous. To transfer 2 mL of resulting solution to 10 mL volumetric flask, add 1 mL of *Internal standard solution*, and top up with *Mobile phase*. To make it homogeneous.

Standard solution: to accurately weigh about 24 mg of pseudoephedrine sulfate SQR and transfer to a 100 mL volumetric flask. To add 10 mL of 0.2 mg/mL loratadine SQR solution in *Mobile phase* and 10 mL of *Internal standard solution*. To top up to volume with *Mobile phase* and make it homogeneous.

To inject replicates of 20 µL of the *Standard solution*. The relative retention times are about 0.4 to pseudoephedrine sulfate, 0.6 to butylparaben, and 1.0 to loratadine. The resolution between butylparaben and loratadine peaks is greater than 2.0. The tailing factor is not greater than 1.6. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2.0 %.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the pseudoephedrine, butylparaben, and loratadine peaks. To calculate the amount of $C_{10}H_{15}NO)_2$, H_2SO_4 and $C_{22}H_{23}ClN_2O_2$ in the oral solution, from the responses to relationship pseudoephedrine/butylparaben and loratadine/butylparaben with *Standard solution* and *Sample solution*.

PACKAGING AND STORAGE

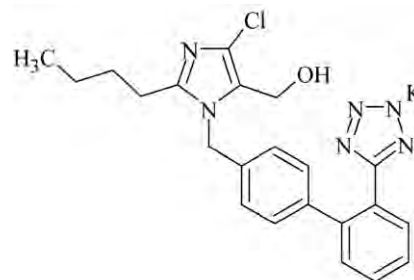
In tightly closed containers.

LABELLING

To comply with the legislation in force.

LOSARTAN POTASSIUM

Losartanum kalicum



$C_{22}H_{22}ClKN_6O$; 461.00

losartan potassium; 05432 of 2-butyl-4-chloro-1-[[2'-(2H-tetrazol-5-yl) [1,1'-biphenyl]-4-yl] methyl]-1H-imidazole-5-methanol potassium salt (1:1) [124750-99-8]

It contains at least 98.50% and at most 101.0% of $C_{22}H_{22}ClKN_6O$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. Crystalline, white or almost white powder.

Solubility. Soluble in water and ethanol, practically insoluble in ethyl acetate, chloroform, and methylene chloride.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maxima only at the same wavelength, with the same relative intensities to those observed in the losartan potassium SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14), from 200 nm to 400 nm, 0.001% solution (w/v) of the sample in methanol has absorption maximum similar to that observed in the spectrum of a similar solution of losartan potassium SQR.

C. It responds to the reactions of potassium ion (5.3.1.1).

PURITY TESTS

Limit for cyclohexane and isopropyl alcohol. To proceed as described in *Gas chromatography* (5.2.17.5). To use gas chromatograph equipped with flame ionization detector, 30 m long capillary column and 0.53 mm inner diameter, filled with phenyl methylpolysiloxane (5:95), with a 1.5 µm film thickness; column temperature as follows: to put it at 50 °C for 5 minutes, increase to 200 °C at 30 °C per minute, and keep it for 5 minutes. To keep injector temperature and detector temperature at 220 °C. To use helium as carrier gas at linear velocity 6 mL/min.

Sample solution: to dissolve in dimethylformamide an accurately weighed amount of the sample, to obtain a 50 mg/mL solution.

Standard solution: to prepare dimethylformamide solution containing 0.05 mg/mL of cyclohexane and 0.05 mg/mL of isopropyl alcohol.

To inject replicates of 1 µL of the *Standard solution*. The retention times are about 2 minutes to isopropyl alcohol, and 4 minutes to cyclohexane. The resolution between cyclohexane and isopropyl alcohol peaks should not be less than 4.0. The relative standard deviation for replicate areas of the peaks recorded should not be greater than 8.0%.

Procedure: to separately inject 1 µL of *Standard solution* and *Sample solution*. To record the chromatograms and measure the areas under the peaks. The areas under the cyclohexane and isopropyl alcohol peaks for the *Sample solution* should not be greater than the area under the cyclohexane and isopropyl alcohol peaks for the *Standard solution*. At most 0.1% of cyclohexane and 0.2% of isopropyl alcohol.

Chromatographic Purity. B. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 220 nm; 250 mm chromatograph column and 4.0 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at room temperature; flow of *Mobile phase* of 1 mL/min.

Eluent A: 0.1% phosphoric acid solution (v/v) in water.

Eluent B: acetonitrile.

Mobile phase gradient: to adopt the gradient system described in the following table:

Time (minutes)	Eluent A (%)	Eluent B (%)	Elution
0 - 25	75 → 10	25 → 90	Linear gradient
25 - 35	10	90	isocratic
35 - 45	10 → 75	90 → 25	Linear gradient
45 - 50	75	25	isocratic

Sample solution: to dissolve 30 mg of the sample in methanol and dilute to 100 mL with the same solvent, thus obtaining 300 µg/mL solution.

Resolution solution: to dissolve an accurately weighed amount of losartan potassium SQR and triphenylmethanol in methanol, and quantitatively diluted to obtain 0.3 mg/mL and 0.002 mg/mL solutions, respectively.

To inject replicates of 20 µL of the *Resolution solution*. The relative retention times are about 1.0 to losartan and 1.9 (about 20 minutes) to triphenylmethanol. The tailing factor for losartan peak is not greater than 1.6.

Procedure: to separately inject 10 µL of *Sample solution*, record the chromatograms, and measure the areas of all the peaks obtained. The area of any secondary peak is not greater than 0.2% of the total area of the peaks obtained. The sum of the areas under the secondary peaks, except the main peak's one, is not greater than 0.5% of the total area of the peaks obtained. Solvent peaks should not be included in the calculations.

Heavy metals (5.3.2.3). To proceed as described in *Reaction methods with tiocetamida*, Method III. At most 0.001% (10 ppm).

Water (5.2.20.1). At most 0.5%.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Titrations in non-aqueous medium* (5.3.3.5). To accurately weigh about 0.18 g of the sample and dissolve in 50 mL of glacial acetic acid. To titrate with 0.1 M SV perchloric acid. To potentiometrically determine the endpoint or use 1-naphtholbenzein SI as an indicator. To perform sample blank correction and adjust it as required. Each mL of 0.1 M SV perchloric acid is equal to 23.050 mg of $C_{22}H_{22}ClKN_6O$.

B. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 254 nm; 250 mm chromatograph column and 4.0 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at 35 °C; flow of *Mobile phase* of 1 mL/min.

Mobile phase: mixture of 0.1% phosphoric acid (v/v) in water and acetonitrile (60:40). To adjust it as required.

Sample solution: to dissolve an accurately weighed amount of the sample in methanol, to obtain 250 µg/mL solution.

Standard solution: to dissolve an accurately weighed amount of losartan potassium SQR in methanol and quantitatively dilute to obtain 250 µg/mL solution.

To inject replicates of 20 µL of the *Standard solution*. The column efficiency should be greater than 4000 theoretical plates. The tailing factor is not greater than 2.0. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2.0 %.

Procedure: to separately inject 10 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_{22}H_{22}ClKN_6O$ from the sample, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers, at room temperature.

LABELLING

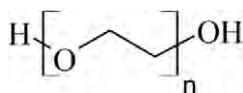
To comply with the legislation in force.

THERAPEUTIC CLASS

Antihypertensive.

MACROGOL

Macrogolum



$\text{H}(\text{OCH}_2\text{CH}_2)_n\text{OH}$

macrogol; 05474

α -Hydro- ω -hydroxypoly (oxy-1,2-ethanediyl)
[25322-68-3]

Macrogol is a polymer of addition of ethylene oxide and water represented by the above formula wherein n is the average number of ethylene oxide groups. The average molecular weight is at least 95.0% and at most 105.0% of the labeled nominal value, if it is less than 1000; at least 90.0% and at most 110.0% of the labeled nominal value when it is 1000 - 7000, and at least 87.5% and at most 112.5% of the labeled nominal value exceeds 7000.

DESCRIPTION

Physical characteristics. Liquid clear or slightly turbid, viscous, colorless, slightly hygroscopic and mild typical odor, or odorless white solid, creamy consistence, in the form of powder or flakes that dissolve in water.

Solubility. Soluble in water, acetone, ethanol; miscible with other glycols and aromatic hydrocarbons; insoluble in ethyl ether and aliphatic hydrocarbons.

Physical and chemical constants.

Viscosity (5.2.7): to determine in capillary viscometer, with flow time of at least 200 seconds and temperature at 98.9 ± 0.3 °C. Viscosity must be within the limits set forth in **Table 1**, according to the average molecular weight of the sample. For samples whose average molecular weight is not in the table, calculate the limits by interpolation.

Table 1 - Viscosity limit for macrogol samples

Average Molecular Weight	Viscosity Range, in centistokes	Average Molecular Weight	Viscosity Range, in centistokes
200	3.9 - 4.8	2200	43.0 - 56.0
300	5.4 - 6.4	2300	46.0 - 60.0
400	6.8 - 8.0	2400	49.0 - 65.0
500	8.3 - 9.6	2500	51.0 - 70.0
600	9.9 - 11.3	2600	54.0 - 74.0
700	11.5 - 13.0	2700	57.0 - 78.0
800	12.5 - 14.5	2800	60.0 - 83.0
900	15.0 - 17.0	2900	64.0 - 88.0
1000	16.0 - 19.0	3000	67.0 - 93.0
1100	18.0 - 22.0	3250	73.0 - 105.0
1200	20.0 - 24.5	3350	76.0 - 110.0
1300	22.0 - 27.5	3500	87.0 - 123.0
1400	24.0 - 30.0	3750	99.0 - 140.0
1450	25.0 - 32.0	4000	110.0 - 140.0
1500	26.0 - 33.0	4250	123.0 - 177.0
1600	28.0 - 36.0	4500	140.0 - 200.0
1700	31.0 - 39.0	4750	155.0 - 228.0
1800	33.0 - 42.0	5000	170.0 - 250.0
1900	35.0 - 45.0	5500	206.0 - 315.0
2000	38.0 - 49.0	6000	250.0 - 390.0
2100	40.0 - 53.0	6500	295.0 - 480.0
		7000	350.0 - 590.0
		7500	405.0 - 735.0
		8000	470.0 - 900.0

IDENTIFICATION

Determination of average molecular weight.

Phthalic anhydride solution: to add 49 g of phthalic anhydride in an amber erlenmeyer flask and dissolve in 300 ml of pyridine freshly distilled in phthalic anhydride. To vigorously stir the flask to obtain total dissolution. To add 7 g of imidazole and carefully mix to fully dissolve it. To let it rest for 16 hours before using it.

Sample preparation for liquid macrogols: to carefully put 25 mL of *Phthalic anhydride solution* into a dry erlenmeyer flask, pressure- and heat-resistant. To add to the flask an amount of sample, accurately weighed, equivalent to its nominal weight divided by 160. To cap the vial and cover it with a cover or safety net.

Sample preparation for solid macrogols: to carefully put 25 mL of *Phthalic anhydride solution* into a dry erlenmeyer flask, pressure- and heat-resistant. To add to the vial amount an amount of sample, accurately weighed, equal to its nominal weight divided by 160 (due to the solubility limit, do not use more than 25 g). To add 25 mL of pyridine freshly distilled in phthalic anhydride. To stir to obtain effective solution. To cap the erlenmeyer flask and cover it with a safety cover.

Procedure: to transfer the erlenmeyer flask to a water bath at 96 °C - 100 °C, so that the height of the water corresponds to the height of the liquid inside the erlenmeyer flask. To take the erlenmeyer flask from the bath, after 5 minutes, without removing the safety cover, and stir for 30 seconds, to ensure homogeneity. To heat for 30 more minutes (60 minutes for macrogol, whose molecular weight is above 3000.) To take the erlenmeyer flask from the bath and wait to cool down at room temperature. To carefully open the vial to eliminate any pressure. To remove the safety cover. To add 10 mL of water and stir. To wait 2 minutes, add 0.5 mL of a mixture of phenolphthalein SI and pyridine (1:99). To titrate with 0.5 M SV sodium hydroxide to pink color persists for 15 seconds. To perform a sample blank correction by using a mixture of 25 mL of *Phthalic anhydride solution* and an amount of pyridine equivalent to that added to the sample.

To calculate the average molecular weight according to the equation:

$$P = \frac{[2000m]}{[B - S]} \times (M)$$

where:

P = average molecular weight in g/mol, m = mass of sample in grams;

B = volume of 0.5 M SV sodium hydroxide consumed by the blank;

S = volume of 0.5 M SV sodium hydroxide consumed by the sample;

M = molarity of the sodium hydroxide solution.

PURITY TESTS

Appearance of solution. The 10% aqueous solution (w/v) is clear (5.2.25) and colorless (5.2.12) to liquid samples and no more than slightly turbid to solid samples.

pH (5.2.19). 4.5 to 7.5. To determine in solution prepared by dissolving 5 g of sample in 100 mL of carbon dioxide-free water and adding 0.3 mL of saturated potassium chloride solution.

Arsenic (5.3.2.5). To proceed as described in *Spectrophotometric method, Method II*. At most 0.0003% (3 ppm).

Heavy metals (5.3.2.3). To mix 4 g of the sample with 5 mL of 0.1 M hydrochloric acid and dilute with water to 25 mL. To proceed as described in *Method I*. At most 0.0005% (5 ppm).

Sulfated ashes (5.2.10). To determine in 25 g of the sample, in platinum crucible. At most 0.1%.

PACKAGING AND STORAGE

In tightly closed containers. Some plastics are softened by macrogol.

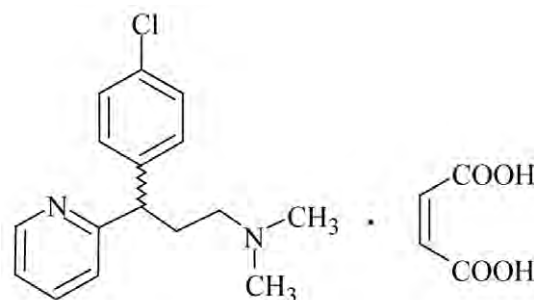
LABELLING

To comply with the legislation in force.

CATEGORY

Pharmaceutic adjuvant.

MALEATE, CHLORPHENIRAMINE Chlorphenamini maleas



$C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$; 390,86

maleato de clorfeniramina; 02442

(2Z)-2-Butenodioato de γ -(4-clorofenil)-N, N-dimetil-2-piridinapropamina (1:1)

[113-92-8]

It contains at least 98.0% and at most 100.5% of $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white or almost white, and odorless powder.

Solubility. Freely soluble in water, soluble in ethanol and chloroform; practically insoluble in ethyl ether.

Physical and chemical constants.

Melting range (5.2.2): 130 °C to 135 °C.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maxima only at the same wavelength, with the same relative intensities to those observed in the chlorpheniramine maleate SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14), ranging from 230 nm to 350 nm, of 0.003% sample solution (w/v) in 0.1 M hydrochloric acid, exhibits maxima at 265 nm.

PURITY TESTS

pH (5.2.19). 4.0 to 5.0. To determine in 2% aqueous solution (w/v).

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1),

by using HF₂₅₄ silica gel as support and a mixture of ethyl acetate, methanol, and *M* acetic acid (50:30:20) as mobile phase. To separately apply to the plate 2 µl of each of the solutions as described below.

Solution (1): 5% solution of the sample (w/v) in chloroform.

Solution (2): 0.01% solution (w/v) of the sample in chloroform.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the two principal spots, corresponding to chlorpheniramine and maleic acid, is not more intense than that obtained with *Solution (2)* (0.2%).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 105 °C for 3 hours. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.2%.

DOSAGE

To accurately dissolve about 0.4 g of the sample, previously desiccated, in 20 mL of glacial acetic acid. To add 2 drops of methylrosanilinium chloride SI and titrate with 0.1 *M* SV perchloric acid to obtain greenish-blue color. To perform sample blank correction and adjust it as required. To potentiometrically determine the endpoint, as an alternative. Each mL of 0.1 *M* perchloric acid is equal to 19.543 mg of C₁₆H₁₉ClN₂C₄H₄O₄.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

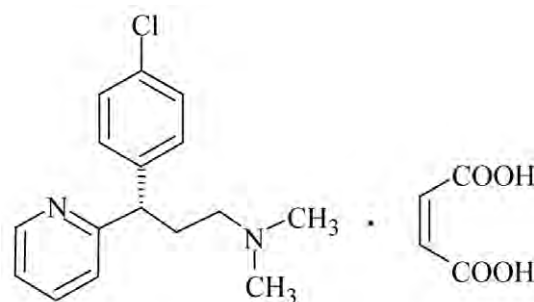
LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antihistamine.

MALEATE, DEXCHLORPHENIRAMINE Dexchlorpheniramini maleas



C₁₆H₁₉ClN₂C₄H₄O₄; 390.86
dexchlorpheniramine maleate; 02839
(2*Z*)-2-butenedioate (y*S*)-y-(4-chlorophenyl)-*N*,
N-dimethyl-2-pyridine propamine (1:1)
[2438-32-6]

It contains at least 98.0% and at most 100.5% of C₁₆H₁₉ClN₂C₄H₄O₄, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White crystalline powder.

Solubility. Freely soluble in water, ethanol, and chloroform.

Physical and chemical constants.

Melting range (5.2.2): 110 °C to 115 °C.

Specific rotation (5.2.8): +39.5° to +43°, in relation to the desiccated substance. To determine in 5% solution (w/v) in dimethylformamide.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the dexchlorpheniramine maleate SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14) ranging from 200 nm to 400 nm, of 0.002% solution (w/v) in water, exhibits maxima and minima only in the same wavelength, with the same relative intensities to those observed in the spectrum of a similar solution of dexchlorpheniramine maleate SQR.

PURITY TESTS

pH (5.2.19). 4.0 to 5.0. To determine in 0.01% aqueous solution (w/v) by using carbon dioxide-free water.

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 65 °C for 4 hours. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.2%.

DOSAGE

To proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. To accurately weigh about 0.4 g of the sample, previously desiccated, and dissolve in 50 mL of glacial acetic acid anhydrous. To titrate with 0.1 M SV perchloric acid, by potentiometrically determining the endpoint or by using methylrosanilinium chloride SI, to blue becomes green. To perform sample blank correction and adjust it as required. Each mL of 0.1 M SV perchloric acid is equal to 19.543 mg of $C_{16}H_{19}ClN_2C_4H_4O_4$.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antiallergic.

MALEATE, DEXCHLORPHENIRAMINE TABLETS

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_{16}H_{19}ClN_2C_4H_4O_4$.

IDENTIFICATION

A. To pulverize to obtain fine powder an amount of tablets equivalent to 150 mg of dexchlorpheniramine maleate. To add 100 mL of *M* acetic acid and mechanically stir for 10 minutes. To filter through a sintered glass funnel. To adjust the pH of the filtrate to 11.0 with 0.1% sodium hydroxide (w/v). To transfer to a separatory funnel and extract with 6 portions (100 mL each) of hexane. To filter each extract by using appropriate means to allow for the efficient separation between the organic phase and aqueous phase. To collect extracts and concentrate it in warm bath, to reduce volume. To transfer to a smaller container and evaporate so as to vapors of hexane are no longer noticeable. To transfer the oily residue by using 4 portions (3 mL each) of dimethylformamide to a 15 mL beaker with lid, top up with the same solvent, and stir. If necessary, centrifuge it. Rotation (5.2.8) is between + 0.24° and + 0.35°.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in *Dosage* corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). At most 1%.

Disintegration test (5.1.4.1). Within 15 minutes in water at 37 °C.

Uniformity of dosage units (5.1.6). It complies with the test.

Procedure for content uniformity. To grind each tablet to obtain fine powder, quantitatively transfer it to a 10 mL volumetric flask, and add 9 mL of a mixture of water and trifluoroacetic acid (100:0.8). To proceed as described in method B., *Dosage*, from “To mechanically stir...”

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 500 mL

Equipment: blades, 50 rpm

Time: 45 minutes

Procedure: after the test, remove aliquot from dissolution medium and filter. To proceed according to chromatographic conditions described in *Dosage*. To prepare *Standard solution* as described below.

Standard solution: to dissolve an accurately weighed amount of dexchlorpheniramine maleate SQR in water and properly dilute to obtain 4 µg/mL solution.

To inject replicates of 40 µL of the *Standard solution*. The relative standard deviation for the replicate areas for the peaks recorded is not greater than 2.0%.

Procedure: to separately inject 40 µL of *Standard solution* and *Sample solution*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of $C_{16}H_{19}ClN_2C_4H_4O_4$ from the responses from the *Standard solution* and *Sample solution*.

Tolerance: not less than 75% (Q) of the amount stated of $C_{16}H_{19}ClN_2C_4H_4O_4$ dissolves within 45 minutes.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 262 nm; 150 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), capped, kept at room temperature; flow of *Mobile phase* of 0.8 mL/min.

Eluent A: mixture of water and trifluoroacetic acid (100:0.05).

Eluent B: mixture of acetonitrile and trifluoroacetic acid (100:0.05).

Mobile phase gradient: to adopt the linear gradient system described in the following table:

Time (minutes)	Eluent A (%)	Eluent B (%)
0	100	0
10	50	50
11	0	100
16	0	100

Sample solution: to weigh and pulverize 20 tablets. To transfer an amount of powder equivalent to 10 mg of dexchlorpheniramine maleate to 50 mL volumetric flask. To add 40 mL of a mixture of water and trifluoroacetic acid (100:0.8) and mechanically stir for 15 minutes. To top up with the same solvent, make it homogeneous, and filter it. To dilute with water, to obtain 40 µg/mL solution.

Standard solution: to dissolve an accurately weighed amount of dexchlorpheniramine maleate SQR in water and properly dilute to obtain 40 µg/mL solution.

To inject replicates of 20 µL of the *Standard solution*. The relative standard deviation for the replicate areas for the peaks recorded is not greater than 2.0%.

Procedure: to separately inject 20 µL of *Standard solution* and *Sample solution*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ in the tablets, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

MALEATE, DEXCHLORPHENIRAMINE, ORAL SOLUTION

It contains at least 90.0 % and at most 110.0 % of the amount stated of dexchlorpheniramine maleate.

IDENTIFICATION

A. To dilute the equivalent to 20 mg of dexchlorpheniramine maleate to 100 mL with hydrochloric acid (1:120). To dilute 10 mL to 100 mL with the same solvent. The ultraviolet absorption spectrum (5.2.14) ranging from 200 nm to 400 nm, of sample solution obtained in *Dosage*

method **A.**, exhibits maxima similar to those observed in the standard solution spectrum.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method **B.** corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

A. To quantitatively transfer the volume of oral solution equivalent to 8 mg of dexchlorpheniramine maleate to a 250 mL separatory funnel and adjust pH to 11.0 with *M* sodium hydroxide. To extract with 2 portions (75 mL each) of hexane and combine the extracts into a second separatory funnel. To repeat the extraction with 3 portions (50 mL each) of hydrochloric acid (1:20), and top up to 200 mL with the same solvent. To weigh 40 mg of dexchlorpheniramine maleate SQR in another container, dissolve it in water, and top up to 100 mL with the same solvent. To transfer 10 mL of this solution to a separatory funnel and adjust pH to 11.0 with *M* sodium hydroxide. To extract with 2 portions (50 mL each) of hexane, by stirring each portion every 2 minutes, before separation of phases. To combine the extracts into a second separatory funnel, extract with 2 portions (40 mL each) of hydrochloric acid (1:20). To combine the extracts into a volumetric flask and top up to 100 mL with the same solvent. To filter the solution, by discarding the first portions of the filtrate. To calculate the content of $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ by absorbance measured, by linking them to the concentrations of the solutions.

B. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 262 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at room temperature; flow of *Mobile phase* of 1.0 mL/min.

Mobile phase: mixture of water, acetonitrile, and trifluoroacetic acid (70:30:0.5).

Sample solution: to transfer known volume of sample to a volumetric flask. To add water and make it homogeneous, to obtain 40 µg/mL solution. To filter it.

Standard solution: to dissolve an accurately weighed amount of dexchlorpheniramine maleate SQR in water, to

obtain 0.4 µg/mL solution. To make it homogenous and filter it.

To inject replicates of 20 µL of the *Standard solution*. The relative standard deviation for the replicate areas for the peaks recorded should not be greater than 2.0%.

Procedure: to separately inject 20 µL of *Standard solution* and *Sample solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_{16}H_{19}ClN_2C_4H_4O_4$ in the tablets, from the responses obtained for the *Sample solution* and *Standard solution*.

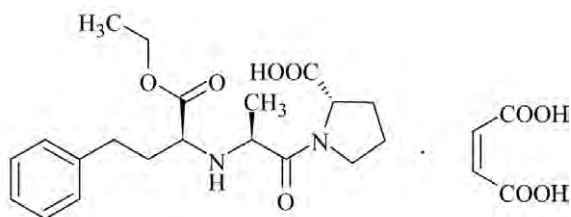
PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

MALEATE, ENALAPRIL Enalapril maleas



$C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$; 492.52
enalapril maleate; 03370

(2Z)-2-butenedioate N-[(1S)-1-(ethoxycarbonyl)-3-phenylpropyl]-L-alanyl-L-proline (1:1)
[76095-16-4]

It contains at least 98.0% and at most 102.0% of $C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white or almost white powder.

Solubility. Slightly soluble in water and methylene chloride, freely soluble in methanol. Soluble in dilute alkalis.

Physical and chemical constants

Melting range (5.2.2): 143 °C to 145 °C.

Specific rotation (5.2.8): -41° to -43.5°, in relation to the desiccated substance. To determine in 1% solution (w/v) in carbon dioxide-free water.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the enalapril maleate SQR spectrum similarly prepared.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage method B*, corresponds to that of main peak of *Standard solution*.

PURITY TESTS

pH (5.2.19). 2.4 to 2.9. To determine in 1% aqueous solution (w/v).

Related substances. To proceed as described in *Dosage method B*. To inject 50 µL of the *Sample solution*. To calculate the percentage of each peak obtained in the chromatogram of the *Sample solution*, by excluding the enalapril maleate peak. Solvent peaks should not be included in the calculations. At most 2.0% of total impurities.

Heavy metals (5.3.2.3). To use *Method III*. To determine in 2 g of the sample. At most 0.001% (10 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 60°C under reduced pressure (up to 5 mmHg) for 2 hours. At most 1.0%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.2%.

DOSAGE

A. To accurately weigh about 0.1 g of sample, transfer to a 250 mL erlenmeyer flask, and dissolve in 30 mL of carbon dioxide-free water. To titrate with 0.1 M SV sodium hydroxide, to potentiometrically determine the endpoint, to the second inflection point. Each mL of 0.1 M SV sodium hydroxide is equal to 16.417 mg of $C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$.

B. To proceed as described in *High performance liquid chromatography (5.2.17.4)*.

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 215 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at 50 °C; flow of *Mobile phase* of 2.0 mL/min.

Mobile phase: mixture of Phosphate buffer pH 2.2 and acetonitrile (75:25).

Enalaprilat solution: to dissolve in water an accurately weighed amount of enalaprilat, to obtain a 0.4 mg/mL solution.

Enalapril diketopiperazine solution: to melt about 20 mg of enalapril maleate SQR at the center of a 100 mL beaker on a heating plate (approximately 5 - 10 minutes heating). To immediately take the beaker from the plate and wait to cool down. To add 50 mL of acetonitrile to the residue and put it in ultrasound for a few minutes to dissolve. Solution generally contains between 0.2 - 0.4 mg/mL of enalapril diketopiperazine.

Sample solution: to dissolve an accurately weighed amount of phosphate buffer pH 2.2, to obtain a 0.3 mg/mL solution. To put it in ultrasound for 15 minutes. To top up the volume with the same solvent. To make it homogeneous.

Standard solution: to dissolve an accurately weighed amount of enalapril maleate SQR in phosphate buffer pH 2.2, to obtain a 0.3 mg/mL solution. To put it in ultrasound for 15 minutes. To top up the volume with the same solvent. To make it homogeneous.

Resolution solution: to prepare 0.3 mg/mL enalapril maleate SQR solution in phosphate buffer pH 2.2 and add appropriate volume of *Enalaprilat solution*, to obtain 0.003 mg/mL enalaprilat SQR solution. To transfer 0.75 mL of *Enalapril diketopiperazine solution* to 25 mL volumetric flask and top up with the solution previously prepared.

To inject replicates of 50 µL of the *Resolution solution*. The column efficiency should be greater than 1000 theoretical plates/meter to enalaprilat, no less than 300 theoretical plates/meter to enalapril, and not less than 2500 theoretical plates/meter to diketopiperazine enalapril. The relative retention times are about 0.3 to maleic acid, 0.5 to enalaprilat, 1 to enalapril, and greater than 1.5 to diketopiperazine enalapril. The tailing factor of enalapril is not greater than 2.0. The resolution is greater than 2.0 between the maleic acid and enalaprilat, enalaprilat and enalapril, and enalapril and diketopiperazine enalapril. The relative standard deviation for the replicate areas for the peaks recorded is not greater than 2.0% to enalapril and 5.0% to enalaprilat.

Procedure: to separately inject 50 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$ from the responses obtained for the *Sample and Standard solutions*.

PACKAGING AND STORAGE

In tightly closed containers.

THERAPEUTIC CLASS

Antihypertensive.

MALEATE, ENALAPRIL TABLETS

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$.

IDENTIFICATION

The retention time of the main peak in the chromatogram of the *Sample solution* obtained in *Dosage* corresponds to that of main peak of *Reference solution*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

Procedure for content uniformity. To transfer each tablet to corresponding volumetric flask, to obtain 0.1 mg/mL solution. To add phosphate buffer pH 2.2 and put it in the ultrasound to obtain total disintegration of the tablet. To proceed as described in *Dosage* from "To mechanically agitate for 30 minutes..." to prepare *Reference solution* in phosphate buffer pH 2.2, to obtain 0.01 mg/mL enalapril maleate SQR solution.

DISSOLUTION TEST (5.1.5)

Dissolution medium: phosphate buffer pH 6.8, 900 mL

Equipment: blades, 50 rpm

Time: 30 minutes

Procedure: after testing, remove aliquot from the dissolution medium and dilute, if necessary, with phosphate buffer pH 6.8, to obtain suitable concentration. To calculate the amount of $C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$ dissolved in the medium, by proceeding as per *Uniformity of dosage units*.

Tolerance: not less than 80% (Q) of the stated amount of $C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$ dissolves within 30 minutes.

PURITY TESTS

Related substances. To proceed as described in *Dosage*. To inject 50 µL of the *Sample solution*. To calculate the percentage of each peak obtained in the chromatogram of the *Sample solution*, by excluding the enalapril maleate peak. Solvent peaks should not be included in the calculations. At most 5.0% of total impurities.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To proceed as described in *High performance liquid chromatography (5.2.17.4)*. To proceed as described in method **B.**, *Dosage*, in the monograph *Maleate, enalapril*. To prepare the solutions as described below.

Sample solution: to weigh and pulverize 20 tablets. To transfer an amount of powder equivalent to 20 mg of enalapril maleate to a 100 mL volumetric flask, by using phosphate buffer pH 2.2. To put it in ultrasound for 15 minutes. To mechanically stir for 30 minutes. To top up to volume with the same solvent, thus obtaining 0.2 mg/mL solution. To make it homogeneous and filter, by discarding the first 5 mL of the filtrate.

Reference solution: to dissolve an accurately weighed amount of enalapril maleate SQR in phosphate buffer pH 2.2, to obtain a 0.2 mg/mL solution. To put it in ultrasound for 15 minutes. To top up the volume with the same solvent. To make it homogeneous.

Resolution solution: to prepare 0.2 mg/mL enalapril maleate SQR solution in phosphate buffer pH 2.2 and add an appropriate volume of *Enalaprilat solution*, to obtain 0.002 mg/mL enalaprilat SQR solution. To transfer 0.5 mL of *Enalapril diketopiperazine solution* to 25 mL volumetric flask and top up with the solution previously prepared.

Procedure: to separately inject 50 µL of *Sample and reference solutions*, record the chromatograms, and measure the area under the peaks. To calculate the amount of $C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$ in the tablets, from the responses obtained for the *Sample and reference solutions*.

PACKAGING AND STORAGE

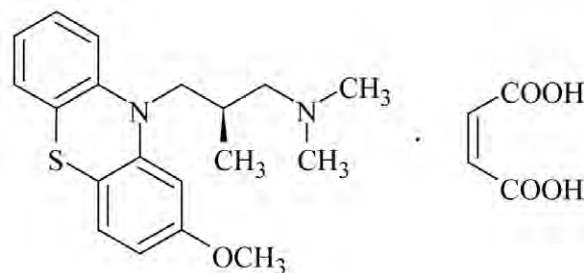
In tightly closed containers.

LABELLING

To comply with the legislation in force.

MALEATE, LEVOMEPRMAZINE

Levomepromazini maleas



$C_{19}H_{24}N_2OS \cdot C_4H_4O_4$; 444.54

levomepromazine maleate; 05265

(2Z)-2-butenedioate (βR)-2-methoxy-N,N,β-trimethyl-10H-phenothiazine-10-propanamine (1:1)
[7104-38-3]

It contains at least 98.5% and at most 101.0% of $C_{19}H_{24}N_2OS \cdot C_4H_4O_4$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white or slightly yellowish powder. It deteriorates when exposed to air and light.

Solubility. Sparingly soluble in water and ethanol, slightly soluble in methylene chloride.

Physical and chemical constants.

Specific rotation (5.2.8): -7.0° to -8.5° , in relation to the desiccated substance. To determine in 5% solution (w/v) in dimethylformamide.

IDENTIFICATION

The identification test A. may be omitted if the tests B. and C. are conducted.

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the levomepromazine maleate SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14) ranging from 230 nm to 350 nm, of 0.001% solution (w/v) in methanol, exhibits maxima at 254 nm and 308 nm. The absorbances are about 0.6 and 0.1, respectively.

C. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF₂₅₄ silica gel as support and a mixture of water, formic acid anhydrous, and isopropyl ether (3:7:90) as mobile phase.

To separately apply to the plate, as 10 mm x 2 mm band, 5 µL of each of the solutions freshly prepared, as described below.

Solution (1): 20 mg/mL solution of the sample in a mixture of water and acetone (1:9).

Solution (2): 5 mg/mL maleic acid SQR solution in a mixture of water and acetone (1:9).

To develop the chromatogram (12 cm). To remove the plate and dry it at 120°C for 10 minutes. To examine under ultraviolet light (254 nm). **Solution (1)** has a spot over the point of application and other principal spot which corresponds in position, color, and intensity to that obtained with **Solution (2)**.

PURITY TESTS

pH (5.2.19). 3.5 to 5.5. To protect from an intense light. To weigh 0.5 of sample and add 25 mL of carbon dioxide-free water. To stir and wait to settle. To check the pH of the supernatant.

Related substances. To protect it from an intense light. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF₂₅₄ silica gel as support and a mixture of acetone, diethylamine, and cyclohexane (10:10:80) as mobile phase. To separately apply to the plate 10 µL of each of the solutions freshly prepared, as described below.

Solution (1): 20 mg/mL solution of the sample in a mixture of water and acetone (1:9).

Solution (2): to dilute 0.5 mL of **Solution (1)** to 100 mL with a mixture of water and acetone (1:9).

To develop the chromatogram (15 cm). To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). Any secondary spot in the chromatogram obtained with **Solution (1)** is not more intense than that obtained with **Solution (2)** (0.5%).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 100 - 105 °C for 3 hours. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. To accurately weigh about 0.35 g of the sample and dissolve in 50 mL of glacial acetic acid anhydrous. To titrate with 0.1 M SV perchloric acid, to potentiometrically determine the endpoint. To perform sample blank correction and adjust it as required. Each mL of 0.1 M SV perchloric acid is equal to 44.454 mg of C₁₉H₂₄N₂OS.C₄H₄O₄.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antipsychotic. Neuroleptic.

PASSION FRUIT, SOUR *Passiflorae acetum folium*

Passiflora edulis Sims - PASSIFLORACEAE

The vegetable drug consists of dried leaves containing at least 1.0% of total flavonoid expressed in apigenin (C₁₅H₁₀O₅, 270.24).

CHARACTERISTICS

Organoleptic characteristics. The leaves have a characteristic odor and sweet taste.

MACROSCOPIC DESCRIPTION

Simple, glabrous, sub-coriaceous, light green leaves. Blades deeply divided into three lobes, rarely bilobed or without lobes, with 7.0 cm to 16.0 cm long and 6.0 cm to 20.0 cm wide, base concave, apex acuminate and serrated margin. Venation palmatinervia, primary and secondary veins more prominent on the abaxial surface. Petiole with 1.0 cm to 4.0 cm, channeled at the top, with a pair of extrafloral. It is common the occurrence of tendrils in the petiole. Differs from *Passiflora alata*, as this presents entire leaf, smooth edge, venation peninervia and is devoid of trichomes on the midrib.

MICROSCOPIC DESCRIPTION

Hypostomatic leaves with dorsiventral mesophyll. The epidermis, in front view, shows polyhedral shaped cells with walls slightly anticlines winding on both sides. The cuticle is smooth. The stomata are of paracitic, anisocytic and anomocytic types. Unicellular trichomes occur on the midrib on the abaxial surface. In cross section, the cuticle is thick, the epidermis is the mesophyll unistratified and is comprised of a three layer multilayer palisade and spongy parenchyma. Calcium oxalate crystals of drusen type occur in the parenchyma. In the central vein, in cross section, the upper side has a bulge and abaxial surface is convex. The epidermis in the region of the bulge, shows unicellular trichomes of smooth wall. Under both the epidermis, collenchyma cells interrupt the chlorenchyma. The vascular system is composed of four vascular bundles arranged centrally. In each vascular bundle is present fascicular rates and idioblasts with drusen occur in the inner portion of the phloem. The petiole, in cross-section has two lobes on the adaxial slightly developed, and the abaxial slightly convex in the central region. Internally in the epidermis occurs filling by collenchyma and the remainder by parenchyma. The vascular system is formed by a vascular bundle in each lobe and the adaxial side of a central group of

bundles, in annular arrangement. Idioblasts containing druses occur internally to the phloem, in smaller numbers, in the parenchyma and collenchyma.

MICROSCOPIC DESCRIPTION OF POWDER

The powder meets all the requirements for the species, unless the macroscopic characters. **CHARACTERISTICS** yellowish-green coloration; fragments of the adaxial epidermal cell as described without stomata; fragments abaxial epidermis cells as described with stomata, as described, fragments of epidermal showing unicellular trichomes; fragments of vascular tissue in transverse or longitudinal sections, with idioblasts containing druses; drusen isolated; fragments of palisade and spongy tissue with rare drusen.

IDENTIFICATION

To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using silica gel, as support and a mixture of ethyl acetate, formic acid, and water (75:5:5) as mobile phase. To separately apply to the plate, as band, 10 μ L of *Solution (1)*, and 5 μ L of *Solution (2)* freshly prepared, as described below.

Solution (1): stir in ultrasound for 10 min a dispersion of 50 mg/mL of the fine powder of the plant drug in a mixture of ethanol and water (1:1). Filtrate

Solution (2): solution at 100 μ g/mL of vitexin and rutin in a mixture of ethanol and water (1:1).

To develop the chromatogram. To remove the plate, and let it air drying. Aerosolize the plate with difenilborato SR aminoethanol, followed by polyethylene glycol 4000 to 5% (w/v) in methanol. To examine under ultraviolet light (365 nm). The spots obtained with *solution (1)* with an R_f of approximately 0.75 and 0.45 correspond in position to those obtained with *solution (2)* relating to vitexin and rutin respectively. Among them, the region of the chromatogram obtained with *solution (1)* presents two fluorescent spots, one orange, R_f of approximately 0.62, and one yellow-green, R_f about 0.53. Above the spot of the vitexin, you have three spots with greenish-yellow *solution (1)* with R_f of approximately 0.8, 0.85 and 1.0. The region of the chromatogram obtained with *solution (1)* also presents a number of fluorescent spots clearly defined between the point of application and the R_f of approximately 0.25. The species *P. alata* does not present the yellow-green fluorescent spots with R_f 0.8 and 0.85 above the spot of the vitexin, observed in *P. edulis*.

B. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 340 nm; 250 mm column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μ m), flow of Mobile phase of 0.8 mL/min.

Mobile phase: mixture of aqueous solution of phosphoric acid 0.05% (v/v) tetrahydrofuran and isopropyl alcohol (80:17:3).

Sample solution: to accurately weigh about 0.5 g of pulverized drug (180 μ m) and transfer to 50 mL volumetric flask. To add about 30 mL of ethanol and water, stir it in ultrasound for about 10 minutes, top up with the same solvent. To stir manually for five minutes and filter the extract with filter paper.

Reference solution (1): To transfer exactly 1 mg of isovitexin to a 10 mL volumetric flask and add about 7 mL of ethanol and water (1:1). To stir it in ultrasound for about 10 minutes

Reference solution (2): To transfer exactly 1 mg of isovitexin to a 10 mL volumetric flask and add about 7 mL of ethanol and water (1:1). To stir it in ultrasound for about 10 minutes Top up the volume with the same solution.

Procedure: to separately inject 20 μ L of *Standard solution* and *Sample solution*. The relative retention times are about 0.75 and 1 for 4-ramnosil vitexin and isovitexin, respectively. It also presents additional peaks characteristic of flavonoid that do not correspond to saponarin, orientin, vitexin or isorientin. It differs from *Passiflora alata* by the absence of isorientin.

PURITY TESTS

Foreign matter (5.4.2.2). At most 2,0%.

Water (5.4.2.3). At most 11%.

Total ash (5.4.2.4). At most 10.0%

Ashes insoluble in acid (5.4.2.5). At most 0.4%.

FOAM INDEX

To proceed as described in *Determination of the foam (5.4.2.10)*, using 1 g of the powdered drug (180 μ m). Calculate the foam index according to the following equation

$$E = \frac{1000}{P \times V}$$

where

IE = foam index;

P = percentage of the drug used in the preparation of the decoction;

V = volume in mL of the decoction used for the preparation of dilution tube of foam 1 cm high.

IE is at most 100.

DOSAGE

Total Flavonoids

To proceed as described in *Visible absorption spectrophotometry (5.2.14)*. To prepare the solutions as described below.

Stock solution: to accurately weigh about 0.4 g of pulverized drug (180 µm) and transfer to a 50 mL round bottom flask. To add 20 mL of ethanol at 50% (v/v) and heat under reflux for 30 minutes. To filter the mixture to a 50 mL volumetric flask. Return to the same cotton reflux flask and adding 20 mL of 50% ethanol (v/v), maintaining at reflux for 30 minutes. Filtering using filter paper to a 50 mL volumetric flask, completing the volume with 50% ethanol (v/v).

Sample solution: To transfer 0.8 mL of the injectable solution to 10 mL volumetric flask. To add 0.8 mL of aluminum chloride, 2% (w/v) in 50% ethanol (v/v), completing the volume with the same solvent.

Blank solution: to transfer 0.8 mL of Stock solution to 10 mL volumetric flask and top up with ethanol at 50% (v/v).

To measure right after 397 minutes the absorbance of *Sample solution* at 1 nm in a 30 cm cuvette, by using the Blank

solution to adjust zero. Calculate the total flavonoids, calculated as apigenin, in percentage (w/w), according to the equation:

$$\text{TFT} = \frac{A \times FD \times 100}{E_{1\text{cm}}^{1\%} \times m \times (100 - PD)}$$

where

A = absorbance,

FD = dilution factor;

$E_{1\text{cm}}^{1\%}$ = specific absorptivity (365.3),

m = mass of the drug (g), *PD* = loss on drying (% w/w).

PACKAGING AND STORAGE

In tightly closed glass container, protected from light and heat

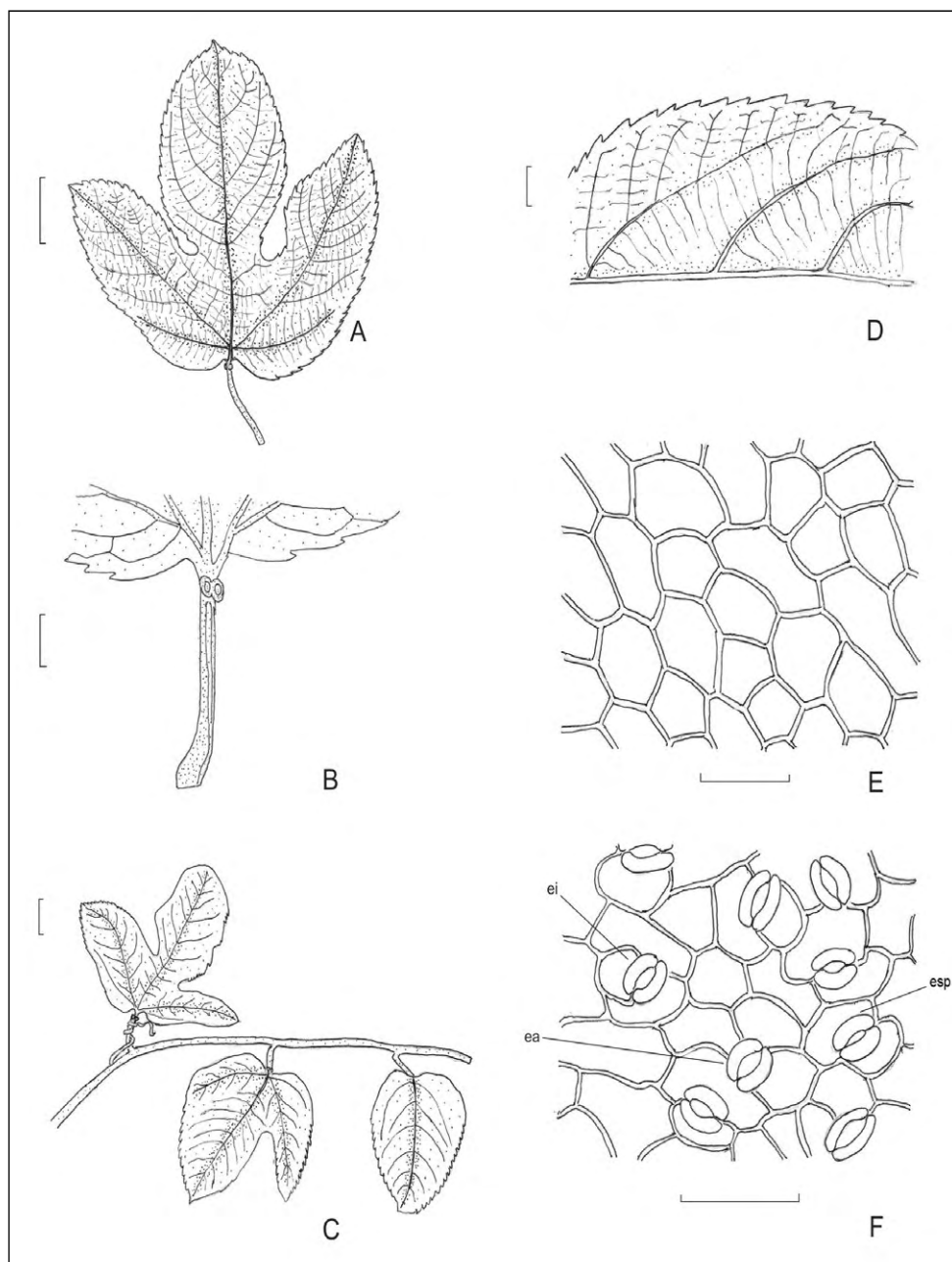


Figure 1 - Macroscopic and microscopic aspects of *Passiflora edulis* Sims

Additional information of Figure 1. Scales correspond to 1cm in A, B, and C; in D, 2 mm, and E, F, G, and H, 100 μ m.

A - general appearance of leaf showing venation digitinervia, apex acuminate, base concave and serrated margin. B - detail of the petiole with a pair of extrafloral. C - detail showing heterophyllous branch and tendril attached to the petiole. D - detail of leaf margin serrated. E — partial detail of the epidermis facing the adaxial face, in front view: F — the epidermis in the leaf blade facing the lower face, in front view: stomata (ea); stomata anisocytic (ei); stomata paracytic (esp).

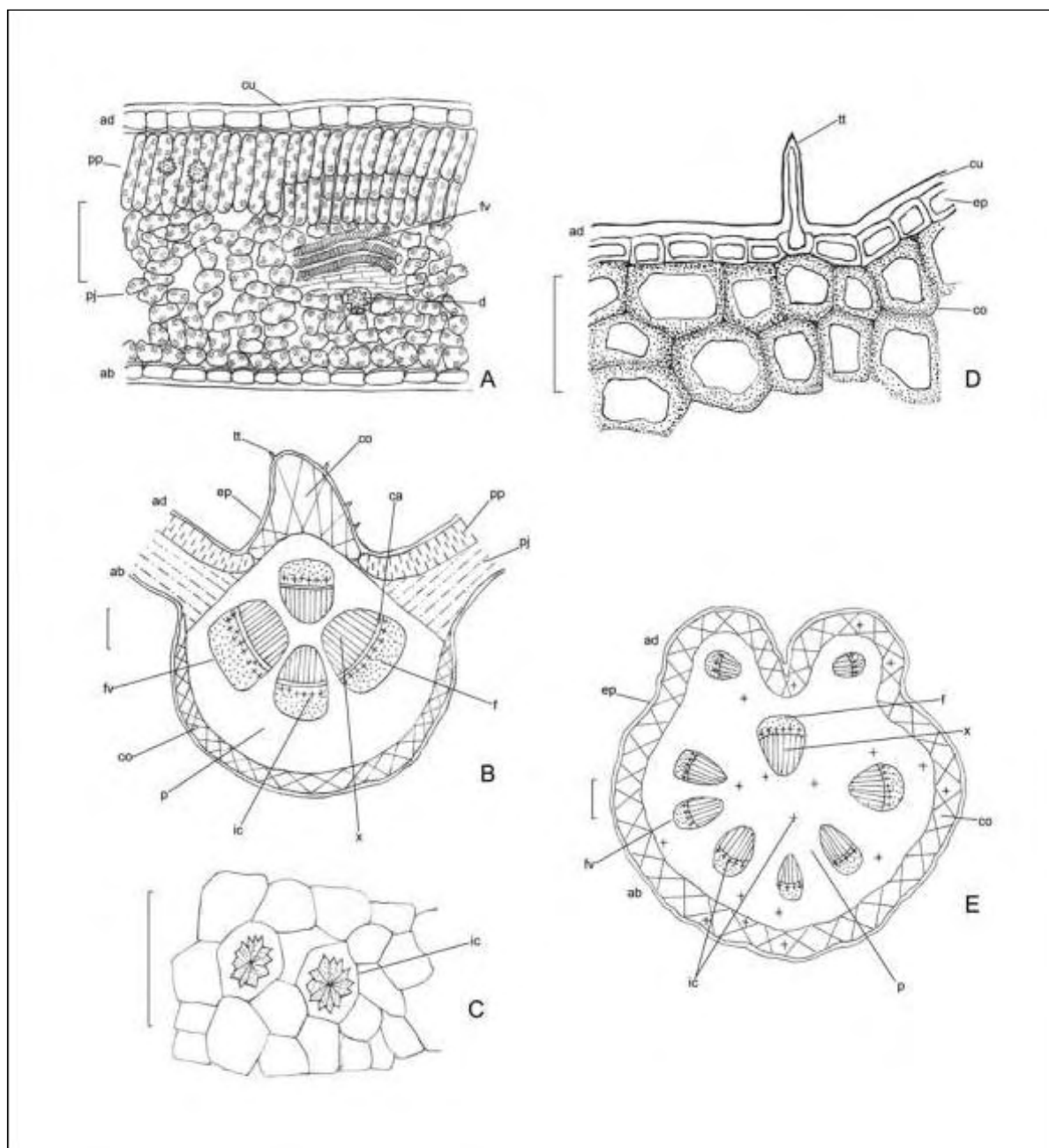


Figure 2 - Microscopic aspects of *Passiflora edulis* Sims

Additional information of Figure 2. Scales correspond to A to 100 μm , in B, C, and D, to 500 μm , and E to 50 μm .

A - cross section of mesophyll: abaxial (ab); adaxial (ad); cuticle (cu); Druze (d); vascular bundle (fv); spongy parenchyma (pj); palisade parenchyma (pp). **B** - scheme of portion of the leaf blade at midrib, in cross-section: abaxial (ab); adaxial (ad); rates (ca); collenchyma (co); epidermis (ep), phloem (f); vascular bundle (fv); cell inclusion (ic); parenchyma (p); spongy parenchyma (pj); palisade parenchyma (pp); tector trichomes (tt); xylem (x). **C** - detail cross section of petiole showing drusen in vascular bundle: cell inclusion (ic). **D** - detail of the adaxial portion of the leaf blade at midrib, in cross section, showing the unicellular tector trichomes: adaxial (ad); collenchyma (co); cuticle (cu); epidermis (ep); tector trichomes (tt). **E** — scheme of general appearance of the petiole in cross-section: abaxial (ab); adaxial (ad); collenchyma (co); epidermis (ep), phloem (f); vascular bundle (fv); cell inclusion (ic); parenchyma (p); xylem (x).

PASSION FRUIT, SWEET

Passiflorae dulcis folium

Passiflora alata Curtis - passifloraceae

The vegetable drug consists of dried leaves containing at least 1.0% of total flavonoid expressed in apigenin ($C_{15}H_{10}O_5$, 270,24)..

CHARACTERISTICS

Organoleptic characteristics. The leaves have a characteristic odor and bitter taste.

MACROSCOPIC DESCRIPTION

Simple, glabrous, sub-coriaceous, light green leaves. Oval or oblong strip of 7.0 cm to 20.0 cm in length and 4.0 cm to 15.0 cm wide, rounded or slightly concave base, acuminate apex and smooth edge. Peninervia veining, veins protruding on abaxial surface. Petiole with 2.0 cm to 7.0 cm, channeled at the top, with a pair of extrafloral. It is common the occurrence of tendrils in the petiole. Differs from *Passiflora edulis*, as this presents leaf lobes, serrated margin, and venation palminervia presents trichomes on the midrib.

MICROSCOPIC DESCRIPTION

Hypostomatic leaves with dorsiventral mesophyll. The epidermis, in front view, shows polyhedral shaped cells with walls slightly anticlines winding on both sides. The cuticle is smooth. The stomata are of paracitic, anisocytic and anomocytic types. In cross section, the cuticle is thick, the epidermis is the mesophyll unistratified and is comprised of a three layer multilayer palisade and spongy parenchyma. Calcium oxalate crystals of drusen type occur in the parenchyma. In the midrib, in cross section, the upper side has little convexity and abaxial has a convexity quite angular. Under both the epidermis, collenchyma cells interrupt the chlorenchyma, with one central vascular ring surrounded by sclerenchyma or continuous vascular ring. The rates are fascicular visible and idioblasts containing druses occur around the fundamental fabric in collenchyma and also in the phloem. The petiole in cross section, presents the adaxial concave, with two lateral projections. The abaxial surface is convex, with a single central projection. Internally in the epidermis occurs filling by collenchyma and the remainder by parenchyma. The vascular system is made up of bundles central and two lateral projections located on the adaxial side. Loads of idioblasts with drusen occurs throughout the collenchyma, parenchyma and vascular bundles.

MICROSCOPIC DESCRIPTION OF POWDER

The powder meets all the requirements for the species, unless the macroscopic characters. **CHARACTERISTICS** yellowish-green coloration; fragments of the adaxial epidermal cell as described without stomata; fragments abaxial epidermis cells as described with stomata, as described,

fragments of epidermal showing unicellular trichomes; fragments of vascular tissue in transverse or longitudinal sections, with idioblasts containing druses; drusen isolated; fragments of palisade and spongy tissue with rare drusen.

IDENTIFICATION

A. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using G silica gel, as support and a mixture of ethyl acetate, formic acid, and water (80:10:10) as mobile phase. To separately apply to the plate, as band, 10 μ l of *Solution (1)*, and 5 μ l of *Solution (2)* freshly prepared, as described below.

Solution (1): stir in ultrasound for 10 min a dispersion of 50 mg/mL of the fine powder of the plant drug in a mixture of ethanol and water (1:1). Filtrate

Solution (2): solution at 100 μ g/mL of vitexin and rutin in a mixture of ethanol and water (1:1).

To develop the chromatogram. To remove the plate, and let it air drying. To nebulize the plate with difenilborato SR aminoethanol, followed by polyethylene glycol 4000 to 5% (w/v) in methanol. To examine under ultraviolet light (365 nm). The region of the chromatogram obtained with *solution (1)* displays orange fluorescence at the finish of the solvent, Rf 1.0. The spots obtained with *solution (1)* with Rf 0.75 and 0.45 correspond in position to those obtained with *solution (2)* relating to vitexin and rutin respectively. Among them, the region of the chromatogram obtained with *solution (1)* presents two fluorescent spots, one orange, Rf 0.62, and one yellow-green, Rf 0.53. The region of the chromatogram obtained with *solution (1)* presents also two fluorescent spots well defined, one yellow-green, Rf 0.29 and another orange, Rf 0.22. It differs from *P. edulis* by the lack of yellowish-green fluorescent spots with Rf 0.85 and 0.8, above the spot of the vitexin.

B. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 340 nm; 250 mm column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μ m), flow of Mobile phase of 0.8 mL/min.

Mobile phase: mixture of aqueous solution of phosphoric acid 0.05% (v/v) tetrahydrofuran and isopropyl alcohol (80:17:3).

Sample solution: to accurately weigh about 0.5 g of pulverized drug (180 μ m) and transfer to 50 mL volumetric flask. To add about 30 mL of ethanol and water (1:1), stir it in ultrasound or 10 minutes and top up with the same solvent. To stir manually for five minutes and filter the extract with filter paper.

Reference solution (1): To transfer exactly 1 mg of isovitexin to a 10 mL volumetric flask and add about 7 mL of ethanol and water (1:1). To stir it in ultrasound for about 10 minutes

Reference solution (2): To transfer exactly 1 mg of isovitexin to a 10 mL volumetric flask and add about 7 mL of ethanol and water (1:1). To stir it in ultrasound for about 10 minutes. Top up the volume with the same solution.

Reference solution (2): To transfer exactly 1 mg of isoorientin to a 10 mL volumetric flask and add about 7 mL of ethanol and water (1:1). To stir it in ultrasound for about 10 minutes.

Procedure: to separately inject 20 µL of *Standard solution* and *Sample solution*. The relative retention times are about 0.75, 0.79 and 1 for 4-ramnosil vitexin, isoorientin and isovitexin, respectively. It also presents two well defined peaks characteristic of flavonoids with relative retention times of less than 0.75. It differs from *Passiflora edulis* by the presence of isorientin.

PURITY TESTS

Foreign matter (5.4.2.2). At most 2,0%.

Water (5.4.2.3). At most 11%.

Total ash (5.4.2.4). At most 10.0%.

Ashes insoluble in acid (5.4.2.5). At most 0.4%.

FOAM INDEX

To proceed as described in *Determination of the foam (5.4.2.10)*, using 0,1 g of the powdered drug (180 µm). Calculate the foam index according to the following

equation:

$$E = \frac{1000}{P \times V}$$

where

IE is at least 5000.

IE = foam index;

P = percentage of the drug used in the preparation of the decoction;

V = volume in mL of the decoction used for the preparation of dilution tube of foam 1 cm high.

DOSAGE

Total flavonoids

To proceed as described in *Visible absorption spectrophotometry (5.2.14)*. To prepare the solutions as described below.

Stock solution: to accurately weigh about 0.4 g of pulverized drug (180 µm) and transfer to a 50 mL round bottom flask. To add 20 mL of ethanol at 50% (v/v) and heat under reflux for 30 minutes. To filter the mixture to a 50 mL volumetric flask. Return to the same cotton reflux flask and adding 20 mL of 50% ethanol (v/v), maintaining at reflux for 30 minutes. Filtering using filter paper to a 50 mL volumetric flask, completing the volume with 50% ethanol (v/v).

Sample solution: To transfer 0,8 mL of the injectable solution to 10 mL volumetric flask. To add 0.8 mL of aluminum chloride, 2% (w/v) in 50% ethanol (v/v), completing the volume with the same solvent.

Blank solution: to transfer 0.8 mL of Stock solution to 10 mL volumetric flask and top up with ethanol at 50% (v/v).

To measure right after 397 minutes the absorbance of *Sample solution* at 1 nm in a 30 cm cuvette, by using the Blank solution to adjust zero. Calculate the total flavonoids, calculated as apigenin, in percentage (w/w), according to the equation:

$$TFT = \frac{A \times FD \times 100}{E_{1\text{cm}}^{1\%} \times m \times (100 - PD)}$$

where

A = absorbance,

FD = dilution factor;

$E_{1\text{cm}}^{1\%}$ = specific absorptivity (365.3),

m = mass of the drug (g),

PD = loss on drying (% w/w).

PACKAGING AND STORAGE

In tightly closed glass container, protected from light and heat

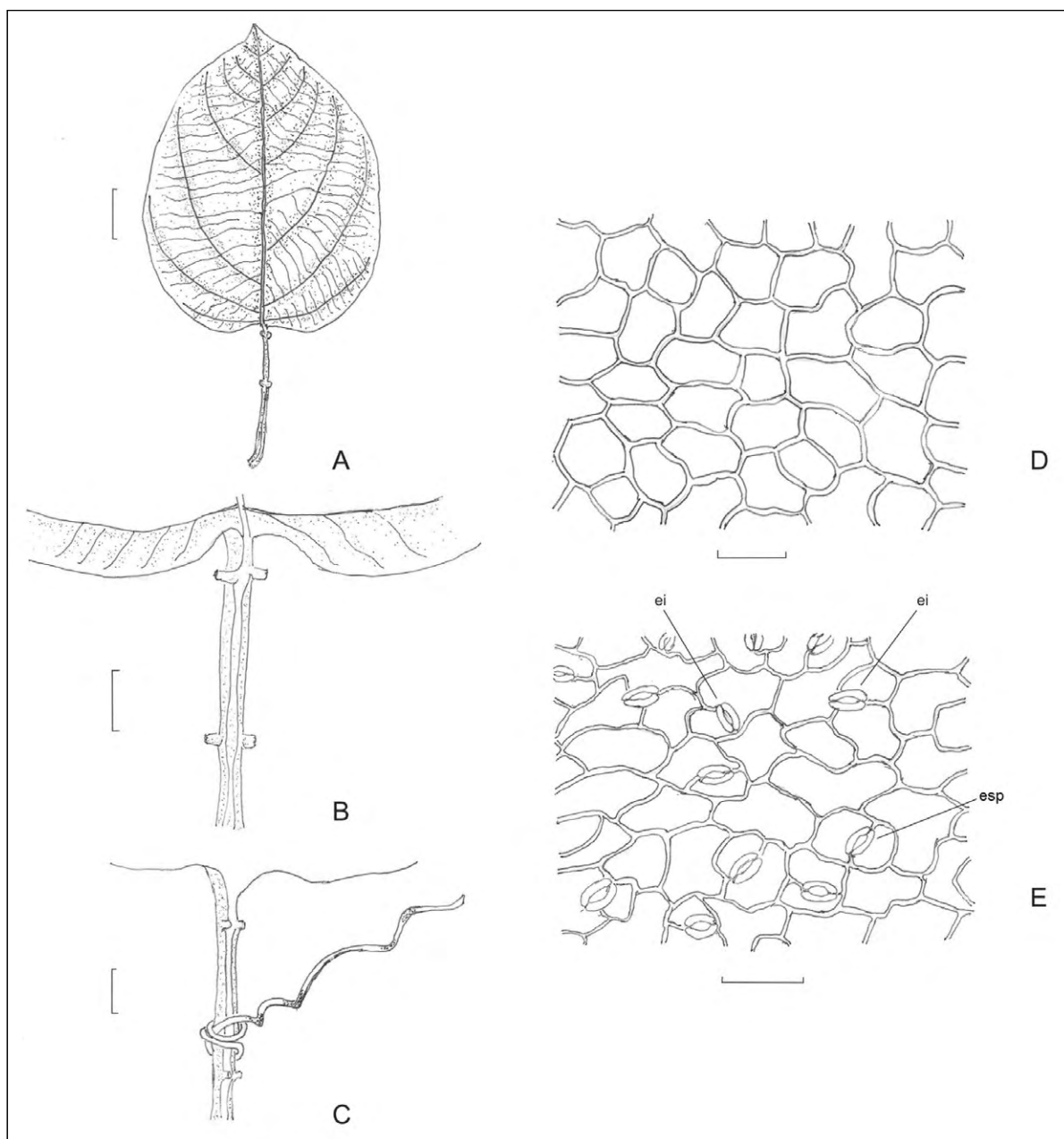


Figure 1 - Macroscopic and microscopic aspects of *Passiflora alata* Curtis

Additional information of Figure 1. Scales correspond to 2cm in A, B, and C; in D, 150 µm; and E and F, 50 µm.

A - general appearance of leaf showing venation digitinervia, apex acuminate, base concave and serrated margin. B - detail of the petiole with a pair of extrafloral. C - detail of the petiole with tendril attached. D — partial detail of the epidermis facing the adaxial face, in front view: E — the epidermis in the leaf blade facing the lower face, in front view: stomata (ea); stomata aniscytic (ei); stomata paracytic (esp).

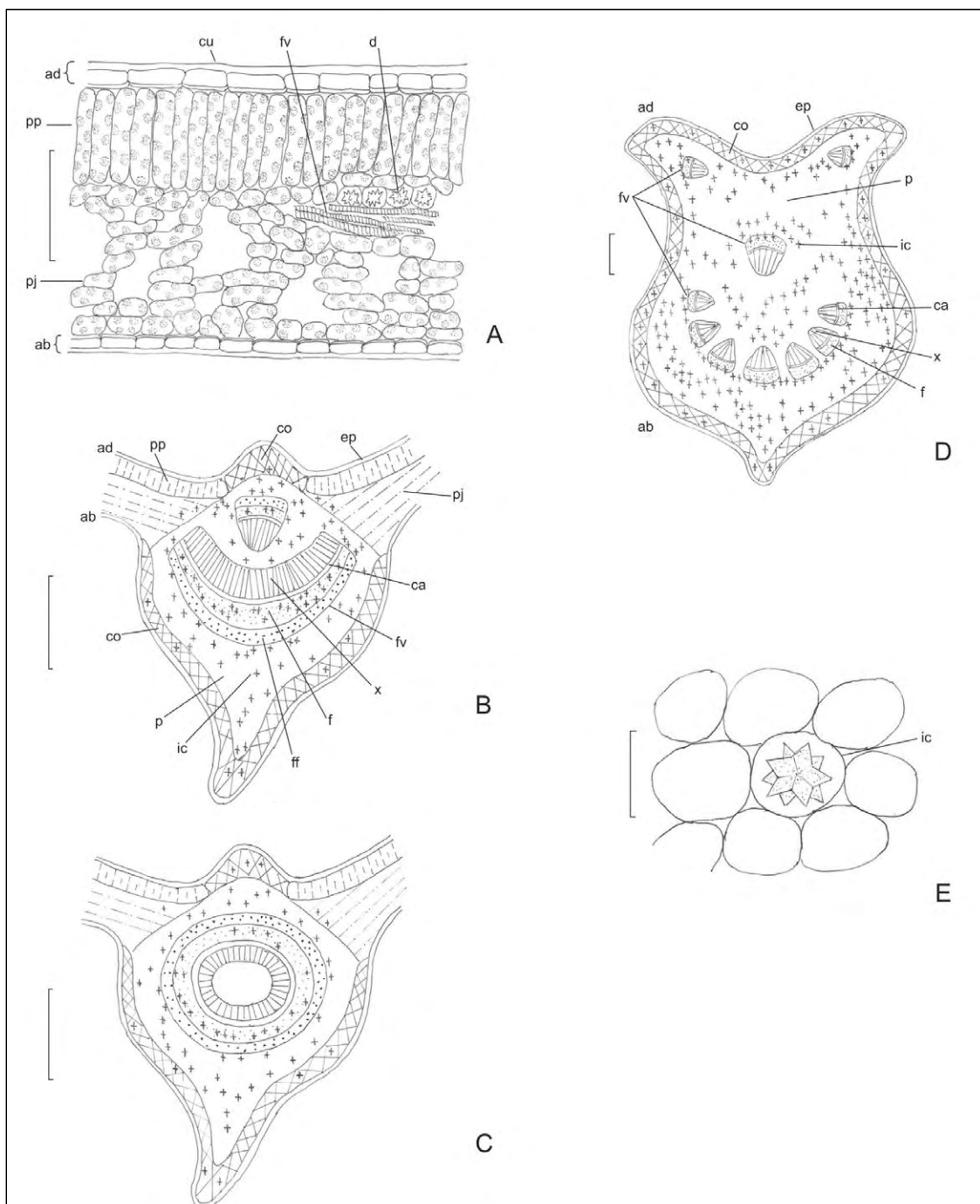


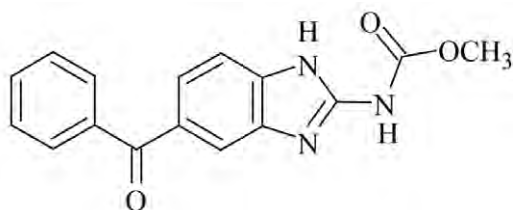
Figure 2 - Macroscopic and microscopic aspects of *Passiflora alata* Curtis

Additional information of Figure 2. Scales correspond to A to 100 μm , in B, C; and D, to 500 μm , and E to 50 μm .

A - cross section of mesophyll: abaxial (ab); adaxial (ad); cuticle (cu), Druze (d); vascular bundle (fv); spongy parenchyma (pj); palisade parenchyma (pp). **B and C** - partial scheme of midrib of the leaf blade, in cross section, showing 3 vascular bundles: abaxial (ab); adaxial (ad); rates (ca); collenchyma (co); epidermis (ep), phloem (f); vascular bundle (fv); inclusion cell (ic); parenchyma (p); spongy parenchyma (pj); palisade parenchyma (pp); tector trichomes (tt); xylem (x). **D** — scheme of general appearance of the petiole in cross-section: abaxial (ab); adaxial (ad); collenchyma (co); epidermis (ep), phloem (f); vascular bundle (fv); cell inclusion (ic); parenchyma (p); xylem (x). **E** - detail cross section of petiole showing Druze in parenchymal cell: cell inclusion (ic).

MEBENDAZOLE

Mebendazolium



$C_{16}H_{13}N_3O_3$; 295,29
mebendazole; 05515

Methyl N-(6-benzoyl-1 *H*-benzimidazol-2-yl) carbamic acid
[31431-39-7]

It contains at least 98.0% and at most 102.0% of $C_{16}H_{13}N_3O_3$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Fine, white to slightly yellow, odorless powder.

Solubility. Practically insoluble in water, chloroform, methylene chloride, ethanol, and ethyl ether. Soluble in formic acid, practically insoluble in mineral acids.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the mebendazole SQR spectrum similarly prepared.

B. To dissolve 30 mg of sample in 2 mL of formic acid anhydrous and successively dilute it with isopropyl alcohol to obtain 0.00075% concentration (w/v). The ultraviolet absorption spectrum (5.2.14), from 230 nm to 320 nm, exhibits maxima at 247 nm and 312 nm similar to those observed in the spectrum of a similar solution of mebendazole SQR.

C. To dissolve 40 mg of sample in 2 mL of formic acid anhydrous and add 5 mL of ethanol acidified with a few drops of hydrochloric acid. To vigorously stir and filter it. To add to the filtrate about 3 mg of *p*-phenylenediamine and stir. To add about 0.1 g of zinc powder and let it rest for 2 minutes. To add 5 mL of ferric ammonium sulfate SR. Violet color develops.

PURITY TESTS

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel as support and a mixture of chloroform, methanol, and formic acid anhydrous (90:5:5) as mobile phase.

To separately apply to the plate 10 µL of each of the solutions freshly prepared, as described below.

Solution (1): to dissolve 50 mg of sample in 1 mL of formic acid anhydrous and top up to 10 mL with chloroform.

Solution (2): to dissolve 50 mg of mebendazole SQR in 1 mL of formic acid anhydrous and top up to 10 mL with chloroform.

Solution (3): to transfer 1 mL of *Solution (2)* to 200 mL volumetric flask and top up with a mixture of chloroform and anhydrous formic acid (9:1). To make it homogeneous.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*. Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (3)* (0.5%).

Heavy metals (5.3.2.3). To use *Method III*. At most 0.002% (20 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 105 °C for 4 hours. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To proceed as described in *Titrations in non-aqueous medium* (5.3.3.5). To accurately weigh about 0.225 g of the sample and dissolve in 30 mL of glacial acetic acid. To titrate with 0.1 *M* SV perchloric acid, to potentiometrically determine the endpoint. To perform sample blank correction and adjust it as required. Each mL of 0.1 *M* SV perchloric acid is equal to 29.529 mg of $C_{16}H_{13}N_3O_3$.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Anthelmintic.

MEBENDAZOLE TABLETS

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_{16}H_{13}N_3O_3$.

IDENTIFICATION

To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using G silica gel as support and a mixture of chloroform, methanol, and 96% formic acid (90:5:5) as

mobile phase. To separately apply to the plate 10 µL of each of the solutions freshly prepared, as described below.

Solution (1): to grind at least 10 tablets to obtain fine powder. To weigh the equivalent to 0.2 g of mebendazole, add 20 mL of a mixture of chloroform and 96% formic acid (w/w) (19:1), to put it in water bath for 1-2 minutes, cool down, and filter it.

Solution (2): to prepare a 10 mg/mL mebendazole SQR solution in a mixture of chloroform and 96% formic acid (w/w) (19:1).

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

Procedure for content uniformity. to transfer each tablet to a 100 mL volumetric flask, add 20 mL of form acid, and wait for total disintegration of the tablet. To heat in water bath for 15 minutes. To cool down and top up with isopropyl alcohol. To make it homogenous and filter it. To successively dilute in isopropyl alcohol to obtain 0.001% concentration (w/v). To prepare a standard solution at similar concentration, under the same conditions. To measure the absorbances of the resulting solutions at 310 nm (5.2.14), by using a mixture of formic acid 96% (w/w) and isopropyl alcohol (1:500) to adjust zero. To calculate the amount of $C_{16}H_{13}N_3O_3$ in the tablets, from the reading obtained.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 1.0% sodium lauryl sulfate (w/v) in 0.1 M hydrochloric acid, 900 mL

Equipment: blades, 75 rpm

Time: 120 minutes

Procedure: after testing, remove aliquot from the dissolution medium, filter, and dilute with *Dissolution medium*, to obtain suitable concentration. To measure the absorbance at 248 nm (5.2.14), by using the *Dissolution medium* to adjust zero.

To calculate the amount of $C_{16}H_{13}N_3O_3$ dissolved in the medium, by comparing the readings obtained with that of 0.0005% mebendazole SQR solution (w/v) prepared in the same solvent.

Tolerance: not less than 75% (Q) of the amount stated of $C_{16}H_{13}N_3O_3$ dissolves within 120 minutes.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

A. To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. To weigh and pulverize 20 tablets. To transfer an amount of powder equivalent to 50 mg of mebendazole to a 100 mL volumetric flask, add 10 mL of formic acid, and mechanically stir for 15 minutes. To top up with isopropyl alcohol. To make it homogenous and filter it. To transfer 15 mL of the filtrate to a 100 mL volumetric flask, add 5 mL of 0.1 M hydrochloric acid, top up with isopropyl alcohol, and make it homogeneous. To prepare a standard solution at similar concentration by using the same solvents. To measure the absorbances of the resulting solutions at 290 nm, by using a mixture of 0.1 M hydrochloric acid and isopropyl alcohol (5:95) to adjust zero. To calculate the amount of $C_{16}H_{13}N_3O_3$ in the tablets, from the readings obtained.

B. To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*.

Sample solution: to weigh and pulverize 20 tablets. To transfer an amount of powder equivalent to 0.1 g of mebendazole to a 100 mL volumetric flask, add 50 mL of formic acid, and heat it in water bath for 15 minutes at 50 °C. To cool down and top up with water. To make it homogeneous and filter through medium porosity glass filter. To transfer 10 mL of filtrate to a separatory funnel, add 50 mL of chloroform and 50 mL of water. To stir for 2 minutes, wait for separation of phases, and transfer the chloroform layer to a second separatory funnel. To wash the aqueous layer with 2 portions (10 mL each) of chloroform, by collecting the chloroform extracts into the second separatory funnel. To discard the aqueous layer. To wash the combined chloroform extracts with a mixture of 0.1 M hydrochloric acid and 10% formic acid (v/v) (4:50). To transfer the chloroform to a 100 mL volumetric flask. To wash the aqueous layer with 2 portions (10 mL each) of chloroform, by collecting the chloroform extracts into the same volumetric flask. To top up with isopropyl alcohol and make it homogeneous. To transfer 5 mL of the solution to a 100 mL volumetric flask, top up with isopropyl alcohol, and make it homogeneous.

Standard solution: to transfer 20 mg of mebendazole SQR to a 100 mL volumetric flask, add 90 mL of chloroform,

7 mL of isopropyl alcohol, and 2 mL of 10% formic acid (v/v). To stir to obtain total dissolution and top up with isopropyl alcohol. To transfer 5 mL of this solution to 200 mL volumetric flask. To top up with isopropyl alcohol and make it homogeneous.

Blank solution: to transfer 45 mL of chloroform to a 100 mL volumetric flask, add 1 mL of 10% formic acid (v/v), top up with isopropyl alcohol, and make it homogeneous. To transfer 5 mL of the solution to a 100 mL volumetric flask, top up with isopropyl alcohol, and make it homogeneous.

To measure the absorbances of the resulting solutions at 274 nm, by using the *Blank solution* to adjust zero. To calculate the amount of $C_{16}H_{13}N_3O_3$ in the tablets, from the readings obtained.

C. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 247 nm; 300 mm chromatograph column and 3.9 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (3 μ m to 10 μ m), kept at 30 °C; flow of *Mobile phase* of 1.5 mL/min.

Mobile phase: mixture of methanol and 0.05 M monobasic potassium phosphate (60:40). To adjust the pH to 5.5 with 0.1 M phosphoric acid or M sodium hydroxide.

Sample solution: to weigh and pulverize 20 tablets. To transfer an amount of powder equivalent to 0.5 g of mebendazole to a 100 mL volumetric flask, add 50 mL of formic acid, and heat it in water bath for 15 minutes at 50 °C. To mechanically stir for 1 hour, top up with water, make it homogeneous, and filter it. To transfer 5 mL of the filtrate to 100 mL volumetric flask, top up with a mixture of formic acid and methanol (1:9), and make it homogeneous. To transfer 5 mL of this solution to 25 mL volumetric flask, top up with *Mobile phase*, make it homogeneous, and filter it.

Standard solution: to transfer 25 mg of mebendazole SQR, accurately weighed, to a 100 mL volumetric flask. To add 10 mL of formic acid and heat it in a water bath at 50 °C for 15 minutes. To mechanically stir for 5 minutes, add 80 mL of methanol, and cool down. To top up with the same solvent and make it homogeneous. To transfer 5 mL of this solution to a 25 mL volumetric flask, top up with *Mobile phase*, make it homogeneous, and filter it.

The column efficiency should be greater than 2500 theoretical plates. The tailing factor should not be greater than 2.0. The relative standard deviation for replicate areas of the peaks recorded should not be greater than 1.0 %.

Procedure: to separately inject 15 μ L of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks.

To calculate the amount of $C_{16}H_{13}N_3O_3$ in the tablets, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

MEBENDAZOLE, ORAL SUSPENSION

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_{16}H_{13}N_3O_3$.

IDENTIFICATION

A. The ultraviolet absorption spectrum (5.2.14) ranging from 200 nm to 400 nm, of sample solution obtained in *Dosage method A.*, exhibits absorption maxima and minima similar to those observed in the standard solution spectrum.

B. The principal spot in the chromatogram of *Solution (1)* in the *Related substances* corresponds in position, color, and intensity to that obtained with the *Solution (2)*.

CHARACTERISTICS

Appearance. To completely empty 10 vials, previously stirred in corresponding, clean, dry beakers with lid, and immediately observe under suitable conditions of visibility. The content flows fluidly, the suspension is homogeneous, viscous, free of lumps, and foreign matter. After 24 hours, it may have slight sedimentation resuspended upon stirring.

Determination of volume (5.1.2). It complies with the test.

pH (5.2.19). 4.0 to 7.5.

PURITY TESTS

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel as support and a mixture of chloroform, methanol, and formic acid (90:5:5) as mobile phase. To separately apply to the plate 50 μ L of each of the solutions freshly prepared, as described below.

Solution (1): To add 1 mL of formic acid to an aliquot equivalent to 10 mg of mebendazole, stir to dissolve, top

up to 10 mL with chloroform, make it homogeneous, and filter.

Solution (2): to weigh 10 mg of mebendazole SQR, add 1 mL of formic acid, stir to dissolve, top up to 10 mL with chloroform, and make it homogeneous.

Solution (3): to transfer 1 mL of *Solution (2)* to 200 mL volumetric flask, top up with a mixture of chloroform and formic acid (9:1), and make it homogeneous.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). Any secondary spot obtained in the chromatogram with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (3)* (0.5%).

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). Total aerobic bacteria: At most 1000 UFC/mL. Fungi and yeasts: At most 100 UFC/mL.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. To transfer the volume of oral suspension equivalent to 100 mg of mebendazole to a 100 mL volumetric flask. To add 30 mL of formic acid and stir to total dissolution. To top up with formic acid and mix it. To transfer 5 mL of this solution to a 50 mL volumetric flask, add 5 mL of 0.1 M hydrochloric acid, stir, and top up with isopropyl alcohol. To simmer and filter it. To cool down and dilute in isopropyl alcohol to obtain 0.001% concentration (w/v). To prepare a standard solution at similar concentration by using the same solvents. To measure the absorbances of the resulting solutions at 310 nm, by using a mixture of 0.1 M hydrochloric acid and isopropyl alcohol (1:9) to adjust zero. To calculate the amount of $C_{16}H_{13}N_3O_3$ in the oral suspension, from the readings obtained.

B. To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. To transfer the volume of oral suspension equivalent to 100 mg of mebendazole to a 100 mL volumetric flask, add 50 mL of formic acid, and put in a water bath at 50 ° C for 15 minutes. To cool down, top up with water, make it homogeneous, and filter through a medium porosity glass filter. To transfer 10 mL of filtrate to a separatory funnel, add 50 mL of chloroform and 50 mL of water, and stir for 2 minutes. To wait for separation of phases, and transfer the chloroform layer to a second separatory funnel. To wash the aqueous layer with 2 portions (10 mL each) of chloroform, and add the chloroform washing to the second separatory funnel.

To wash the combined chloroform extracts with a mixture of M hydrochloric acid and 10% formic acid (v/v) (4:50). To transfer the chloroform layer to a 100 mL volumetric flask. To wash the aqueous layer with 2 portions (10 mL each) of chloroform, add the extract to the volumetric flask, top up with isopropyl alcohol, and mix it. To successively dilute in isopropyl alcohol to obtain 0.0005% concentration (w/v). To prepare a standard solution at similar concentration by using the same solvents. To prepare the blank as described below. To transfer 45 mL of chloroform to a 100 mL volumetric flask, add 1 mL of 10% formic acid (v/v), top up with isopropyl alcohol, make it homogeneous, transfer 5 mL of this solution to a 100 mL volumetric flask, top up with isopropyl alcohol, and make it homogeneous. To measure the absorbances of the resulting solutions at 274 nm, by using the blank to adjust zero. To calculate the content of $C_{16}H_{13}N_3O_3$ in the oral suspension, from the readings obtained.

PACKAGING AND STORAGE

In tightly closed, amber glass containers.

LABELLING

To comply with the legislation in force.

HENBANE Hyoscyami folium

Hyoscyamus niger L.- SOLANACEAE; 09910

The drug consists of dried leaves and must provide at least 0.05% of total alkaloids expressed as hyoscyamine ($C_{17}H_{23}NO_3$; 289.4). The alkaloids are primarily hyoscyamine together with scopolamine (hyoscine) in varying proportions

CHARACTERISTICS

Organoleptic characteristics. Odor slightly nauseous.

MACROSCOPIC DESCRIPTION

Leaves entire, up to 30 cm long and 10 cm wide, ovate to oblong-ovate, acute apex and base cordate leaves sessile and toned leaves petiolate, maple lobed, irregularly toothed, greenish yellowish to brownish green; midrib broad and well developed, secondary veins forming sharp angle with the midrib, ending at the end of the lobes. Heavily pubescent leaf blades and sticky on both sides. Friable leaves often broken

MICROSCOPIC DESCRIPTION

The leaf blade is amphistomatic and dorsiventral symmetry. The leaf epidermis, in front view, exhibits cell of sinuous walls more pronounced in the lower face. The trichomes are tectors and glandular. The trichomes are smooth, thick-walled, long, tapered and multicellular, usually with 2-4 cells. The glandular can provide pedicel long, unicellular

or multicellular and uniseriate, with a small glandular bicellular head, exuding a viscous or a large elliptical multicellular glandular head and other times are very short, formed by a small pedicel which supports a large claviform and multicellular gland. The stomata are anisocytic, elliptical and accompanied by 3-4 subsidiary cells, one of which is always smaller than the other; occur in greater amount on the abaxial surface. The epidermis, in cross section, is uniseriate and covered with a smooth and thin cuticle. The mesophyll is composed of a single layer of palisade parenchyma, followed by a spongy parenchyma which occur mainly in the region closest to the palisade, idioblasts with calcium oxalate crystals, usually prismatic. The midrib is biconvex and vascular bundle vascular bundles bicollateral main features, the secondary beams are also bicollateral and surrounded by a lignified pericycle bit.

MICROSCOPIC DESCRIPTION OF POWDER

The powder meets all the requirements for the species, unless the macroscopic characters. Characteristics: grayish green color, showing fragments of epidermis cells sinuous walls smooth cuticle, stomata anisocytic more abundant on the abaxial surface; trichomes multicellular, uniseriate glandular trichomes and as described; mesophyll fragments, as described, a single layer of palisade cells and a spongy parenchyma containing single or double prisms of calcium oxalate; vessel elements with thickening ringed or helical.

MICROSCOPIC DESCRIPTION OF IMPURITIES IN POWDER

The powder may also provide glass fiber and crosslinked elements of the stem, subspherical pollen with a diameter which may reach 60 microns three germ pores three grooves and a nearly smooth exine; corolla epidermis fragments papillae, seed coat fragments containing sclereids integument thick-walled, sinuous, yellowish-brown color and crystals of calcium oxalate cuneiform.

IDENTIFICATION

To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel as support and a mixture of concentrated ammonia solution, acetone, chloroform, and 1-butanol (3:7:90) as mobile phase. To separately apply to the plate, as band, 20 mm for 3 m, 1 cm of distance, 10 µL of *Sample solution* and 10 20µL of *Standard solution*, as described below.

Sample solution: To 2 g of pulverized sample add 20 mL of 0.05 M sulfuric acid. To stir for 15 minutes, and filter. To wash the filter with 0.05 M sulfuric acid to obtain 25 mL of filtrate. To add 1 mL of concentrated ammonia solution to the filtrate and stir twice with 10 mL of peroxide-free ethyl ether each time. To separate by centrifugation, if necessary. To gather ether layers and dry with anhydrous sodium sulphate. To filter and evaporate the filtrate in a water bath to obtain a residue. To dissolve the residue in 0.5 mL of methanol.

Standard solution: to dissolve 50 mg of hyoscyamine sulfate SQR in 9 mL of methanol. To dissolve 15 mg of scopolamine hydrobromide SQR in 10 mL of methanol. To mix 3.8 mL of hyoscyamine sulfate solution and 4.2 mL of scopolamine hydrobromide solution and top up with 10 mL of methanol.

To develop the chromatogram. To remove the plate, allow to air dry. To nebulize with potassium iodide and bismuth subnitrate SR and watch the orange spots. Then the plate with nebulized sodium nitrite 5% (w/v) until the gel becomes transparent and examined after 15 minutes. The color of the bands corresponding to hyoscyamine in the chromatograms obtained with the *Sample solution* are brown and become reddish-brown, but they not change to blue-gray (atropine). The sequence of bands present in the chromatograms obtained with the *Standard solution* and the *Sample solution* is similar to the chromatograms of the bands corresponding to the same volume of the *Standard solution*. May appear weak secondary bands, particularly at the center of the chromatogram with 20 µL of *Standard solution*, or near the line of application in the chromatogram obtained with 10 µL of *Sample solution*.

B. To stir 3 g of pulverized drug with 30 mL of 0.05 M sulfuric acid for 2 minutes, and filter. To alkalize the filtrate with 3 mL of ammonium hydroxide and add 15 mL of water through the filter. To transfer the alkaline solution to a separatory funnel and successively extract with three aliquots of chloroform (15 mL). To gather chloroform phases and add anhydrous sodium sulfate. To filter and divide the filtrate into two porcelain capsules, by proceeding to solvent evaporation.

Vitali-Morin reaction

In a porcelain capsule, add 0.5 mL of fuming nitric acid and evaporate to dryness in a water bath. To add 2 mL of acetone to the residue and drip a 3% potassium hydroxide solution (w/v) in ethanol. An intense violet color develops. Develops violet color, featuring the presence of atropine and/ or hysciamina.

Wasicky Reaction

To add a drop of p-dimethylaminobenzaldehyde (SR2 Reagent Wasicky) in the second capsule and heat

of density less than water. To extract the solution at least three times, by using 20 mL of 0.25 M sulfuric acid solution each time. To separate by centrifugation the phases, if necessary, and transfer the acid phase to another separatory funnel. The acid phase basified with ammonium hydroxide to pH 8-9 and extracted with chloroform three times with 30 mL aliquots. To gather the chloroform phases and remove the residual water, by adding 4 g of anhydrous sodium sulfate. Let it rest for 30 minutes by occasionally stirring. To remove the chloroform phase and wash the remaining sodium sulfate with three aliquots of 10 mL of chloroform. To gather the chloroform extracts and evaporate to dryness in a water bath. To heat the residue in oven at 100 °C - C-105

°C for 15 minutes. To dissolve the residue in 5 mL of chloroform, add 20 mL of 0.01 M SV sulfuric acid, and remove the chloroform by evaporation in a water bath. To titrate the acid in excess with a solution of 0.02 M SV sodium hydroxide by using methyl red as indicator.

To calculate the content in percentage of total alkaloids, expressed in boldine, according to the equation:

slightly. Forms a reddish purple color at first edges and subsequently drop across characterized by the presence of atropine and/or hyoscyamine.

PURITY TESTS

Foreign matter (5.4.2.2). At most 2,0% of stems having a diameter greater than 7 mm.

Water (5.4.2.3). At most 12.0%.

Total ash (5.4.2.4). At most 3.0%

Ashes insoluble in acid (5.4.2.5). At most 12.0%.

DOSAGE TOTAL ALKALOIDS

To weigh about 40 g of pulverized sample (180 µm) and moisten with 5 mL of ammonium hydroxide. To add 10 mL

of ethanol and 30 mL of peroxide-free ethyl ether carefully mixed. To transfer the mixture to a percolator, if necessary, by using an extraction solution. To macerate for 4 hours and percolate the mixture with a mixture of chloroform and peroxide-free ethyl ether (1:3) until complete extraction of alkaloids. To evaporate to dryness 1 mL of leachate and dissolve the residue in 0.25 M sulfuric acid. Then, check the absence of alkaloids with mercury alkali potassium iodide SR. To reduce the volume of leachate to 50 mL and transfer to a separatory funnel with the aid of peroxide-free ethyl ether. To add peroxide-free ethyl ether to the liquid obtained 2.5 times the volume of the percolator to obtain a liquid of density less than water.

$$\% \text{ Alkaloids} = \frac{57,88 \times (20 - n)}{(100 - d) \times m}$$

where

d = loss on drying (%)

n = number of mL of 0.02 M sodium hydroxide spent;

m = mass of the test portion in g.

PACKAGING AND STORAGE

In tightly closed containers, protected from light and heat.

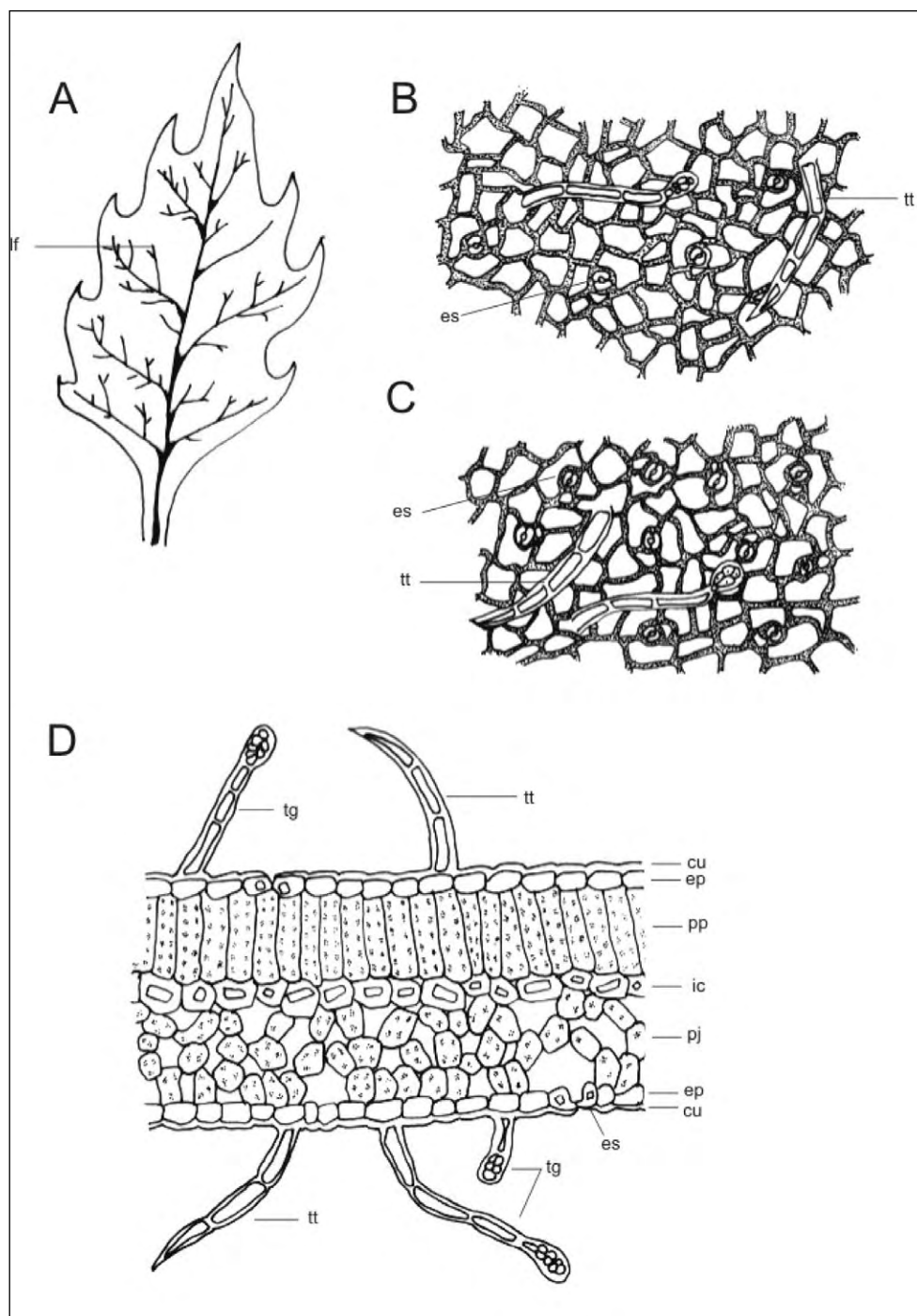


Figure 1 — Macroscopic and microscopic aspects in *Hyoscyamus niger* L.

Additional information of Figure 1.

A — Schematic representation of the leaf: leaf blade (lf). **B** — detail of the portion of the epidermis facing the adaxial side, in front view: stomata (es); tector trichomes (tt). **C** — detail of portion of the mesophyll, in cross-section: stomata (es); tector trichomes (tt). **D** — detail of the portion of the epidermis facing the lower side, in front view: glandular trichomes (tg); cuticle (cu); epidermis (ep); palisade parenchyma (pp); idioblast containing microcrystals of calcium oxalate (ic); vascular bundle (fv); spongy parenchyma (pj); epidermis (ep); tector trichomes (tt); stomata (es).

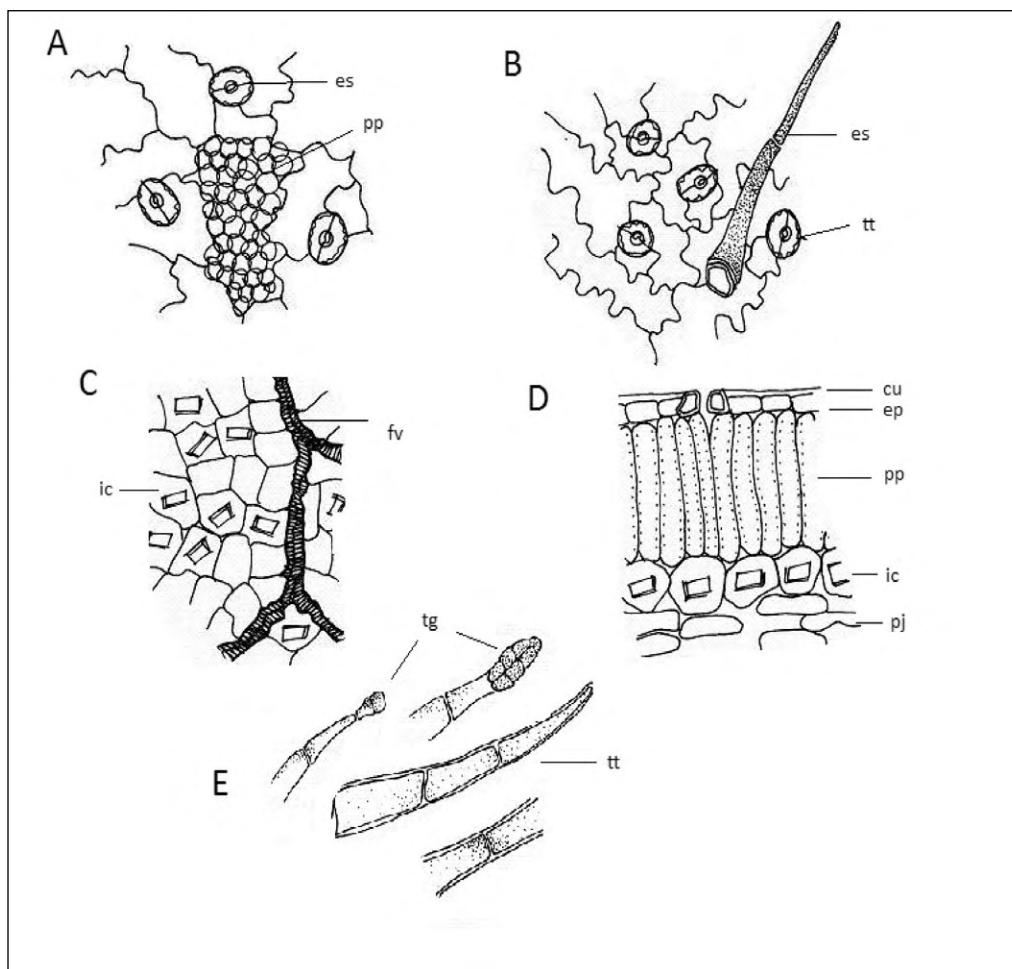


Figure 2 - Aspects of microscopic dust in *Hyoscyamus niger* L.

Additional information of Figure 2.

A, B, C, D and E — schematic representation of the powder. **A** — fragment of the epidermis in surface view, on the adaxial: stomata anisocytic type (s); palisade parenchyma (pp). **B** — fragment of the epidermis in surface view, on the adaxial: stomata (es); tector trichomes (tt). **C** - fragment of the epidermis in front view, in the lower face, showing crystals and portions of vessel elements by transparency: crystal idioblast (ic); vascular bundle (fv). **D** — fragment of portion of the mesophyll, in cross-section: cuticle (cu); epidermis (ep); palisade parenchyma (pp); crystal idioblast (ic); spongy parenchyma (pj). **E** — trichomes or their portions, isolated: glandular trichomes (tg); tector trichomes (tt).

MELISSA

Melissae folium

Melissa officinalis L. - LAMIACEAE; 09913 The drug consists of dried leaves containing at least 4.0% of total hydroxycinnamic derivatives and at least 2.0% of rosmarinic acid and at least 0.6% volatile oil.

CHARACTERISTICS

Organoleptic characteristics. The crushed leaves have a strong odor, aroma, similar to citral flavor and pleasant aroma and slightly bitter, slightly astringent.

MACROSCOPIC DESCRIPTION

Leaves entire, membranous, wrinkled, opposite-cross, brittle, petiolate, dark green and shiny on the adaxial surface and pale green on the abaxial surface, when dry vinosas sometimes, especially in the region close to the petiole and on the veins of the abaxial, with rare and glandular trichomes on the adaxial and numerous glandular and glandular trichomes on the abaxial surface, the latter seeming small dots, visible with magnifying glass six times; venation Camptodrome-reticulous, veins prominent on the adaxial and the abaxial, ribbed lower order forming characteristic meshes. Blade ovate to ovate-cordate, based oval, rounded or cordate, apex obtuse margin and irregularly sawn, finely ciliated, measuring from 4.0 cm to 8.0 cm long and 3.0 cm to 5.0 cm width. Petiole of 0.3 cm to 5.0 cm long, green or vinous when dry, concave on the adaxial side, convex on the abaxial and two lateral ribs; adaxial surface covered by long trichomes, the ribs visible to the naked eye.

MICROSCOPIC DESCRIPTION

Leaf blade of dorsiventral symmetry, hypostomatic with anomocytic stomata. In front view, the cuticle is smooth and epidermal cells have anticlinal walls with wavy contour in the region between the ribs and straight walls on the ribs. The epidermis of the leaf blade features up to six types of trichomes: (1) the glandular tapered triangular dentiform, unicellular, rarely glandular, short, warty walls and thick cuticle, (2) glandular uniseriate multicellular, three to five cells, with the apical apex acute, uncinat aspect, thick walls and cuticle rough, warty or striated; (3) glandular uniseriate multicellular, three to nine cells, very long, thick walls and cuticle rough, warty or striated; (4) glandular, uniseriate multicellular, three to nine cells, very long and broad base formed by a crown of cells, (5) glandular unicellular or bicellular head, rounded and pedicel unicellular or bicellular tricellular; (6) peltate glandular, nearly sessile, with unicellular pedicel and located below ordinary epidermal cells and secretory head octocellular, dilated cuticle, showing coloration generally brown. In cross section, the cuticle is thick, rough, striated and epidermis is uniseriate with transversely flattened cells on the adaxial surface, larger than those of the abaxial; anthocyanins are visible, especially in the cells of the abaxial surface of young leaves, the stomata are designed; trichomes of type 1 occur in greater numbers in the abaxial and type 2 are more common on the ribs on the adax-

ial; trichomes of type 3 occur mainly on the adaxial surface and are more common on the ribs, the trichomes type 4 are occurring on the abaxial region of the ribs and intercostal region of adaxial; glandular trichomes of types 5 and 6 are most common on the abaxial surface. The palisade parenchyma is compact and unistratified occupying nearly half of the section and the spongy parenchyma is little loose and bistratified or tristratified; leaf margin in the region of these tissues are more compact; starch grains present in all tissues; drops of oil absent; calcium oxalate crystals absent. The main vein in cross section, presents smooth cuticle on the adaxial side and ribbed on the abaxial epidermal cells are isodiametric, the collenchyma is angular, unistratified along the abaxial and with three to four layers along the adaxial surface, followed by the chlorenchyma of isodiametric cells with one to two layers with the abaxial side up to six layers, and with the adaxial surface, and also parenchyma cells isodiametric, thin-walled, with higher and higher development intercellular spaces along the abaxial face

The vascular system is formed by a single rule side beam, two or three rare, surrounded by a continuous endoderm or not, the exchange fascicular is evident. The petiole in cross section, has a thick cuticle, rough and grooved uniseriate epidermis of isodiametric cells, which contain anthocyanins, the stomata are designed; trichomes are the same as mentioned for the blade; regions of lateral prominences, it is quite common the occurrence of trichomes, long and broad-based (type 4 - quoted for leaf), rare on the abaxial surface. The trichomes, uniseriate and long (type 3), occur mainly on the adaxial trichomes and conical dentiform (type 1), more frequently occur on the abaxial surface. The glandular octocellular (type 6) are more common on the abaxial surface. The collenchyma is angular, and this has chloroplastidials distributed throughout the length of the petiole, or unistratified bistratified on the adaxial and abaxial tristratified in; in the ribs occur up to seven layers. Chlorenchyma is followed by a more compact and more chloroplastidials along the ribs and formed by parenchyma cells isodiametric, thin-walled, small intercellular spaces and few chloroplastidials. The vascular system is formed by three to five side beams, each surrounded by endoderm; phloem may lodge next to the stone cells and xylem fiber has radial distribution, the fascicular exchange is obvious. Starch occurs in all tissues in higher density in the chlorenchyma and endoderm.

MICROSCOPIC DESCRIPTION OF POWDER

The powder meets all the requirements for the species, unless the macroscopic characters. CHARACTERISTICS odor of citral; greenish; fragments of leaf epidermal cells with sinuous anticlinal walls and stomata diacytic and scars of trichomes type dentiform; lot of trichomes as those described; mesophyll fragments as described; crystals of calcium oxalate absent

MACROSCOPIC DESCRIPTION OF IMPURITIES

The stems, branches, flowers, fruits, and seeds of the species itself, if considered an impurity, are characterized by:

quadrangular stem, hairy when young, small flowers, stipitate and protected by bracts foliaceous, similar to other leaves; calyx pubescent, tubular-campanulate, bilabiate, upper lip and lower tridentate bifid; corolla white to yellowish or pinkish, tube bent and limbo with two unequal lobes, the upper erect, bifid and the lower extended lobed, with obtuse lobes, the middle one being the longest, four stamens, didynam, conniving under the upper lip of the corolla, anthers with thecae divergent; ovarian superoinferior, four lobes, locules with monospermic; stylus gynobasic, bifid; tetracheni fruit, brown color.

MICROSCOPIC DESCRIPTION OF IMPURITY FOR THE STEM

The stems of the species itself, if present as an impurity present in primary structure, thickened and striated cuticle, epidermis with polyhedral cells, stomata distributed near the ribs and located high above the epidermal cells, many trichomes, most commonly the type 6, in addition to the types 2 and 5 and the type 4 are distributed in the ribs. The cortex presents angular collenchyma distributed throughout the length and more developed in the ribs, cortical parenchyma and chlorenchyma formed by isodiametric cells with large intercellular spaces. The endoderm has a large amount of starch grains and involves the four side beams. The pith is formed by large-volume isodiametric cells and thin walls. In secondary structure, the epidermis and cortex maintains its characteristics except clear reduction of trichomes and the common occurrence of stone cells in the cortical parenchyma. The phloem has lots of fiber, the vascular cambium and xylem course features lots of starch grains. These grains occur in all tissues except in the epidermis and in greater amount when the secondary structure.

IDENTIFICATION

To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ with 250 µm thick as support and a mixture of toluene and ethyl acetate (90:10) as mobile phase. To apply separately to the plate-shaped band 20 µL of *solution (1)* and 10 µL of *solution (2)*, freshly prepared as described below.

Solution (1): To transfer approximately 2 g of the drug ground to a round bottom flask of 250 mL, add 100 mL of water. To add 0.5 mL of xylene by distillation k lateral opening for one hour as described in *Determination of volatile oils in herbal drugs* (5.4.2.6). After distillation, the organic phase transferred to a volumetric flask of 1 mL graduated tube of the washing machine with a little xylene and complete 1 mL of the same solvent.

Solution (2): To dissolve 1 µg of citronellal, and 10 µg citral in 25 mL of xylene.

To develop the chromatogram. To remove the plate, allow to air dry. To nebulize with anisaldehyde SR and to heat in an oven at 105 °C - 105 °C for 5-10 minutes The chromatogram obtained with *solution (2)* shows, in the lower third,

a slick double spot violet-gray to bluish-violet (citral) and above this, a patch of gray color violet-gray (citronellal). The chromatogram obtained with *solution (1)* has similar spots in position and color to the spots obtained in the chromatogram of *solution (2)*, and between these spots, a spot reddish-violet (epoxy caryophyllene). Other spots can be observed.

PURITY TESTS

Foreign matter (5.4.2.2). At most 10.0 %.

Water (5.4.2.3). At most 10.0%. To determine in 1 g of the ground sample (355 µm) in an oven at 100 °C - 105 °C for 2 hours.

Total ash (5.4.2.4). At most 12.0%

DOSAGE

Total hydroxycinnamic derivatives

To proceed as described in *Visible absorption spectrophotometry* (5.2.14). To prepare the solutions as described below.

Solution (1): to weigh 0,2 g of the pulverized drug and transfer to a round bottom flask. To heat under reflux in water bath for 10 minutes. Cool and filter. To wash the clot with 10 mL of 50% ethanol (v/v). to transfer 0.8 mL of Stock solution to 10 mL volumetric flask and top up with ethanol at 50% (v/v).

Solution (2) in a test tube, adding 1 mL of *Solution (1)* 2 mL of 0.5 M hydrochloric acid, 2 mL of a solution prepared by dissolving 10 g of sodium nitrite and 10 g of sodium molybdate in 100 mL of water and, after 2 mL of 2 M sodium hydroxide and dilute to 10 mL with water and mix.

Blank solution: in another test tube, adding 1 mL of *Solution (1)* 2 mL of 0.5 M hydrochloric acid, 2 mL of 2 M sodium hydroxide and dilute to 10 mL with water.

To measure the absorbance (2) of the solution at 505 nm. To use the white solution for zero adjustment. Calculate the content by percentage of total hydroxycinnamic derivatives expressed as rosmarinic acid, taking 400 as the value of rosmarinic acid-specific absorbance at 505 nm according to the equation:

$$DHC = \frac{A \times 5}{m}$$

where

DHC = total hydroxycinnamic derivatives expressed as rosmarinic acid (%)

A = absorbance of the solution;

m = mass of drug by considering the determination of water.

ACID, ROSMARINIC

To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 332 nm; 150 mm chromatograph column and 3.9 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at room temperature; flow of Mobile phase of 0.6 mL/min.

Eluent A: water and trifluoroacetic acid (100:0,1).

Eluent B: acetonitrile and trifluoroacetic acid (100:0,1).

Mobile phase gradient: to adopt the gradient system described in the following table:

<i>Time (minutes)</i>	<i>Eluent A (%)</i>	<i>Eluent B (%)</i>	<i>Elution</i>
0 - 14	90 → 61	10 → 39	Linear gradient
14 - 16	61 → 50	39 → 50	Linear gradient
16 - 18	50 → 90	50 → 10	Linear gradient
18 - 23	90	10	isocratic

Sample solution: weigh accurately about 0.1 g of dried and milled drug (800 | m) and place in centrifuge tube closed. To add 5 mL of ethanol 40% (v/v) and lead to ultrasound bath for 10 minutes. To centrifuge for 10 minutes at 1500 rpm. Separating the supernatant by transferring it to a 10 mL volumetric flask. Re-extract the residue of the drug with 4 mL of 40% ethanol (v/v) in ultrasonic bath for 5 minutes. To centrifuge, transfer 10 mL of the supernatant to a 40% mL volumetric flask, and top up with Mobile phase. To dilute 50 µL of the resulting solution in 0.3 mL of water.

Stock Standard solution: To dissolve 10 mg of rosmarinic acid in 10 mL of methanol.

Solutions for analytical curve: to dilute 500 µL of Stock Standard solution by half, so as to obtain a 0.50 mg/mL solution. To perform serial dilutions prior to dilution in methanol to obtain concentrations of 7.80 mg/mL, 15.60 mg/mL, 31.25 mg/mL, 62.50 mg/mL 125 mg/mL, and 250 mg/mL

Procedure: to separately inject 10 µL of Solutions for Analytical curve and *Sample solution*. To record the chromatograms and measure the areas under the peaks. The retention time is approximately 11.4 minutes to 3-methyl-2-pentanone. Calculate the content of rosmarinic acid in the sample from the equation of the line obtained from the calibration curve. The result is expressed as the mean of the

determinations in grams for chlorogenic acid per 100 g of drug (%), by taking into account the water determination.

Volatile oils

To proceed as described in *Determination of volatile oils in herbal drugs* (5.4.2.7). To use 1000 mL flask containing 500 mL of distilled water as the liquid. To add 0.5 mL of xylene through the opening k. To use dried scratched plant. To immediately proceed to determine the volatile oil from 20 g of the scratched drug. To distill for 4 hours.

CHROMATOGRAPHIC PROFILE

To proceed as described in *Gas chromatography* (5.2.17.5). To use chromatograph equipped with flame ionization detector, by using mixture of nitrogen, synthetic air, and hydrogen (1:1:10) as the auxiliary gas to flame detector; 30 m long capillary column and 0.25 mm inner diameter, filled with poly (dimethyl/diphenyl siloxane), with a film thickness of 0.25 µm; column temperature from 60 °C to 300 °C, at 3 °C per minute (total: 80 minutes), injector temperature at 220 °C and detector temperature at 250 °C; use helium at 80 kPa as carrier gas; flow of gas of 1 mL/minute.

Sample solution: to dilute the volatile oil in ethyl ether (2:100).

Procedure: to inject 1 µL of *Sample solution* into the gas chromatograph, by using 1:50 flow division. The relative retention index of the oil constituents is calculated in relation to homologous series of hydrocarbons and compared to reference samples. The relative concentration is obtained by normalization (integrating manually or electronically).

To calculate the Relative Retention Index (RRI), according to the equation:

$$K = 100 \times n + \frac{100 \times (tr_x - tr_z)}{(tr_{z+1} - tr_z)}$$

where

n = number of carbon atoms in the alkane with retention time immediately before the constituent "x" to be characterized;

tr_x = retention time of constituent "x" (intermediate to tr_z and tr_{z+1});

tr_z = retention time of the alkane immediately before the constituent "x";

tr_{z+1} = retention time of the alkane with "n + 1" carbons (immediately after the constituent "x").

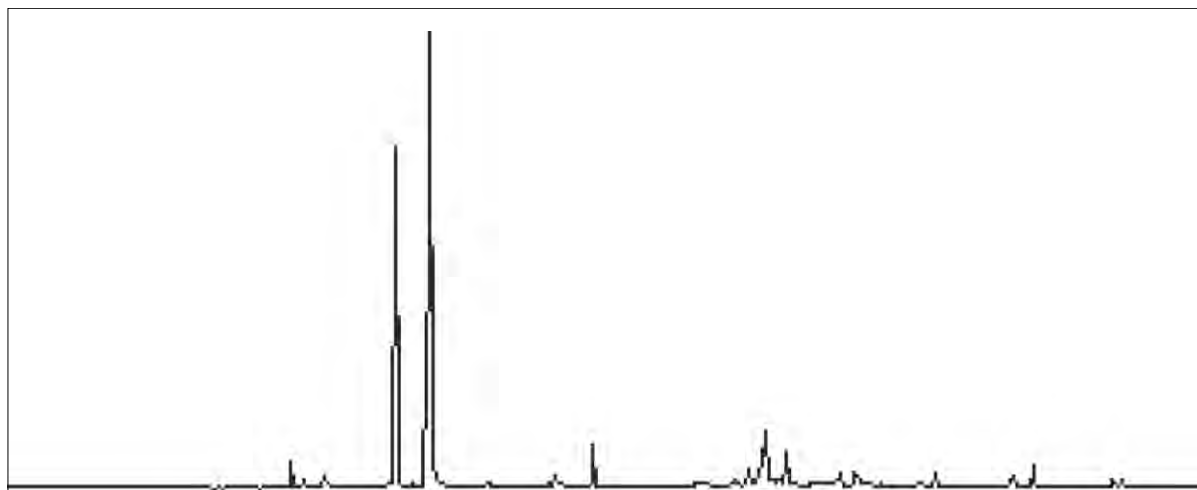


Figure 1 - Chromatogram obtained with volatile oil of *Melissa officinalis* L.

The percentages of the main constituents are within the following ranges:

<i>Peak</i>	<i>Retention index</i>	<i>Constituent</i>	<i>Content (%)</i>
1	1234	Neral (cital b)	30,4 – 32,9
2	1265	Geranial (cital a)	49,0 – 53,3
3	1404	Beta-caryophyllene	2,6 -3,1
4	1579	oxide of caryophyllen	3,9 – 6,4

PACKAGING AND STORAGE

In tightly closed glass container, protected from light and heat.

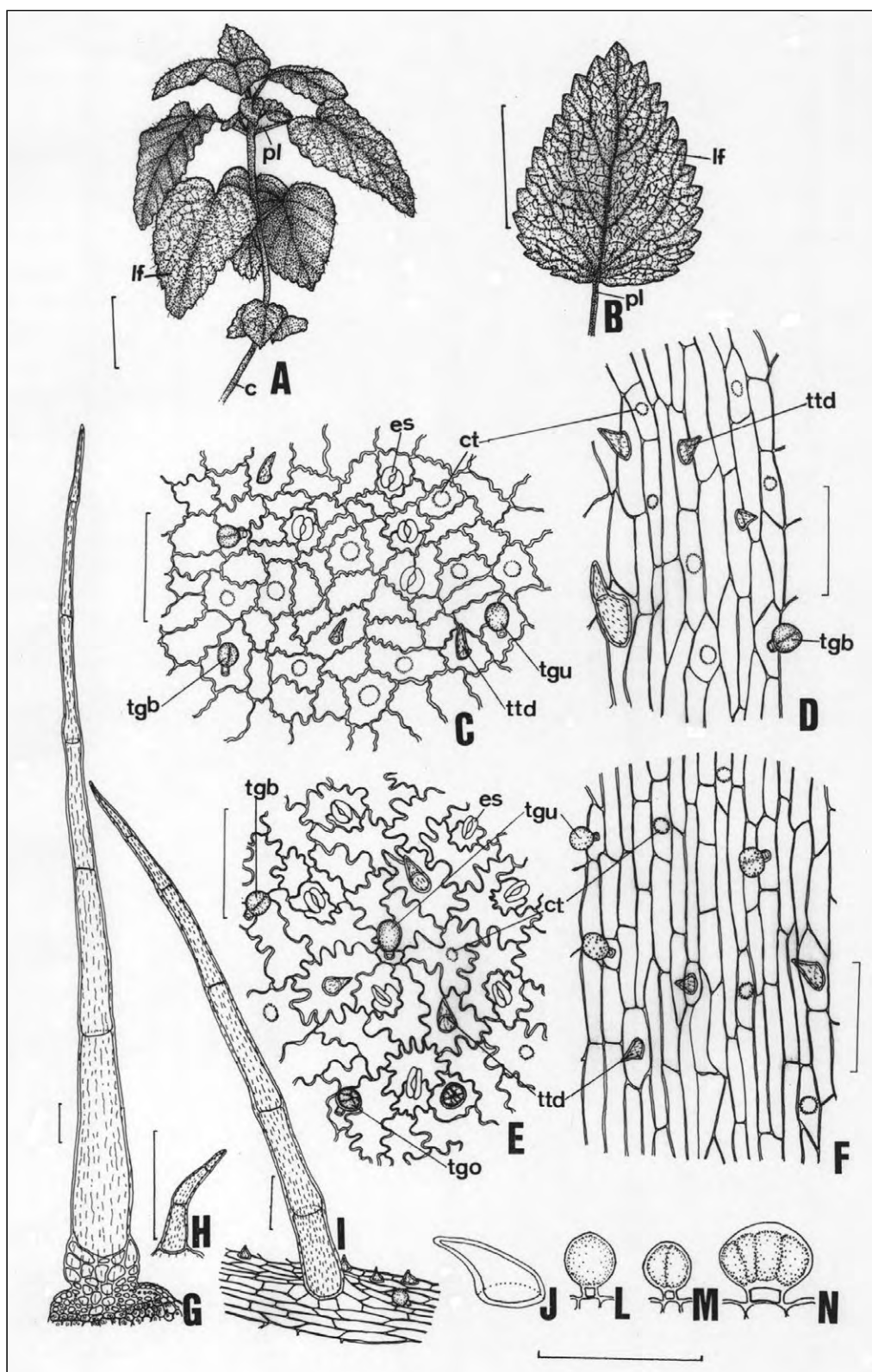


Figure 2 - Macroscopic and microscopic aspects in *Melissa officinalis* L.

Additional information of Figure 2. The scales correspond in A and B to 3 cm, C, D, E, F, G, H, I, J, L, M and N to 100 μ m

A — general appearance of a branch: stem (c), leaf blade (lf); petiole (pl). **B** — view of the adaxial face of leaf: leaf blade (lf); petiole (pl). **C** — detail of a portion of the adaxial face of epidermis in the leaf blade, in the intercostal region, in front view: tector trichomes scar type dentiform (ct); stoma (s), glandular trichomes with bicellular head, type 5 (tgb); glandular trichomes with unicellular head, type 5 (tgu); tector trichomes dentiform type, type 1 (ttd). **D** — detail of a portion of the adaxial face of epidermis in the leaf blade, on the midrib, in front view: tector trichomes scar dentiform type, type 1 (ct); glandular trichomes with bicellular head, type 5 (tgb); tector trichomes dentiform type, type 1 (ttd). **E** — detail of a portion of the lower face of epidermis in the leaf blade, in the intercostal region, in front view: tector trichomes scar dentiform type, type 1 (ct); stoma (s), glandular trichomes with bicellular head, type 5 (tgb); glandular trichome head octacellar, type 6 (tgo); glandular trichomes with unicellular head, type 5 (tgu); tector trichomes dentiform type, type 1 (ttd). **F** — detail of a portion of the lower face of epidermis in the leaf blade, on the midrib, in front view: tector trichomes scar

dentiform type, type 1 (ct); glandular trichomes with unicellular head, type 5 (tgo); tector trichomes dentiformde type, type 1 (ttd). **G** — detail of a multicellular uniseriate trichomes tector crown with basal cells, type 4, side view. **H** — detail of a multicellular uniseriate trichomes tector crown with basal cells, type 2, side view. **I** — detail of a multicellular uniseriate trichomes tector crown with basal cells, type 3, side view. **J** — Detail of a dentiform tector trichomes, unicellular, type 1, side view. **L** — Detail of a dentiform tector trichomes, unicellular, type 5, side view. **M** — Detail of a dentiform tector trichomes, unicellular, type 5, side view. **N** — detail of a glandular trichomes with secretory head octocelular, type 6, side view.

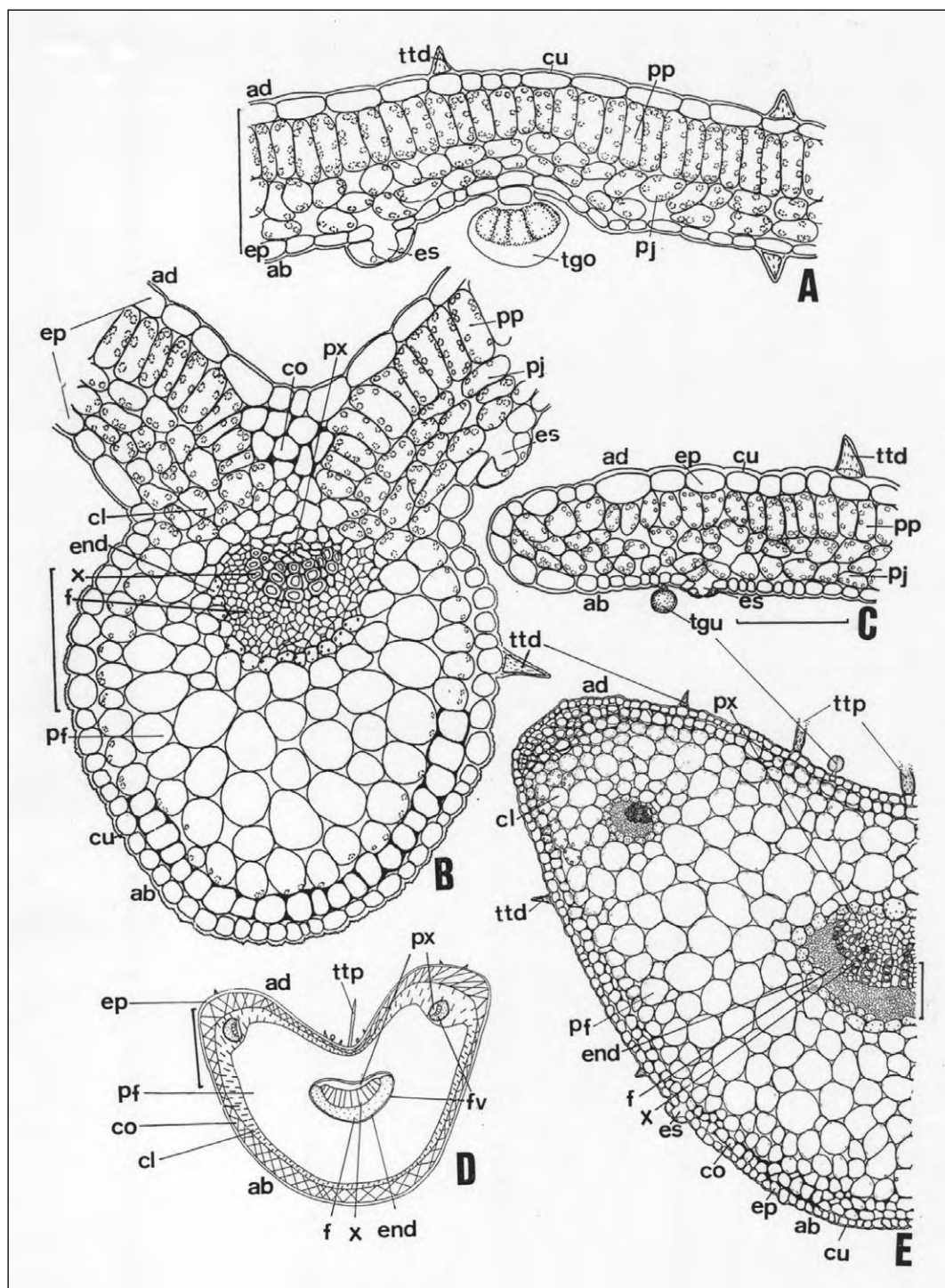


Figure 3 - Microscopic aspects of *Melissa officinalis* L.

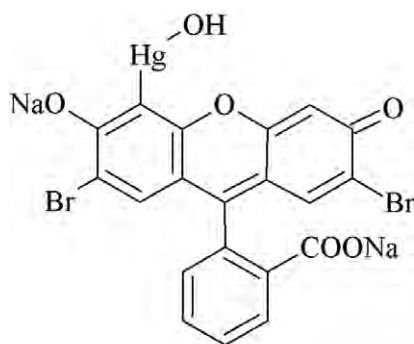
Additional information of Figure 3. Scales correspond to 100 µm in A, B, and D, and C and E, 400 µm.

A — detail of the portion of the mesophyll in the middle region of the leaf blade, in cross section: abaxial (ab); adaxial (ad); cuticle (cu); epidermis (ep); stoma (s), spongy parenchyma (pj); palisade parenchyma (pp); tector trichomes dentiform type, type 1 (ttd) **B** — detail of region of the midrib and the portion of intercostal region, in cross-section: abaxial (ab); adaxial (ad); chlorenchyma (cl); collenchyma (co); cuticle (cu), endoderm (end); epidermis (ep); stoma (s), phloem (f); spongy parenchyma (pj); parenchyma (p); palisade parenchyma (pp); xylem parenchyma (px); tector type trichomes dentiform, type 1 (ttd), xylem (x). **C** — detail of a portion of the leaf margin at

cross section: abaxial (ab); adaxial (ad); cuticle (cu); epidermis (ep); stoma (s), spongy parenchyma (pj); palisade parenchyma (pp); tector trichomes dentiform type, type 1 (ttd) **D** — scheme of general appearance of the petiole in cross-section: abaxial (ab); adaxial (ad); chlorenchyma (cl); collenchyma (co); endoderm (end); epidermis (ep), phloem (f); vascular bundle (fv); fundamental parenchyma (pf); wood parenchyma (px); tector trichomes multicellular uniseriate type 3 (ttp), xylem (x). **E** — detail of the portion of the petiole in cross section: abaxial (ab); adaxial (ad); chlorenchyma (cl); collenchyma (co); cuticle (cu), endoderm (end); epidermis (ep); stoma (s), phloem (f); fundamental parenchyma (pf); wood parenchyma (px); glandular trichomes with bicellular head, type 5 (tgo); tector trichomes dentiform type, type 1 (ttd); tector trichomes multicellular uniseriate type 3 (ttp), xylem (x).

MERBROMINE

Merbrominum



$C_{20}H_8Br_2HgNa_2O_6$; 750.65

merbromine; 05676

(2'7'-Dibromo-3', 6'-dihydroxy-3-oxospiro
[isobenzofuran-1 (3H), 9'-[9H] xanthen]-4'-yl) hydroxy
mercury sodium salt (2:1)
[129-16-8]

It contains at least 22.4% and at most 26.7% of mercury (Hg = 200.59) and at least 18.0% and at most 22.4% of bromine (Br = 79.90), in relation to desiccated substance.

DESCRIPTION

Physical characteristics. Metallic green to reddish-brown scale or granule.

Solubility. Freely soluble in water, but sometimes small amounts of insoluble material are left; practically insoluble in ethanol, acetone, ethyl ether, and chloroform.

IDENTIFICATION

A. The 0.05% solution (w/v) is red and has yellowish-green fluorescence.

B. To add 3 drops of sulfuric acid SR to 5 mL of 0.4% solution (w/v). Reddish-orange precipitate is produced.

C. To heat 0.1 g of sample with small crystals of iodine in the test tube. Red crystals are sublimated on top of the tube. In case of yellow crystals, rub it with a glass rod. Crystals become red.

D. To weigh 0.1 g of the sample and add 12 mL of 16.67% sodium hydroxide (w/v). To evaporate to dryness, by stirring it, and incinerate at 600 °C for 1 hour.

To dissolve the residue in 5 mL of water and acidify with hydrochloric acid. To add 3 drops of chlorine SR and 2 mL of chloroform and stir. The chloroform layer becomes yellowish-tan.

PURITY TESTS

Appearance of solution. To dissolve 0.4 g of the sample in 20 mL of water, add 3 mL of sulfuric acid SR, and filter it.

The color of the filtrate is not more intense than the color of *Standard solution color SC C (5.2.12)*.

Soluble halogens

Sample preparation: to dissolve 5 g of the sample in 80 mL of water, add 10 mL of 10% nitric acid (w/v), and dilute to 100 mL with water. To make it homogenous and filter it. To transfer 40 mL of the filtrate to a Nessler tube, add 6 mL of 10% nitric acid (w/v), and dilute to 50 mL with water.

Standard preparation: To add 0.25 mL of 0.01 M hydrochloric acid and 6 mL of 10% nitric acid (w/v) to a Nessler tube and dilute to 50 mL with water.

Procedure: to add 1 mL of 0.1 M silver nitrate to the tubes, mix well, and let it rest for 5 minutes protected from light. Any turbidity developed in the *Sample preparation* is not more intense than that obtained with *Standard preparation*.

Soluble mercury salts

Sample preparation: to transfer 5 mL of the filtrate obtained *Appearance of solution* to the test tube and add 5 mL of water.

Standard preparation: to dissolve 40 mg of mercury chloride (II), accurately weighed, in water and dilute to 1000 mL with the same solvent. To add 3 mL of sulfuric acid SR to 20 mL of this solution. To transfer 5 mL of the previous solution to the test tube and add 5 mL of water.

Procedure: To add a drop of sodium sulfide SR to the tubes. Any color developed in the *Sample preparation* is not more intense than that obtained with *Standard preparation*.

Insoluble mercury compounds. To dissolve 2.5 g of the sample in 50 mL of water and let it rest for 24 hours protected from light. To centrifuge and wash the precipitate with water until the last washing becomes colorless. To transfer the precipitate to vial with polished stopper, accurately add 5 mL of 0.05 M SV iodine, and let it rest for 1 hour, by frequently stirring. To add dropwise, by stirring, 4.3 mL of 0.1 M SV sodium thiosulfate. To add 1 mL of starch SI. The blue color develops.

Loss on drying (5.2.9). To determine in 2 g of the sample. To desiccate in an oven at 105 °C for 5 hours. At most 5.0%.

DOSAGE

Mercury

To accurately weigh about 0.6 g of the sample, previously pulverized and desiccated, transfer to a vial with lid, and dissolve in 50 mL of water. To add 8 mL of glacial acetic acid, 20 mL of chloroform, and accurate 30 mL of 0.05 M SV iodine. To tightly close and let it rest for 1 hour, by vigorously and frequently stirring. To titrate the iodine in excess with 0.1 M SV sodium thiosulfate by vigorously stirring, by using 1 mL of starch SI. To perform sample

blank correction and adjust it as required. Each mL of 0.05 M SV iodine is equal to 10.030 mg of Hg.

Bromine

To accurately weigh, in a porcelain crucible, about 0.5 g of the sample previously pulverized and desiccated, add 2 g of potassium nitrate, 3 g of potassium carbonate anhydrous, 3 g of sodium carbonate anhydrous, and make it homogeneous. To cover the surface of the mixture with 3 g of equal parts of potassium carbonate anhydrous and sodium carbonate anhydrous, and calcine at 400 °C - 500 °C for 1 hour. To cool down, dissolve, and quantitatively transfer to the erlenmeyer flask the calcined mixture, by using 80 mL of hot water and acidify with nitric acid. To add 25 mL of 0.1 M SV silver nitrate and stir. To titrate the silver nitrate in excess with 0.1 M SV ammonium thiocyanate, by using 2 mL of ferric ammonium sulfate SR as an indicator. To perform sample blank correction and adjust it as required. Each mL of 0.1 M SV silver nitrate is equivalent to 7.990 mg of Br.

PACKAGING AND STORAGE

In tightly closed opaque containers.

LABELLING

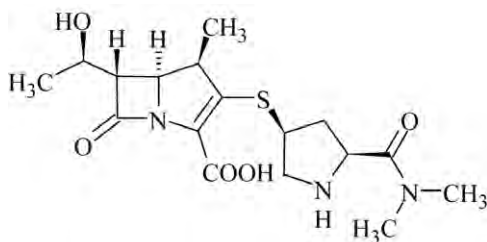
To comply with the legislation in force.

CATEGORY

Preservative.

MEROPENEM

Meropenémum



$C_{17}H_{25}N_3O_5S$; 383.46

$C_{17}H_{25}N_3O_5S_3H_2O$; 437.51

meropenem; 05688

meropenem trihydrate; 09494

(4R, 5S, 6S) -3 - [[(3S, 5S) -5 - [(dimethylamino) carbonyl] - 3-pyrrolidinyl] thio] -6 - [(1R)-1-hydroxyethyl]-4-methyl -7-oxo-1-azabicyclo [3.2.0] hept-2-ene-2-carboxylic acid
[96036-03-2]

(4R, 5S, 6S) -3 - [[(3S,5S) -5 - [(dimethylamino) carbonyl] - 3-pyrrolidinyl] thio] -6 - [(1R)-1-hydroxyethyl]-4-methyl -7-oxo-1-azabicyclo [3.2.0] hept-2-ene-2-carboxylic acid hydrate (1:3)
[119478-56-7]

It contains at least 98.0% and at most 101.0% of $C_{17}H_{25}N_3O_5S$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. Crystalline, white to pale yellow powder.

Solubility. Sparingly soluble in water, soluble in dimethylformamide, very slightly soluble in ethanol, practically insoluble in acetone and ethyl ether. Soluble in 5% mono-basic potassium phosphate (w/v).

Physical and chemical constants.

Melting range (5.2.2): 230 °C to 240 °C, with decomposition.

Specific rotation (5.2.8): -17° to -21°. To determine in 0.5% aqueous solution (w/v) at 20 °C.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the meropenem SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14) ranging from 200 nm to 400 nm, of 0.003% solution (w/v) in water, exhibits maxima and minima only at the same wavelength of a similar meropenem SQR solution.

PURITY TESTS

pH (5.2.19). 4.0 to 6.0. To determine in 1% aqueous solution (w/v).

Related substances. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 220 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at 40 °C; flow of *Mobile phase* of 1.6 mL/min. If necessary, adjust the flow of *Mobile phase*, to retention time of meropenem be 5 - 7 minutes.

Mobile phase: mixture of *Diluent* and acetonitrile (1000:70).

Diluent: to add 1 mL of triethylamine to 900 mL of water, adjust the pH to 5.0 + 0.1 with 10% phosphoric acid (v/v), and dilute to 1000 mL with water.

Solution (1): to dissolve an accurately weighed amount of the sample in the *Diluent* and quantitatively dilute to obtain a 5 mg/mL solution. To immediately inject after preparation.

Solution (2): to dissolve an accurately weighed amount of meropenem SQR in the *Diluent* and quantitatively dilute to obtain 25 µg/mL solution. To immediately inject after preparation or store in refrigerator up to 24 hours.

The column efficiency is greater than 2500 theoretical plates. The tailing factor is not greater than 1.5. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2.0 %.

Procedure: to separately inject 10 µL of *Solution (1)* and *Solution (2)*. Then, record the chromatograms at least three times the retention time of the meropenem peak. The main impurities are observed in the retention times 0.45 and 1.9 for the meropenem peak. To calculate the percentage of each Impurity in the sample according to the equation:

$$\left(\frac{C_p}{C_a} \right) \times P \times \left(\frac{A_i}{A_p} \right)$$

where

C = concentration in mg/mL of meropenem SQR in the Standard solution;

Ca = concentration in mg/mL of meropenem in the sample solution;

P = stated potency of meropenem, in anhydrous basis, in chemical reference;

Ai = area under the peak corresponding to any individual impurity in the sample solution;

Ap = area under the peak corresponding to meropenem in the standard solution;

At most 0.3% of either of the two main impurities, in relation to the anhydrous substance.

At most 0.1% of any individual impurity, in relation to the anhydrous substance. The sum of all other impurities, calculated on an anhydrous basis, except for the main impurities, should not be greater than 0.3%.

Water (5.2.20.1). 11.4% to 13.4%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. To incinerate at (500 ± 50) °C. At most 0.1%.

BIOLOGICAL SAFETY TEST

When the label states that the substance is sterile, the sample complies with Bacterial endotoxins and Sterility tests. Should the substance be indicated to sterilization for sterile preparations, the sample complies with Bacterial endotoxins tests.

Sterility (5.5.3.2.1). It complies with the test. To use the *Membrane filtration method*.

Bacterial endotoxins (5.5.2.2). At most 0.125 UE/ mg of meropenem.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 298 nm; 250 mm column and 4 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (3 µm to 10 µm), kept at room temperature; flow of *Mobile phase* of 1.0 mL/minute.

Buffer pH 3.0: to prepare 0.03 M monobasic potassium phosphate solution. To adjust the pH to 3.0 ± 0.1 with phosphoric acid.

Mobile phase: mixture of *Buffer pH 3.0* and acetonitrile (90:10).

Note: to inject the solutions described below immediately after preparation and kept under refrigeration up to 24 hours.

Sample solution: to dissolve an accurately weighed amount of the sample in water and dilute to obtain 0.2 mg/mL meropenem (C₁₇H₂₅N₃O₅S) solution. To transfer 5 mL to 25 mL volumetric flask and top up with water, thus obtaining 40 µg/mL solution.

Standard solution: to dissolve an accurately weighed amount of meropenem SQR in water and dilute to obtain 0.2 mg/mL meropenem (C₁₇H₂₅N₃O₅S) solution. To transfer 5 mL to 25 mL volumetric flask and top up with water, thus obtaining 40 µg/mL solution.

The column efficiency is greater than 4000 theoretical plates to meropenem peak. The tailing factor is not greater than 2.0. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2.0 %.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of C₁₇H₂₅N₃O₅S from the sample, from the responses obtained for the *Sample solution* and *Standard solution*.

B. To proceed as described in *Microbiological test of antibiotics (5.5.3.3)* by using cylinders with the agar diffusion method.

Micro-organism: *Micrococcus luteus* ATCC 9341.

Culture media: culture medium number 1, to maintain the micro-organism; sterile saline solution to the standardization of the inoculum; and culture medium number 11, to the base layer and inoculum preparation.

Sample solution: to weigh an amount of sample equivalent to 30 mg of meropenem, transfer to a 100 mL volumetric flask, and top up with sterile water. To successively dilute,

by using sterile water as diluent, to obtain 1.5 µg/mL, 3.0 µg/mL, and 6.0 µg/mL concentrations.

Standard solution: to weigh an amount of meropenem SQR equivalent to 30 mg of meropenem, transfer to a 100 mL volumetric flask, and top up with sterile water. To successively dilute, by using sterile water as diluent, to obtain 1.5 µg/mL, 3.0 µg/mL, and 6.0 µg/mL concentrations.

Procedure: to add 20 mL of culture medium number 11 in each plate, wait to solidify, add 5 mL of 0.5% inoculum, and proceed as described in *Microbiological test of antibiotics (5.5.3.3.1)*, by adding 0.2 mL of freshly prepared solutions to the cylinders. To calculate the potency of the sample, in µg/mg of meropenem, from the potency of the standard and responses obtained with the *Standard and sample solutions*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light, at room temperature.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antibiotic.

MEROPENEM TRIHYDRATE POWDER FOR INJECTABLE SOLUTION

It contains at least 90.0 % and at most 120.0 % of the amount stated of meropenem ($C_{17}H_{25}N_3O_5S$). Meropenem powder for injectable solution is a sterile dry blend of meropenem trihydrate and sodium carbonate.

IDENTIFICATION

A. To individually weigh three units, remove the contents, wash corresponding vials, dry, and weigh them again. To make the content of the vials homogeneous. To stir an amount of powder with water and quantitatively dilute with the same solvent to obtain 0.002% concentration (w/v). To filter, if necessary. The ultraviolet absorption spectrum (5.2.14), from 200 nm to 400 nm, has maximum at 298 nm, similar to those observed in the spectrum of a similar solution of meropenem SQR.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

pH (5.2.19). 7.3 to 8.3. To determine in 5% aqueous solution (w/v).

Determination of weight (5.1.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

PURITY TESTS

Related substances. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 220 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at 40 °C; flow of *Mobile phase* of 1.6 mL/min. If necessary, adjust the flow of *Mobile phase*, to retention time of meropenem be 5 - 7 minutes.

Diluent: to add 1 mL of triethylamine to 900 mL of water, adjust the pH to 5.0 ± 0.1 with 10% phosphoric acid (v/v), and dilute to 1000 mL with water.

Mobile phase: mixture of *Diluent* and acetonitrile (1000:70).

Sample solution: to individually weigh three units, remove the contents, wash corresponding vials, dry, and weigh them again. To make the content of the vials homogeneous. To stir an accurately weighed amount of powder with *Diluent* and quantitatively dilute with the same solvent, to obtain 5 mg/mL solution. To immediately inject after preparation.

Standard solution: to dissolve an accurately weighed amount of meropenem SQR in the *Diluent* and quantitatively dilute with the same solvent to obtain 29 µg/mL solution. To immediately inject after preparation or store in refrigerator up to 24 hours.

To inject replicates of 10 µL of the *Standard solution*. The column efficiency is greater than 2500 theoretical plates. The tailing factor is not greater than 1.5. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2.0 %.

Procedure: to separately inject 10 µL of *Standard solution* and *Sample solution*. Then, record the chromatograms at least three times the retention time of the meropenem peak. The main impurities are observed in the retention times 0.45 and 1.9 for the meropenem peak. To calculate the percentage of each Impurity in the sample according to the equation:

$$10 \times \left(\frac{C \times P}{Ca} \right) \times \left(\frac{A_i}{A_p} \right)$$

where

C = concentration in mg/mL of meropenem SQR in the *Standard solution*;

P = stated potency of meropenem SQR, in anhydrous basis;

m = amount of meropenem in mg, in the mass of powder to prepare the *Sample solution*;

A_i = area of the peak corresponding to any individual impurity in the *Sample solution*;

A_p = area under the peak corresponding to meropenem in the *Standard solution*;

At most 0.8% of impurities with relative retention time of 0.45 relative to a meropenem peak. At most 0.6% of impurities with retention time of 1.9 relative to a meropenem peak.

Sodium. To proceed as described in *Atomic absorption spectrometry with flame (5.2.13.1.1)*. To use flame spectrophotometer with a mixture of air-acetylene, hollow sodium cathode lamp, with light emission at 589.6 nm.

Potassium chloride solution: to dissolve 38.1 g of potassium chloride and dilute to 1000 mL with the same solvent.

Sample solution: to individually weigh three units, remove the contents, wash corresponding vials, dry, and weigh them again. To make the content of the vials homogeneous. To transfer an amount of powder equal to 25 mg of meropenem to a 200 mL volumetric flask and top up with water. To transfer 5 mL to 50 mL volumetric flask, add 5 mL of *Potassium chloride solution*, and top up with water.

Standard sodium solution: to dissolve 28.67 mg of sodium chloride, previously desiccated at 105 °C for 2 hours, to obtain 28.67 µg/mL sodium chloride solution. To transfer 5 mL to 50 mL volumetric flask, add 5 mL of *Potassium chloride solution*, and top up with water.

Blank: to transfer 5 mL of *Potassium chloride solution* to 50 mL volumetric flask and top up with water.

Procedure: to measure the absorbance of *Standard sodium solution* and *Sample solution*, by using *Blank* to adjust zero.

To calculate the amount of sodium, in mg, in the injectable solution of meropenem, from the readings obtained. It contains 80% - 120% of the stated amount of sodium.

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 65°C under reduced pressure for 6 hours. From 9.0% to 12.0%.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test. To use the *Membrane filtration method*.

Bacterial endotoxins (5.5.2.2). At most 0.125 UE/ mg of meropenem.

DOSAGE

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 298 nm; 250 mm column and 4 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (3 µm to 10 µm), kept at room temperature; flow of *Mobile phase* of 1 mL/minute.

Buffer pH 3.0: to prepare 0.03 M monobasic potassium phosphate solution. To adjust the pH to 3.0 ± 0.1 with phosphoric acid.

Mobile phase: mixture of *Buffer pH 3.0* and acetonitrile (90:10).

Note: to inject the solutions described below immediately after preparation and kept under refrigeration up to 24 hours.

Sample solution: to individually weigh 20 units, remove the contents, wash corresponding vials, dry, and weigh them again. To make the content of the vials homogeneous. To dissolve an accurately weighed amount of powder in water, to obtain 0.2 mg/mL meropenem ($C_{17}H_{25}N_3O_5S$) solution. To transfer 5 mL to 25 mL volumetric flask and top up with water, thus obtaining 40 µg/mL solution.

Standard solution: to dissolve an accurately weighed amount of meropenem SQR in water and dilute to obtain 0.2 mg/mL meropenem ($C_{17}H_{25}N_3O_5S$) solution. To transfer 5 mL to 25 mL volumetric flask and top up with water, thus obtaining 40 µg/mL solution.

To inject replicates of 20 µL of the *Standard solution*. The column efficiency is greater than 4000 theoretical plates to meropenem peak. The tailing factor is not greater than 2.0. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2.0 %.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas of the peaks. To calculate the amount of $C_{17}H_{25}N_3O_5S$ in the product, from the responses from the *Standard solution* and *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light, at room temperature.

LABELLING

To comply with the legislation in force.

METABISULFITE, SODIUM

Natrii metabisulfis

Na₂S₂O₅; 190.11
 SO₂; 64.06
 sodium metabisulfite; 05711
 disulfur acid sodium salt (2:1)
 [7681-57-4]

Sodium metabisulphite contains an amount of Na₂S₂O₅ equivalent to at least 65.0% and at most 67.4% of SO₂.

DESCRIPTION

Physical characteristics. White crystals, almost white or transparent, odorless or slight sulfur odor. It is efflorescent.

Solubility. Sparingly soluble in water, slightly soluble in ethanol.

IDENTIFICATION

The 5% solution (w/v) responds to the reactions of sodium ion and sulfite ion (**5.3.1.1**).

PURITY TESTS

pH (5.2.19). 3.5 to 5.0. To determine in 5% solution (w/v) in carbon dioxide-free water.

Thiosulfate. To mix 2.2 g of sample with 10 mL of *M* hydrochloric acid. To gently heat for 5 minutes, cool down, and transfer to a small test tube. In case of turbidity, this is not greater than that produced by 0.1 mL of 0.1 *M* sodium thiosulfate under the same conditions (0.05%).

Arsenic (5.3.2.5). To mix 0.2 g of the sample with 2 mL of water in a beaker. To add dropwise 1.5 mL of nitric acid. To evaporate to dryness in water bath. To heat over the flame until vapor ceases. To transfer the residue and top up to 25 mL. To proceed as described in *Method I*. At most 0.0005% (5 ppm).

Heavy metals (5.3.2.3). To dissolve 1 g of the sample in 10 mL of water, add 5 mL of hydrochloric acid, and evaporate to dryness in a water bath. To dissolve the residue in 25 mL of water. At most 0.002% (20 ppm).

Iron (5.3.2.4). To dissolve 0.5 g of the sample in 14 mL of diluted hydrochloric acid (2 : 7), and evaporate to dryness in a water bath. To dissolve the residue in 7 mL of diluted hydrochloric acid (2 : 7) and evaporated to dryness again. To dissolve the residue in a mixture of 2 mL of hydrochloric acid and 20 mL of water, add 3 drops of bromine water SR. To heat to boiling to expel bromine. To cool down. To dilute to 40 mL with water. At most 0.002% (20 ppm).

DOSAGE

To dissolve 0.2 g of the sample in 50 mL of 0.05 *M* SV iodine. Let it rest for 5 minutes protected from light. To

add 1 mL of hydrochloric acid and titrate the iodine in excess with 0.1 *M* SV sodium thiosulphate, by using 3 mL of iodine starch SI solution as an indicator, which must be added near the end of the titration. Each mL of 0.05 *M* SV iodine is equal to 3.203 mg of SO₂.

PACKAGING AND STORAGE.

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

CATEGORY

Antioxidant.

METAPHOSPHATE, POTASSIUM

Kalli metaphosphas

(KPO₃)_x
 potassium metaphosphate; 05721
 Metaphosphoric acid potassium salt (1:1)
 [7790-53-6]

Potassium metaphosphate is a linear chain polymer with a high degree of polymerization. It contains at least 59.0% and at most 61.0% of P₂O₅.

DESCRIPTION

Physical characteristics. White, odorless powder.

Solubility. Insoluble in water. Soluble in diluted aqueous solutions of alkali metals salts (except potassium).

Physical and chemical constants.

Viscosity (5.2.7): to mix 0.3 g of sample with 200 mL of 0.35% sodium pyrophosphate (w/v), by using a magnetic stirrer.

To determine the viscosity of the clear solution obtained or liquid phase of the mixture obtained after 30 minutes of continuous stirring. From 6.5 cP to 15 cP.

IDENTIFICATION

A. To slowly add 1 g of the finely pulverized sample, by vigorously stirring, to 100 mL of 2% sodium chloride (w/v). A gelatinous mass is formed.

B. To heat to boiling, for 30 minutes, a mixture of 0.5 g of the sample, 10 mL of nitric acid, and 50 mL of water, and cool down. The resulting solution responds to the reactions of phosphate ion (**5.3.1.1**) and potassium ion (**5.3.1.1**).

PURITY TESTS

Limit for fluoride. To transfer 5 g of sample, 25 mL of water, 50 mL of perchloric acid, 5 drops of 50% silver nitrate (w/v) and some glass beads to a 250 mL distillation flask connected with condenser provided with thermometer and capillary tube, both in contact with the liquid. To connect the small addition funnel filled with water or steam generator with the capillary tube. To adapt the bottle to the distillation system with 1/3 of the bottom of the bottle in the flame. To distill to a 250 mL volumetric vial until the temperature reaches 135 °C. To add water through the addition funnel or inject steam through a capillary, to maintain at 135 °C - 140 °C. To continue the distillation to collect 225 mL - 240 mL, top up with water, and make it homogeneous. To transfer 50 mL of this solution to a Nessler tube. To transfer 50 mL of water to standard Nessler. To add 0.1 mL of filtered alizarin SI solution and 1 mL of 0.025% hydroxylamine hydrochloride solution (w/v), freshly prepared, to each tube and make it homogeneous. To add dropwise, by stirring, 0.05 M sodium hydroxide to the tube containing sample until the color matches that of the standard tube (slightly pink). To add 1 mL of 0.1 M hydrochloric acid to each tube and make it homogeneous. By using burette (0.05 mL graduation), slowly add to the tube containing the sample a sufficient amount of 0.025% thorium nitrate (w/v), so that after homogenization the color of the liquid becomes pink. Record the volume of solution added, add the same volume accurately measured to the standard tube, and make it homogeneous. By using the burette, add sodium fluoride SR (10 µg F/mL) to become similar to the color of the both tubes, after dilution to the same volume. To make it homogeneous and allow air bubbles to escape prior to final comparison of color. To check the endpoint by adding 1-2 drops of sodium fluoride SR to the standard tube. A distinct coloration is observed. The volume of sodium fluoride required for the reference solution should not exceed 1 mL (0.001%).

Arsenic (5.3.2.5). To dissolve 1 g of sample in 15 mL of 3 M hydrochloric acid and proceed as described in *Spectrophotometric method, Method I*. At most 0.0003% (3 ppm).

Lead (5.3.2.1). To dissolve 1 g of the sample in 10 mL of 3 M hydrochloric acid and proceed as described in *Limit test for lead*. At most 0.0005% (5 ppm).

Heavy metals (5.3.2.3). To use *Method I*. To add 10 mL of 3 M hydrochloric acid to 1 g of the sample and heat until it no longer dissolves. To add 15 mL of water, make it homogeneous, and filter. At most 0.002% (20 ppm).

DOSAGE

To mix 0.2 g of sample with 15 mL of nitric acid and 30 mL of water, boil it for 30 minutes, cool down, and dilute with water to approximately 100 mL. To heat at 60 °C, add ammonium molybdate SR1 in excess, and heat it at 50 °C for 30 minutes. To filter and wash the precipitate firstly with 0.5 M nitric acid and then with 1% potassium nitrate (w/v) to observe no acid residue in the filtrate (use litmus paper).

To add 25 mL of water to the precipitate, dissolve in 50 mL of M SV sodium hydroxide, add phenolphthalein SI, and titrate the M SV sodium hydroxide in excess with 0.5 M SV sulfuric acid. Each mL of M SV sodium hydroxide is equal to 3.086 of P₂O₅.

PACKAGING AND STORAGE

In tightly closed containers.

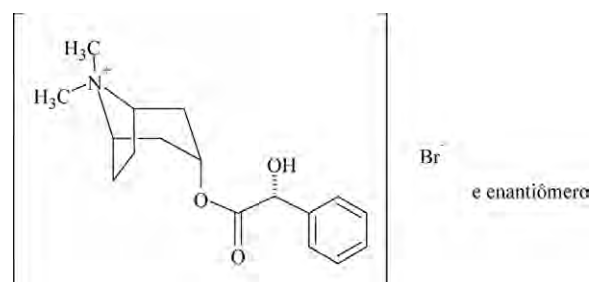
LABELLING

To comply with the legislation in force.

CATEGORY

Buffering agent.

METHYLBROMIDE, HOMATROPINE Homatropini methylbromidum



C₁₇H₂₄BrNO₃; 370.28
 homatropine methylbromide; 04747
 (3-endo) -3 - [(2-hydroxy-2-phenylacetyl) oxy]
 -8,8-dimethyl-8-azoniabicyclo [3.2.1] octane bromide
 (1:1)
 [80-49-9]

It contains at least 98.5% and at most 100.5% of C₁₇H₂₄BrNO₃, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White, crystalline powder, or colorless crystals. Melting range (5.2.2): around 190 °C.

Solubility. Freely soluble in water, soluble in ethanol, practically insoluble in ethyl ether.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the homatropine methylbromide SQR spectrum similarly prepared. If there are differences between in the spectra, individually dissolve the sample and standard in methanol and recrystallize it by adding dioxane to each solution.

B. The ultraviolet absorption spectrum (**5.2.14**), from 200 nm to 400 nm, 0.1% sample solution (w/v) in ethanol, has maximum at 258 nm, similar to those observed in the spectrum of a similar solution of homatropine methylbromide SQR.

C. To dissolve 0.1 g of sample in 5 mL of water and add mercury potassium iodide SR. White or slightly yellowish precipitate is produced. No precipitate is produced by adding alkalis or carbonates, even at concentrated solutions of the sample (most distinction in alkaloids).

D. To dissolve 0.1 g of the sample in 5 mL of water and add 3 ammonium reineckate SR. Red precipitate is produced.

E. The 5% aqueous solution (w/v) of the sample responds to the reactions of bromide ion (**5.3.1.1**).

PURITY TESTS

Appearance of solution. The 5% solution (w/v) of the sample in carbon dioxide-free water is clear (**5.2.25**) and colorless (**5.2.12**).

pH (5.2.19). 4.5 to 6.5. To determine in 1% solution (w/v) in carbon dioxide-free water.

Related substances. To proceed as described in *Thin-layer chromatography* (**5.2.17.1**) by using G silica gel as support and a mixture of formic acid Anhydrous, water, and ethyl acetate (16.5:16.5:67) as mobile phase. To separately apply to the plate 5 µL of each of the solutions freshly prepared, as described below.

Solution (1): to dissolve 0.2 g of the sample in a mixture of water and methanol (1:9) and dilute to 5 mL with the same solvent.

Solution (2): to dilute 0.5 mL of *Solution (1)* to 100 mL with a mixture of water and methanol (1:9).

To develop the chromatogram. To remove the plate, dry it in an oven at 100-105 °C until the solvent odor is no longer perceptible. To cool down.

To nebulize with diluted potassium iodobismuthate SR followed by 3% hydrogen peroxide SR (w/v). Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (2)* (0.5%).

Volatile organic impurities. To proceed as described in *Gas Chromatography* (**5.2.17.5**). To use gas chromatograph equipped with flame ionization detector; melted silica chromatographic column (30 m long, 0.53 mm inner diameter), coated with silica chemically bonded to phenylmethylpolysiloxane (5:95) (5 µm); 5 m long pre-column and 0.53 mm inner diameter with silica deactivated with phenylmethylsiloxane To set the column temperature according to the following parameters: to keep at 35 °C for 5 minutes, increase to 175 °C at 8 °C per minute, then to 260

°C at 35 °C per minute. To keep it for at least 16 minutes. To keep injector temperature and detector temperature at 70 °C and 260 °C, respectively. To use helium as carrier gas at linear velocity 35 cm/second.

Sample solution: to dissolve in water free of organic compound an accurately weighed amount of the sample, to obtain a 20 mg/mL solution.

Standard solution: to prepare a solution in water free of organic compounds containing 0.04 µg/mL of benzene, 12.0 µg/mL of methylene chloride, 1.2 µg/mL of chloroform, 7.6 µg/mL of dioxane, and 1.6 µg/mL of trichlorethylene.

To inject replicates of 1 µL of the *Reference solution*. The resolution between any component should not be greater than 1.0. The relative standard deviation for replicate areas of the peaks recorded should not be greater than 15.0%.

Procedure: to separately inject 1 µL of *Standard solution* and *Sample solution*. To record the chromatograms and measure the areas under the peaks. The areas under the benzene, methylene chloride, chloroform, dioxane, and trichlorethylene peaks obtained with the *Sample solution* should not be greater than the areas under the benzene, methylene chloride, chloroform, dioxane, and trichlorethylene peaks obtained with the *Standard solution*, corresponding to at most 2 ppm, 600 ppm, 60 ppm, 380 ppm, and 80 ppm, respectively.

Loss on drying (5.2.9). To determine in an oven at 105 °C for 3 hours. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.2%.

DOSAGE

To employ one of the methods described below.

A. To dissolve 0.3 g of the sample in 10 mL of water. To titrate with 0.1 M SV silver nitrate. To potentiometrically determine the endpoint by using silver indicator electrode and the silver/silver chloride reference electrode. Each mL of 0.1 M SV silver nitrate is equivalent to 37.028 mg of $C_{17}H_{24}BrNO_3$.

B. To proceed as described in *Titrimetric methods in non-aqueous medium* (**5.3.3.5**). To accurately weigh about 0.7 g of sample dissolved in a mixture of 50 mL of glacial acetic acid and 10 mL of mercury acetate SR. To add a drop of methylrosanilinium chloride SI and titrate with 0.1 M SV perchloric acid to obtain bluish-green color. To perform sample blank correction and adjust it as required. Each mL of 0.1 M SV perchloric acid is equal to 37.028 mg of $C_{17}H_{24}BrNO_3$.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

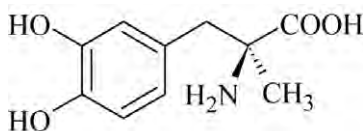
LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Anticholinergic.

METHYLDOPA Methyldopum



$C_{10}H_{13}NO_4$; 211.21

$C_{10}H_{13}NO_4 \cdot 1 \frac{1}{2} H_2O$; 238.24

methyldopa; 05799

methyldopa sesquihydrate; 09496

3-Hydroxy- α -methyl-L-tyrosine

[555-30-6]

3-Hydroxy- α -methyl-L-tyrosine hydrate (2:3)

[41372-08-1]

It contains at least 98.0% and at most 101.0% of $C_{10}H_{13}NO_4$ in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. Crystalline, white or yellowish-white powder, or colorless or almost colorless crystals.

Solubility. Sparingly soluble in water, glacial acetic acid and methanol, very slightly soluble in ethanol, practically insoluble in ethyl ether. Soluble in dilute mineral acid solutions.

Physical and chemical constants.

Specific rotation (5.2.8): -25° to -28° in relation to the anhydrous substance. To determine in 4.4% solution (w/v) in aluminum chloride SR.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the methyldopa SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14), ranging from 200 nm to 400 nm, of 0.004% solution (w/v) in 0.1 M hydrochloric acid, exhibits maxima at 280 nm, calculated on the anhydrous basis. The absorbance does not differ more than 3% compared to the methyldopa SQR similarly prepared.

C. To Add 3 drops of 0.4% ninhydrin (w/v) in sulfuric acid to 10 mg of the sample. Dark violet develops after 10 - 15 minutes. To add 3 drops of water. Color turns pale yellowish-brown.

PURITY TESTS

Acidity. To dissolve under heating 1 g of the sample in carbon dioxide-free water. To add a drop of methyl red SI and titrate with 0.1 M sodium hydroxide to yellow color develops. At most 0.5 mL of titrant is spent to change color.

Limit for Methyl 3-O-methyldopa. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using chromatographic cellulose, 0.25 mm thickness, as a support, and a mixture of 1-butanol, glacial acetic acid, and water (65:15:25) as mobile phase. To separately apply to the plate 20 μ L of *Solution (1)* and 10 μ L of *Solution (2)* freshly prepared, as described below.

Solution (1): to dissolve about 0.1 g of the sample in methanol and dilute to 10 mL with the same solvent, thus obtaining 10 mg/mL solution.

Solution (2): to dissolve 5 mg of Methyl 3-O-methyldopa SQR in methanol and dilute to 50 mL with the same solvent, thus obtaining 0.1 mg/mL solution.

To develop the chromatogram. To remove the plate, and dry in warm air. To nebulize with *p*-nitroaniline and sodium nitrite SR and dry in warm air. To nebulize with 20% sodium carbonate decahydrate (w/v). Any spot corresponding to methyl 3-O-methyldopa obtained in the chromatogram with *Solution (1)* is not more intense than that obtained with *Solution (2)* (0.5%).

Heavy metals (5.3.2.3). To use *Method III*. At most 0.001% (10 ppm).

Water (5.2.20.1). To determine in 0.2 g of the sample. From 10.0% to 13.0%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To proceed as described in *Titrations in non-aqueous medium* (5.3.3.5). To accurately weigh about 0.2 g of the sample and dissolve in 25 mL of glacial acetic acid, by heating it, if necessary. To add 50 mL of acetonitrile, 0.1 mL of methylrosanilinium chloride SI and titrate with 0.1 M SV perchloric acid to it turns blue. To perform sample blank correction and adjust it as required. Each mL of 0.1 M SV perchloric acid is equal to 21.121 mg of $C_{10}H_{13}NO_4$.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antihypertensive.

METHYLDOPA TABLETS

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_{10}H_{13}NO_4$. Tablets must be coated.

IDENTIFICATION

A. To weigh and pulverize the tablets. To use an amount of powder equivalent to 10 mg of methyldopa. To add 3 drops of 0.4% ninhydrin (w/v) in sulfuric acid. Dark violet develops after 10 - 15 minutes. To add 3 drops of water. The color becomes pale yellowish-brown.

B. To weigh and pulverize the tablets. To use an amount of powder equivalent to 10 mg of methyldopa, add 2 mL of 0.05 M sulfuric acid, 2 mL of ferrous tartrate SR, and 0.25 mL of 6 M ammonium hydroxide. Dark violet color develops.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 900 mL

Equipment: blades, 50 rpm

Time: 20 minutes

Procedure: after testing, remove aliquot from the dissolution medium, filter, and dilute in 0.1 M hydrochloric acid, to obtain suitable concentration. To measure the absorbances of the solutions at 280 nm (5.2.14), by using the same solvent to adjust zero. To calculate the amount of $C_{10}H_{13}NO_4$ dissolved in the medium, by comparing the readings obtained with that of 0.005% methyldopa SQR solution (w/v) prepared in the same solvent.

Tolerance: not less than 80% (Q) of the amount stated of $C_{10}H_{13}NO_4$ dissolves within 20 minutes.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To proceed as described in *Visible absorption spectrophotometry* (5.2.14). To weigh and pulverize 20 tablets. To transfer an amount of powder equivalent to 0.1 g of methyldopa to a 100 mL volumetric flask, add 50 mL of 0.05 M sulfuric acid, and mechanically stir for 15 minutes. To top up the volume with the same solvent. To make it homogenous and filter it. To accurately weigh about 50 mg of methyldopa SQR, transfer to a 50 mL volumetric flask, dissolve, and top up with 0.05 M sulfuric acid, and make it homogeneous. To individually transfer 5 mL of *Standard and sample solutions* to a 100 mL volumetric flasks. To simultaneously prepare a blank by using 5 mL of 0.05 M sulfuric acid. To add 5 mL of ferrous tartrate SR to each flask and top up with ammonium acetate buffer pH 8.5. To measure the absorbances of the resulting solutions at 520 nm, by using the blank to adjust zero. To calculate the amount of $C_{10}H_{13}NO_4$ in the tablets, from the readings obtained.

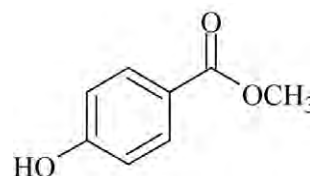
PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

METHYLPARABEN
Methylis parahydroxybenzoas



$C_8H_8O_3$; 152.15
 methylparaben; 05809
 4-hydroxybenzoic acid methyl ester
 [99-76-3]

It contains at least 98.0% and at most 102.0% of $C_8H_8O_3$.

DESCRIPTION

Physical characteristics. Crystalline, white or colorless powder.

Solubility. Sparingly soluble in water, freely soluble in acetone, ethanol, and ethyl ether.

Physical and chemical constants. *Melting range* (5.2.2): 125 °C to 128 °C.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the methylparaben SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14) ranging from 230 nm to 280 nm, of 0.0005% solution (w/v) in ethanol, exhibits maxima at 258 nm. The absorbance at 258 nm is between 0.52 and 0.56.

PURITY TESTS

Appearance of solution. To dissolve 1 g of the sample in ethanol and dilute to 10 mL with the same solvent. The solution is clear (5.2.25) and colorless (5.2.12).

Acidity. To add 3 mL of ethanol, 5 mL of carbon dioxide-free water, and 0.1 mL of bromocresol green SI to 2 mL of solution obtained in *Appearance of solution*. At most 0.1 mL of 0.1 M sodium hydroxide is spent to change the indicator.

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel as support and a mixture of formic acid anhydrous, ethyl acetate, and methylene chloride (2:10:88) as mobile phase. To separately apply to the plate 5 µL of each of the solutions freshly prepared, as described below.

Solution (1): to dissolve 0.1 g of the sample in methanol and dilute to 5 mL with the same solvent.

Solution (2): to dilute 1 mL of *Solution (1)* to 100 mL with methanol.

To develop the chromatogram. To remove the plate, and allow it to dry in hot air. To examine under ultraviolet light (254 nm). Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (2)* (1%).

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To accurately weigh about 1 g of the sample, transfer to erlenmeyer flask fitted with ground stopper, and add 20 mL of *M* SV sodium hydroxide. To adapt reflux condenser and heat at 70 °C for 1 hour. To cool down at room temperature. To titrate the *M* SV sodium hydroxide in excess with 0.5 M SV sulfuric acid. To potentiometrically determine the endpoint, and proceed titration to the second inflection point. To perform sample blank correction and adjust it as required. Each mL of *M* SV sodium hydroxide is equal to 152.1 mg of C₆H₉N₃O₃.

PACKAGING AND STORAGE

In tightly closed containers.

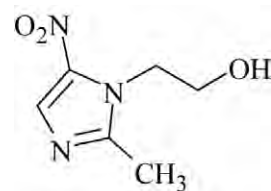
LABELLING

To comply with the legislation in force.

CATEGORY

Preservative.

METRONIDAZOLE
Metronidazolum



C₆H₉N₃O₃; 171.15

metronidazole; 05902

2-Methyl-5-nitro-1*H*-imidazole-1-ethanol
[443-48-1]

It contains at least 99.0% and at most 101.0% of C₆H₉N₃O₃, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white or slightly yellowish powder.

Solubility. Slightly soluble in water and ethanol, sparingly soluble in ethyl ether and methylene chloride.

Physical and chemical constants. *Melting range* (5.2.2): 159 °C to 163 °C.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the metronidazole SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14), ranging from 230 nm to 350 nm, of 0.002% sample solution (w/v) in 0.1 M hydrochloric acid, exhibits maxima at 277 nm and minima at 240 nm. The absorbance at 277 nm is approximately 0.76.

C. To weigh approximately 10 mg of sample and heat it for 5 minutes in a water bath with 10 mg of granulated zinc, 1 mL of water, and 0.25 mL of hydrochloric acid. To cool down in an ice bath and add 0.5 mL of nitric acid SR. To

remove nitrite in excess with sulfamic acid. To add 0.5 mL of 2-naphthol SR and 2 mL of 0.5 M sodium hydroxide. Reddish-orange color is developed.

PURITY TESTS

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel as support and a mixture of chloroform and diethylamine (90:10) as mobile phase. To separately apply to the plate 5 µl of each of the solutions as described below.

Solution (1): 2% solution (w/v) of the sample in acetone.

Solution (2): 0.01% solution (w/v) of the sample in acetone.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (2)* (0.5%).

Non-basic substances. 1 g of the sample completely dissolves in 10 mL of 50% hydrochloric acid (v/v).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 105 °C for 3 hours. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To proceed as described in *Titrations in non-aqueous medium* (5.3.3.5). To accurately dissolve about 0.3 g of the sample, previously desiccated, in 20 mL of acetic anhydride, and slowly heat it, if necessary. To cool down, add a drop of green malachite SI, and titrate with 0.1 M SV perchloric acid, by using micro-burette to it becomes greenish-yellow. To perform sample blank correction and adjust it as required. To potentiometrically determine the endpoint, as an alternative. Each mL of 0.1 M SV perchloric acid is equal to 17.115 mg of C₆H₉N₃O₃.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antimicrobial.

METRONIDAZOLE TABLETS

It contains at least 90.0 % and at most 110.0 % of the amount stated of C₆H₉N₃O₃. Tablets may be coated.

IDENTIFICATION

The identification test B. may be omitted if the tests A., C., and D. are conducted. The identification tests C. and D. can be omitted if the tests A. and B. are carried out.

A. To weigh and pulverize the tablets. To stir for 15 minutes an amount of powder equivalent to 0.1 g of metronidazole with 40 mL of chloroform. To filter and evaporate the filtrate to dryness. To proceed as described in *Identification*, test A., in the monograph *Metronidazole*.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method **B.** corresponds to that of main peak of *Standard solution*.

C. To weigh and pulverize the tablets. To use an amount of powder equivalent to 0.1 g of metronidazole, heat it for 5 minutes in water bath with 10 mg of zinc powder, 1 mL of water, and 0.25 mL of hydrochloric acid. To cool down in an ice bath and add 0.5 mL of sodium nitrite SR. To remove nitrite in excess with sulfamic acid. To add 0.5 mL of 2-naphthol SR1 and 2 mL of 0.5 M sodium hydroxide..

The red-orange color develops.

D. To weigh and pulverize the tablets. To stir an amount of powder equivalent to 0.2 g of metronidazole with 4 mL of 0.5 M sulfuric acid. To filter it. To add 10 mL of picric acid SR to the filtrate and let it rest. After being washed with water and dried at 105 °C, the melting point of the precipitate is about 150 °C.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

Procedure for content uniformity. To transfer each tablet to 250 mL volumetric flask To add 100 mL of 1% hydrochloric acid (v/v) and stir it for 30 minutes. To top up with the same solvent and make it homogeneous. To filter it by discarding the first 15 mL of the filtrate. To proceed as described in method A., Dosage, from "To successively dilute..."

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 1000 mL

Equipment: baskets, 100 rpm

Time: 60 minutes

Procedure: after testing, remove aliquot from the dissolution medium, filter, and dilute in 0.1 M hydrochloric acid, to obtain suitable concentration. To measure the absorbances at 274 nm (5.2.14), by using the same solvent to adjust zero. To calculate the amount of $C_6H_9N_3O_3$ dissolved in the medium, by comparing the readings obtained with that of 0.002% standard solution (w/v) prepared in the same solvent.

Solution (2): 0.2 mg/mL 2-methyl-5-nitroimidazole SQR solution in a mixture of chloroform and methanol (1:1).

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). Any 2-methyl-5-nitroimidazole spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (2)* (0.5%).

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. To weigh and pulverize 20 tablets. To transfer an amount of powder equivalent to 0.2 g of metronidazole to 100 mL volumetric flask and add 70 mL of 1% hydrochloric acid (v/v). To mechanically stir for 15 minutes and top up with the same solvent. To filter it. To successively dilute to obtain 0.002% concentration (w/v) by using 1% hydrochloric acid (v/v) as solvent. To prepare standard solution under the same conditions. To measure the absorbances of the solutions at 278 nm, by using 1% hydrochloric acid (v/v) to adjust zero. To calculate the amount of $C_6H_9N_3O_3$ in the tablets, from the readings obtained.

B. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 254 nm; 150 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octylsilane group (3 μ m to μ m), kept at room temperature; flow of *Mobile phase* of 1.0 mL/min.

Tolerance: not less than 85% (Q) of the amount stated of $C_6H_9N_3O_3$ dissolves within 60 minutes.

PURITY TESTS

Limit for 2-methyl-5-nitroimidazole. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using F_{254} silica gel as support and a mixture of chloroform, dimethyl formamide, and 90% formic acid (v/v) (80:25:5) as mobile phase. To separately apply to the plate 20 μ L of each of the solutions freshly prepared, as described below.

Solution (1): to weigh and pulverize 20 tablets. To stir for 5 minutes an amount of powder equivalent to 0.2 g of metronidazole with 5 mL of a mixture of chloroform and methanol (1:1). To filter it.

Sample solution: to weigh and pulverize 20 tablets. To transfer an amount of powder equivalent to 0.5 g of metronidazole to 50 mL volumetric flask, add 30 mL of methanol, and mechanically stir for 30 minutes. To top up with methanol and wait to decant. To transfer 5 mL of the supernatant to a 100 mL volumetric flask and top up with *Mobile phase*.

Standard solution: 0.5 mg/ mL metronidazole SQR solution in *Mobile phase*.

To inject replicates of 10 μ L of the *Standard solution*. The tailing factor should not be greater than 2.0. The relative standard deviation for the replicate areas for the peaks recorded is not greater than 2.0%.

Procedure: to separately inject 10 μ L of *Standard solution* and *Sample solution*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of $C_6H_9N_3O_3$ in the tablets, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

METRONIDAZOLE INJECTABLE SOLUTION

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_6H_9N_3O_3$.

IDENTIFICATION

A. To transfer volume of injectable solution equivalent to about 0.1 g of metronidazole to the separatory funnel and stir with 9 g of sodium chloride, for 5 minutes. To extract with 20 mL of acetone. To separate the upper layer and

evaporate it to dryness. The infrared absorption spectrum (5.2.14) of the residue, dispersed in potassium bromide, has absorption maxima only at the same wavelength, with the same relative intensities to those observed in the metronidazole SQR spectrum similarly prepared.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method **B.** corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

pH (5.2.19). 4.5 to 7.0.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

Bacterial endotoxins (5.5.2.2). At most 0.35 UE/ mg of metronidazole.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To transfer a volume of injectable solution containing the equivalent to 50 mg of metronidazole to a 100 mL volumetric flask and top up with 0.1 M hydrochloric acid. To successively dilute in 0.1 M hydrochloric acid to obtain 0.002% concentration (w/v). To prepare standard solution under the same conditions.

To measure the absorbances of the resulting solutions at 277 nm, by using 0.1 M hydrochloric acid to adjust zero. To calculate the amount of $C_6H_9N_3O_3$ in the injectable solution, from the readings obtained.

B. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 320 nm; 250 mm column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (3 μ m to 10 μ m), flow of *Mobile phase* of 2.0 mL/min.

Mobile phase: mixture of 0.073% monobasic potassium phosphate (w/v) and methanol (93:7). To adjust pH to 4.0 $\pm$ 0.5 with 0.1 M phosphoric acid.

Sample solution: to transfer volume of injectable solution equivalent to 25 mg of metronidazole to 25 mL volumetric flask and top up with water. To transfer 2 mL of the solution obtained to 10 mL volumetric flask containing 2 mL of methanol and top up with *Mobile phase*.

Standard solution: to accurately transfer about 25 g of metronidazole SQR to 25 mL volumetric flask, dissolve in

methanol, and top up the volume with the same solvent. To transfer 2 mL of the resulting solution to 10 mL volumetric flask containing 2 mL of water and top up with *Mobile phase*.

To inject replicates of 20 μ L of the *Standard solution*. The tailing factor is not greater than 2.0. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2.0 %.

Procedure: to separately inject 20 μ L of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_6H_9N_3O_3$ in the injectable solution, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

MIXTURES, SURPLUS HUMAN PLASMA, VIRUS INACTIVATION TREATMENT

Plasma Humanum Collectum Excederem Deinde Conditum ad Viros Exstinguendos

Frozen or lyophilized, sterile, pyrogen-free preparation from human plasma from donors having the same ABO and Rh(D_u) blood group. The preparation is thawed or reconstituted prior to use, in order to obtain an injectable solution. The human plasma used should meet the requirements of the monograph *Human Plasma for Fractionating*.

The plasma units for production are frozen at -30 °C or below within 6 hours following the separation of blood cell fractions, within 24 hours after collection. The mixture is prepared from plasma units having the same ABO and Rh (D_u) blood group.

The mixture of plasma is examined by employing appropriate sensitivity and specificity methods for the presence of the surface antigen of hepatitis B virus (HBsAg), hepatitis C antibodies, and HIV antibodies. In these tests, the mixture of plasma must submit negative results.

The mixture of plasma must also be submitted to RNA research of hepatitis C as described in *Amplification Techniques for Nucleic Acids* (5.5.1.10) duly validated. The test includes a positive pattern of 100 IU/mL RNA of hepatitis C virus and to identify the presence of inhibitors, an internal standard is prepared by adding an appropriate marker in the sample of plasma mixture. The test is only valid if the positive pattern is reactive or if the result obtained with the internal standard does not indicate the presence of inhibitors.

The mixture complies with the test if it is not reactive to RNA of hepatitis C.

The mixture of plasma must also be submitted to DNA research of B19 virus, as described in *Amplification Techniques for Nucleic Acids* (5.5.1.10) duly validated. The pool should not exceed 10 IU per microliter. A positive control of 10 IU per microliter of DNA for B19 virus and to identify the presence of inhibitors, an internal standard is prepared by adding an appropriate marker in the sample of plasma mixture. The test is only valid if the positive pattern is reactive or if the result obtained with the internal standard does not indicate the presence of inhibitors.

The method of preparation is carried out to avoid activating any clotting factor and thus limit their potential thrombogenic action. It comprises one or more stages related to the elimination or inactivation of infectious agents known. Should substances be used for viral inactivation during the production, the subsequent purification process must be validated to demonstrate that the concentration of these substances is at an appropriate level and any residues do not compromise the safety of the preparation.

The typical method used for the inactivation of enveloped viruses is the solvent-detergent process, which comprises a treatment with a mixture of tributyl phosphate and octoxynol 10. Subsequently, these reagents are removed by solid or oily phase extraction, so that the residual content in the final product is less than 2 µg/mL for tributyl phosphat, and 5 µg/mL for octoxynol 10. No antimicrobial preservative must be added.

The solution is filtered through a sterilizing filter and aseptically distributed in the final containers and frozen immediately. The final containers are composed of plastic material and comply with the requirements for *Plastic containers* (6.2), or glass, by complying with the requirements for *Glass containers* (6.1). It may then be lyophilized.

IDENTIFICATION

To reconstitute or thaw the sample as stated on the label immediately before carrying out identification tests and trials.

A. To perform electrophoresis examination by comparing the sample with normal human plasma. The electrophoretograms have the same bands.

B. To perform precipitation tests from a suitable range of sera specific for domestic animal. The test should be performed with specific plasma protein sera from each of the domestic species commonly used in the country, to prepare biological products. The sample contains human proteins and it gives negative results to specific sera with plasma protein from other species.

C. The mixture complies with the Determination of titer *anti-A and anti-B Hemagglutinins* (see *Dosage*).

CHARACTERISTICS

Appearance. After thawing it, the liquid solution is clear or slightly opalescent, free from solid particles, and gelatinous. The lyophilized preparation is a white or light yellow powder or friable solid.

pH (5.2.19). 6.5 to 7.6.

Osmolality (5.2.28). At least 240 mosmol/kg.

PHYSICAL AND CHEMICAL TESTS

Water. To determine by using one of the following methods: *Determination of water by semi-micro method* (5.2.20.3), *Loss on drying* (5.2.9), or *Infrared absorption spectrophotometry* (5.2.14). The content is within the limits approved by the competent authorities.

Citrate. At most 25 mmol/L. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 215 nm; 300 mm column and 7.8 mm inner diameter, packed with cation-exchange resin (9 µm), flow of *Mobile phase* of 0.5 mL/min.

Mobile phase: 0.051% sulfuric acid solution (w/v).

Sample solution: to dilute the sample with an equal volume of a 0.9% sodium chloride solution (w/v). To filter through 0.45 µm filter.

Standard solution: to dissolve 0.3 g of sodium citrate in water and dilute to 1000 mL with the same solvent.

Procedure: to separately inject 10 µL of *Standard solution* and *Sample solution*. The retention time of citrate is about 10 minutes. Column balance time: 15 minutes

Calcium. To proceed as described in *Atomic absorption spectrometry* (5.2.13.1). To determine the wavelength at 622 nm. At most 5 mmol/L.

Potassium. To proceed as described in *Atomic absorption spectrometry* (5.2.13.2). To determine in the wavelength. 766.5 nm. At most 5 mmol/L.

Sodium. To proceed as described in *Atomic absorption spectrometry* (5.2.13.2). To determine in the wavelength at 589 nm. At most 200 mmol/L.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

Pyrogens (5.5.2.1). It complies with the test. To inject into each rabbit, per kilogram of body weight, volume corresponding to 3 mL of sample.

DOSAGE**Irregular erythrocyte antibodies.**

When examined by indirect antiglobulin test, the undiluted sample shows no signs of irregular erythrocyte antibodies.

Hepatitis A antibodies.

At least 2 IU/mL determined by *suitable Immunochemical methods (5.6)*. The pattern of human immunoglobulin for Hepatitis A is suitable for use as a reference preparation.

Anti-A and anti-B hemagglutinins.

To proceed as described in *Determination of Anti-A and Anti-B hemagglutinin (5.5.1.9)*. The presence of the Hemagglutinins (anti-A or anti-B) corresponds to blood group on the label.

Activated Clotting Factors.

To proceed as described in *Determination of activated clotting factors (5.5.1.8)*. To perform the test with 0.1 mL of sample instead of dilutions at 1/10 and 1/100. The clotting time related to the tube containing the sample is not less than 150 seconds. It complies with the test.

Factor V.

To prepare 3 dilutions at 1/10 and 1/40 of the sample by using imidazole buffer, pH 7.4, preferably prepared in duplicate. To proceed as follows for each dilution: to mix 0.1 mL of substrate for Factor V deficient plasma, 0.1 mL of dilution from sample, 0.1 mL of thromboplastin reagent, and 0.1 mL of 0.35% calcium chloride solution (w/v). To record the clotting time, that is, the time interval between the addition of calcium chloride solution and the first signs of formation of fibrin.

To observe by using an appropriate equipment. To determine in duplicate and under the same conditions, the clotting times of 4 dilutions between 1/10 and 1/80 of normal human plasma in the imidazole buffer pH 7.4. A unit of Factor V corresponds to activity of 1 mL of normal human plasma. The normal human plasma is prepared from a mixture of plasma units from at least 30 donors and kept at -30 °C or below. To check the validity of the test and calculate the activity of the sample through *Statistical procedures applicable to biological tests (8)*. The activity determined is not less than 0.5 units/mL. The confidence interval ($P = 0.95$) of the activity determined does not exceed 80% - 120%.

Factor VIII.

Proceed as described in *Determination of Blood Clotting Factor VIII, lyophilized (5.5.1.7)* by using a standard plasma calibrated against the International Standard Factor VIII for human blood clotting. The activity determined is

not less than 0.5 IU/mL. The confidence interval ($P = 0.95$) of the activity determined does not exceed 80% - 120%.

Total protein

To dilute the sample in 0.9% sodium chloride solution (w/v), to obtain a solution containing about 15 mg of protein in 2 mL. To add 2 mL of this solution into round bottom centrifuge tube. To add 2 mL of 7.5% sodium molybdate solution (w/v) and 2 mL of a mixture of nitrogen-free sulfuric acid and water (1:30). To stir, centrifuge for 5 minutes, decant the supernatant, and invert the tube on a filter paper to drain it. To perform dosage of nitrogen in the residue by the digestion method with sulfuric acid, as described in *Determination of nitrogen by the Kjeldahl method (5.3.3.2)* and calculate the protein content by multiplying the result by 6.25. The total protein content is not less than 45 g/L.

LABELLING

The label should indicate the ABO and Rh (D_u) blood group and the method used for viral inactivation. To comply with the legislation in force.

**MIXTURES, HUMAN PLASMA, VIRUS
INACTIVATION TREATMENT**

**Plasma Humanum Collectum Deinde Conditum
ad Viros Exstinguendos**

Frozen or lyophilized, sterile, pyrogen-free preparation from human plasma from donors having the same ABO and Rh(D_u) blood group. The preparation is thawed or reconstituted prior to use, in order to obtain an injectable solution. The human plasma used should meet the requirements in the monograph for *Human Plasma for Fractionating*.

The plasma units for production are frozen at -30 °C or below within 6 hours following the separation of blood cell fractions, within 24 hours after collection. The mixture is prepared from plasma units having the same ABO and Rh (D_u) blood group.

The mixture of plasma is examined by employing appropriate sensitivity and specificity methods for the presence of the surface antigen of hepatitis B virus (HBsAg), hepatitis C antibodies, and HIV antibodies. In these tests, the mixture of plasma must submit negative results.

The mixture of plasma must also be submitted to RNA research of hepatitis C as described in the monograph *Amplification Techniques for Nucleic Acids (5.5.1.10)* duly validated. The test includes a positive pattern of 100 IU/mL RNA of hepatitis C virus and to identify the presence of inhibitors, an internal standard is prepared by adding an appropriate marker in the sample of plasma mixture. The test is only valid if the positive pattern is reactive or if the result obtained with the internal standard does not indicate the presence of inhibitors.

The mixture complies with the test if it is not reactive to RNA of hepatitis C.

The mixture of plasma must also be submitted to DNA research of B19 virus as described in the monograph *Amplification Techniques for Nucleic Acids* (5.5.1.10) duly validated. The pool should not exceed 10 IU per microliter. A positive control of 10 IU per microliter of DNA for B19 virus and to identify the presence of inhibitors, an internal standard is prepared by adding an appropriate marker in the sample of plasma mixture. The test is only valid if the positive pattern is reactive or if the result obtained with the internal standard does not indicate the presence of inhibitors.

The method of preparation is carried out to avoid activating any clotting factor and thus limit their potential thrombogenic action. It comprises one or more stages related to the elimination or inactivation of infectious agents known. Should substances be used for viral inactivation during the production, the subsequent purification process must be validated to demonstrate that the concentration of these substances is at an appropriate level and any residues do not compromise the safety of the preparation.

The typical method used for the inactivation of enveloped viruses is the solvent-detergent process, which comprises a treatment with a mixture of tributyl phosphate and octoxynol 10. Subsequently, these reagents are removed by solid or oily phase extraction, so that the residual content in the final product is less than 2 µg/mL for tributyl phosphat, and 5 µg/mL for octoxynol 10. No antimicrobial preservative must be added.

The solution is filtered through a sterilizing filter and aseptically distributed in the final containers and frozen immediately. The final containers are composed of plastic material and comply with the requirements for Plastic containers (6.2), or glass, by complying with the requirements for Glass containers (6.1). It may then be lyophilized.

IDENTIFICATION

To reconstitute or thaw the sample as stated on the label immediately before carrying out identification tests and trials.

A. To perform electrophoresis examination by comparing the sample with normal human plasma. The electrophoretograms have the same bands.

B. To perform precipitation tests from a suitable range of sera specific for domestic animal. The test should be performed with specific plasma protein sera from each of the domestic species commonly used in the country, to prepare biological products. The sample contains human proteins and it gives negative results to specific sera with plasma protein from other species.

C. C. The mixture complies with the *Determination of titer anti-A and anti-B Hemagglutinins* (see *Dosage*).

CHARACTERISTICS

Appearance. After thawing it, the liquid solution is clear or slightly opalescent, free from solid particles, and gelatinous. The lyophilized preparation appears is a white or light yellow powder or friable solid.

pH (5.2.19.). 6.5 to 7.6.

Osmolality (5.2.28). At least 240 mosmol/kg.

PHYSICAL AND CHEMICAL TESTS

Water. To determine by using one of the following methods: *Determination of water by semi-micro method* (5.2.20.3), *Loss on drying* (5.2.9), or *Infrared absorption spectrophotometry* (5.2.14). The content is within the limits approved by the competent authorities.

Citrate. At most 25 mmol/L. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 215 nm; 300 mm column and 7.8 mm inner diameter, packed with cation-exchange resin (9 µm), flow of *Mobile phase* of 0.5 mL/min.

Mobile phase: 0.051% sulfuric acid solution (w/v).

Sample solution: to dilute the sample with an equal volume of a 0.9% sodium chloride solution (w/v). To filter through 0.45 µm filter.

Standard solution: to dissolve 0.3 g of sodium citrate in water and dilute to 1000 mL with the same solvent.

Procedure: to separately inject 10 µL of *Standard solution* and *Sample solution*. The retention time of citrate is about 10 minutes. The column balance time is about 15 minutes.

Calcium. To proceed as described in *Atomic absorption spectrometry* (5.2.13.1). To determine the wavelength at 622 nm. At most 5 mmol/L.

Potassium. To proceed as described in *Atomic absorption spectrometry* (5.2.13.2). To determine in the wavelength at 766.5 nm. At most 5 mmol/L.

Sodium. To proceed as described in *Atomic absorption spectrometry* (5.2.13.2). To determine in the wavelength at 589 nm. At most 200 mmol/L.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

Pyrogens (5.5.2.1). To inject into each rabbit, per kilogram of body weight, volume corresponding to 3 mL of sample. It complies with the test.

DOSAGE

Irregular erythrocyte antibodies.

When examined by indirect antiglobulin test, the undiluted sample shows no signs of irregular erythrocyte antibodies.

Hepatitis A antibodies.

At least 2 IU/mL determined by suitable *Immunochemical method* (5.6). The pattern of human immunoglobulin for Hepatitis A is suitable for use as a reference preparation.

Anti-A and anti-B hemagglutinins.

To proceed as described in *Determination of Anti-A and Anti-B hemagglutinin* (5.5.1.9). The presence of the Hemagglutinins (anti-A or anti-B) corresponds to blood group on the label.

Activated Clotting Factors.

To proceed as described in *Determination of activated clotting factors* (5.5.1.8). To perform the test with 0.1 mL of sample instead of dilutions at 1/10 and 1/100. The clotting time related to the tube containing the sample is not less than 150 seconds. It complies with the test.

Factor V.

To prepare 3 dilutions at 1/10 and 1/40 of the sample by using imidazole buffer, pH 7.4, preferably prepared in duplicate. To proceed as follows for each dilution: to mix 0.1 mL of substrate for Factor V deficient plasma, 0.1 mL of dilution from sample, 0.1 mL of thromboplastin reagent, and 0.1 mL of 0.35% calcium chloride solution (w/v). To record the clotting time, that is, the time interval between the addition of calcium chloride solution and the first signs of formation of fibrin. To observe by using an appropriate equipment. To determine in duplicate and under the same conditions, the clotting times of 4 dilutions between 1/10 and 1/80 of normal human plasma in the imidazole buffer pH 7.4. A unit of Factor V corresponds to activity of 1 mL of normal human plasma. The normal human plasma is prepared from a mixture of plasma units from at least 30 donors and kept at -30 °C or below. To check the validity of the test and calculate the activity of the sample through *Statistical procedures applicable to biological tests* (8). The activity determined is not less than 0.5 units/mL. The confidence interval ($P = 0.95$) of the activity determined does not exceed 80% - 120%.

Factor VIII.

Proceed as described in *Determination of Blood Clotting Factor VIII, lyophilized* (5.5.1.7) by using a standard plasma calibrated against the International Standard Factor VIII for human blood clotting. The activity determined is

not less than 0.5 IU/mL. The confidence interval ($P = 0.95$) of the activity determined does not exceed 80% - 120%.

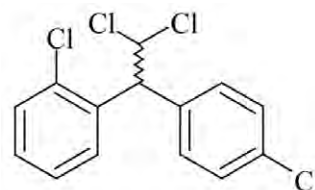
Total protein.

To dilute the sample in 0.9% sodium chloride solution (w/v), to obtain a solution containing about 15 mg of protein in 2 mL. To add 2 mL of this solution into round bottom centrifuge tube. To add 2 mL of 7.5% sodium molybdate solution (w/v) and 2 mL of a mixture of nitrogen-free sulfuric acid and water (1:30). To stir, centrifuge for 5 minutes, decant the supernatant, and invert the tube on a filter paper to drain it. To perform dosage of nitrogen in the residue by the digestion method with sulfuric acid, as described in *Determination of nitrogen by the Kjeldahl method* (5.3.3.2) and calculate the protein content by multiplying the result by 6.25. The total protein content is not less than 45 g/L.

LABELLING

The label should indicate the ABO and Rh (D_u) blood group and the method used for viral inactivation. To comply with the legislation in force.

MITOTANE Mitotanium



$C_{14}H_{10}Cl_4$; 320.04

mitotane; 06020

1-Chloro-2-[2,2-dichloro-1-(4-chlorophenyl) ethyl]

benzene

[53-19-0]

It contains at least 97.0% and at most 103.0% of $C_{14}H_{10}Cl_4$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystals of pentane or methanol.

Solubility. It is practically insoluble in water. Soluble in ethanol, ethyl ether, methanol, isooctane, carbon tetrachloride, hexane, and fixed and fatty oils.

Physical and chemical constants.

Melting range (5.2.2): 75 °C to 81 °C.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium chloride, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the mitotane SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14) ranging from 200 nm to 400 nm, of 0.02% solution (w/v) in methanol, exhibits maxima and minima only in the same wavelength of a similar mitotane SQR solution.

PURITY TESTS

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in a vacuum oven at 60 °C for 2 hours. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.5%.

DOSAGE

To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To accurately weigh about 0.1 mg of the sample and dissolve in methanol. To successively dilute in the same solvent to obtain 0.02% concentration (w/v).

To prepare a standard solution at the same concentration by using the same solvent. To measure the absorbance of the resulting solutions at 268 nm, by using methanol to adjust zero. To calculate the content of $C_{14}H_{10}Cl_4$ in the sample from the readings obtained.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force. To handle it with special care.

THERAPEUTIC CLASS

Antineoplastic.

MITOTANE TABLETS

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_{14}H_{10}Cl_4$.

IDENTIFICATION

To weigh and pulverize the tablets. To stir an amount of powder equivalent to 0.5 g of mitotane with 10 mL of wa-

ter. To filter through a sintered glass funnel and wash the residue with 2 portions (5 mL each) of water. To transfer the residue to a small beaker, add 4 mL of ethanol, simmer, and immediately filter it. To cool down, filter the crystals of mitotane, wash once with 2 mL of ethanol, and carry out vacuum dry at 60 °C for 2 hours. The infrared absorption spectrum (5.2.14) of the residue, dispersed in mineral oil, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the mitotane acid SQR spectrum similarly prepared.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). Within 15 minutes.

Uniformity of dosage units (5.1.6). It complies with the test.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To weigh and pulverize 20 tablets. To transfer an amount of powder equivalent to 0.1 g of mitotane to a 250 mL volumetric flask, add 100 mL of methanol, stir for 5 minutes, top up with methanol, make it homogeneous, and filter it. To transfer 25 mL of the filtrate to a 50 mL volumetric flask, top up with methanol, and make it homogeneous, to obtain 0.02% solution (w/v). To prepare a standard solution at the same concentration by using the same solvent. To measure the absorbances of the resulting solutions at 268 nm, by using methanol to adjust zero. To calculate the amount of $C_{14}H_{10}Cl_4$ in the tablets, from the readings obtained.

PACKAGING AND STORAGE

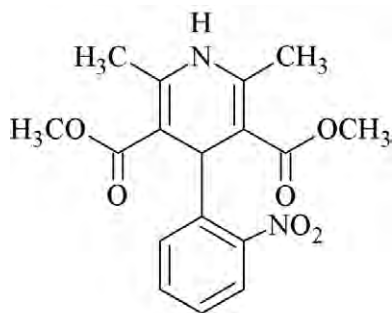
In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

NIFEDIPINE

Nifedipinum



C₁₇H₁₈N₂O₆; 346,34
nifedipine; 06352
1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-
pyridinedicarboxylic 3,5-dimethyl ester
[21829-25-4]

It contains at least 98.0% and at most 102.0% of C₁₇H₁₈N₂O₆,
in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Yellow, odorless, and tasteless crystals.

Solubility. Practically insoluble in water; soluble in ethyl acetate, slightly soluble in ethanol, sparingly soluble in acetone and chloroform.

Physical and chemical constants.

Melting range (5.2.2): 171 °C to 175 °C.

IDENTIFICATION

The identification tests **C.** and **D.** may be omitted if the tests **A.** and **B.** are conducted.

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the nifedipine SQR spectrum similarly prepared.

B. To dissolve 50 mg of nifedipine in 1 mL of dimethylsulfoxide. Yellow color with maximum absorption at 330 nm is developed. This color becomes red with by adding 5 drops of sodium hydroxide SR, absorbing at 451 nm.

C. To add a mixture of 2 mL of glacial acetic acid, 2 mL of dimethylsulfoxide, 5 drops of 2% chromium oxide solution (0.2 g of chromium oxide in 10 mL of acetic acid) to 30 mg of nifedipine acidic solution. This solution becomes yellowish-green.

D. To dissolve 25 mg of sample in 1 mL of 90% ethanol (v/v), 5 mL of 1% calcium chloride (w/v), and 19 mg of zinc powder.

To vigorously stir and then heat it in a water bath at 80 °C, for 10 minutes. To filter and add 5 drops of benzoyl solution to 3 mL of the filtrate. To stir for 1 minute and then add 10 drops of ferric chloride SR by stirring it. Upon adding hydrochloric acid, yellow and red color alternate.

PURITY TESTS

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel as support and a mixture of cyclohexane and ethyl acetate (6:4) as mobile phase. To separately apply to the plate 5 µL of each of the solutions freshly prepared, as described below.

Solution (1): 1 mg/mL solution of the sample in methanol.

Solution (2): 1 mg/mL nifedipine SQR solution in methanol.

Solution (3): 10 µg/mL solution of the sample in methanol.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (3)* (1.0%).

Chlorides (5.3.2.1). At most 0.02% (200 ppm).

Heavy metals (5.3.2.3). At most 0.001% (10 ppm).

Sulfates (5.3.2.2). At most 0.05% (500 ppm).

Loss on drying (5.2.9). To determine in 2 g of the sample. To desiccate in an oven at 60°C under reduced pressure for 3 hours. At most 1.0%.

Sulfated ashes (5.2.10). At most 0.1 %.

DOSAGE

To employ one of the methods described below.

B. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 235 nm; 250 mm chromatograph column and 4.0 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at room temperature; flow of *Mobile phase* of 1.0 mL/min.

Mobile phase: to prepare a mixture of water, acetonitrile, and methanol (50:25:25).

Sample solution: to accurately transfer about 25 mg of the sample to a 250 mL volumetric flask. To dissolve it in 25

mL of methanol and top up with *Mobile phase* to obtain known 0.1 mg/mL concentration.

Standard solution: to dissolve accurately weighed amount of nifedipine SQR in *Mobile phase*, to obtain a 0.1 mg/mL solution.

To inject replicates of the *Standard solution*. The column efficiency is greater than 16000 theoretical plates/meter. The tailing factor is not greater than 1.5. The standard deviation of the response of the main peak is not greater than 1%.

Procedure: to separately inject 25 µL of *Sample and standard solutions*. To record the chromatograms and measure the areas under the peaks. To calculate the amount of $C_{17}H_{18}N_2O_6$ from the responses from the *Standard solution* and *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Vasodilator.

NIFEDIPINE CAPSULES

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_{17}H_{18}N_2O_6$.

IDENTIFICATION

To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using G silica gel with 0.5 mm thickness as support and a mixture of ethyl acetate and cyclohexane (1:1) as mobile phase. To separately apply to the plate 0.5 µL of each of the solutions freshly prepared, as described below.

Solution (1): 1.2 mg/mL nifedipine SQR solution in dichloromethane.

Solution (2): to transfer the content of 3 capsules to centrifuge tube and wash inside the capsules with 20 mL of 0.1 M sodium hydroxide. To volumetrically add 20 mL of dichloromethane to the tube and close it. To gently stir and release the pressure inside the tube. To tightly close and stir for 1 hour. To centrifuge for 10 minutes at 2000-2500 rpm. To remove the supernatant aqueous phase by using a syringe and transfer 5 mL of the clear bottom layer to a beaker.

Developing solution: to dissolve 3 g of bismuth subnitrate and 30 g of potassium iodide in 10 mL of 3 M hydrochloric acid and transfer to a 100 mL volumetric flask. To top

up with water and make it homogeneous. To transfer 10 mL to a 100 mL volumetric flask. To add 10 mL of 3 M hydrochloric acid and top up with water. To make it homogeneous.

To develop the chromatogram protected from direct light. To remove the plate, and let it drying. To immediately examine under ultraviolet light (254 nm).

The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*. To nebulize the plate with *Developing solution*. Each chromatogram shows bright orange band over a yellow background.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

Procedure for content uniformity. To transfer the content of each capsule to a 200 mL volumetric flask. To wash inside the capsules with small portions of methanol, by collecting the washings in the flask. To top up to volume with methanol and make it homogeneous. To transfer 5 mL to 100 mL volumetric flask and top up with methanol. To prepare a standard solution at the same concentration by using the same solvent. To measure the absorbances of the resulting solutions at 350 nm (5.2.14), by using methanol to adjust zero. To calculate the amount of $C_{17}H_{18}N_2O_6$ in the capsules, from the readings obtained.

DISSOLUTION TEST (5.1.5)

Dissolution medium: simulated gastric fluid (no pepsin), 900 mL

Equipment: baskets, 50 rpm

Time: 20 minutes

Procedure: after testing, remove aliquot from the dissolution medium, filter, and dilute with *Dissolution medium*, to obtain suitable concentration. To measure the absorbances of solutions at 340 nm (5.2.14), by using the *Dissolution medium* to adjust zero. To calculate the amount of $C_{17}H_{18}N_2O_6$ dissolved in the medium, by comparing the readings obtained with that of 0.005% nifedipine SQR solution (w/v) prepared in the same solvent.

Tolerance: not less than 80% (Q) of the amount stated of $C_{17}H_{18}N_2O_6$ dissolves within 20 minutes.

PURITY TESTS

Related substances. To proceed as described in *Dosage, method B.*, in the monograph *Nifedipine*. To prepare Test solutions as described below.

Note: to immediately proceed to test after preparation of *Solution (1)* and *Solution (5)*, by protecting them from direct light.

Solution (1): to dissolve accurately weighed amount of nifedipine SQR in methanol, to obtain a 1 mg/mL solution. To dilute with *Mobile phase*, to obtain 0.3 mg/mL solution.

Solution (2): to dissolve accurately weighed amount of nitrophenyl pyridine SQR in methanol, to obtain a 1 mg/mL solution.

To dilute with *Mobile phase*, to obtain 6 µg/mL solution.

Solution (3): to dissolve accurately weighed amount of nitrosophenyl pyridine SQR in methanol, to obtain a 1 mg/mL solution. To dilute with *Mobile phase*, to obtain 1.5 µg/mL solution.

Solution (4): to mix 5 mL of *Solution (2)* with 5 mL of *Solution (3)* and 5 mL of *Mobile phase*. To make it homogeneous.

Solution (5): to proceed as described for *Sample solution* in method **B.**, *Dosage*.

Solution (6): to mix equal volumes of *Solution (1)*, *Solution (2)*, and *Solution (3)*.

To inject replicates of 25 µL of *Solution (6)*. The relative retention times are about 0.8 to nitrophenyl pyridine, 0.9 to nitrosophenyl pyridine, and 1.0 to nifedipine. The resolution between nitrophenyl pyridine and nitrosophenyl pyridine is greater than 1.5. The resolution between nitrosophenyl pyridine and nifedipine is greater than 1.0. The relative standard deviation for replicate areas of the peaks recorded corresponding to nitrophenyl pyridine and nitrosophenyl pyridine is not greater than 10.0 %.

Procedure: to separately inject 25 µL of *Solutions (4)* and *(5)*, record the chromatograms, and measure the areas under the main peaks. To calculate the amount, in mg, of impurities nitrophenyl pyridine and nitrosophenyl pyridine in the capsules, according to the equation:

$$\left(\frac{V}{5}\right) \times C \times \left(\frac{r_5}{r_4}\right)$$

where

V = total volume of *Solution (5)*, in mL

C = concentration of nitrophenyl pyridine or nitrosophenyl pyridine of *Solution (4)*, in mg/mL

r_5 = response of the nitrophenyl pyridine or nitrosophenyl pyridine peak in the chromatogram obtained with *Solution (5)*

r_4 = response of the nitrophenyl pyridine or nitrosophenyl pyridine peak in the chromatogram obtained with *Solution (4)*

At most 2.0% of nitrophenyl pyridine and 0.5% of nitrosophenyl pyridine in relation to the content of nifedipine.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. To protect it from direct light. To transfer the content of 10 capsules to a 100 mL volumetric flask. To wash inside the capsules with small portions of methanol, by collecting the washings in the flask. To top up with the same solvent and make it homogeneous. To dilute with methanol to obtain 0.005% concentration (w/v). To prepare a standard solution at the same concentration by using the same solvent. To measure the absorbances of the resulting solutions at 350 nm, by using methanol to adjust zero. To calculate the amount of $C_{17}H_{18}N_2O_6$ in the capsules, from the readings obtained.

B. To proceed as described in *Dosage*, method **B.**, in the monograph *Nifedipine*. To prepare the *Sample solution* as described below.

Sample solution: to transfer the content of 5 capsules to 100 mL volumetric flask by using small portions of methanol. To top up with the same solvent and make it homogeneous. To successively dilute in *Mobile phase*, to obtain 0.1 mg/mL nifedipine solution.

Procedure: to separately inject 25 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the main peaks. To calculate the amount of $C_{17}H_{18}N_2O_6$ in the capsules from the responses obtained from *Sample and standard solutions*.

PACKAGING AND STORAGE

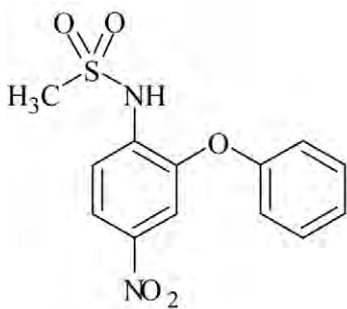
In airtight closed containers, protected from light, at 15 °C - 25 °C.

LABELLING

To comply with the legislation in force.

NIMESULIDE

Nimesulidum



$C_{13}H_{12}N_2O_5S$; 308.31

nimesulide; 06391

N-(4-Nitro-2-phenoxyphenyl) methanesulfonamide
[51803-78-2]

It contains at least 98.5% and at most 101.0% of $C_{13}H_{12}N_2O_5S$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Pale yellow powder, crystalline, slightly unctuous to the touch, odorless. It is not hygroscopic.

Solubility. Practically insoluble in water, freely soluble in ethanol and methanol; very soluble in chloroform, acetone, acetonitrile, and dimethylformamide. Soluble in alkalis. Insoluble in acidic solutions.

Physical and chemical constants.

Melting range (5.2.2): 143.3 °C to 144.5 °C.

IDENTIFICATION

The Identification test **C**. may be omitted if the tests **A**. and **B**. are conducted. The Identification test **B**. can be omitted if the tests **A**. and **C**. are conducted.

A. The infrared absorption spectrum (5.2.14) of the sample, desiccated at 105 °C to constant weight and dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the nimesulide SQR spectrum similarly prepared.

B. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel activated in an oven for 30 minutes at 105 °C as support and a mixture of methanol and acetonitrile (80:20) as mobile phase. To separately apply to the plate 4 µL of each of the solutions freshly prepared, as described below.

Solution (1): to accurately weigh about 75 mg of the sample, transfer to a 100 mL volumetric flask, and top up the volume with acetone. To transfer 1 mL of this solution to 10 mL volumetric flask and top up with the same solvent.

Solution (2): to accurately weigh about 75 mg of nimesulide SQR, transfer to a 100 mL volumetric flask, and top up the volume with acetone. To transfer 1 mL of this solution to 10 mL volumetric flask and top up with the same solvent.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm and 365 nm). The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

C. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in *Dosage method c.* corresponds to that of main peak of *Standard solution*.

PURITY TESTS

Heavy metals (5.3.2.3). To use *Method IV*. At most 0.002% (20 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 105 °C for 4 hours. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To employ one of the methods described below.

A. To accurately weigh about 0.24 g of sample, dissolve in 30 mL of acetone, previously neutralized, and add 20 mL of water. To titrate with 0.1 M SV sodium hydroxide, to potentiometrically determine the endpoint. Each mL of 0.1 M SV sodium hydroxide is equal to 30.831 mg of $C_{13}H_{12}N_2O_5S$.

B. To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To accurately transfer about 0.1 g of the sample to a 100 mL volumetric flask, dissolve, and top up with 0.01 M sodium hydroxide. To successively dilute with the same solvent, to obtain 0.00015% concentration (w/v). To prepare a standard solution at the same concentration by using the same solvent. To measure the absorbances of the resulting solutions at 392 nm, by using 0.01 M sodium hydroxide to adjust zero. To calculate the content of $C_{13}H_{12}N_2O_5S$ in the sample from the readings obtained.

C. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 220 nm; 150 mm chromatograph column and 3.9 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at room temperature; flow of *Mobile phase* of 1.8 mL/min.

Mobile phase: mixture of water and acetonitrile (50:50).

Sample solution: To accurately transfer about 50 mg of the sample to a 100 mL volumetric flask, dissolve in *Mobile phase*, and top up the volume with the same solvent. To successively dilute in *Mobile phase*, to obtain 20 µg/mL solution.

Standard solution: to dissolve accurately weighed amount of nimesulide SQR in *Mobile phase*, to obtain 20 µg/mL solution.

Procedure: to separately inject 20 µL of *Sample and standard solutions*, record the chromatograms, and measure the area under the peaks. To calculate the content of $C_{13}H_{12}N_2O_5S$ from the sample, from the responses obtained for the *Sample and Standard solutions*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Anti-inflammatory

NIMESULIDE TABLETS

It contains at least 95.0 % and at most 105.0 % of the amount stated of $C_{13}H_{12}N_2O_5S$.

IDENTIFICATION

A. To weigh and pulverize the tablets. To prepare 0.01% nimesulide solution (w/v) in chloroform. To filter it. To evaporate the filtrate to dryness in water bath. To desiccate the residue at 105 °C, to obtain constant weight. To proceed as described in *Identification test A.*, in the monograph *Nimesulide*.

B. The ultraviolet absorption spectrum (5.2.14) ranging from 200 nm to 400 nm, of sample solution obtained in *Dosage method A.*, exhibits maxima at 212 nm and 392 nm similar to those observed in the standard solution spectrum.

C. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage method B.* corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test. To proceed as described in *Dosage method A.*

DISSOLUTION TEST (5.1.5)

Dissolution medium: potassium phosphate buffer pH 7.4 with 2% polysorbate 80 (v/v), 900 mL

Equipment: blades, 75 rpm

Time: 45 minutes

Procedure: after testing, remove aliquot from the dissolution medium, filter, and dilute with water to obtain suitable concentration. To measure the absorbances at 392 nm (5.2.14), by using the same solvent to adjust zero. To calculate the amount of $C_{13}H_{12}N_2O_5S$ dissolved in the medium, by comparing the readings obtained with 0.0015% nimesulide SQR solution (w/v) similarly prepared.

Tolerance: not less than 85% (Q) of the amount stated of $C_{13}H_{12}N_2O_5S$ dissolves within 45 minutes.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. To weigh and pulverize 20 tablets. To transfer an amount of powder equivalent to 0.1 g of nimesulide to a 100 mL volumetric flask, add 60 mL of 0.01 M sodium hydroxide, and stir for 40 minutes. To top up with the same solvent and filter it. To successively dilute to obtain 0.002% concentration (w/v), by using 0.01 M sodium hydroxide. To prepare a standard solution at the same concentration by using the same solvent. To measure the absorbances of the resulting solutions at 392 nm, by using 0.01 M sodium hydroxide to adjust zero. To calculate the amount of $C_{13}H_{12}N_2O_5S$ in the tablets, from the readings obtained.

B. To proceed as described in *Dosage, method C.*, in the monograph *Nimesulide*. To prepare the *Sample solution* as described below.

Sample solution: To weigh and pulverize 20 tablets. To transfer an amount of powder equivalent to 50 mg of nimesulide to a 100 mL volumetric flask, add 60 mL of *Mobile phase*, and mechanically stir for 40 minutes. To top up to volume with *Mobile phase* and filter it. To successively dilute in *Mobile phase*, to obtain 20 µg/mL solution.

Procedure: to separately inject 20 µL of *Sample and standard solutions*, record the chromatograms, and measure the area under the peaks. To calculate the amount of C₁₃H₁₂N₂O₅S in the tablets, from the responses obtained for the *Sample solution* and *Standard solution*.

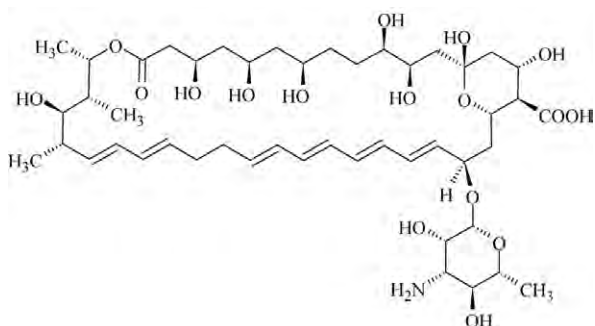
PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

NYSTATIN Nystatinum



C₄₇H₇₅N₀O₁₇; 926.09
nystatin; 06410
Nystatin
[1400-61-9]

Nystatin is a substance or mixture of two or more substances produced by *Streptomyces noursei* Brown et al. (Streptomycetaceae). It has at least 4400 IU/mg of nystatin, or 5000 IU/mg of nystatin for manufacturing powder for oral suspension.

DESCRIPTION

Physical characteristics. Hygroscopic powder, fine, yellow or brown.

Solubility. Practically insoluble in water, freely soluble in dimethylformamide, sparingly soluble in methanol, practically insoluble in chloroform, ethanol, and ethyl ether.

IDENTIFICATION

A. To accurately weigh the equivalent to 100000 IU of sample and dissolve in a mixture of 5 mL of glacial acetic acid with 50 mL of methanol. To top up to 100 mL with methanol. To successively dilute in methanol to obtain 40 UI/mL concentration. The ultraviolet absorption spectrum (5.2.14), ranging from 220 nm to 350 nm, of the resulting solution, exhibits maxima at 230 nm, 291 nm, 305 nm, and 319 nm. The ratio between the absorbance values at 291 nm and 319 nm, relative to maximum absorbance at 305 nm, is between 0.61 and 0.73 and between 0.83 and 0.96,

respectively. The ratio between the absorbance values measured at 230 nm and 280 nm is between 0.83 and 1.25.

B. To add 0.1 mL of hydrochloric acid to 2 mg of sample. Brown color is produced.

C. To add 0.1 mL of sulfuric acid to 2 mg of sample. Brown color is produced. After resting, violet color is developed.

PURITY TESTS

pH (5.2.19). 6.0 to 8.0. To determine in 3% aqueous suspension (w/v).

Crystallinity. To suspend in mineral oil some particle from the sample. Then, transfer it to a microscope slide and examine by polarized light microscope. The particles exhibit birefringence, which is extinguished by micrometric adjustment.

Nystatin A. To proceed as described in *High performance liquid chromatography* (5.2.17.4). To protect from direct light. To use chromatograph equipped with an ultraviolet detector at 304 nm; 150 mm column and 4.6 mm inner diameter, packed with deactivated silica chemically bonded to the octadecylsilane group (5 µm), kept at 30 °C; flow of *Mobile phase* of 1.0 mL/min.

Eluent A: 0.05 M ammonium acetate and acetonitrile (71:29).

Eluent B: 0.05 M ammonium acetate and acetonitrile (40:60).

Mobile phase gradient: to adopt the gradient system described in the following table:

Time Min.	Eluent A (%)	Eluent B (%)	Elution
0-25	100	0	Isocratic
25-35	100→0	0→100	Linear Gradient
35-40	0	100	Isocratic
40-45	0→100	100→0	Linear Gradient
45-60	100	0	balance

Sample solution: to dissolve accurately weighed amount of the sample in dimethylsulfoxide, to obtain a 0.4 mg/mL solution. To use within 24 hours after preparation, kept under refrigeration.

Standard solution: to dissolve accurately weighed amount of nystatin SQR in dimethylsulfoxide, to obtain a 0.4 mg/mL solution. To use within 24 hours after preparation, kept under refrigeration.

Resolution solution: to dissolve 20 mg of nystatin SQR in methanol, dilute in water to 50 mL, and make it homogeneous. To add 2 mL of hydrochloric acid in 10 mL of

the solution prepared above and wait for 1 hour, at room temperature, to use.

To inject replicates of 20 µL of the *Resolution solution*. The average retention time for nystatin A is 14 minutes. The resolution between the two highest peaks should not be less than 3.5. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2.0 %.

Procedure: to separately inject 20 µL of *Sample and standard solutions*, record the chromatograms, and measure the area under the peaks. To disregard peaks obtained before 2 minutes. At least 85% of nystatin A. At most 4% of any component.

Heavy metals (5.3.2.3). To prepare the standard solution by using 2 mL of *Standard lead solution (10 ppm Pb)*.

To proceed as described in *Method IV*. At most 0.002% (20 ppm).

Loss on drying (5.2.9). To determine in 0.1 g of the sample. To desiccate in an oven at 60°C under reduced pressure for 3 hours. At most 5.0%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 3.5%.

Nystatin for powder for oral suspension complies with the following additional test.

Dispersibility. To accurately transfer about 0.2 g of the sample to a beaker containing 200 mL of water and slowly disperse with a glass rod. Let it rest for 2 minutes. Material should be suspended and there will be at most a small sediment. In case of sedimentation, determine the potency of the suspended portion, as described in Microbiological test of antibiotics (5.5.3.3). To volumetrically transfer the suspended sample to a high speed blender and add dimethylformamide, to obtain 400 IU/mL concentration, and stir for 3 - 5 minutes. To dilute with 1% *Potassium phosphate buffer, sterile, pH 6.0 (Solution 1)*, to obtain solution with concentration similar to that of standard curve. At least 90.0% of the expected potency of nystatin.

BIOLOGICAL SAFETY TEST

Nystatin for powder for oral suspension complies with the following additional test.

Toxicity (5.5.2.3). To intraperitoneally inject an amount equivalent to 600 IU suspended in 0.5 mL of 0.5% acacia (w/v) in water.

DOSAGE

To proceed as described in *Microbiological test of antibiotics (5.5.3.3)* by using agar diffusion method.

Sample solution: To protect it from direct light. To dissolve accurately weighed amount of sample in dimethylforma-

mid. To successively dilute with a mixture of dimethylformamide and 1% *Potassium phosphate buffer, sterile, pH 6.0 (Solution 1)* (5:95), to obtain solutions at 10 IU/mg - 40 IU/mg concentrations.

Standard solution: To protect it from direct light. To dissolve accurately weighed amount of nystatin SQR in dimethylformamide. To successively dilute with a mixture of dimethylformamide and 1% *Potassium phosphate buffer, sterile, pH 6.0 (Solution 1)* (5:95), to obtain solutions at 10 IU/mg - 40 IU/mg concentrations.

Procedure: to proceed as described in *Microbiological agar diffusion test (5.5.3.3.1)*. To calculate the potency of the sample in µg/mg of nystatin from the potency of the standard and the responses obtained with the *Standard and sample solutions*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light, at 2 °C - 8 °C.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antifungal.

NYSTATIN VAGINAL TABLETS

It contains at least 90.0 % and at most 140.0 % of the amount stated of nystatin. The vaginal tablets contain binder, diluent, and lubricant agents.

IDENTIFICATION

To weigh and pulverize the vaginal tablets. To transfer an amount of powder equivalent to 300000 IU of nystatin to a 100 mL volumetric flask. To add 5 mL of glacial acetic acid and 50 mL of methanol. To make it homogeneous. To add a sufficient amount of methanol to obtain 100 mL. To filter it. To transfer 1 mL of this solution to 100 mL volumetric flask and top up with methanol. To prepare the blank sample correction by using the same solvent and omitting the addition of the sample. The ultraviolet absorption spectrum (5.2.14), ranging from 250 nm to 350 nm, of the resulting solution, exhibits maxima at 291 nm, 305 nm, and 319 nm. The ratio between the absorbance values at 291 nm and 319 nm, relative to maximum absorbance at 305 nm, is between 0.61 and 0.73 and between 0.83 and 0.96, respectively.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.2). Within 60 minutes.

Uniformity of dosage units (5.1.6). It complies with the test.

PURITY TESTS

Loss on drying (5.2.9). To weigh and pulverize the vaginal tablets. To determine in 0.1 g of the sample, in a vacuum oven, at 60 °C for 3 hours. At most 5.0%.

DETERMINATION OF POTENCY

To protect it from direct light. To weigh and pulverize 20 vaginal tablets. To mix an amount of powder equivalent to 200000 IU with 50 mL of dimethylformamide for 1 hour. To centrifuge it.

To transfer 10 mL of the supernatant to 200 mL volumetric flask and dilute with 9.56% monobasic potassium phosphate solution (w/v) and 11.5% *M* potassium hydroxide (v/v). To proceed as described in *Microbiological agar diffusion test (5.5.3.3.1)*. The accuracy of the test is such that the lower limit is 95.0%, and the upper limit is 105.0% of the estimated potency. The lower limit is 97.0% and the upper limit is 110.0% of the prescribed or declared amount of IU.

PACKAGING AND STORAGE

In airtight closed containers, protected from moisture, at temperature below 25 °C.

LABELLING

To comply with legislation in force.

NYSTATIN VAGINAL CREAM

It contains at least 90.0 % and at most 130.0 % of the amount stated of $C_{47}H_{75}NO_{17}$.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). Total bacteria: At most 100 UFC/g. Fungi and yeasts: At most 10 UFC/g.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To proceed as described in *Dosage*, in the monograph *Nystatin*. To prepare the *Sample solution* as described below.

Sample solution: to accurately mix an amount of vaginal cream with dimethylformamide at high speed blender, to obtain 400 IU/mL nystatin. To successively dilute this solution in 1% *Potassium phosphate buffer, sterile, pH 6.0 (Solution 1)*, to obtain solutions at the concentration range appropriate for the standard curve.

PACKAGING AND STORAGE

In airtight closed containers, protected from moisture, at temperature below 25 °C.

LABELLING

To comply with the legislation in force.

NYSTATIN ORAL SUSPENSION

It contains at least 90.0 % and at most 130.0 % of the amount stated of nystatin. The oral suspension contains flavoring, preservative, and dispersing agents.

IDENTIFICATION

To transfer an amount of oral solution containing 300000 IU of nystatin to 100 mL volumetric flask and add a mixture of glacial acetic acid and methanol (5:50). To make it homogeneous. To top up with the methanol and filter it. To transfer 1 mL of this solution to 100 mL volumetric flask and top up with methanol. To prepare the blank sample correction by using the same solvents and omitting the addition of the sample. The ultraviolet absorption spectrum (5.2.14), ranging from 250 nm to 350 nm, of the resulting solution, exhibits maxima at 291 nm, 305 nm, and 319 nm. The ratio between the absorbance values at 291 nm and 319 nm, relative to maximum absorbance at 305 nm, is between 0.61 and 0.73 and between 0.83 and 0.96, respectively.

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

pH (5.2.19). 4.5 to 6.0. If the product contains glycerin, the pH should be between 6.0 and 7.5.

Uniformity of dosage units (5.1.6). It complies with the test. It must be performed for single doses packaging.

Procedure for content uniformity. To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. To transfer the volume of oral suspension solution to a 100 mL volumetric flask, top up with methanol, and make it homogeneous. To quantitatively dilute the solution in methanol,

to obtain 25 IU/mL nystatin solution. To simultaneously prepare nystatin SQR solution in methanol at 25 IU/mL nystatin. To measure the absorbances of the solutions at 304 nm, by using methanol to adjust zero. To calculate the content of nystatin in the oral suspension, from the readings obtained.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Dosage*, in the monograph *Nystatin*. To prepare the *Sample solution* as described below.

Sample solution: to protect it from direct light. To accurately transfer volume of oral suspension to a volumetric flask and dilute in dimethylformamide, to obtain suitable concentration. To mix it for 3 - 5 minutes. To dilute volume of this solution in dimethylformamide, to obtain 400 IU/mL nystatin solution. To successively dilute in 1% *Potassium phosphate buffer, sterile, pH 6.0 (Solution 1)*, to obtain solutions at the concentration range appropriate for the standard curve.

B. To protect it from light during dosage. To dissolve an amount containing 200,000 IU of nystatin in dimethylformamide to obtain 50 mL, dilute 10 mL to 200 mL with 9.56% monobasic potassium phosphate solution (w/v) and 11 5% *M* potassium hydroxide (v/v), and proceed as described in *Microbiological agar diffusion test (5.5.3.3.1)*. The accuracy of the test is such that the error limit is greater than 95% and at most 105% of the estimated potency. The lower limit is not less than 95% and upper one at most 120% in relation to number of IU.

PACKAGING AND STORAGE

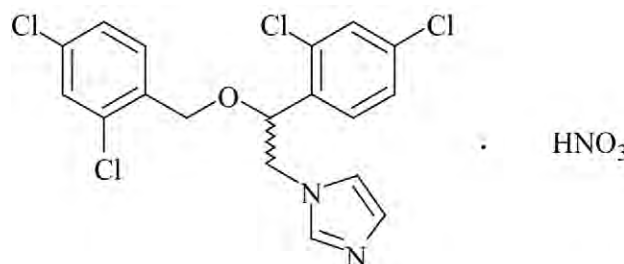
In airtight closed containers, protected from moisture, at temperature below 25 °C.

LABELLING

To comply with the legislation in force.

NITRATE, MICONAZOLE

Miconazoli nitras



$C_{18}H_{14}Cl_4N_2O \cdot HNO_3$; 479,14
miconazole nitrate; 05929

1-[2-(2,4-dichlorophenyl)-2-[(2,4-dichlorophenyl) methoxy] ethyl]-1*H*-imidazole nitrate (1:1)
[22832-87-7]

It contains at least 98.0% and at most 101.0% of $C_{18}H_{14}Cl_4N_2O \cdot HNO_3$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White crystalline powder.

Solubility. Very slightly soluble in water, slightly soluble in methanol, sparingly soluble in ethanol.

Physical and chemical constants.

Melting range (5.2.2): 178 °C to 184 °C.

Specific rotation (5.2.8): -0.10° a $+0.10^\circ$, in relation to the desiccated substance. To determine in 1% solution (w/v).

IDENTIFICATION

The test A. may be omitted if the tests B., C., and D. are conducted. The test B. can be omitted if the tests A., C., and D are carried out.

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the miconazole nitrate SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14), ranging from 200 nm to 400 nm, of 0.04% solution (w/v) in a mixture of 0.1 *M* hydrochloric acid and isopropyl alcohol (1:10), exhibits maxima and minima only in the same wavelength of a similar miconazole nitrate SQR solution.

C. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using octadecylsilane silica gel as support and a mixture of ammonium acetate SR, dioxane, and methanol (20:40:40) as mobile phase. To separately apply to the plate 5 µL of each of the solutions freshly prepared, as described below.

Solution (1) = sample solution at 6 mg/mL concentration in mobile phase.

Solution (2) = 6 mg/mL miconazole nitrate SQR solution in mobile phase.

Solution (3): to dissolve 30 mg of miconazole nitrate SQR and 30 mg of econazole nitrate SQR in 5 mL of mobile phase.

To develop the chromatogram. To remove the plate, let it drying, and apply iodine vapor. To examine under visible light. The principal spot obtained with *Solution (1)* is similar in position, color, and intensity to that obtained with *Solution (2)*. The test is valid only if the chromatogram obtained with *Solution (3)* presents two clearly separated spots.

D. It responds to the reactions of nitrate (5.3.1.1).

PURITY TESTS

Related substances. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 235 nm; 100 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octylsilane group (3 µm), at room temperature; flow of *Mobile phase* of 2.0 mL/min.

Mobile phase: To weigh 6 g of ammonium acetate and transfer to 1000 mL volumetric flask, add 300 mL of acetonitrile and 320 mL of methanol, and top up with water.

Solution (1): to accurately transfer about 0.1 mg of the sample to a 10 mL volumetric flask and top up the volume with *Mobile phase*. To make it homogeneous.

Solution (2): to accurately transfer about 25 mg of miconazole nitrate SQR and 25 mg of econazole nitrate SQR to 25 mL volumetric flask and top up with *Mobile phase*. To make it homogeneous. To dilute 2.5 mL to 100 mL volumetric flask and top up with the same diluent.

Solution (3): to dilute 1 mL of *Solution (1)* to 100 mL volumetric flask and top up with *Mobile phase*.

To balance the chromatographic system for 30 minutes. To inject 10 µL of *Solution (3)*. To adjust the chromatographic system so that the height of the main peak in the chromatogram obtained with *Solution (3)* be less than 50% of full scale. To inject 10 µL of *Solution (3)*. The retention time of miconazole nitrate is approximately 20 minutes and 10 minutes for econazole nitrate. The resolution between the peaks obtained with the *Solution (3)* is not less than 10. If necessary, adjust the composition of *Mobile phase*.

Procedure: to separately inject 10 µL of *Solutions (1)* and (3), record the chromatograms, and measure the areas under the peaks. In the chromatogram obtained with *Solution (1)*, the area of all peaks, other than the main peak, is not

greater than the area under the main peak obtained with *Solution (3)*. The sum of the areas of all peaks obtained is not greater than twice the area under the main peak obtained with *Solution (3)*. To disregard all peaks with area less than 0.2 times the main peak obtained with *Solution (3)* and nitrate ion peaks.

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 100-105 °C for 2 hours. At most 0.25%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To proceed as described in *Titrations in non-aqueous medium* (5.3.3.5). To accurately weigh about 0.35 g of the sample, previously desiccated, and dissolve it in 50 mL of glacial acetic acid, by gently heating it, if necessary, and potentiometrically titrate with 0.1 M SV perchloric acid. To perform sample blank correction and adjust it as required. Each mL of 0.1 M SV perchloric acid spent corresponds to 47.914 mg of $C_{18}H_{14}Cl_4N_2O.HNO_3$.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antifungal.

NITRATE, SILVER

Argentí nitrás

AgNO₃; 16987
silver nitrate; 06427
Nitric acid silver (1+) salt (1:1)
[7761-88-8]

It contains at least 99.0% and at most 100.5% of AgNO₃, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Large colorless crystals, clear, or small white crystals.

Solubility. Very soluble in water, soluble in ethanol, slightly soluble in ammonia water and ethyl ether, sparingly soluble in acetone.

IDENTIFICATION

A. To add a drop of diphenylamine SR to 10 mL of 10% solution (w/v) and make it homogeneous. To carefully pour the solution to a test tube containing 2 mL of sulfuric acid. Blue color is developed on the interface.

B. The 2% solution (w/v) responds to the reactions of silver ion (5.3.1.1).

C. The 2% solution (w/v) responds to the reactions of nitrate ion (5.3.1.1).

PURITY TESTS

Appearance of solution. The 10% aqueous solution (w/v) is clear (5.2.25) and colorless (5.2.12).

Acidity and alkalinity. To add 0.1 mL of bromocresol green SI to 2 mL of 4% solution (w/v). The blue color develops. To add 0.1 mL of phenol red SI to 2 mL of 10% solution (w/v). The yellow color develops.

Aluminum, copper, lead, and bismuth. To dissolve 5 g of sample in a mixture of 4 mL of 13.5 M ammonia with 6 mL of water. The solution is clear (5.2.25) and colorless (5.2.12).

Evaporation residue. To add 7.5 mL of diluted hydrochloric acid to 30 mL of 4% solution (w/v), vigorously stir, heat for 5 minutes in a water bath, and filter it.

To evaporate 20 mL of the filtrate in water bath and desiccate the residue at 100 °C - 105 °C in an oven. At most 2 mg (0.3%).

DOSAGE

To previously desiccate the sample over silica for 4 hours, protected from light. To accurately weigh about 0.3 g of the desiccated sample and dissolve in 50 mL of water. To add 2 mL of nitric acid and 2 mL of ferric ammonium sulfate SR and make it homogeneous. To titrate with 0.1 M SV ammonium thiocyanate to obtain reddish-yellow color. Each mL of 0.1 M SV ammonium thiocyanate is equal to 16.987 mg of AgNO_3 .

PACKAGING AND STORAGE

In tightly closed, nonmetallic containers; protected from light.

THERAPEUTIC CLASS

Antiinfective.

LABELLING

To comply with the legislation in force.

NITRATE, SILVER, OPHTHALMIC SOLUTION

It contains at least 95.0% and at most 105.0% AgNO_3 . The ophthalmic solution is buffered by adding sodium acetate.

IDENTIFICATION

A. The ophthalmic solution responds to the reactions of silver ion (5.3.1.1).

B. The ophthalmic solution responds to the reactions of nitrate ion (5.3.1.1).

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test. pH (5.2.19). 4.5 to 6.0.

PURITY TESTS

Appearance of solution. The ophthalmic solution is clear (5.2.25) and colorless (5.2.12).

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

DOSAGE

To transfer volume of ophthalmic solution equivalent to 50 mg of silver nitrate to erlenmeyer flask, dilute in 20 mL of water, add 1 mL of nitric acid and 1 mL of ferric ammonium sulfate SR, and make it homogeneous. To titrate with 0.02 M SV ammonium thiocyanate to obtain reddish-yellow color. Each mL of 0.02 M SV ammonium thiocyanate is equal to 3.397 mg of AgNO_3 .

PACKAGING AND STORAGE

In tightly closed, inert containers; protected from light.

LABELLING

To comply with the legislation in force.

NITRITO DE SÓDIO

Natrii nitris

NaNO_2 ; 69.00
sodium nitrite; 06433
Sodium salt of nitrous acid (1:1)
[7632-00-0]

It contains at least 97.0% and at most 101.0% of NaNO_2 , in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Granular powder, or clear hexagonal crystals, colorless, or white, opaque, and deliquescent mass. Odorless and slightly saline taste.

Solubility. Freely soluble in water, sparingly soluble in ethanol, insoluble and ethyl ether.

IDENTIFICATION

A. The 10% solution (w/v) responds to the reactions of nitrite ion (5.3.1.1).

B. The 10% solution (w/v) responds to the reactions of sodium ion (5.3.1.1).

PURITY TESTS

Heavy metals (5.3.2.3). To use *Method III*. To determine in 1 g of the sample. At most 0.002% (20 ppm).

Loss on drying (5.2.9). To determine in an oven at 105 °C for 4 hours. At most 0.25%.

DOSAGE

To exactly weigh about 1 g of the sample, transfer it to 100 mL volumetric flask, dissolve in water, and top up the volume with the same solvent.

To transfer 15 mL of this solution to vial containing a mixture of 50 mL of 0.02 M SV potassium permanganate, 100 mL of water, and 5 mL of sulfuric acid. By adding the sample solution, immerse the tip of the pipette under the surface of the mixture of permanganate. To heat the mixture at 40 °C, let it rest for 5 minutes, and add 25 mL of 0.05 M SV oxalic acid. To heat the mixture at 80 °C (maximum) and perform hot titration with 0.02 M SV potassium permanganate. Each mL of 0.02 M SV potassium permanganate is equal to 3.450 mg of NaNO₂.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

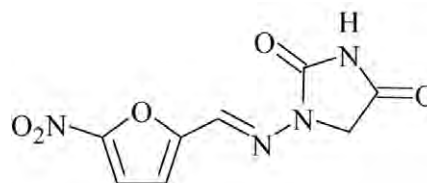
To comply with the legislation in force.

THERAPEUTIC CLASS

Vasodilator; antidote for cyanide poisoning.

NITROFURANTOIN

Nitrofurantoinum



C₈H₆N₄O₅; 238.16

C₈H₆N₄O₅·H₂O; 256.17

nitrofurantoin; 06438

1-[[[(5-nitro-2-furanyl)methylene]amino]-2,4-imidazolidinedione

[67-20-9]

1-[[[(5-nitro-2-furanyl)methylene]amino]-2,4-imidazolidinedione hydrate (1:1)

[17140-81-7]

It contains at least 98.0% and at most 102.0% of C₈H₆N₄O₅, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. Yellow crystalline powder or yellow crystals. In solid form or solution, alkalis, exposure to light, and decomposition by contact with metals, other than stainless steel and aluminum, discolor it.

Solubility. Very slightly soluble in water, soluble in dimethylformamide, sparingly soluble in ethanol.

IDENTIFICATION

The identification tests B., C, and E. may be omitted if the tests A. and D. are conducted. The identification tests A. and D. can be omitted if the tests B., C., and D. are carried out.

A. The infrared absorption spectrum (5.2.14) of the sample, desiccated at 140 °C to constant weight and dispersed in mineral oil, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the nitrofurantoin SQR spectrum similarly prepared.

B. To protect it from intense light. The ultraviolet absorption spectrum (5.2.14) ranging from 220 nm to 400 nm, of sample solution obtained in *Dosage* method A., exhibits maxima at 266 nm and 367 nm. The ratio between the absorbance values measured at 367 nm and 266 nm is between 1.36 and 1.42.

C. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method B. corresponds to that of main peak of *Standard solution*.

D. To dissolve 10 mg of the sample in 10 mL of dimethylformamide. To transfer 1 mL of solution to test tube

and add 0.1 mL of 0.5 M ethanolic potassium hydroxide. Brown color develops.

E. To dissolve 5 mg of the sample in 5 mL of 0.1 M sodium hydroxide. A intense yellow solution is produced, which then becomes reddish-orange.

PURITY TESTS

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica as support and a mixture of nitromethane and methanol (9:1) as mobile phase. To separately apply to the plate 10 µL of each of the solutions freshly prepared, as described below.

Solution (1): to dissolve 0.25 g of the sample in dimethylformamide and top up to 10 mL with acetone.

Solution (2): to dilute 1 mL of *Solution (1)* to 100 mL with acetone.

To develop the chromatogram. To remove the plate, allow it to dry in air, and heat it at 110 °C - 105 °C for 5 minutes. To examine under ultraviolet light (254 nm). To nebulize with phenylhydrazine hydrochloride SR. To heat the plate again at 110 °C - 105 °C for 10 minutes. Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (2)* (1%).

Water (5.2.20). To desiccate at 140 °C for 30 minutes. At most 1% to anhydrous form, and between 6.5% and 7.5% to monohydrate form.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

A. To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To protect it from intense light. To accurately weigh about 0.12 g of the sample and dissolve in 50 mL of dimethylformamide. To top up the volume to 500 mL with water. To transfer 2.5 mL of solution to 100 mL volumetric flask and top up to volume with solution containing 1.8% sodium acetate (w/v) and 0.14% glacial acetic acid (v/v). To prepare a standard solution at the same concentration by using the same solvent. To measure the absorbance of the resulting solution at 367 nm by using the sodium acetate and acetic acid solution, as described above, to adjust zero. To calculate the content of C₈H₆N₄O₅ in the sample from the readings obtained.

B. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 254 nm; 300 mm chromatograph column and 3.9 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at room temperature; set the operating parameters so that the retention time of the nitrofurantoin peak be 8 minutes.

Phosphate Buffer pH 7.0: to dissolve 6.8 g of monobasic potassium phosphate in 500 mL of water and adjust pH to 7.0 with M ammonium hydroxide. To top up to 1000 mL with water and make it homogeneous.

Mobile phase: mixture of *Phosphate buffer pH 7.0* and tetrahydrofuran (9:1). To filter and degas it.

Internal standard solution: to dissolve accurately weighed amount of acetanilide in water and properly dilute to obtain a 1 mg/mL solution.

Sample solution: to accurately dissolve about 25 mg of the sample in 20 mL of dimethylformamide. To add 25 mL of *Internal standard solution*, top up to 50 mL with dimethylformamide, and make it homogeneous.

Standard solution: to accurately dissolve about 25 mg of nitrofurantoin SQR in 20 mL of dimethylformamide. To add 25 mL of *Internal standard solution*, top up to 50 mL with dimethylformamide, and make it homogeneous.

The resolution factor between acetanilide and nitrofurantoin should not be less than 3. The relative standard deviation for replicate areas of the peaks recorded should not be greater than 2 %.

Procedure: to separately inject equal volumes (5 µL or 10 µL) of *Sample and standard solutions*, record the chromatograms, and measure the area under the peaks. To calculate the content of C₈H₆N₄O₅ from the sample, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In airtight closed containers, protected from light, at temperature below 25 °C.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antibacterial.

NITROFURANTOIN TABLETS

It contains at least 90.0 % and at most 110.0 % of the amount stated of C₈H₆N₄O₅. Tablets must be coated.

IDENTIFICATION

The identification test A. may be omitted if the tests B. and C. are conducted. The identification tests B. and C. can be omitted if the test A. is carried out.

A. To weigh and pulverize the coated tablets. To stir an amount of powder equivalent to 0.1 g of nitrofurantoin

with 10 mL of 6 M acetic acid. To heat for a few minutes and perform hot filtering. To cool down at room temperature, collect the precipitate, and desiccate at 105 °C for 1 hour, to obtain constant weight. The residue responds to test **A.**, *Identification*, in the monograph *Nitrofurantoin*.

B. The ultraviolet absorption spectrum (5.2.14) ranging from 220 nm to 400 nm, of sample solution obtained in *Dosage* method **A.**, exhibits maxima at 266 nm and 367 nm. The ratio between the absorbance values measured at 367 nm and 266 nm is between 1.36 and 1.42.

C. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method **B.** corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: phosphate buffer pH 7.2, 900 mL

Equipment: baskets, 100 rpm

Time: 60 minutes and 120 minutes

Procedure: after testing, remove aliquot from the dissolution medium, filter, and dilute with Dissolution medium, to obtain suitable concentration.

To measure the absorbances of the solutions at 375 nm (5.2.14), by using the same solvent to adjust zero. To calculate the amount of $C_8H_6N_4O_5$ dissolved in the medium, by comparing the readings obtained with that of 0.001% nitrofurantoin SQR solution (w/v) prepared in the same solvent.

Tolerance: no less than 25% (Q) of the stated amount of $C_8H_6N_4O_5$ dissolves within 60 minutes, and not less than 85% (Q) of the stated amount of $C_8H_6N_4O_5$ dissolves within 120 minutes.

PURITY TESTS

Related substances. To proceed as described in *Related substances*, in the monograph *Nitrofurantoin*. To prepare *Solution (1)* as described below.

Solution (1): to weigh and pulverize the coated tablets. To dissolve an amount of powder equivalent to 0.1 g of nitrofurantoin in 10 mL of a filtered mixture of dimethylformamide and acetone (1:9).

PACKAGING AND STORAGE

In tightly closed containers, protected from light, at temperature below 25 °C.

LABELLING

To comply with the legislation in force.

KOLA NUT Colae semen

Cola nitida (Vent.) A.Chev. - STERCULIACEAE; 09902

The vegetable drug consists of the dried cotyledons, containing at least, 1.7% of total tannins and 2.0% caffeine.

SCIENTIFIC SYNONYMY

Cola vera K.Schum.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To weigh and pulverize 20 coated tablets. To proceed as described in *DOSAGE* method **A.**, in the monograph *Nitrofurantoin*, by using an amount of powder equivalent to 0.12 g of nitrofurantoin. To calculate the amount of $C_8H_6N_4O_5$ in the coated tablets, from the reading obtained.

To proceed as described in *DOSAGE* method **B.**, in the monograph *Nitrofurantoin*. To prepare the *Sample solution* as described below.

Sample solution: to weigh and pulverize 20 coated tablets. To transfer an amount of powder equivalent to 50 mg of nitrofurantoin to a 100 mL volumetric flask, add 40 mL of dimethylformamide, and mechanically stir for 15 minutes. To add 50 mL of *Internal standard solution*, make it homogeneous, and let cool down at room temperature. To top up with dimethylformamide and make it homogeneous. To filter through 0.45 µm nylon filter.

Procedure: to separately inject equal volumes (5 µL or 10 µL) of *Standard and sample solutions*, record the chromatograms, and measure the areas under the peaks corresponding to nitrofurantoin and acetanilide. To calculate the amount of $C_8H_6N_4O_5$ in coated tablets from the responses obtained for relationship nitrofurantoin/ acetanilide, in the *Standard solution* and *Sample solution*.

CHARACTERISTICS

Organoleptic characteristics. The cotyledons have an astringent and bitter taste and almost no odor.

MACROSCOPIC DESCRIPTION

The cotyledons are usually in two, found in trade already separated. They are rough and uneven, solid, irregular, reddish-brown color, highly variable in size, 2 to 5 cm in length and about 2 cm width and up to 1 cm thick. The apex of the cotyledon is wider than its base and both are rounded. The margin is entire. The outer surface of each cotyledon is convex or slightly depressed and roughened, with brown to reddish brown color. The inner surface is flat or depressed, more or less smooth, usually irregular, and the base has a small cavity, containing sometimes the radicle and plumule, or traces of these. The fracture surface is uniform and bright brown.

MICROSCOPIC DESCRIPTION

The cotyledons are surrounded by a skin formed by rectangular and small cells which are slightly elongated in the radial direction and they are constituted by a homogeneous parenchyma of polygonal cells, sometimes irregular. The innermost cells are larger, with thick walls which have pits, with brown color, containing phenolic compounds, fatty matter and abundant starch grains. The latter are mainly distributed in the central cells and are unequal, spherical, oval, oval-rounded, oblong, reniform, ellipsoidal or pyriform, with branched, central or eccentric hilum, almost always melted, star-shaped or cross-shaped and their concentric strips are barely visible.

The grain size ranges from 5 μm to 35 μm , rarely 45 μm .

MICROSCOPIC DESCRIPTION OF THE POWDER

The powder meets all the requirements established for the species, unless the macroscopic characters. The characteristics are: reddish-brown moderately to yellowish-brown; fragments of epidermis and parenchyma with polygonal cells, with brown or red-brown walls containing numerous and varied starch grains as described; few fragments of small fibrovascular bundles. The starch grains, when observed in polarized light, exhibit a cross in the hilar region.

IDENTIFICATION

A. To Proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF_{254} silica gel as support and a mixture of ethyl acetate, methanol and water (100:13.5:10) as mobile phase. To separately apply, band-shaped, 5 μL to 10 μL of *Sample solution* and 2 μL to 3 μL of the *Standard solution*, prepared as described below.

Sample solution: to previously extract the pulverized drug under reflux for 15 minutes, at concentration equal to 2% (w/v) by using ethanol as extraction liquid. To filter and apply to the chromatoplate.

Standard solution: to dissolve 10 mg of caffeine SQR in 2 mL of absolute ethanol.

To develop the chromatogram. To remove chromatoplate and allow it to air dry for a few minutes. To observe it under ultraviolet light (254 nm). The spot with R_f about 0.50 corresponds to caffeine. To nebulize with potassium iodide and bismuth subnitrate SR. To additionally nebulize with an aqueous solution of 5% sodium nitrite (w/v). The stain corresponds to caffeine and has a reddish brick color.

PURITY TESTS

Foreign matter (5.4.2.2). At most 3.0%.

Water (5.4.2.3). At most 15.0%.

Total ashes (5.4.2.4). At most 5.0%.

DOSAGE

Methylxanthines

To proceed as described in the *Ultraviolet absorption spectrophotometry* (5.2.14). To prepare solutions described as follows:

Sample solution: to accurately weigh about 0.25 g of the pulverized sample. To extract with 20 mL of 2.5% sulfuric acid (v/v) solution under magnetic stirring for 15 minutes, four times. To filter the portions to 100 mL volumetric

flask. To top up with 2.5% sulfuric acid (v/v) solution and transfer 10 mL of this solution to 100 mL volumetric flask. To top up with the same 2.5% sulfuric acid (v/v) solution, obtaining thus the theoretical concentration around 15 $\mu\text{g/mL}$.

Standard solution: To accurately weigh about 25 mg of caffeine SQR and transfer to a 100 mL volumetric flask. To top up with 2.5% sulfuric acid (v/v) acid to obtain a stock solution of 250 $\mu\text{g/mL}$ caffeine.

Solutions for analytical curve: to dilute aliquots of the *Standard solution* to obtain the following concentrations: 2.5 $\mu\text{g/mL}$; 5.0 $\mu\text{g/mL}$; 10.0 $\mu\text{g/mL}$; 15.0 $\mu\text{g/mL}$ and 20.0 $\mu\text{g/mL}$ using 2.5% sulfuric acid (v/v) solution to top up to the volume.

To measure the absorbance of the solutions at 271 nm, employ 1 cm cuvettes. To use 2.5% sulfuric acid (v/v) solution for zero adjustment. To calculate the content of caffeine (methylxanthines) in the sample from the equation of the line obtained with the analytical curve of caffeine.

Total tannins

Note: to protect light samples during extraction and dilution. To use water free of carbon dioxide in all operations.

n

Stock solution: To weigh 0.75 g of pulverizes drug, transfer to Erlenmeyer flask and add 150 mL of water. To heat to boil and keep in a water bath at 80°C to 90°C for 30 minutes. To cool down with running water, transfer the mixture to a volumetric flask and dilute to 250 mL of water. To decant the sediment and filter by using filter paper. To discard the first 50 mL of the filtrate.

Sample solution for total polyphenols: to dilute 5 mL of the filtrate to 25 mL with water. To mix 5 mL of this solution with 2 mL of phosphotungstic acid SR and dilute at 50 mL with sodium carbonate SR. To measure the absorbance of the solution (A_1) at 715 nm (**5.2.14**) exactly 3 minutes after adding the last reagent, by using water.

Sample solution for polyphenols not adsorbed by hide powder: to add 20 mL of the filtrate 0.2 g of hide powder SQR skin and vigorously stir for 60 minutes. To filter. To dilute 5 mL of the filtrate at 25 mL in water. To mix 5 mL of this solution with 2 mL of phosphotungstic acid SR and dilute 50 mL in sodium carbonate SR. To measure the absorbance of the solution at 715 nm (A_2) (**5.2.14**) exactly 3 minutes after adding the last reagent, by using water.

Standard solution: To dissolve 50 mg of pyrogallol in water and dilute at 100 mL. To dilute 5 mL of this solution at

100 mL in water. To mix 5 mL of this solution with 2 mL of phosphotungstic acid SR and dilute at 50 mL with sodium carbonate SR. To measure the absorbance of the solution at 715 nm (A_3) (**5.2.14**) 3 minutes after the addition of the last reagent for 15 minutes from the dissolution of pyrogallol, by using water as blank.

To calculate the content, according to the equation:

$$TT = \frac{13,12 \times (A_1 - A_2)}{A_3 \times m}$$

where

m = mass of the sample in grams.

A_1 = absorbance measured from the *Sample solution for total polyphenols*;

A_2 = absorbance measured from the *Sample solution* for polyphenols not adsorbed by hide powder;

A_3 = absorbance measured for the *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light and heat.

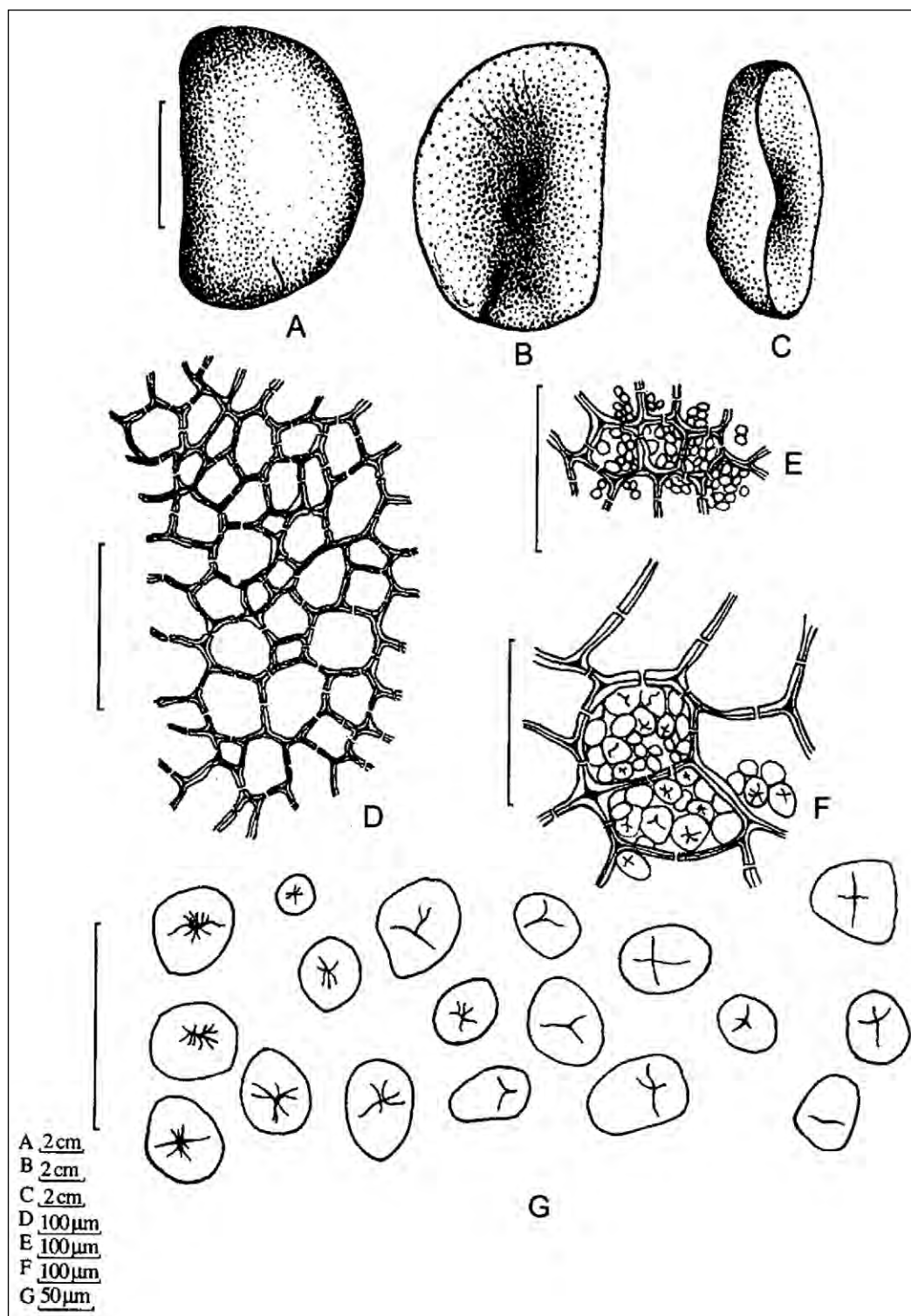
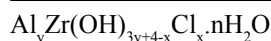


Figure 1 - Macroscopic and microscopic aspects, and aspects of the microscopy of powder in *Cola nitida* (Vent.) A. Chev.

Additional information of **Figure 1**. Scales correspond in **A**, **B** and **C** to 2 cm; **D**, **E** and **F** to 100 μm; **G** to 50 μm.

A - aspect of the outer face of the cotyledon. **B** — aspect of the inner face of the cotyledon. **C** — cotyledon in equatorial view. **D**, **E**, **F** and **G** - details of the powder. **D**, **E** and **F** — fragments of parenchyma, showing varying sizes and wall with pits. **G** — detail of starch grains, showing variability in shape, grain size and aspect of the hilum.

OCTA HYDROCHLORIDE, ALUMINUM AND ZIRCONIUM



aluminum and zirconium octa hydrochloride; 09831

Aluminum and zirconium chloride hydroxide

[57158-29-9]

Aluminum and zirconium octa hydrochloride is a complex polymer hydrated of basic aluminum and zirconium chloride. It contains at least 90.0% and at most 110.0% of aluminum and zirconium octa hydrochloride, in relation to the anhydrous substance.

IDENTIFICATION

A. The 10% aqueous solution (w/v) of the sample responds to the reactions of chloride ion (**5.3.1.1**).

PURITY TESTS

pH (5.2.19). 3.0 to 5.0. To determine in 15% aqueous solution (w/v).

Aluminum content. To weigh 0.15 g of the sample, transfer to 150 mL beaker, and add 5 mL of water and 15 mL of hydrochloric acid. To keep boiling for 5 minutes. To add 40 mL of water and 30 mL of 0.05 M SV disodium edetate. To boil again for 5 minutes. To wait to cool down, add 15 mL of acetic acid-ammonium acetate buffer, and adjust pH to 4.5 with concentrated ammonia solution. To add 20 mL of ethanol and adjust the pH to 4.6 with concentrated ammonia solution. To add 10 drops of 0.025% dithizone (w/v) in ethanol. To titrate with 0.1 M SV zinc sulfate to obtain pink-purple color. To perform sample blank correction. To calculate the percentage of aluminum:

$$\frac{2,698 [15\text{Me} - (z\text{Mz} + \text{Ze})]}{W}$$

where

Me = molarity of the volumetric solution of disodium edetate;

z = consumed volume of volumetric solution of zinc sulfate in mL;

Mz = molarity of volumetric solution of zinc sulfate;

W = amount of sample, in g;

Z = volume equivalent of disodium edetate consumed by the amount of zirconium calculated according to the equation:

$$\left(\frac{\text{Zr}}{\text{Me}} \right) \times \left(\frac{W}{92,97} \right)$$

where

Zr = percentage of zirconium determined in the test *Zirconium content*

92.97 = atomic mass of zirconium corrected for the 2% hafnium content.

The result must be used to calculate the atomic ratio of aluminum/zirconium and the atomic ratio of (aluminum + zirconium)/chloride.

Zirconium content. To weigh 250 mg of the sample, transfer to 150 mL beaker, and add 5 mL of water and 15 mL of hydrochloric acid. To keep boiling for 6 - 8 minutes. Then add 30 - 40 mL of water and 5 mL of hydrochloric acid and heat to boiling. To add a drop of 0.1% xylenol orange solution (w/v) and titrate the solution, still warm, with 0.05 M SV disodium edetate to change from yellow to pink. To perform sample blank correction. Each mL of 0.05 M SV disodium edetate is equal to 46.48 mg of zirconium. It contains at least 12.8% and at most 15.4% of zirconium. The result must be used to calculate the atomic ratio of aluminum/zirconium and the atomic ratio of (aluminum + zirconium)/chloride.

Atomic ratio of aluminum/zirconium. To divide the percentage of aluminum found in the test for *Aluminum Content* by the percentage of zirconium found in the test for *Zirconium Content* and multiply by 92.97/26.98. Where 92.97 is the atomic mass of zirconium corrected to 2% of hafnium and 26.98 is the atomic mass of aluminum. At least 6:1 and at most 10:1.

Chloride content. To weigh 250 mg of the sample, transfer to a 250 mL beaker, and add 120 mL of water and 20 mL of *M* nitric acid. To stir to obtain solubilization and titrate with 0.1 M silver nitrate, thus potentiometrically determining the endpoint. Each mL of 0.1 M silver nitrate is equivalent to 3.546 mg of chloride. It contains at least 16.5% and at most 19.0% of chloride.

The result must be used to calculate the atomic ratio of aluminum/zirconium and the atomic ratio of (aluminum + zirconium)/chloride.

Atomic ratio (aluminum + zirconium)/chloride. To calculate the atomic ratio (aluminum + zirconium)/chloride, according to the equation:

$$\frac{\left(\frac{\text{Al}}{26,98} \right) + \left(\frac{\text{Zr}}{92,97} \right)}{\left(\frac{\text{Cl}}{35,453} \right)}$$

where

Al, Zr, and Cl = percentage of aluminum, zirconium, and chloride, determined in tests for *Aluminum Content*, *Zirconium Content*, and *Chloride Content*, respectively, 26.98 = atomic mass of aluminum; 92.97 = atomic mass of zirconium corrected for 2% of hafnium.

35.453 = atomic mass of chlorine.

The ratio is between 1.5:1 and 0.9:1.

Arsenic (5.3.2.5). To determine in 1,5 g of the sample. To proceed as described in *Limit test for arsenic*. At most 0.0002% (2 ppm).

Iron (5.3.2.4). To use *Method I*. To weigh 0.667 g of sample and add 40 mL of water. To proceed as described in *Limit test for iron*. At most 0.015% (150 ppm).

Heavy Metals (5.3.2.3). To use *Method I*. To weigh 1 g of the sample and add 40 mL of water. Should the preparation be not clear, heat it at 80 °C for a few minutes, then wait to cool down. Should the preparation be cloudy, repeat the process by adding 3 mL of hydrochloric acid. To adjust the pH to 3 - 4 with 6 M ammonium hydroxide. To proceed as described in *Limit test for heavy metals*, by using 1 mL of a standard lead solution (20 ppm). At most 0.002% (20 ppm).

DOSAGE

To calculate the percentage of aluminum and zirconium octa hydrochloride anhydrous and aluminum and zirconium octa hydrochloride, according to the equation:

$$Al \left(\frac{\left\{ 26,98y + 17,01 \left[3y + 4 - \frac{(y+1)}{z} \right] + 35,453 \frac{(y+1)}{z} \right\}}{26,98y} \right)$$

where

Al = percentage of aluminum found in the test for *Aluminum Content*;

y = atomic ratio of aluminum/zirconium found in the test for *Atomic ratio of aluminum/zirconium*;

z = atomic ratio (aluminum + zirconium)/chloride found in the test for *Atomic ratio of (aluminum + zirconium)/chloride*;

26.98 = atomic mass of aluminum;

92.97 = atomic mass of zirconium corrected for 2% of hafnium.

17.01 = molecular weight of hydroxide anion (OH)

35.453 = atomic mass of chlorine (Cl).

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

ALUMINUM AND ZIRCONIUM OCTA HYDROCHLORIDE SOLUTION

It contains at least 90.0 % and at most 110.0 % of the amount stated of aluminum octa hydrochloride anhydrous. Aluminum and zirconium octa hydrochloride is a basic complex polymer of aluminum chloride. It has atomic ratio of aluminum/zirconium between 6:1 and 10:1, and (aluminum + zirconium)/chloride between 1.5:1 and 0.9:1.

The following solvents may be used: water, propylene glycol, or dipropylene glycol.

IDENTIFICATION

A. For solution prepared in propylene glycol, add about 10 mL of isopropyl alcohol of 2 g solution. To mix and filter it. To evaporate the filtrate in a water bath to obtain 1 mL of volume. The infrared absorption spectrum (5.2.14) of a film of this solution, dispersed in silver chloride, has absorption maxima only at the same wavelengths to those observed in the propylene glycol spectrum film prepared similarly.

B. For solution prepared in dipropylene glycol, add about 10 mL of isopropyl alcohol of 2 g solution. To mix and filter it. To evaporate the filtrate in a water bath to obtain 1 mL of volume. The infrared absorption spectrum (5.2.14) of a film of this solution, dispersed in silver chloride, has absorption maxima only at the same wavelengths to those observed in the dipropylene glycol spectrum film prepared similarly.

C. The solution containing the equivalent of about 100 mg/mL of aluminum and zirconium octa hydrochloride responds to the reactions of chloride ion (5.3.1.1).

CHARACTERISTICS

pH (5.2.19). 3.0 to 5.0. To determine in a solution prepared by diluting 3 g of solution in water, to top up to 10 mL.

PURITY TESTS

Arsenic (5.3.2.5). To use *Method I*. To determine in 1.5 g of the sample. At most 0.0002% (2 ppm).

Iron (5.3.2.4). To use *Method III*. To transfer 5.3 g of aluminum and zirconium octa hydrochloride solution to 100 mL volumetric flask and top up with water. To transfer 5 mL of the sample solution and 2 mL of the standard solution to individual beakers. To add 5 mL of 6 M nitric acid to each beaker. To cover with a watch glass and boil both solutions for 3-5 minutes. To cool down. At most 0.0075% (75 ppm).

Heavy metals (5.3.2.3). To use *Method I*. To weigh 2 g of aluminum and zirconium octa hydrochloride solution and add 40 mL of water. Should the preparation be not clear, heat it at 80 °C for a few minutes, then wait to cool down. Should the preparation be cloudy, repeat the process by adding 3 mL of hydrochloric acid. To adjust the pH to 3 - 4 with 6 M ammonium hydroxide. To use 1 mL of *Standard lead solution (10 ppm)*. At most 0.001% (10 ppm).

Aluminum content. To weigh 0.15 g of the sample, transfer to 150 mL beaker, and add 5 mL of water and 15 mL of hydrochloric acid. To keep boiling for 5 minutes. To add 40 mL of water and 30 mL of 0.05 M SV disodium edetate.

To keep boiling for 5 minutes. To wait to cool down, add 15 mL of acetic acid-ammonium acetate buffer, and adjust pH to 4.5 with concentrated ammonia solution. To add 20 mL of ethanol and adjust the pH to 4.6 with concentrated ammonia solution. To add 10 drops of 0.025% dithizone (w/v) in ethanol. To titrate with 0.1 M SV zinc sulfate to obtain pink-purple color. To perform sample blank correction. To calculate the percentage of aluminum:

$$Al = \frac{2,698 [15Me - (zMz + Ze)]}{W}$$

where, M_e is the molarity of 0.05 M SV sodium edetate, z is volume consumed of 0.1 M SV zinc sulfate, in mL, M is the molarity of 0.1 M SV zinc sulfate, W is the amount, in g, of the sample and Z_e is the equivalent volume of sodium edetate consumed by the amount of zirconium, calculated according to the following equation:

$$Z_e \left(\frac{Zr}{Me} \right) \times \left(\frac{W}{92,97} \right)$$

where, Z_r is the percentage of zirconium determined in the test *Zirconium content*, 92.97 is the atomic mass of zirconium corrected for the content of 2% of hafnium. To use the result to calculate the atomic ratio of aluminum/zirconium and the atomic ratio of (aluminum + zirconium)/chloride.

Zirconium content. To weigh 500 mg of the sample, transfer to 150 mL beaker, and add 5 mL of water and 15 mL of hydrochloric acid. To keep boiling for 6-8 minutes. Then add 30 - 40 mL of water and 5 mL of hydrochloric acid and heat to boiling. To add a drop of xylenol orange SI and titrate the solution, still warm, with 0.05 M SV disodium edetate to change from pink to yellow. To perform sample blank correction. Each mL of 0.05 M SV sodium edetate is equal to 46.48 mg of zirconium. It contains at least 12.8% and at most 15.4% of zirconium. To use the result to calculate the atomic ratio of aluminum/zirconium and the atomic ratio of (aluminum + zirconium)/chloride.

Atomic ratio of aluminum/zirconium. To divide the percentage of aluminum found in the test for *Aluminum Content* by the percentage of zirconium found in the test for *Zirconium Content* and multiply by 92.97/26.98. Where 92.97 is the atomic mass of zirconium corrected to 2% of hafnium and 26.98 is the atomic mass of aluminum. At least 6:1 and at most 10:1.

Chloride content. To weigh 500 mg of the sample, transfer to a 250 mL beaker, and add 120 mL of water and 20 mL of M nitric acid. To stir to obtain solubilization and titrate with 0.1 M SV silver nitrate, thus potentiometrically determining the endpoint. Each mL of 0.1 M SV silver nitrate is equivalent to 3.546 mg of chloride. It contains at least 16.5% and at most 19.0% of chloride. To use the result obtained to calculate the atomic ratio of aluminum/

zirconium and atomic ratio of (aluminum + zirconium)/chloride.

Atomic ratio (aluminum + zirconium)/chloride. To calculate the atomic ratio (aluminum + zirconium)/chloride, according to the equation:

$$\frac{\left(\frac{Al}{26,98} \right) + \left(\frac{Zr}{92,97} \right)}{\left(\frac{Cl}{35,453} \right)}$$

where, Al , Zr , and Cl = percentage of aluminum, zirconium, and chloride, determined in tests for *Aluminum Content*, *Zirconium Content*, and *Chloride Content*, respectively. 26.98 is the atomic mass of aluminum, 92.97 is the atomic mass of zirconium corrected for 2% hafnium and 35.453 is the atomic mass of chlorine. The ratio is between 1.5:1 and 0.9:1.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To calculate the percentage of aluminum and zirconium octa hydrochloride anhydrous and the solution, according to the equation:

$$Al \times \left(\frac{26,98y + 92,97 + 17,01 \left[3y + 4 - \left(\frac{y+1}{z} \right) \right] + 35,453 \left(\frac{y+1}{z} \right)}{26,98y} \right)$$

where, Al is the percentage of aluminum found in the test *Aluminum content*, y is the atomic ratio aluminum/zirconium found in the test *Atomic ratio of aluminum/zirconium*, z is the atomic ratio of (aluminum + zirconium)/chloride found in the test *Atomic ratio of (aluminum + zirconium)/chloride*, 26.98 is the atomic mass of aluminum, 92.97 is the atomic mass of zirconium corrected for 2% of hafnium, 17.01 is the molecular mass of hydroxide anion (OH), and 35.453 is the atomic mass of chlorine (Cl).

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

OFLOXACIN

Ofloxacinum



$C_{18}H_{20}FN_3O_4$; 361.37
 ofloxacin; 06574
 9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido [1,2,3-de] -1,4-benzoxazine 6-carboxylic acid
 [83380-47-6]

It contains at least 98.5% and at most 101.5% of $C_{18}H_{20}FN_3O_4$, in relation to the desiccated substance.

DESCRIPTION

Physical and chemical characteristics. Crystals as colorless needles. Melting range (5.2.2): 250 °C with decomposition.

Physical and chemical constants.

Rotation (5.2.8): 1.0° to +1.0°, in relation to the desiccated substance. To determine in 1% solution (w/v) in chloroform.

IDENTIFICATION

The infrared absorption spectrum (5.2.14) of the desiccated sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the ofloxacin SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14), from 200 nm to 400 nm, 0.00067% solution (w/v) prepared in 0.1 M hydrochloric acid, has absorption maximum similar to those observed in the spectrum of a similar solution of ofloxacin SQR.

PURITY TESTS

Arsenic (5.3.2.5). To use *Method III*. At most 0.0001% (1 ppm).

Heavy metals (5.3.2.3). To use *Method III*. At most 0.001% (10 ppm).

Loss on drying (5.2.9). To determine in 2 g of the sample. To desiccate in oven at 105 °C for 4 hours. At most 0.2%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. To dissolve about 0.1 mg of the sample previously desiccated in 400 mL beaker and add 275 mL of acetic anhydride to dissolve. To titrate with 0.1 M SV perchloric acid, to potentiometrically determine the endpoint. To use the first inflection point. To perform sample blank correction and adjust it as required. Each mL of 0.1 M SV perchloric acid is equal to 36.138 mg of $C_{18}H_{20}FN_3O_4$.

B. To proceed as described in *Microbiological test of antibiotics (5.5.3.3.1)* by using cylinders with the agar diffusion method.

Micro-organism: *Micrococcus luteus* ATCC 9341.

Culture media: culture medium number 1, to maintain the micro-organism; sterile saline solution to the standardization of the inoculum; and culture medium number 11, to the base layer and inoculum layer in the plate.

Sample solution: To accurately weigh about 0.25 g of sample, transfer to a 250 mL volumetric flask, by using 100 mL of 0.1 M Potassium phosphate buffer, sterile, pH 8.0 (Solution 2), stir for 30 minutes, and top up with the same solvent. To successively dilute to obtain 20 µg/mL, 30 µg/mL, and 45 µg/mL concentrations, by using the 0.1 M Potassium phosphate buffer, sterile, pH 8.0 (Solution 2) as diluent.

Standard solution: to accurately weigh about 50 mg of ofloxacin SQR, transfer to 50 mL volumetric flask, and top up with 0.1 M Potassium phosphate buffer, sterile, pH 8.0 (Solution 2). To successively dilute to obtain 20 µg/mL, 30 µg/mL, and 45 µg/mL concentrations, by using 0.1 M Potassium phosphate buffer, sterile, pH 8.0 (Solution 2) as diluent.

Procedure: to add 20 mL of culture medium number 11 in each plate, wait to solidify, add 5 mL of 0.5% inoculum, and proceed as described in *Microbiological test of antibiotics (5.5.3.3.1)*, by adding 0.2 mL of freshly prepared solutions to the cylinders. To calculate the potency of the sample, in µg, of ofloxacin per mg, from the potency of the standard and responses obtained with the *Standard and sample solutions*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antimicrobial.

OFLOXACIN TABLETS

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_{18}H_{20}FN_3O_4$.

IDENTIFICATION

A. To weigh and pulverize the tablets. To prepare 0.00067% solution (w/v) in 0.1 M hydrochloric acid, mechanically stir for 20 minutes, and filter. The ultraviolet absorption spectrum (5.2.14), from 200 nm to 400 nm, has absorption maximum similar to those observed in the spectrum of a similar solution of ofloxacin SQR.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

PURITY TESTS

Related substances. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 294 nm; 100 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group, kept at room temperature; flow of *Mobile phase* of 1 mL/min.

Buffer solution: to dissolve 2.72 g of monobasic potassium phosphate in 1000 mL of water, adjust the pH to 3.3 ± 0.1 with phosphoric acid SR.

Eluent A: mixture of *Buffer solution* and acetonitrile (88:12). To degas and filter it.

Eluent B: mixture of acetonitrile and *Buffer solution* (60:40). To degas and filter it.

Mobile phase: to use several mixtures of *Eluents A and B*, as follows.

Time (Minutes)	Eluent A (%)	Eluent B (%)	Elution
0-8	100	0	Isocratic
8-25	100→40	0→60	Linear Gradient
25-26	40→100	60→0	Linear Gradient
26-40	100	0	Isocratic

Sample solution: to weigh and grind at least 20 tablets. To transfer amount of powder equivalent to 100 mg of ofloxacin to a 100 mL volumetric flask, add about 70 mL of methanol, and put the flask in ultrasonic bath for 20 minutes. To top up with methanol and filter through 0.45 µm filter, by discarding the first 5 mL of filtrate.

Standard solution: to dissolve accurately weighed amount of ofloxacin SQR in methanol and dilute to obtain 4 µg/mL solution.

To inject replicates of 10 µL of the *Standard solution*. The tailing factor for ofloxacin peak is not greater than 2.0. The relative standard deviation for replicate of the peaks recorded is not greater than 5.0 %.

Procedure: to separately inject 10 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the percentage of each Impurity in the sample according to the equation:

$$100 (1/F) (A_i/A_p) (C_p/C_a)$$

where

F = relative response factor for each impurity;

A_i = area under the peak corresponding to any individual impurity in the sample solution;

A_p = area under the peak corresponding to ofloxacin in the standard solution;

C = concentration in mg/mL of ofloxacin in the Standard solution;

C_a = concentration in mg/mL of ofloxacin in the sample solution, based on the stated value.

The limits for impurities are as follows:

Impurity	Relative Retention Time	Relative Response Factor	Limit (%)
Impurity A (2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido [1,2,3-de] -1,4-benzoxazine-6-carboxylic acid)	0.5	1	0.3
Impurity B (9,10-difluoro-3-methyl-7-oxo-2,3-dihydro-7 H-pyrido [1,2,3-de] -1,4-benzoxazine-6-carboxylic acid)	3.6	0.22	0.3
Other individual impurities	-	1	0.2
Impurities - Total	-	-	1.0

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Microbiological test of antibiotics (5.5.3.3.1)* by using cylinders with the agar diffusion method.

Micro-organism: *Kocuria rhizophila* ATCC 9341.

Culture media: culture medium number 1, to maintain the micro-organism; sterile saline solution to the standardization of the inoculum; and culture medium number 11, to the base layer and inoculum layer in the plate.

Sample solution: To weigh and pulverize 20 tablets. To use amount of powder equivalent to 0.25 g of ofloxacin, quantitatively transfer to a 250 mL volumetric flask, by using 100 mL of 0.1 M Potassium phosphate buffer, sterile, pH 8.0 (Solution 2), stir for 30 minutes, top up with the same diluent, and filter it. To successively dilute to obtain 0.05 µg/mL, 0.10 µg/mL, and 0.20 µg/mL concentrations, by using the potassium phosphate buffer, sterile, pH 8.0 (Solution 2) as diluent.

Standard solution: to accurately weigh about 25 mg of ofloxacin SQR, transfer to a 250 mL volumetric flask, and top up with potassium phosphate buffer solution, sterile, pH 8.0 (Solution 2), and stir. To successively dilute to obtain 0.05 µg/mL, 0.10 µg/mL, and 0.20 µg/mL concentrations, by using the potassium phosphate buffer, sterile, pH 8.0 (Solution 2) as diluent.

Procedure: to add 20 mL of culture medium number 11 in each plate, wait to solidify, add 5 mL of 0.5% inoculum, and proceed as described in *Microbiological test of antibiotics (5.5.3.3)*, by adding 0.2 mL of freshly prepared solutions to the cylinders. To calculate the amount in mg of ofloxacin in the tablets, from the potency of the standard and responses obtained with the *Standard and sample solutions*.

B. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 294 nm; 100 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group, kept at room temperature; flow of *Mobile phase* of 1 mL/min.

Buffer solution: to dissolve 2.72 g of monobasic potassium phosphate in 1000 mL of water, adjust the pH to 3.3 ± 0.1 with phosphoric acid SR.

Mobile phase: mixture of *Buffer solution* and acetonitrile (88:12). To degas and filter it.

Diluent 1: mixture of methanol and glacial acetic acid (75:25).

Diluent 2: mixture of water and acetonitrile (90:10).

Standard solution: to transfer about 25 mg of ofloxacin SQR to a 25 mL volumetric flask and dissolve in *Diluent 1* (1 mg/mL).

To transfer 2 mL of this solution to 100 mL volumetric flask and top up with *Diluent 2* (20 µg/mL).

Sample solution: to weigh and grind at least 20 tablets. To transfer an amount of powder equivalent to 100 mg of ofloxacin to a 100 mL volumetric flask, add about 70 mL of *Diluent 1*, and put the flask in ultrasonic bath for 20 minutes. To top up with *Diluent 1* and filter it by using 0.45 µm filter. To transfer 2 mL of filtered solution to 100 mL volumetric flask top up with *Diluent 2*, and stir.

To inject replicates of 20 µL of the *Standard solution*. The tailing factor for ofloxacin peak is not greater than 2.0. The relative standard deviation for replicate of the peaks recorded is not greater than 2.0 %.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of $C_{18}H_{20}FN_3O_4$ in the tablets, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

OFLOXACIN OPHTHALMIC SOLUTION

It contains at least 90.0% and at most 110.0 % of the amount stated of $C_{18}H_{20}FN_3O_4$.

IDENTIFICATION

A. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF₂₅₄ silica gel as support and a mixture of chloroform, methanol, and solution (1:30) of ammonium hydroxide (150:75:15) as mobile phase. To apply separately to the plate 2 µL of each of the solutions as described below.

Solution (1): to dilute accurately measured amount of ophthalmic solution in a mixture of chloroform and methanol (1:1), to obtain 0.3 mg/mL ofloxacin solution.

Solution (2): to dissolve amount of ofloxacin SQR in a mixture of chloroform and methanol (1:1), to obtain 0.3 mg/mL ofloxacin solution.

To develop the chromatogram. To remove the plate, and let it air drying. To examine under ultraviolet light (254 nm). The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

pH (5.2.19). 6.0 to 6.8.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

DOSAGE

To employ one of the methods described below:

A. To proceed as described in *Microbiological test of antibiotics* (5.5.3.3.1) by using cylinders with the agar diffusion method.

Micro-organism: *Kocuria rhizophila* ATCC 9341.

Culture media: culture medium number 1, to maintain the micro-organism; sterile saline solution to the standardization of the inoculum; and culture medium number 11, to the base layer and inoculum layer in the plate.

Sample solution: to dilute the ophthalmic solution to obtain 0.05 µg/mL, 0.10 µg/mL, and 0.20 µg/mL concentrations, by using the *Potassium phosphate buffer, sterile, pH 8.0* (*Solution 2*) as diluent.

Standard solution: to accurately weigh about 25 mg of ofloxacin SQR, transfer to a 250 mL volumetric flask, and top up with potassium phosphate buffer solution, sterile, pH 8.0 (*Solution 2*), and stir. To successively dilute to obtain 0.05 µg/mL, 0.10 µg/mL, and 0.20 µg/mL concentrations, by using the *potassium phosphate buffer, sterile, pH 8.0* (*Solution 2*) as diluent.

Procedure: to add 20 mL of culture medium number 11 in each plate, wait to solidify, add 5 mL of 0.5% inoculum, and proceed as described in *Microbiological test of antibiotics* (5.5.3.3), by adding 0.2 mL of freshly prepared solutions to the cylinders. To calculate the amount in mL of ofloxacin in the ophthalmic solution, from the potency of the standard and responses obtained with the *Standard and sample solutions*.

B. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 294 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at 35 °C; flow of *Mobile phase* of 1.5 mL/min.

Mobile phase: mixture of 0.24% sodium lauryl sulfate (w/v), acetonitrile, and glacial acetic acid (580:400:20). To degas and filter it.

Sample solution: to transfer an amount of ophthalmic solution equivalent to 3 mg of ofloxacin to a 50 mL volumetric flask, dilute with 0.05 M hydrochloric acid, and stir.

Standard solution: to dissolve accurately weighed amount of ofloxacin SQR and dilute in 0.05 M hydrochloric acid, to obtain 60 µg/mL solution.

Resolution solution: to prepare a solution containing about 0.1 mg of ofloxacin SQR and about 2.4 mg of propylparaben in each mL of acetonitrile.

To inject 20 µL of *Resolution solution*. The resolution between propylparaben and ofloxacin peaks is not less than 2. To inject replicates of 20 µL of *Standard solution*. The tailing factor for ofloxacin peak should not be greater than 2.0. The relative standard deviation for replicate of the peaks recorded should not be greater than 2.0 %.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of C₁₈H₂₀FN₃O₄ in the ophthalmic solution, from the responses from the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

PEANUT OIL

Arachidis oleum

Peanut oil; 09887
[8002-03-7]

Refined fixed oil obtained from the seeds of one or more cultivated varieties of *Arachis hypogaea* L. – LEGUMINOSAE.

DESCRIPTION

Physical characteristics. Oil almost colorless or slightly yellowish, viscous, odorless or slight pleasant odor.

Solubility. Insoluble in water, soluble in benzene, carbon tetrachloride and mineral oils, sparingly soluble in ethanol, miscible with ethyl ether, petroleum ether, chloroform, and carbon disulfide.

Physical and chemical constants.

Relative density (5.2.5): 0.912 to 0.920.

Refractive index (5.2.6): 1.462 to 1.464. Determine at 40°C.

Solidification temperature (5.2.29.3): 26°C to 33°C. To determine in the dry mixture of fatty acids.

IDENTIFICATION

To saponify 5 g of sample with 2.5 mL of sodium hydroxide and 7.5 M and 12.5 mL of ethanol by heating to boiling. To evaporate the ethanol, dissolve the soap in 50 mL of hot water and add hydrochloric acid let the free fatty acids separate as an oily layer. To cool down the mixture, remove the fatty acids separated and dissolve them in 75 mL of ethyl ether. To add the heated solution of 10% lead acetate (w/v) in ethanol to the ether solution. Let it rest for 18 hours. To filter the supernatant and transfer the precipitate to the filter with the aid of ethyl ether. To place the precipitate in a mixture of 40 mL of 3 M hydrochloric acid and 20 mL of water. To heat until the oil layer becomes completely clear. To cool down, decant the aqueous solution and boil fatty acids with water acidified with hydrochloric acid until the lead is eliminated. [Fatty acids are lead-free when 100 mg, dissolved in 10 ml of ethanol, are not darkened by the addition of 2 drops of sodium sulfate at 10% (w/v)]. To allow fatty acids to solidify and press them between filter papers on a cold surface to dry. To dissolve the solid fatty acid in 25 mL of 90% ethanol (v/v) moderately heat, cool down at 15°C and maintain this temperature until the fatty acids to crystallize. To filter fatty acids obtained. To recrystallize them with 90% hot ethanol (v/v) and dry in a vacuum desiccator for 4 hours. The arachidonic acid thus obtained melts between 73°C and 76°C.

PURITY TESTS

Index of acidity (5.2.29.7). At most 2mL of 0.02 M sodium hydroxide SV are spent to neutralize the free fatty acids present in 10 g of the sample

Index of Iodine (5.2.29.10). 84 to 100.

Index of Saponification (5.2.29.8.). 185 to 195.

Unsaponifiable matter (5.2.29.14.). At most 1.5%.

Cottonseed oil. In a test tube, stir 5 mL of the sample with 5 mL of *n*-amyl alcohol mixture and 1% sulfur 1% (w/v) in carbon disulfide (1:1). To carefully heat to evaporation of the carbon disulphide. To submerge the tube to one third of its depth in a saturated solution of boiling sodium chloride. Reddish color is not developed for 15 minutes.

Sesame oil. To stir the sample with an equal volume of a mixture of 90% ethanol (v/v) and ammonium hydroxide (9:1). Heat in water bath until elimination of ethanol and ammonia. To mix 2 mL of the residue with 1 mL of 1% sucrose (w/v) in hydrochloric acid and let it rest for 5 minutes. The acid layer should not color of pink or if pink color appears, it should be at most equal to that obtained by repeating the test with sucrose.

Other vegetable oils. In a flask with reflux condenser, saponify 1 mL of the sample with 5 mL of 2 M ethanolic

potassium hydroxide for 5 minutes. To add 1.5 mL of glacial acetic acid and 50 ml of 70% ethanol (v/v). To heat until the solution becomes clear. To slowly cool down, and measure the liquid temperature. Turbidity occurs at a temperature not lower than 39°C.

Rancidity. To mix 1 mL of 10% sample (v/v) in ethyl ether with 1 mL of hydrochloric acid and add 1 mL of 0.1% phloroglucinol (w/v) in ethyl ether. No red or pink color is developed.

Heavy metals (5.3.2.3). To use Method III. At most 0.001% (10 ppm).

PACKAGING AND STORAGE

In airtight closed containers, protected from light, avoid exposure to excessive heat.

LABELLING

To comply with the legislation in force.

CATEGORY

Pharmaceutical adjuvant.

SESAME OIL

Sesami oleum

Glyceride and fatty sesame oils; 09888
[8008-74-0]

It is the refined fixed oil obtained from the seed of one or more cultivated varieties of *Sesamum indicum* L. – PEDALIACEAE.

DESCRIPTION

Physical characteristics. Pale-yellow oil, almost odorless, mild flavor. Mainly composed by oleic and linoleic acids.

Solubility. Insoluble in water, soluble in chloroform, ethyl ether and petroleum ether, sparingly soluble in ethanol.

Physical and chemical constants.

Relative density (5.2.5): 0.916 to 0.921.

Solidification temperature (5.2.29.3): 20°C to 25°C. Use the dry mixture of fatty acids.

IDENTIFICATION

To stir 1 mL of the sample and 10 ml of 1% sucrose (w/v) in hydrochloric acid for 30 seconds. The acid layer becomes pink and turns red at rest (distinction from most other fixed oils).

PURITY TESTS

Index of Iodine (5.2.29.10). 103 to 116.

Index of saponification (5.2.29.8). 188 to 195.

Unsaponifiable matter (5.2.29.14). At most 1.5%.

Index of acidity (5.2.29.7). Not more than 2 mL of 0.02 *M* sodium hydroxide SV are spent to neutralize the free fatty acids present in 10 g of the sample.

Cottonseed oil. In a test tube, stir 5 mL of the sample and 5 mL of *n*-amyl alcohol mixture and 1% sulfur 1% (w/v) in carbon disulfide (1:1). Carefully heat, to evaporation, of the carbon disulphide. To submerge the tube to one third of its depth in a saturated solution of boiling sodium chloride. Reddish color is not developed for 15 minutes.

Heavy metals (5.3.2.3). at most 0.001% (10 ppm). To use *Method III*. No

Triglyceride	Relative retention time	Composition (%)
LLL	0,55	7.0 to 19.0%
OLL	0,65	13.0 to 30.0%
PLL	0,69	5.0 to 9.0%
OOL	0,77	14.0 to 25.0%
POL	0,82	8.0 to 16.0%
OOO	0,93	5.0 to 14.0%
SOL	0,97	2.0 to 8.0%
POO	1,0	2.0 to 8.0%

Composition of triglycerides. To proceed as described in *High performance liquid chromatography (5.2.17.4)*. To use a chromatograph equipped with refractive index detector; two columns in series of 250 mm length and 4.6 mm internal diameter packed with silica chemically bonded to the group to the octylsilane group (5 µm), kept at 30°C; flow of *Mobile phase* of 1.0 mL/min.

Mobile phase: mixture of acetonitrile and methylene chloride (60:40).

Sample solution: to accurately transfer about 0.2 g of sample to 10 mL volumetric flask and top up to volume with *Mobile phase*. The fatty acids present in the sample are: linoleic acid (L), oleic acid (O), palmitic acid (P) and stearic acid (S). The triglycerides in the sample are: trilinolein (LLL), 1,2-dilinoyleoyl-3-oleyl-rac-glycerol (OLL), 1,2-dilinoyleoyl-3-palmitoyl-rac-glycerol (PLL), 1,2-di-oleoyl-3-rac-linoleoyl-rac-glycerol (OOL), 1-palmitoyl-2-oleyl-3-linoleoyl-rac-glycerol (POL), triolein (OOO), 1-linoleoyl-2-oleyl-3- estearoil-rac-glycerol (SOL) and 1,2-dioleoyl-3-palmitoyl-rac-glycerol (POO).

Resolution solution: to transfer 30 mg of OLL and 30 mg of PLL, accurately weighed, to 10 mL volumetric flask and top up to *Mobile phase*.

To inject replicates of 20 µL of *Resolution solution*. The relative retention times are about 0.93 to OLL and 1.0 to PLL. The resolution between the peaks of OLL and PLL is not less than 1.8. The relative standard deviation of the replicate areas of peaks recorded is not greater than 1.5%

Procedure: to inject 20 µL of *Sample solution*, record the chromatograms, and measure the areas under the peaks of triglycerides. To calculate the percentage of each triglyceride in the sample of sesame oil, relative to the sum of the peaks observed. To disregard peaks relative to the solvents. The table below shows the relative retention times and percent composition of the triglycerides.

PACKAGING AND STORAGE

In airtight closed containers, protected from light, by avoiding exposure to excessive heat.

LABELLING

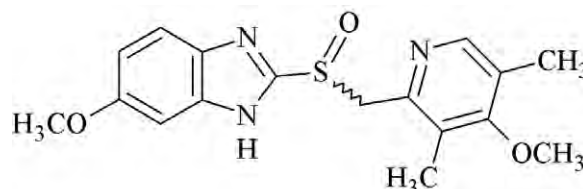
To comply with the legislation in force.

CATEGORY

Excipient pharmacotechnical.

OMEPRAZOLE

Omeprazolium



C₁₇H₁₉N₃O₃S; 345,42

omeprazole; 06602

6-Methoxy-2-[[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl] sulfinyl]-1*H*-benzimidazole
[73590-58-6]

It contains at least 98.0% and at most 102.0% of C₁₇H₁₉N₃O₃S, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or almost white powder. It shows polymorphism.

Solubility. Very slightly soluble in water, soluble in methylene chloride, slightly soluble in methanol and ethanol. Soluble in dilute alkalis.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength,

with the same intensities to those observed in the omeprazole SQR spectrum. If there are differences between the spectra, separately dissolve sample and omeprazole SQR in methanol and evaporated to dryness. To obtain new spectra from these residues.

B. The ultraviolet absorption spectrum (5.2.14), ranging from 230 nm to 350 nm, 0.002% solution (w/v), in 0.1 M sodium hydroxide, exhibits absorption maxima at 276 nm and 305 nm. The ratio between the absorbance values measured at 305 nm and 276 nm is between 1.6 and 1.8.

C. The principal spot in the chromatogram of *Solution (2)* in the *Related substances 1* corresponds in position, color, and intensity to that obtained with *Solution (3)*.

PURITY TESTS

Appearance of solution. The 2% solution (w/v) in methylene chloride is clear (5.2.25) and colorless (5.2.12).

Absorption of light. The absorbance of 2% solution (w/v) in methylene chloride measure at 440 nm is not greater than 0.10.

Related substances 1. To proceed as described in *Thin-layer chromatography (5.2.17.1)*, by using F₂₅₄ silica gel as support and a mixture of isopropyl alcohol, methylene chloride, and methylene chloride saturated with ammonia (1:2:2) as mobile phase. To separately apply to the plate 10 µL of each of the solutions freshly prepared, as described below.

Solution (1): to dissolve 100 mg of the sample in 2 mL of a mixture of methanol and methylene chloride (1:1).

Solution (2): to dilute 1 mL of *Solution (1)* to 10 mL with methanol.

Solution (3): to dissolve 10 mg of omeprazole SQR in 2 mL of methanol.

Solution (4): to dilute 1 mL of *Solution (1)* to 10 mL with a mixture of methanol and methylene chloride (1:1). To dilute 1 mL of the resulting solution to 100 mL with the same diluent.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (4)* (0,1%).

Related substances 2. To proceed as described in *Dosage method B*. To prepare the Sample solution as described below.

Test solution: to dissolve 16 mg of the sample in *Mobile phase* and dilute to 10 mL with the same solvent. To dilute 1 mL of the resulting solution to 10 mL with the *Mobile phase*.

Procedure: To separately inject 40 µL of *Test solution*, record the chromatograms at least twice the retention time of the main peak. To measure the areas of all peaks obtained.

The area of any secondary peak, other than the main peak's one, is not greater than 0.3% of the total area of the peaks obtained with the *Test solution*. The sum of the areas under the secondary peaks, except the main peak's one, is not greater than 1.0% of the total area of the peaks obtained with *Test solution*. Solvent peaks should not be included in the calculations.

Volatile organic impurities. To proceed as described in *Gas chromatography (5.2.17.5)*. To use gas chromatograph equipped with flame ionization detector, by using helium as carrier gas; 30 m long melted silica capillary column; and 0.53 mm inner diameter, filled with polycyanopropyl-phenyldimethylsiloxane, 1.8 µm film thickness; column temperature at 40 °C for 20 minutes, rapidly raised to 240 °C and kept for 20 minutes, injector temperature at 140 °C; and detector temperature at 260 °C.

Sample solution: To transfer 100 mg of sample to a 10 mL vial, add 5 mL of dimethylacetamide and 1 g of sodium sulfate anhydrous, close, and heat at 80 °C for 1 hour.

Reference solution: to prepare a solution in dimethylacetamide containing 12.0 µg/mL methylene chloride, 1.2 µg/mL chloroform, 7.6 µg/mL dioxane, and 1.6 µg/mL trichlorethylene. To transfer 5 mL of the resulting solution to a 10 mL volumetric flask, add 1 g of sodium sulfate anhydrous, close, and heat at 80 °C for 1 hour.

To inject replicates the *Reference solution* by using an appropriate "headspace." The resolution between any components should not be less than 3. The relative standard deviation for replicate areas of the peaks recorded should not be greater than 15%.

Procedure: To separately inject, by an appropriate "headspace", the *Reference solution* and *Sample solution*. To record the chromatograms and measure the areas under the peaks. The areas under the methylene chloride, chloroform, dioxane, and trichlorethylene peaks obtained for the *Sample solution* should not be greater than those of area under the methylene chloride, chloroform, dioxane, and trichlorethylene peaks obtained for the *Reference solution*, corresponding at most 600 ppm, 60 ppm, 380 ppm, and 80 ppm, respectively.

Heavy metals (5.3.2.3). To use the *Method I*. At most 0.5%.

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 60°C under reduced pressure for 4 hours. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

A. To accurately weigh about 1.1 g of sample and dissolve in 50 mL of a mixture of water and ethanol (1:4). To titrate with 0.5 M SV sodium hydroxide. To potentiometrically determine the endpoint. Each mL of 0.5 M SV sodium hydroxide is equal to 0.1727 mg of $C_{17}H_{19}N_3O_3S$.

B. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 280 nm; 150 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at room temperature; flow of *Mobile phase* of 0.8 mL/min.

Phosphate buffer pH 7.6: to dissolve 0.725 g of monobasic sodium phosphate and 4.472 g of dibasic sodium phosphate anhydrous in 300 mL of water, dilute to 1000 mL with the same solvent, and make it homogeneous. To transfer 250 mL of the resulting solution to a 1000 mL volumetric flask, add about 980 mL of water, adjust the pH to 7.6 with phosphoric acid, and top up with water.

Mobile phase: mixture of *Phosphate buffer pH 7.6* and acetonitrile (3:1).

Reference solution (a): to dissolve accurately weighed amount of omeprazole SQR in a mixture of 0.01 M sodium borate and acetonitrile (3:1) and diluted with the same solvent to obtain 200 µg/mL solution.

Reference solution (b): to transfer 5 mL of *Reference solution (a)* to a 10 mL volumetric flask, top up with a mixture of 0.01 M sodium borate and acetonitrile (3:1), thus obtaining 100 µg/mL solution.

Sample solution: to dissolve accurately weighed amount of the sample in a mixture of 0.01 M sodium borate and acetonitrile (3:1) and diluted with the same solvent to obtain 200 µg/mL solution.

To inject replicates of 20 µL of the *Reference solution (b)*. The column efficiency should be greater than 3000 theoretical plates. The capacity factor is greater than 6.0. The tailing factor is not greater than 1.5. The relative standard deviation for replicate areas of the peaks recorded should not be greater than 1.0 %.

Procedure: to separately inject 20 µL of *Reference solution (a)* and *Sample solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_{17}H_{19}N_3O_3S$ from the sample, from the responses obtained for the *Reference solution (a)* and *Sample solution*.

PACKAGING AND STORAGE

In airtight containers, protected from light and moisture, at 2 °C - 8 °C.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antisecretory

OXIDE, MAGNESIUM Magnesii oxidum

MgO; 40.30
magnesium oxide; 06728 Magnesium oxide
[1309-48-4]

It contains at least 96.0% and at most 100.5% of MgO, in relation to the incinerated substance.

DESCRIPTION

Physical characteristics. Amorphous, fine, white powder.

Solubility. It is practically insoluble in water. Soluble in diluted acids, producing slight effervescence.

IDENTIFICATION

To dissolve about 15 mg of the sample in 2 mL of 20% nitric acid (w/v) and neutralize with 8.5% sodium hydroxide (w/v). The solution responds to the reactions of magnesium ion (**5.3.1.1**).

PURITY TESTS

Appearance of solution. To dissolve 5 g of sample in a mixture of 70 mL of acetic acid SR and 30 mL of water. To heat to boiling for 2 minutes, cool down, and top up to 100 mL with 0.045 M acetic acid. To filter, if necessary, by using silica or porcelain filter, previously calcined and tared, whose porosity provides a clear filtrate. To store any residue. The reference solution is a mixture (1:1) of the solution described below and 1% hydrochloric acid (w/v): to mix 3 mL of *Cobaltous chloride base solution II*, 3 mL of *Ferric chloride base solution*, and 2.4 mL of *Cupric sulfate base solution*, prepared as described in *Color of liquids (5.2.12)*, and 1.6 mL of 1% hydrochloric acid (w/v). 10 mL of sample of the solution is not more colored than 10 mL of reference solution.

Substances insoluble in acetic acid. To wash, dry, and calcine at 600 °C any residue collected during the preparation of the solution of the sample in *Appearance of solution*. The mass of the residue is not greater than 5 mg, which represents at most 0.1%.

Soluble substances and alkali free. To add 100 mL of water to 2 g of the sample and heat to boiling for 5 minutes. To perform hot filtering through a porous glass filter. To add 2 drops of methyl red SI to 50 mL of the filtrate and titrate with 0.05 M SV sulfuric acid. At most 2 mL of 0.05 M SV sulfuric acid are consumed. To evaporate 25 mL of the filtrate to dryness and dry at 100 - 105 °C. The mass of the residue is less than 10 mg. At most 2.0%.

Arsenic (5.3.2.5). To determine in 5 mL of the sample solution obtained in *Appearance of solution*. To proceed as described in *Visual method*. At most 0.0004% (4 ppm).

Calcium (5.3.2.7). To dilute 1.3 mL of sample solution obtained in *Appearance of solution* to 150 mL with water. To use 15 mL of this solution and proceed as described in *Limit test for calcium*. At most 1.5%.

Iron (5.3.2.4). To dissolve 40 mg of sample in 5 mL of 2 M nitric acid, heat to boiling for 1 minute, and dilute to 50 mL with water. To dilute 25 mL of the resulting solution to 45 mL with water, add 2 mL hydrochloric acid, and proceed as described in *Method I*. To use 1 mL of *Standard iron solution* (1 ppm Fe). At most 0.05% (500 ppm).

Heavy metals (5.3.2.3). To dissolve 2 g of the sample in 35 mL of 3 M hydrochloric acid and evaporate to dryness in a water bath. About to end the evaporation, continuously stir the residue to disintegrate it, to obtain a fine dry powder. To dissolve the residue in 20 mL of water and evaporate to dryness. To dissolve the residue again in 20 mL of water, filter, if necessary, and dilute to 40 mL with water. To dilute 20 mL of the resulting solution to 25 mL with water and proceed as described in *Method I*. At most 0.002% (20 ppm).

Sulfates (5.3.2.2). To weigh 2 g of the sample and add 30 mL of hydrochloric acid SR. To filter, if necessary, add 1 mL of barium chloride SR, top up to 50 mL with water, and heat in a water bath for 10 minutes. Any opalescence obtained is not more intense than that presented by 0.2 mg of sulfate ion in an equal volume of liquid containing the same amount of reagents. At most 0.01% (100 ppm).

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 10.0%.

DOSAGE

To proceed as described in *Complexometric titrations for magnesium* (5.3.3.4). To incinerate the sample at 800 °C for 1 hour. To accurately weigh about 0.32 g of the sample, dissolve in 7 mL of 3 M hydrochloric acid, and dilute to 100 mL with water. To titrate 20 mL of the resulting solution by using 0.1 M SV disodium edetate. Each mL of 0.1 M SV disodium edetate is equal to 4.030 mg of MgO.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force. To state whether magnesium oxide is light or heavy.

CATEGORY

Pharmacotechnical adjuvant, antacid, hyperosmotic saline laxative.

OXIDE, ZINC

Zinci oxidum

ZnO; 81.41
zinc oxide; 06730
Zinc oxide
[1314-13-2]

It contains at least 99.0% and at most 100.5% of ZnO, in relation to the incinerated substance.

DESCRIPTION

Physical characteristics. Fine, amorphous, white or slightly yellowish powder.

Solubility. Insoluble in water and ethanol. Soluble in acetic acid and ammonia. Soluble in diluted mineral acids.

IDENTIFICATION

A. It becomes yellow when subjected to intense heating. Such color fades out after cooling.

B. To dissolve 0.1 g of the sample in 1.5 mL of hydrochloric acid SR and dilute to 5 mL with water. Solution responds to the reactions of zinc ion (5.3.1.1).

PURITY TESTS

Appearance of solution. To dissolve 1 g of the sample in 15 mL of hydrochloric acid SR. No effervescence is observed during dissolution. The resulting solution is colorless (5.2.12) and is not more opalescent than the *Reference suspension II* (5.2.25).

Alkalinity. To stir 1 g of the sample with 10 mL of boiling water. To add 2 drops of phenolphthalein SI and filter. More than 0.3 mL of 0.1 M hydrochloric acid is not necessary to change the indicator, for red filtrate.

Cadmium. To proceed as described in *Atomic absorption spectrometry* (5.2.13.1). To prepare the *Standard and sample solutions* as described below.

Sample solution: To dissolve 2 g of sample in 14 mL of a mixture of water and cadmium- and lead-free nitric acid (1:1). To boil for 1 minute, cool down, and dilute to 100 mL with water.

Standard solution: to prepare the standard solution by using standard cadmium solution (0.1% Cd) and diluting 3.5% cadmium- and lead-free nitric acid (v/v).

To measure the absorbance of the resulting solutions at 228.8 nm by using hollow-cathode cadmium lamp as source of radiation and propane or acetylene flame. At most 0.001% (10 ppm).

Lead. To dissolve 2 g of the sample in 20 mL of water and add 5 mL of glacial acetic acid in a water bath to obtain total dissolution.

To add 5 drops of potassium chromate SR. The resulting solution produces no turbidity or precipitate.

Arsenic (5.3.2.5). To determine in 0.6 g of the sample. To proceed as described in *Spectrophotometric method, Method I*. At most 0.0005% (5 ppm).

Iron (5.3.2.4). To dissolve 0.5 g of the sample in 1 mL of hydrochloric acid SR and dilute to 10 mL with water. To proceed as described in *Method I*. To use *Standard iron solution (100 ppm Fe)*. At most 0.02% (200 ppm).

Sulfated ashes (5.2.10). To determine in 1 g of the sample at 500 °C. At most 1.0%.

DOSAGE

To accurately dissolve about 0.8 g of the sample previously calcined at 850 °C for 1 hour in 2 mL of water and 3 mL of hydrochloric acid, transfer to a 100 mL volumetric flask, and top up with water.

To pipette 10 mL of this solution and add 80 mL of water. Then, add a sodium hydroxide solution (1 : 50) until the solid particles in suspension appear. To add 5 mL of ammonia chloride buffer pH 10.7 and 2 drops of eriochrome black T SI, and titrate with 0.05 M SV disodium edetate. Each mL of 0.05 M SV disodium edetate is equal to 4.071 mg of ZnO.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

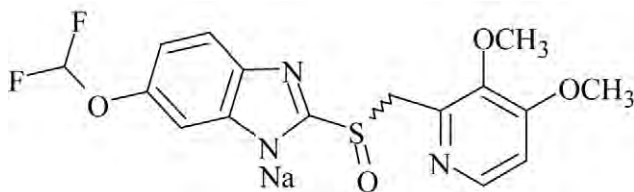
To comply with the legislation in force.

THERAPEUTIC CLASS

Dermatological protectors.

PANTOPRAZOLE SODIUM

Pantoprazoli natricum



$C_{16}H_{14}F_2N_3NaO_4S$; 405.35

$C_{16}H_{14}F_2N_3NaO_4S \cdot 1.5H_2O$; 432.37

pantoprazole sodium; 06819

Pantoprazole sodium hydrated sesqui; 09514 Sodium salt of 6 - (difluoromethoxy) -2 - [[(3,4-dimethoxy-2-pyridinyl) methyl] sulfinyl]-1H-benzimidazole (1:1) [138786-67-1]

Sodium salt of 6 - (difluoromethoxy) -2 - [[(3,4-dimethoxy-2-pyridinyl) methyl] sulfinyl]-1H-benzimidazol hydrate (2:2:3) [164579-32-2]

It contains at least 98.0% and at most 102.0% of $C_{16}H_{14}F_2N_3NaO_4S$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. Crystalline, white or almost white powder; hygroscopic.

Solubility. Freely soluble in water, and soluble in ethanol and methanol.

Physical and chemical constants.

Melting range (5.2.2): 150 °C to 160 °C, with decomposition.

Specific rotation (5.2.8): -1.0° to +1.0°, in relation to the anhydrous substance. To determine in 5% aqueous solution (w/v).

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the pantoprazole sodium SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14) ranging from 210 nm to 360 nm, of 0.001% solution (w/v) in methanol, exhibits maxima at 289 nm.

C. To solubilize 20 mg of sample in 1 mL of water. Solution responds to the reactions of sodium ion (5.3.1.1).

PURITY TESTS

Appearance of solution. The 10% aqueous solution (w/v) is clear (5.2.25) and slightly yellowish (5.2.12).

pH (5.2.19). 9.0 to 11.5. To determine in 2% aqueous solution (w/v).

Absorption of light. The maximum absorbance of the 2% aqueous solution (w/v), measured at 440 nm, is 0.025. The minimum transmittance, measured at 650 nm, is 97%. The maximum absorbance of the 10% aqueous solution (w/v), measured at 440 nm, is 0.1. The minimum transmittance, measured at 650 nm, is 95%.

Heavy metals (5.3.2.3). To use *Method III*. To determine in 1 g of the sample, in a vacuum oven, at 60 °C for 4 hours. At most 0.002% (20 ppm).

Water (5.2.20.1). 4.5% to 8.0%.

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in a vacuum oven at 60 °C for 4 hours. At most 1.0%.

DOSAGE

To employ one of the methods described below.

A. To accurately dissolve about 0.35 g of the sample in 50 mL of ethanol. To titrate with 0.1 M SV hydrochloric acid. To potentiometrically determine the endpoint or use bromophenol blue SI as indicator, to green color. To perform sample blank correction and adjust it as required. Each mL of 0.1 M SV hydrochloric acid is equal to 40.535 mg of $C_{16}H_{14}F_2N_3NaO_4S$.

B. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 290 nm; 150 mm column and 4 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), flow of *Mobile phase* of 1.0 mL/min.

Mobile phase: mixture of acetonitrile and water (75:25).

Sample solution: to dissolve accurately weighed amount of the sample in 0.1 M sodium hydroxide, to obtain 1 mg/mL $C_{16}H_{14}F_2N_3NaO_4S$ solution. To dilute in phosphate-sodium lauryl sulfate buffer pH 6.8 to obtain 0.1 mg/mL concentration. To dilute the resulting solution in a mixture of acetonitrile and water (50:50) to obtain 10 µg/mL concentration.

Standard solution: to dissolve an accurately weighed amount of pantoprazole sodium in 0.1 M sodium hydroxide, to obtain 1 mg/mL $C_{16}H_{14}F_2N_3NaO_4S$ solution. To dilute in phosphate-sodium lauryl sulfate buffer pH 6.8 to obtain 0.1 mg/mL concentration. To dilute the resulting solution in a mixture of acetonitrile and water (50:50) to obtain 10 µg/mL concentration.

To inject replicates of 20 µL of the *Standard solution*. The relative standard deviation for the replicate areas for the peaks recorded is not greater than 2.0%.

Procedure: to separately inject 20 µL of *Sample and standard solutions*, record the chromatograms, and measure the area under the main peaks. To calculate the content of $C_{16}H_{14}F_2N_3NaO_4S$ from the sample, from the responses obtained for the *Sample and standard solutions*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light under refrigeration.

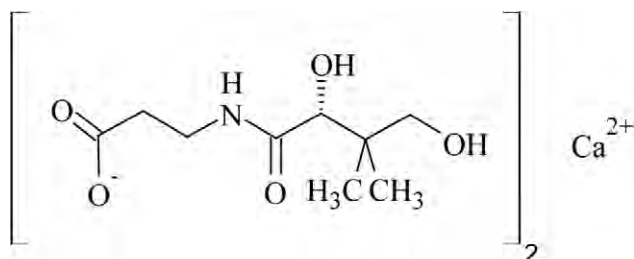
LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antisecretory

PANTOTHENATE, CALCIUM Calcii pantothenas



$C_{18}H_{32}CaN_2O_{10}$; 476,53

calcium pantothenate; 00317

Calcium salt of *N*-(2*R*)-2,4-dihydroxy-3,3-dimethyl-1-oxobutyl]-6-alanine (1:2)

[137-08-6]

It contains at least 98.0% and at most 101.0% of $C_{18}H_{32}CaN_2O_{10}$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White powder, slightly hygroscopic.

Solubility. Freely soluble in water; soluble in glycerol, sparingly soluble in acetone and ethanol; practically insoluble in ethyl ether.

Physical and chemical constants.

Specific rotation (5.2.8): +25.0° to + 27.5°, in relation to the desiccated substance.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium chloride, has absorption maximum only at the same wavelength, with the same relative

intensities to those observed in the calcium pantothenate SQR spectrum similarly prepared.

B. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using G silica gel as support and a mixture of water, glacial acetic acid, and ethanol (20:30:50) as mobile phase.

To separately apply to the plate 5 µL of each of the solutions freshly prepared, as described below.

Solution (1): to dissolve 0.2 g of the sample in water and top up to 5 mL with the same solvent.

Solution (2): to dilute 1 mL of *Solution (1)* to 10 mL with water.

Solution (3): to dissolve 20 mg of calcium pantothenate SQR in water and dilute to 5 mL with the same solvent.

To develop the chromatogram. To remove the plate and allow it to air dry, and nebulize it with ninhydrin SR. To heat at 110 °C for 10 minutes. The principal spot in the chromatogram obtained with *Solution (2)* corresponds in position, color, and intensity to that obtained with *Solution (3)*.

C. To dissolve 2.5 g of the sample in 50 mL of water. To transfer 1 mL of this solution to a test tube and add 1 mL of sodium hydroxide SR and 0.1 mL of cupric sulfate SR.

D. It responds to the reactions of calcium ion (5.3.1.1).

PURITY TESTS

Appearance of solution. The 5% solution (w/v) in water is clear and colorless.

pH (5.2.19). 6.8 to 8.0. To determine in 5 % solution (w/v).

3-amino propionic acid To proceed as described in *Identification*, test **B**. To prepare *Solution (4)* as described below.

Solution (4): to dissolve 10 mg of 3-amino propionic acid in water and dilute to 50 mL with the same solvent.

To develop the chromatogram. To remove the plate and allow it to air dry, and nebulize it with ninhydrin SR. To heat at 110 °C for 10 minutes. The 3-amino propionic acid spot in the chromatogram obtained with *Solution (1)* is no more intense than the corresponding spot in the chromatogram obtained with *Solution (4)*. At most 0.5%.

Volatile organic impurities. To proceed as described in *Gas chromatography (5.2.17.5)*. To use chromatograph equipped with flame ionization detector, by using mixture of nitrogen, synthetic air, and hydrogen (1:1:10) as the auxiliary gas to flame detector; 30 m long capillary column and 0.53 mm inner diameter, filled with stationary linked phase of 5% phenyl polysiloxane and 95% methyl polysiloxane, with a film thickness of 5 µm; column temperature from 35 °C to 260 °C (35 °C kept for 5 minutes, then

rises to 175 °C at 8 °C per minute, and rises to 260 °C at 35 °C and kept at this temperature for at least 16 minutes); injector temperature at 70 °C and detector temperature at 260 °C; use helium as carrier gas; flow of carrier gas of 1.0 mL/minute.

Sample solution: to accurately dissolve about 1 g of sample in 50 mL of water, free of organic compounds.

Standard solution: to prepare a solution in water free of organic compounds containing, in each mL, 10 µg of methylene chloride, 1 µg of chloroform, 2 µg of benzene, 2 µg of dioxane, and 2 µg of trichlorethylene.

Procedure: To separately inject 1 µL of *Sample solution* and *Standard solution* into the gas chromatograph. To obtain the chromatograms and measure the area under the peaks. Based on the retention time, identify any peak in the chromatogram of the *Sample solution*. To compare the chromatograms of the *Sample solution* and *Standard solution* to establish the presence and identification of peaks in the chromatogram. Limit: Benzene (2 ppm), chloroform (50 ppm), dioxane (100 ppm), methylene chloride (500 ppm), and trichlorethylene (80 ppm). It complies with the test.

Chlorides (5.3.2.1). To dissolve 1.8 g of the sample in 40 mL of water and proceed as described in *Limit test for chlorides*. At most 0.02% (200 ppm).

Heavy Metals (5.3.2.3). To use the *Method I*. To dissolve 0.5 g of the sample in 10 mL of water and use 1 mL of *Standard lead solution (10 ppm Pb)*. At most 0.002% (20 ppm).

Loss on drying (5.2.9). To determine in 2 g of the sample. To desiccate in an oven at 105 °C for 3 hours. At most 3%.

DOSAGE

To proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. To dissolve 180 mg of the sample in 50 mL of glacial acetic acid. To titrate with 0.1 M SV perchloric acid. To potentiometrically determine the endpoint. Each mL of 0.1 M SV perchloric acid is equal to 23.826 mg of C₁₈H₃₂CaN₂O₁₀.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

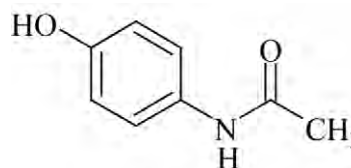
LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Food supplement.

PARACETAMOL Paracetamolum



C₈H₉NO₂; 151.16
paracetamol; 06827
N-(4-hydroxyphenyl) acetamide
[103-90-2]

It contains at least 98.0% and at most 101.0% of C₈H₉NO₂, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. Crystalline, white, and odorless powder; slight bitter taste.

Solubility. Slightly soluble in water; soluble in boiling water; freely soluble in ethanol; practically insoluble in chloroform and ethyl ether. Soluble in *M* sodium hydroxide.

Physical and chemical constants.

Melting range (5.2.2): 168 °C to 172 °C.

IDENTIFICATION

The Identification test B. may be omitted if the tests A. and C. are conducted. The identification test A. can be omitted if the tests B. and C. are conducted.

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the paracetamol SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14), ranging from 200 nm to 400 nm, of 0.0005% solution (w/v) in a mixture of 0.1 M hydrochloric acid and methanol (1:100), exhibits maxima and minima only in the same wavelength of a similar paracetamol SQR solution.

C. To add a drop of ferric chloride SR to 10 mL of 1% solution (w/v) of the sample. The blue-violet color develops.

PURITY TESTS

pH (5.2.19). 5.3 to 6.5. To determine in saturated solution.

Aminophenol-free. To dissolve 0.5 g of the sample in a mixture of methanol and water (1:1) and top up to 10 mL with the same mixture of solvents.

To prepare 10 mL of the standard solution from 0.5 g of paracetamol SQR, 4-aminophenol-free, and 0.5 mL of 0.005% 4-aminophenol (w/v) in the same mixture of solvents. To simultaneously add 0.2 mL of 1% sodium carbonate anhydrous solution (w/v), freshly prepared,, to sample solution and standard solution. To make it homogeneous and let it rest for 30 minutes. The blue color in the sample solution is not more intense than that of the Standard solution.

Chloroacetanilide. To proceed as described in *Thin-layer chromatography* (5.2.17.1). To prepare a stationary phase by dissolving 8 mg of sodium acetate in 50 mL of water, by adding 20 g of GF₂₅₄ silica gel. To prepare the plates with 0.5 mm thickness. To use a mixture of chloroform, benzene, and acetone (65:10:25) as mobile phase. To separately apply to the plate 100 µL of *Solution (1)* and 20 µL of *Solution (2)* described below.

Solution (1): to transfer 1 g of the sample to a 15 mL centrifuge tube with lid, add 5 mL of ethyl ether, mechanically stir it for 30 minutes, and centrifuge at 1000 rpm for 15 minutes or obtain clear separation.

Solution (2): 10 µg/mL p-chloroacetanilide solution in ethanol.

To develop the chromatogram in an open system to the mobile phase reaches at least 12 cm from the origin. To remove the plate, allow it to dry in air for 30 minutes, and examine it under ultraviolet light (254 nm) 4 cm away. To find the spots under 365 nm UV light. Any blue fluorescent spot produced by the *Solution (1)*, R_f 0.5 - 0.6, is not greater or more intense than that produced by the *Solution (2)*. At most 0.001%.

Readily carbonisable substances. To dissolve 0.5 g of the sample in 5 mL of sulfuric acid. The color of the solution is not more intense than the color of *Standard solution color SC A* (5.2.12).

Chlorides (5.3.2.1). To stir 1 g of sample with 25 mL of water, filter, and 1 mL of 2 M nitric acid. At most 0.014% (140 ppm).

Sulfates (5.3.2.2). To stir 1 g of the sample with 25 mL of water and quantitatively filter to Nessler tube. At most 0.02% (200 ppm).

Sulfides. To weigh about 2.5 g of the sample in 50 mL beaker. To add 5 mL of ethanol and 1 mL of M hydrochloric acid. To moisten with water a filter paper impregnated with lead acetate and place it on the watch glass. To cover the beaker with a watch glass such that one end of the paper lies on the opening of the vial. To heat on a electric plate until boiling. No spot or color appears on lead acetate paper.

Heavy Metals (5.3.2.3). To dissolve 1 g of the sample in a mixture of acetone and water (85:15) and dilute to 20 mL with the same mixture of solvents. To transfer 12 mL of the

resulting solution to Nessler tube and proceed as described in *Method III*. At most 0.002% (20 ppm).

Water (5.2.20.1). At most 0.5%.

Sulfated ashes (5.2.10). At most 0.1%.

DOSAGE

To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To accurately weigh about 0.15 g of sample, dissolve in 50 mL of 0.1 M sodium hydroxide, add 100 mL of water, mechanically stir for 15 minutes, and add enough water to 200 mL. To make it homogenous and filter it. To dilute 10 mL of filtrate to 100 mL with water. To transfer 10 mL of the resulting solution to 100 mL volumetric flask, add 10 mL of 0.1 M sodium hydroxide, and top up with water. To prepare a standard solution at the same concentration by using 0.01 M sodium hydroxide as solvent. To measure the absorbances of the resulting solutions at 257 nm, by using 0.01 M sodium hydroxide to adjust zero. To calculate the content of C₈H₉NO₂ in the sample from the readings obtained. Alternatively, make the calculations by taking into account that A (1%, 1 cm) = 715 at 257 nm.

PACKAGING AND STORAGE

In tightly closed, opaque containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Analgesic and antipyretic.

PARACETAMOL TABLETS

It contains at least 95.0 % and at most 105.0 % of the amount stated of C₈H₉NO₂.

IDENTIFICATION

A. To weigh and pulverize the tablets. To extract an amount of powder equivalent to 0.5 g of paracetamol and 20 mL of acetone. To filter, evaporate the filtrate, and dry at 105 °C. The infrared absorption spectrum (5.2.14) of the residue, dispersed in potassium bromide, has absorption maxima only at the same wavelength, with the same relative intensities to those observed in the paracetamol SQR spectrum similarly prepared.

B. To heat to boiling, for 3 minutes, 0.1 g of the residue obtained in Test A. *Identification* with 1 mL of hydrochloric acid, and add 10 mL of water and cool down. No precipitate is formed. To add 0.05 mL of 0.0167 M potassium dichromate. Violet color is developed, which does not turn red.

C. The melting point (5.2.2) of the residue obtained in Test A. *Identification* is approximately 169 °C.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: phosphate buffer pH 5.8, 900 mL

Equipment: blades, 50 rpm

Time: 30 minutes

Procedure: after testing, remove aliquot from the dissolution medium, filter, and dilute with phosphate buffer pH 5.8 to obtain suitable concentration. To measure the absorbances of the resulting solutions at 243 nm (5.2.14) compared with the 0.0017% paracetamol solution SQR (w/v) in phosphate buffer pH 5.8. To use the same solvent to adjust zero. To calculate the amount of $C_8H_9NO_2$ dissolved in the medium, from the readings obtained.

Tolerance: not less than 80% (Q) of the amount stated of $C_8H_9NO_2$ dissolves within 30 minutes.

PURITY TESTS

4-Aminophenol. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 272 nm; 200 mm column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (10 µm), flow of *Mobile phase* of 2 mL/min.

Mobile phase: to prepare 0.01 M sodium butane sulphate solution by using a mixture of water, methanol, and formic acid (85:15:0.4) as solvent.

Solution (1): to weigh and pulverize the tablets. To transfer an amount of powder equivalent to 1 g of paracetamol to 100 mL volumetric flask, add 15 mL of methanol, and stir. To top up to volume with water, make it homogeneous, and filter it.

Solution (2): to prepare 0.001% 4-Aminophenol solution (w/v) in 15% methanol (v/v).

Procedure: to separately inject 20 µL of *Solutions (1) and (2)*, record the chromatograms, and measure the areas under the peaks. The area under 4-Aminophenol peak obtained in

the chromatogram with *Solution (1)* is not greater than the main peak obtained in the chromatogram with *Solution (2)*.

Related substances. To proceed as described in *Thin-layer chromatography* by using GF_{254} silica gel as support and a mixture of chloroform, acetone, and toluene (65:25:10) as mobile phase. To separately apply to the plate 200 µL of *Solution (1)* and 40 µL of *Solutions (2), (3) and (4)*, as described below.

Solution (1): to weigh and pulverize the tablets. To transfer an amount of powder equivalent to 1 g of paracetamol to a 15 mL centrifuge tube with polished glass lid. To add 5 mL of ethyl ether and mechanically stir for 30 minutes. To centrifuge at 1000 rpm for 15 minutes or obtain clear supernatant. To use the supernatant.

Solution (2): to dilute 1 mL of *Solution (1)* to 10 mL with ethanol.

Solution (3): 0.005% *p*-chloroacetanilide solution (w/v) in ethanol.

Solution (4): to dissolve 0.25 g of *p*-chloroacetanilide and 0.1 g of paracetamol in ethanol and top up to 100 mL.

To develop the chromatogram in unsaturated tank, to mobile phase reaches 14 cm from the origin. To remove the plate, allow it to dry in hot air, and examine it under ultraviolet light (254 nm). Any spot corresponding to *p*-chloroacetanilide obtained in the chromatogram with *Solution (1)* is not more intense than the spot obtained in the chromatogram with *Solution (3)*. Any secondary spot obtained in the chromatogram with *Solution (2)*, *R_f* lower than that of *p*-chloroacetanilide, is not more intense than the spot obtained in the chromatogram with *Solution (3)*. The test is valid only if the chromatogram obtained with *Solution (4)* show two clearly separated principal spots. The spot corresponding to *p*-chloroacetanilide presents higher *R_f* value.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To weigh and pulverize 20 tablets. To transfer an amount of powder equivalent to 0.15 g of paracetamol to a 200 mL volumetric flask. To add 50 mL of 0.1 M sodium hydroxide and 100 mL of water, mechanically stir for 15 minutes, and top up with water. To make it homogeneous, filter, and dilute 10 mL of filtrate to 100 mL with water. To transfer 10 mL of the resulting solution to 100 mL volumetric flask, add 10 mL of 0.1 M sodium hydroxide, and top up with water. To prepare a standard paracetamol solution in 0.01

M sodium hydroxide at the same final concentration. To measure the absorbance of the resulting solutions at 257 nm (5.2.14),

by using 0.01 *M* sodium hydroxide to adjust zero. To calculate the amount of $C_8H_9NO_2$ in the tablets, from the readings obtained. Alternatively, make the calculations by taking into account that $A(1\%, 1\text{ cm}) = 715$, at 257 nm, in 0.01 *M* sodium hydroxide.

B. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 243 nm; 300 mm chromatograph column and 3.9 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μm), kept at room temperature; flow of *Mobile phase* of 1.5 mL/min.

Mobile phase: mixture of water and methanol (75:25).

Sample solution: to dissolve accurately weighed amount of the sample in *Mobile phase*. To mechanically stir for 10 minutes and put it in ultrasound for 5 minutes. To dilute with the same solvent to obtain 10 $\mu\text{g/mL}$ concentration.

Standard solution: to dissolve an accurately weighed amount of paracetamol SQR in *Mobile phase* to obtain 10 $\mu\text{g/mL}$ solution.

The column efficiency should be greater than 1000 theoretical plates/meter. The tailing factor is not greater than 2. The relative standard deviation for replicate areas of the peaks recorded for *Standard solution* is not greater than 2.0 %.

Procedure: to separately inject 10 μL of *Sample and standard solutions*, record the chromatograms, and measure the areas of the peaks. To calculate the content of $C_8H_9NO_2$ from the sample, from the responses obtained for the *Sample and Standard solutions*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

PARACETAMOL ORAL SOLUTION

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_8H_9NO_2$.

IDENTIFICATION

The Identification test A. may be omitted if the tests B. and C. are conducted. The identification test C. can be omitted if the tests A. and B. are conducted.

A. The ultraviolet absorption spectrum (5.2.14) ranging from 200 nm to 400 nm, of sample solution obtained in *Dosage* method A., exhibits absorption maxima at 249 nm similar to those observed in the standard solution spectrum.

B. To proceed as described in *Thin-layer chromatography* (5.2.17.1), by using GF_{254} silica gel

as support and a mixture of methylene chloride and methanol (4:1) as mobile phase. To separately apply to the plate 20 μL of each of the solutions freshly prepared, as described below.

Solution (1): to dilute the oral solution in methanol to obtain 1 mg/mL solution.

Solution (2): to dissolve 10 mg of paracetamol SQR in methanol and top up to 10 mL with the same solvent.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light. The principal spot obtained with *Solution (1)* corresponds in position to that obtained with *Solution (2)*.

C. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method B. corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

Dripping test (5.1.8). It complies with the test.

pH (5.2.19). 3.8 to 6.5.

PURITY TESTS

4-Aminophenol. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 272 nm; 200 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (10 μm), kept at 25 °C; flow of *Mobile phase* of 2.0 mL/min.

Mobile phase: to prepare 0.01 *M* sodium butane sulphonate solution by using a mixture of water, methanol, and formic acid (85:15:0.4) as solvent.

Solution (1): to prepare a 4.8 mg/mL solution of the sample in *Mobile phase*. To filter, if necessary.

Solution (2): to prepare a 24 $\mu\text{g/mL}$ 4-Aminophenol SQR solution in *Mobile phase*.

Procedure: to separately inject 20 μL of *Solutions (1) and (2)*, record the chromatograms, and measure the areas under the peaks. The area under 4-Aminophenol peak obtained in the chromatogram with *Solution (1)* is not greater than the

main peak obtained in the chromatogram with *Solution (2)* (0.5%). In the chromatogram obtained with *Solution (1)*, the peaks with a long retention time may occur because the presence of preservatives in the formula.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. To transfer volume of oral solution containing to volumetric flask and dilute in methanol, to obtain 1 mg/mL solution. To transfer 1 mL of the resulting solution to a 100 mL volumetric flask, add 1 mL of 0.1 M hydrochloric acid, top up with methanol, and make it homogeneous. To prepare a standard solution at similar concentration by using the same solvents. To measure the absorbances of the resulting solutions at 249 nm, by using 0.1 M methanolic hydrochloric acid solution to adjust zero. To calculate the amount of $C_8H_9NO_2$ in the oral solution, from the readings obtained. Alternatively, make the calculations by taking into account that $A(1\%, 1\text{ cm}) = 880$ at 249 nm.

B. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 243 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μm), kept at 25 °C; flow of *Mobile phase* of 1.0 mL/min.

Mobile phase: mixture of water and methanol (75:25).

Sample solution: to transfer an accurately measured volume of oral solution equivalent to 200 mg of paracetamol to a 200 mL volumetric flask, top up with *Mobile phase*, and make it homogeneous. To transfer 1 mL of this solution to 100 mL volumetric flask, top up with *Mobile phase*, make it homogeneous, and filter it.

Standard solution: to dissolve an accurately weighed amount of paracetamol SQR in *Mobile phase* to obtain 0.01 mg/mL final concentration.

The relative standard deviation for the replicate areas for the peaks recorded is not greater than 2.0%.

Procedure: to separately inject 10 μL of *Standard solution* and *Sample solution*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of $C_8H_9NO_2$ in oral solution from the responses from the *Standard solution* and the *Sample solution*.

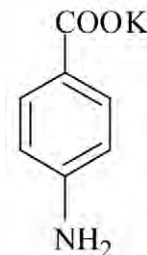
PACKAGING AND STORAGE

To protect it from light.

LABELLING

To comply with the legislation in force.

PARA-AMINO BENZOATE, POTASSIUM Kalli 4-aminobenzoas



$C_7H_6KNO_2$; 175,23
Potassium salt of 4-aminobenzoic acid (1:1)
[138-84-1]

It contains at least 98.5% and at most 101.0% of $C_7H_6KNO_2$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White, crystalline powder.

Solubility. Freely soluble in water, sparingly soluble in ethanol, practically insoluble in ethyl ether.

IDENTIFICATION

A. The ultraviolet absorption spectrum (5.2.14) ranging from 200 nm to 400 nm, of 0.001% solution (w/v) in 0.001 M sodium hydroxide, exhibits maxima and minima only at the same wavelength of a similar potassium para-amino benzoate SQR solution.

B. To dissolve about 400 g of sample in 10 mL of water. To add 1 mL of 3 M hydrochloric acid, filter, and wash the precipitate with 2 aliquots (5 mL each) of cold water. To wash with ethanol and wait to recrystallize. To desiccate the precipitate obtained at 110 °C for 1 hour. The melting point (5.2.2) of para-aminobenzoic acid is between 186.0 °C and 189.5 °C.

C. The 1% solution (w/v) of the sample responds to the reactions of potassium ion (5.3.1.1).

PURITY TESTS

pH (5.2.19). 8.0 to 9.0. To determine in 5% solution w/v).

Volatile diazotized substances.

Solution (1): to dissolve 10 mg of p-toluidine in 5 mL of methanol in a 100 mL volumetric flask. To top up with water and make it homogeneous. To transfer 1 mL of this solution to 100 mL volumetric flask, top up with the same solvent, and make it homogeneous.

Solution (2): to transfer 5 g of potassium aminobenzoate to a 50 mL volumetric vial. To add an amount of *M* sodium hydroxide sufficient to dissolve the sample and to alkalize the medium in relation to phenolphthalein. To top up the volume to 50 mL.

To distill in the distillation system, by using steam carrier, and collect approximately 95 mL of distillate in a 100 mL flask. To top up with water and mix.

Procedure: To individually transfer 20 mL of *Solution (1)* and 20 mL of *Solution (2)* to 100 mL volumetric flasks. To perform sample blank correction by transferring 20 mL of water to a third 100 mL flask. To add 5 mL of *M* hydrochloric acid to each of the volumetric flasks and cool down in an ice bath. To add dropwise, by stirring, 2 mL of 0.1 *M* SV sodium nitrite. To let it rest for 5 minutes to complete the diazotization reaction. To rapidly add 10 mL of cooled guaiacol solution, freshly prepared by dissolving 0.2 g of guaiacol in 100 mL of *M* sodium hydroxide. To make it homogeneous and let it rest for 30 minutes. To determine the absorbance (5.2.14) of the solutions at 405 nm, by using sample blank correction to adjust zero. The absorbance of *Solution (2)* should not be greater than the absorbance of *Solution (1)* (0.002% ppm).

Heavy metals (5.3.2.3). To use *Method IV*. To use 1 g of the sample in silica crucible. At most 0.002% (20 ppm).

Chlorides (5.3.2.1). To dissolve 1.8 g of the sample in 40 mL of water and proceed as described in *Limit test for chlorides*. At most 0.02% (200 ppm).

Sulfates (5.3.2.2). To dissolve 6 g of the sample in 40 mL of water and proceed as described in *Limit test for sulfates*. At most 0.02% (200 ppm).

Loss on drying (5.2.9). To accurately weigh about 1-2 g of the sample and perform vacuum drying at 105 °C for 2 hours. At most 1.0%.

DOSAGE

To accurately weigh about 500 mg of the sample and transfer to a beaker. To add 25 mL of water and 25 mL of 3 *M* hydrochloric acid. To mix it and cool down in an ice bath. To titrate with 0.1 *M* SV sodium nitrite. To potentiometrically determine the endpoint by using a platinum-calomel electrode. Each mL of 0.1 *M* SV sodium nitrite is equal to 17.523 mg of $C_7H_6KNO_2$.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Analgesic.

PERCHLORATE, POTASSIUM
Kalli perchloras

KClO₄; 138.55
 potassium perchlorate; 09834
 Potassium salt of perchloric acid (1:1)
 [7778-74-7]

It contains at least 99.0% and at most 100.5% of KClO₄, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White, crystalline powder, or colorless crystals.

Solubility. Slightly soluble in water, practically insoluble in ethanol.

IDENTIFICATION

A. To prepare a 10% solution (w/v) of the sample in water and add a few drops of methylthioninium chloride SR. Violet precipitate is produced.

B. To dissolve 0.1 g of the sample in 5 mL of water. To add 5 mL of indigo carmine SR and heat until boiling. The color of the solution does not fade out.

C. It responds to the reactions of potassium ion (5.3.1.1).

D. It responds to the reactions of chloride ion (5.3.1.1).

E. It responds to the reactions of chlorate ion (5.3.1.1).

PURITY TESTS

Appearance of solution. The 1% aqueous solution (w/v) is clear (5.2.25) and colorless (5.2.12).

pH (5.2.19). 5.0 to 6.5. To determine in 1.4% aqueous solution (w/v).

Acidity or alkalinity. To accurately weigh about 5 g of the sample and add 90 mL of water. To heat to boiling. To cool down and filter it. To dilute the filtrate to 100 mL with carbon dioxide-free water. To add 5 mL of water and 0.1 mL of phenolphthalein SI to 5 mL of this solution. No more

than 0.25 mL of 0.01 *M* SV sodium hydroxide is required to change the color of the indicator. To add 5 mL of water and 0.1 mL of bromocresol green SI solution to 5 mL of this solution. No more than 0.25 mL of 0.01 *M* hydrochloric acid is required to change the color of the indicator.

Volatile organic impurities. To proceed as described in *Gas chromatography* (5.2.17.5). To use chromatograph equipped with flame ionization detector, by using mixture of nitrogen, synthetic air, and hydrogen (1:1:10) as the auxiliary gas to flame detector; 30 m long capillary column and 0.53 mm inner diameter,

filled with stationary linked phase of 5% phenyl polysiloxane and 95% methyl polysiloxane, with a film thickness of 5 µm; column temperature from 35 °C to 260 °C (35 °C kept for 5 minutes, then rises to 175 °C at 8 °C per minute, and rises to 260 °C at 35 °C and kept at this temperature for at least 16 minutes); injector temperature at 70 °C and detector temperature at 260 °C; use helium as carrier gas; flow of carrier gas of 1 mL/minute.

Sample solution: To accurately dissolve about 1 g of sample in 50 mL of water, free of organic compounds.

Standard solution: to prepare a solution in water free of organic compounds containing, in each mL, 10 µg of methylene chloride, 1 µg of chloroform, 2 µg of benzene, 2 µg of dioxane, and 2 µg of trichlorethylene.

Procedure: To separately inject 1 µL of *Sample solution* and *Standard solution* into the gas chromatograph. To obtain the chromatograms and measure the area under the peaks. Based on the retention time, identify any peak in the chromatogram of the *Sample solution*. To compare the chromatograms of the *Sample solution* and *Standard solution* to establish the presence and identification of peaks in the chromatogram. Limit: Benzene (2 ppm), chloroform (50 ppm), dioxane (100 ppm), methylene chloride (500 ppm), and trichlorethylene (80 ppm). It complies with the test.

Insoluble substances. To dissolve 20 mg of the sample in 150 mL of warm water. To filter through medium porosity filter previously weighed. To wash with 3 portions (50 mL each) of warm water. To dry the residue at 105 °C for 3 hours. The weight of the residue does not exceed 0.005% (50 ppm).

Chlorides (5.3.2.1). To weigh 10 g of the sample and proceed as described in *Limit test for chlorides*. At most 0.003% (30 ppm).

Chlorides and chlorates (5.3.2.1). To accurately weigh about 3.5 g of sample, and add 30 mL of water, 1 mL of nitric acid, and 0.1 g of sodium nitrite. To proceed as described in *Limit test for chlorides*. At most 0.01% (100 ppm) (calculated as chloride).

Sodium. To prepare a 10% solution of the sample. To dip a platinum loop in this solution and direct to flame. No pronounced yellow color fades in the flame.

Sulfates (5.3.2.2). To accurately weigh about 1 g of the sample and dissolve in 40 mL of water. To proceed as described in *Limit test for sulfates*. To use 0.25 mL of the standard solution. At most 0.012% (120 ppm).

Calcium. The opalescence developed, after 15 minutes, in *Sample preparation*, is not more intense than in the *Standard preparation* similarly prepared. At most 100 ppm.

Sample preparation: to accurately weigh about 5 g of the sample, transfer to a beaker, and 90 mL of water. To heat to boiling. To wait to cool down, filter to a 100 mL volumetric flask, and top up with carbon dioxide-free distilled water.

In a Nessler tube, mix 0.2 mL of standard solution of ethanolic calcium (100 ppm Ca) and 1 mL of ammonium oxalate SR. After a minute, add 1 mL of 2 *M* acetic acid and 15 mL of the solution containing the sample prepared above.

Standard preparation: to transfer 0.2 mL of standard solution of ethanolic calcium (100 ppm Ca) to a Nessler tube (50 mL capacity and 22 mm inner diameter) and mix with 1 mL of ammonium oxalate SR. To wait 1 minute, add 7.5 mL of the standard solution of calcium (10 ppm Ca) 1 mL of diluted acetic acid, and 7.5 mL of distilled water.

Heavy Metals (5.3.2.3). To dissolve 2 g of sample in 25 mL of water, adjust the pH to 3.0 - 4.0 with *M* acetic acid and 6 *M* ammonium hydroxide, dilute with water, and make it homogeneous. To proceed as described in *Limit test for heavy metals, Method I*. At most 0.001% (10 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample, in silica gel. To desiccate for 12 hours. At most 0.5%.

DOSAGE

To proceed as described in *Chromatography column for ion-exchange* (5.2.17.3). To use chromatograph equipped with conductivity detector, 7.5 cm long chromatographic column and 4.6 mm inner diameter, packed with polyacrylate or polymethacrylate porous gel with quaternary ammonia groups of about 10 µm; flow of *Mobile phase* of 1.2 mL/min.

Mobile phase: to dissolve 1.66 g of phthalic acid in 1000 mL of water. To adjust pH to 4.5 with approximately 0.45 g of lithium hydroxide. To filter it.

Sample solution: to dissolve in water an accurately weighed amount of the sample, to obtain a 0.5 mg/mL solution. To transfer 20 mL of this solution to 100 mL volumetric flask and top up with water, thus obtaining 0.1 mg/mL solution.

Standard solution: to dissolve in water an accurately weighed amount of potassium perchlorate SQR, to obtain a 0.5 mg/mL solution. To transfer 20 mL of this solution

to 100 mL volumetric flask and top up with water, thus obtaining 0.1 mg/mL solution.

Procedure: to separately inject 50 µL of *Standard solution* and *Sample solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of KClO₄ from the sample, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Anti-thyroid medication

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate under reduced pressure over silica gel for 18 hours. At most 0.5%.

PERMANGANATE, POTASSIUM

Kalli permanganas

KMnO₄; 158.03
potassium permanganate; 07000
Potassium salt of permanganic acid (1:1)
[7722-64-7]

It contains at least 99.0% and at most 100.5% of KMnO₄, in relation to the desiccated substance.

Caution: *dangerous explosions may occur if placed in contact with organic or easily oxidizable substances, both in solution and dry state.*

DOSAGE

To accurately weigh about 0.125 g of the sample and dissolve in 25 mL of water. To add a previously prepared solution of 2 mL of sulfuric acid in 5 mL of water and 50 mL of 0.05 M SV oxalic acid. To heat the solution at about 80 °C. To titrate the excess of oxalic acid with 0.02 M SV potassium permanganate to obtain pale pink color, persisting for 15 seconds. Each mL of 0.05 M SV oxalic acid is equivalent to 3.161 mg of KMnO₄.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

DESCRIPTION

Physical characteristics. Dark violet crystals, metallic luster, odorless, stable to air.

Solubility. Freely soluble in boiling water, soluble in cold water

IDENTIFICATION

A. To dissolve 50 mg of the sample in 5 mL of water. To add 1 mL of ethanol and 0.3 mL of sodium hydroxide SR. The green color develops. To heat to boiling. Dark brown precipitate is formed.

B. It responds to the reactions of permanganate ion (5.3.1.1).

C. To filter the mixture obtained in test **A.**, *Identification*. Filtrate responds to the reactions of potassium ion (5.3.1.1).

PURITY TESTS

Appearance of solution. To dissolve 0.75 g of the sample in 2 mL of water and add 3 mL of ethanol. To heat to boiling for 2 minutes. To cool down and top up with water to 30 mL. To filter it. The solution is colorless (5.2.12).

Substances insoluble in water. To dissolve by heating 0.5 g of the sample in 50 mL of water. To filter through a medium porosity glass filter, previously weighed and washed with water, to obtain a colorless filtrate. To collect the residue and dry it in oven at (102.5 ± 2.5) °C. The mass of the residue is not greater than 5mg (1.0 %).

Chlorides (5.3.2.1). To top up to 15 mL with water to 10 mL of the resulting solution in *Appearance of solution*. At most 0.02% (200 ppm).

Sulfates (5.3.2.2). To top up to 15 mL with water to 12 mL of the resulting solution in *Appearance of solution*. At most 0.05% (500 ppm).

LABELLING

To comply with the legislation in force

THERAPEUTIC CLASS

Antifungal, topic.

PETROLATUM, WHITE

white petrolatum; 09104

Purified mixture of semisolid hydrocarbons from petroleum. It may contain suitable stabilizer.

DESCRIPTION

Physical characteristics. Unctuous mass, semi-solid, white or slightly yellowish, practically odorless and tasteless.

Solubility. Practically insoluble in water; soluble in benzene, chloroform, ethyl ether, petroleum ether, carbon disulfide, oils; practically insoluble in ethanol and glycerol.

Physical and chemical constants.

Relative density (5.2.5): 0.815 to 0,880. To determine at 60 °C. **Melting range (5.2.2):** 38 °C to 60 °C.

PURITY TESTS

Color of liquids (5.2.12). To melt about 10 g of the sample in a water bath and transfer 5 mL of the liquid to a test tube, transparent glass, approximately 16 mm diameter x 150 mm long. The hot molten liquid should not be darker than a solution prepared by mixing 1.6 mL of *Ferric chloride base solution* and

3.4 mL of water in a similar tube. To compare it against a white background, and the tubes should be held directly against the bottom to avoid fluorescence.

Acidity or alkalinity. To weigh 35 g of sample in a beaker and add 100 mL of boiling water. To cover, put on a hot plate, and keep the boiling temperature of water for 5 minutes. Then, let it rest to obtain phase separation. To remove the aqueous layer and transfer it to an erlenmeyer flask. To wash the sample with two additional portions (50 mL each) of boiling water. To collect the three aqueous layers (the first one plus washings), add 0.1 mL of phenolphthalein SI, and heat to boiling. The solution should not become pinkish. If the solution remains colorless, add 0.1 mL of methyl orange SI. Solution does not become reddish or pinkish.

Consistency

Equipment: To determine the consistency by using a penetrometer, which must have a polished metallic piston, 150 g, cone-shaped, and a detachable steel tip. The tip of the cone should have an angle of 30° and the truncated end must have a (0.381 ± 0.025) mm diameter. The diameter of the base of the tip is (8.38 ± 0.05) mm and the length of it is (14.94 ± 0.05) mm. The remaining portion of the cone should have an angle of 90°, 28 mm high, and about 65 mm maximum diameter at the base. The containers for the test must be flattened bottom metallic cylinders, whose diameter must be (100 ± 6) mm and a minimum 65 mm high. They must be manufactured from 1.6 mm metal and well sealed and watertight lids.

Procedure: to heat in an oven a sufficient amount of sample at (82 ± 2.5) °C and transfer to preheated cylinder at the same temperature, filling up to 6 mm from the edge. To cool down at (25 ± 2.5) °C for at least 16 hours, by protecting it from environment. Two hours before the test, place the cylinder in a water bath at (25 ± 0.5) °C. If the temperature is below 23.5 °C or above 26.5 °C, adjust the cone temperature at (25 ± 0.5) °C by placing it in a water bath. Without causing disturbances on the surface of the sample, place the cylinder on the table of the penetrometer cone and lower the cone until the tip touches the surface

of the sample at a point of 25 mm to 38 mm, below the edge of the cylinder. To zero the scale of the equipment and quickly release the piston, and keep it for 5 seconds. To attach the piston and take a reading. To make three or more readings, at intervals set, to avoid overlapping in areas of penetration. When the penetration exceeds 20 mm, use a separate container with test sample. To make a reading of penetration up to tenths of a millimeter. To calculate the average of three or more readings and perform up to 10 tests, if individual results differ from the average by more than 3%. The average of all the tests should be at least 10 mm and at most 30 mm, by indicating a consistency value between 100 and 300.

Organic acids. To weigh 20 g of sample and add 100 mL of a mixture of ethanol, previously neutralized, with 0.1 M sodium hydroxide and water (1:2).

To stir the solution and heat to boiling. To add 1 mL of phenolphthalein SI and rapidly titrate with 0.1 M SV sodium hydroxide, by vigorously stirring, to obtain pinkish color in the hydroalcoholic layer. At most 0.4 mL of 0.1 M SV sodium hydroxide is required to change the indicator.

Fixed oils, fats, and rosin. To heat 10 g of sample with 50 mL of 5 M sodium hydroxide at 100 °C for 30 minutes. To separate the aqueous layer and acidify it with 2.5 M sulfuric acid. No separation of solid or oily material should occur.

Sulfated ashes (5.2.10). To determine in 2 g of the sample. No releasing of irritating odor during heating should occur. At most 0.05%.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

CATEGORY

Excipient.

PETROLATUM, LIQUID

Paraffinum liquidum

liquid petrolatum; 09388

Paraffin oils
[8012-95-1]

Mixture of liquid hydrocarbons from petroleum. DESCRIPTION

Physical characteristics. Liquid oily, clear, colorless, non-fluorescent in daylight.

Solubility. Practically insoluble in water, sparingly soluble in ethanol, miscible with hydrocarbons.

Physical and chemical constants.

Relative density (5.2.5): 0.827 to 0.890.

Viscosity (5.2.7): 110 mPa.s to 230 mPa.s.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample has absorption maximum at the same wavelength, with the same relative intensities to those observed in the liquid pet-rolatum SQR similarly prepared.

B. In a test tube, carefully boil 1 mL of the sample with 1 mL of 0.1 M sodium hydroxide, by continuously stirring, for about 30 seconds. To cool down at room temperature. Two phases should be formed. To add 0.1 mL of phenolphthalein SI to the aqueous phase. Pink color is produced.

PURITY TESTS

Acidity or alkalinity. To vigorously stir 10 mL of sample with 20 mL of boiling water for 1 minute. To separate the aqueous phase and filter. To add 0.1 mL of phenolphthalein SI to 10 mL of filtrate. The solution is colorless. No more than 0.1 mL of 0.1 M sodium hydroxide is required to change the color of the indicator to pink.

Polycyclic aromatic hydrocarbons. To use reagents for spectrophotometry. To put 25 mL into a 125 mL separatory funnel with ground stopper. To stir twice 25 mL of hexane with 5 mL of dimethyl sulfoxide and add it. To mix and add 5 mL of dimethyl sulfoxide. To vigorously stir for 1 minute and let it rest to obtain two phases. To transfer the lower layer to a second separatory funnel, add 2 mL of hexane, and vigorously stir it. To let it rest to obtain two phases. To separate the lower layer and measure the absorbance (5.2.14) at 260 nm - 420 nm. To simultaneously prepare blank by using the lower layer from vigorously stirring at separatory funnel with 5 mL of dimethyl sulfoxide and 25.0 mL of hexane. To prepare a standard solution of 7 mg/L naphthalene in isooctane and measure the absorbance at 275 nm, by using isooctane as a blank. In no wavelength at 260 nm - 420 nm, the absorbance of the test solution exceeds one third of the absorbance of the standard solution at 275 nm.

Carbonizable substances. To use a tube with ground stopper of approximately 125 mm long and 18 mm inner diameter, graduated in 5 mL and 10 mL. To wash with chromic acid cleaning solution, rinse with water, and dry it. To put 5 mL of the sample and 5 mL of nitrogen-free sulfuric acid. To insert the stopper and vigorously stir as much as possible in the longitudinal direction of the tube for 5 seconds. After removal of the lid, immediately place the tube in a water bath, by avoiding the tube to get contact with the bottom or side of the bath. To heat it. After 2 minutes, 4 minutes, 6 minutes, and 8 minutes, remove the tube from

the bath and vigorously stir as much as possible in the longitudinal direction of the tube, for 5 seconds. After 10 minutes of heating, take the tube from the water bath and wait for 10 minutes. To centrifuge at 2000 g for 5 minutes. To transfer 4 mL of the upper layer to a clean test tube. The color is not more intense (5.2.12) than 4 mL of a mixture of 0.6 mL of Standard brown solution and 9.4 mL of 1% hydrochloric acid solution (w/v). The color of the lower phase is not more intense than a mixture of 0.5 mL of *Cupric sulfate base solution*, 1.5 mL of *Cobaltous chloride base solution*, 3 mL of *Ferric chloride base solution*, and 2 mL of 1% hydrochloric acid (v/v).

Solid paraffins. To dry an amount of sample sufficient to heat at 100 °C for 2 hours and cool down in a desiccator with sulfuric acid. To store in a glass tube (25 mm inner diameter), close the tube, and put it in an ice water bath. After 4 hours, the liquid is sufficiently translucent to easily see a black line (0.5 mm wide) on a white background, when vertically placed behind the tube.

PACKAGING AND STORAGE

In tightly closed containers, protected from light

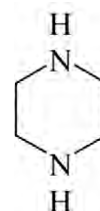
LABELLING

To comply with the legislation in force.

CATEGORY

Pharmaceutical adjuvant.

PIPERAZINE
Piperazinum



$C_4H_{10}N_2$; 86.14
piperazine; 07099
Piperazine
[110-85-0]

It contains at least 98.0% and at most 101.0% of $C_4H_{10}N_2$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. Lumps or white or whitish flakes, ammonium smell.

Solubility. Soluble in water and ethanol; insoluble in ethyl ether.

Physical and chemical constants. Melting range (5.2.2): 109 °C to 113 °C.

IDENTIFICAÇÃO

A. To dissolve about 0.2 g of sample in 5 mL of diluted hydrochloric acid and add 1 mL of 50% sodium nitrite solution (w/v), by stirring it. To cool down in an ice bath for 15 minutes and stir, if necessary, to induce crystallization. To filter the precipitate through fritted funnel, wash the precipitate with 10 mL of cold water, and desiccate at 105 °C:

the resulting *N, N'*-dinitroso-piperazine melts at 156 °C - 160 °C.

B. It responds to the reactions of citrate ion (5.3.1.1).

PURITY TESTS

Appearance of solution. To dissolve 10 g of the sample in water and dilute to 50 mL with the same solvent. The resulting solution is not colored (5.2.12) than the reference solution prepared by adding 2 mL of *Ferric chloride base solution* in water and diluted to 50 mL with the same solvent when compared in Nessler tubes.

Primary amines and ammonia. To dissolve 0.2 g of sample in 10 mL of water, add 1 mL of acetone and 0.5 mL of freshly prepared 10% sodium nitroprusside solution (w/v). To mix it and let it rest for 10 minutes. To measure the absorbance (5.2.14) of this solution at 520 nm and 600 nm, by preparing the blank as the solution described above, except by the sample. The ratio of absorbance at 600 nm and absorbance at 520 nm is at most 0.5 (equivalent to approximately 0.7% of primary amines and ammonia).

Water (5.2.20.1). At most 2%.

DOSAGE

To proceed as described in *Titration in non-aqueous medium* (5.3.3.5). To accurately weigh about 0.15 g of the sample and dissolve in 75 mL of glacial acetic acid. To potentiometrically titrate with 0.1 M SV perchloric acid, by using the silver-glass electrode system. When approaching the turning point, heat the solution at 68-70 °C, then complete the titration. To perform sample blank correction and adjust it as required. Each mL of 0.1 M SV perchloric acid is equal to 4.307 mg of C₅H₅N₃O.

PACKAGING AND STORAGE

In airtight closed containers, protected from light.

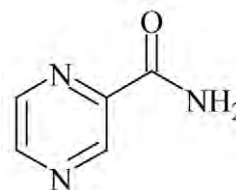
LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Anthelmintic.

PYRAZINAMIDE Pyrazinamidum



C₅H₅N₃O; 123.11
pyrazinamide; 07141
2-pyrazinecarboxamide
[98-96-4]

It contains at least 99.0% and at most 101.0% of C₅H₅N₃O, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. Crystalline, white or almost white powder. Odorless or almost odorless.

Solubility. Slightly soluble in water; sparingly soluble in ethanol, ethyl ether, and chloroform.

Physical and chemical constants.

Melting range (5.2.2): 188 °C to 191 °C.

IDENTIFICATION

The test A. may be omitted if the tests B., C., and D. are conducted. The tests B. and C. can be omitted if the tests A. and D are carried out.

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the pyrazinamide SQR spectrum similarly prepared.

B. The ultraviolet absorption spectra (5.2.14), from 200 nm to 400 nm, 0.001% solution (w/v) in water, exhibits maxima and minima similar to those observed in the spectrum of a similar solution of pyrazinamide SQR.

C. To dissolve 0.1 g of the sample in 5 mL of water. To add 1 mL of acidified ferrous sulfate SR. Orangish color develops. To add 1 mL of sodium hydroxide SR. Solution becomes dark blue.

D. To heat to boiling 20 mg of sample with 5 mL of 5 M sodium hydroxide. Typical ammonia odor is released.

PURITY TESTS

Appearance of solution. To dissolve 0.5 g of the sample in carbon dioxide-free water and dilute to 50 mL with the same solvent.

The solution is clear (5.2.25) and colorless (5.2.12).

Acidity or alkalinity. To add 0.5 mL of phenolphthalein SI and 0.2 mL of 0.01 M sodium hydroxide to 25 mL of the solution obtained in *Appearance of solution*. Solution becomes pinkish. To add 1.0 mL of 0.01 M hydrochloric acid. Solution becomes colorless. To add 0.12 mL of methyl red SI. The solution becomes red.

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel as support and a mixture of glacial acetic acid, water, and 1-butanol (20:20:60) as mobile phase. To separately apply to the plate 50 µL of each of the solutions freshly prepared, as described below.

Solution (1): to transfer 0.1 mg of the sample to a 10 mL volumetric flask, dilute in a mixture of methanol and methylene chloride (1:9), and top up with the same solvent.

Solution (2): to transfer 1 mL of *Solution (1)* to 50 mL volumetric flask, and top up with a mixture of methanol and methylene chloride (1:9). To transfer 1 mL of this solution to 10 mL volumetric flask and top up with the same solvent.

Solution (3): to transfer 10 mg of nicotinic acid SQR to 10 mL volumetric flask, dissolve in a mixture of methanol and methylene chloride (1:9), add 1 mL of *Solution (1)*, and top up with the same solvent.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (2)* (0.2%). The test is valid only if the chromatogram obtained with *Solution (3)* shows two principal spots clearly separated.

Heavy metals (5.3.2.3). To use *Method III*. At most 0.001% (10 ppm).

Water (5.2.20.1). At most 0.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To proceed as described in *Titrations in non-aqueous medium* (5.3.3.5). To accurately dissolve about 0.1 g of the sample in 50 mL of acetic anhydride. To titrate with 0.1 M SV perchloric acid, to potentiometrically determine the endpoint. Each mL of 0.1 M SV perchloric acid is equal to 12.311 mg of C₅H₅N₃O.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Tuberculostatic.

PYRAZINAMIDE, TABLETS

It contains at least 93.0 % and at most 107.0 % of the amount stated of C₅H₅N₃O.

IDENTIFICATION

The identification test A. may be omitted if the tests B., C., and D. are conducted. The identification test B. can be omitted if the tests A., C., and D. are carried out.

A. To weigh and pulverize the tablets. To stir an amount of powder equivalent to 50 mg pyrazinamide with 50 mL of absolute ethanol. To filter and evaporate the filtrate to dryness. To dry the residue in an oven at 105 °C for 30 minutes. The residue responds to test **A.**, *Identification*, in the monograph *Pyrazinamide*.

B. The ultraviolet absorption spectrum (5.2.14) ranging from 200 nm to 400 nm, of sample solution obtained in *Dosage* method **A.**, exhibits absorption maxima similar to those observed in the standard solution spectrum.

C. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method **B.** corresponds to that of main peak of *Standard solution*.

D. To weigh and pulverize the tablets. To boil an amount of powder equivalent to 20 mg pyrazinamide with 5 mL of 5 M sodium hydroxide. Typical ammonia odor is released.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

Procedure for content uniformity. To transfer each tablet to a 500 mL volumetric flask containing 350 mL of water and wait for total disintegration of the tablet. To proceed as described in Method **A.**, *Dosage*, from "To put it in ultrasound for 15 minutes..."

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL

Equipment: blades, 50 rpm

Time: 45 minutes

Procedure: after testing, remove aliquot from the dissolution medium and dilute, if necessary, in water, to obtain suitable concentration. To measure the absorbances at 268 nm (5.2.14), by using the same solvent to adjust zero. To calculate the amount of $C_5H_5N_3O$ dissolved in the medium, by comparing the readings obtained with that of 0.001% pyrazinamide SQR solution (w/v) prepared in the same solvent. Alternatively, make the calculations by taking into account that $A(1\%, 1\text{ cm}) = 650$, at 268 nm, in water.

Tolerance: not less than 75% (Q) of the amount stated of $C_5H_5N_3O$ dissolves within 45 minutes.

PURITY TESTS

Related substances. To proceed as described in *Related substances*, in the monograph *Pyrazinamide*. To prepare the solutions as described below.

Solution (1): to weigh and pulverize the tablets. To dissolve an amount of powder equivalent to 0.1 g of pyrazinamide in 50 mL of a mixture of methanol and chloroform (1:9), and stir for 15 minutes. To filter and evaporate the filtrate to dryness and dissolve the residue with the same solvent to obtain 10 mL.

Solution (2): to transfer 1 mL of *Solution (1)* to 50 mL volumetric flask, and top up with a mixture of methanol and chloroform (1:9). To transfer 1 mL of this solution to 10 mL volumetric flask and top up with the same solvent.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). Any spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (2)* (0.2%).

Water (5.2.20.1). At most 3.0%.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. To weigh and pulverize 20 tablets.

To transfer an amount of powder equivalent to 0.1 g of pyrazinamide to 500 mL volumetric flask containing 350 mL of water.

To leave it in ultrasound for 15 minutes and mechanically stir for 15 minutes. To top up the volume with the same solvent. To make it homogenous and filter it. To dilute in water to obtain 0.001% concentration (w/v). To prepare a standard solution at the same concentration by using the same solvent. To measure the absorbances of the resulting solutions at 268 nm wavelength, by using water to adjust zero. To calculate the amount of $C_5H_5N_3O$ in the tablets, from the readings obtained. Alternatively, make the calculations by taking into account that $A(1\%, 1\text{ cm}) = 650$, at 268 nm, in water.

B. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 270 nm; 150 mm chromatograph column and 3.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μm), kept at room temperature; flow of *Mobile phase* of 1.0 mL/min.

Mobile phase: to transfer 0.6805 g of monobasic potassium phosphate to a 250 mL volumetric flask, dissolve in water, and top up with the same solvent. To transfer the resulting solution to 1000 mL volumetric flask. To add 18 mL of 0.2 M sodium hydroxide and top up with water. To adjust the pH to 3.0 ± 0.2 with phosphoric acid. To mix 10 mL of acetonitrile with 1000 mL of this solution, filter, and degas it.

Sample solution: to weigh and pulverize 20 tablets. To transfer an amount of powder equivalent to 0.1 g of pyrazinamide to 100 mL volumetric flask and add 70 mL of water. To leave it in ultrasound for 15 minutes and mechanically stir for 15 minutes. To top up the volume with the same solvent. To make it homogenous and filter it. To transfer 1 mL of filtrate to 25 mL volumetric flask and top up with water.

Standard solution: to transfer 50 mg of pyrazinamide SQR to a 50 mL volumetric flask, dissolve in water, and top up with the same solvent. To transfer 1 mL of this solution to a 25 mL volumetric flask and top up with water, thus obtaining 40 $\mu\text{g/mL}$ solution.

Resolution solution: To transfer 1 mL of hydrochloric acid to a 5 mL volumetric flask and top up with *Standard solution*. To put this solution in a boiling water bath for 5 minutes, to produce pyrazinoic acid. To cool down.

To inject replicates of 20 μL of the *Standard solution*. The column efficiency should be greater than 2500 theoretical plates. The tailing factor should not exceed 1.3. To inject replicates of 20 μL of the *Resolution solution*. The relative retention times are about 0.45 to pyrazinoic acid and 1.0 to pyrazinamide. The resolution between the pyrazinoic acid and pyrazinamide should not be less than 6.0.

Procedure: to separately inject 20 µL of *Sample and standard solutions*, record the chromatograms, and measure the area under the peaks. To calculate the amount of $C_5H_5N_3O$ in the tablets, from the responses obtained for the Sample and standard solutions.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

PYRIMETHAMINE Pirimethaminum



$C_{12}H_{13}ClN_4$; 248.71
pyrimethamine; 07170
5 - (4-Chlorophenyl)-6-ethyl-2,4-pyrimidinediamine
[58-14-0]

It contains at least 99.0% and at most 101.0% of $C_{12}H_{13}ClN_4$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Almost white, crystalline powder, or colorless crystals.

Solubility. Practically insoluble in water, slightly soluble in ethanol, very slightly soluble in ethyl ether.

Physical and chemical constants.

Melting range (5.2.2): 239 °C to 243 °C.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the pyrimethamine SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14), ranging from 200 nm to 400 nm, of 0.001% solution (w/v) in 0.1 M hydrochloric acid, prepared by heating, if necessary, exhibits maxima and minima only in the same wavelength of a similar pyrimethamine SQR solution.

C. The principal spot in the chromatogram of *Solution (2)* in the *Related substances* corresponds in position and intensity to that obtained with *Solution (3)*.

D. To transfer approximately 1 g of the sample and 5 g of sodium carbonate anhydrous to a crucible. To mix and heat to ignite. To cool down, add 5 mL of hot water, put it in ultrasound for 5 minutes, filter, and neutralize the filtrate with nitric acid. The resulting solution responds to the reactions of chloride ion (5.3.1.1).

PURITY TESTS

Acidity or alkalinity. To transfer 1 g of the sample to a 50 mL volumetric flask, add 30 mL of water, stir for 2 minutes, top up with the same solvent, and filter. To add 0.5 mL of phenolphthalein SI to 10 mL of the solution. At most 0.2 mL of 0.01 M sodium hydroxide is spent to change the indicator. To add 0.05 mL of methyl red SI and 0.4 mL of 0.01 M hydrochloric acid. Orangish or red color is developed.

Related substances. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF_{254} silica gel as support and a mixture of chloroform, n-propyl alcohol, glacial acetic acid, and toluene (4:8:12:76) as mobile phase. To separately apply to the plate 20 µL of each of the solutions freshly prepared, as described below.

Solution (1): 10 mg/mL solution of the sample in a mixture of methanol and chloroform (1:9).

Solution (2): to transfer 1 mL of *Solution (1)* to 10 mL volumetric flask and top up with a mixture of methanol and chloroform (1:9).

Solution (3): 1 mg/mL pyrimethamine SQR solution in a mixture of methanol and chloroform (1:9).

Solution (4): to transfer 2.5 mL of *Solution (1)* to 100 mL volumetric flask, and top up with a mixture of methanol and chloroform (1:9). To transfer 1 mL of this solution to 10 mL volumetric flask and top up with the same mixture of solvents.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (4)* (0.25%).

Sulfates (5.3.2.2). To transfer 1 g of the sample to a 50 mL volumetric flask, add 30 mL of water, stir for 2 minutes, top up with the same solvent, and filter. To use 15 mL of the filtrate. To prepare a 0.001% standard sulfate solution (10 ppm). At most 0.008% (80 ppm).

Loss on drying (5.2.9). To determine in 0.5 g of the sample. To desiccate in an oven at 100°C - 105 °C for 4 hours. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. To accurately weigh about 0.2 g of the sample and dissolve in 25 mL of glacial acetic acid, by gently heating it. To cool down and titrate with 0.1 M SV perchloric acid, to potentiometrically determine the endpoint. To perform sample blank correction and adjust it as required. Each mL of 0.1 M SV perchloric acid is equal to 24.871 mg of $C_{12}H_{13}ClN_4$.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antimalarial and anti-toxoplasmosis.

PYRIMETHAMINE TABLETS

It contains at least 93.0 % and at most 107.0 % of the amount stated of $C_{12}H_{13}ClN_4$.

IDENTIFICATION

A. To weigh and pulverize the tablets. To transfer an amount of powder equivalent to 0.2 g of pyrimethamine to a beaker and add 25 mL of acetone, heat to boiling for 2 minutes, and filter through a sintered glass crucible. To repeat 3 times this procedure with portions (25 mL each) of acetone. To evaporate to dryness the filtrates combined in a water bath by using current of air. The infrared absorption spectrum (5.2.14) of the residue, dispersed in potassium bromide, has absorption maxima only at the same wavelength, with the same relative intensities to those observed in the pyrimethamine SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14) ranging from 250 nm to 300 nm, of sample solution obtained in *Dosage*, exhibits absorption maximum only at the same wavelength, with the same relative intensities to those observed in the standard solution spectrum similarly prepared.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test

Friability test (5.1.3.2). It complies with the test.s

Disintegration test (5.1.4.1). It complies with the test.
Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 900 mL

Equipment: blades, 50 rpm

Time: 45 minutes

Procedure: after testing, remove aliquot from the dissolution medium, filter, and dilute in 0.1 M hydrochloric acid, to obtain suitable concentration. To measure the absorbances at 272 nm (5.2.14), by using the same solvent to adjust zero. To calculate the amount of $C_{12}H_{13}ClN_4$ dissolved in the medium, by comparing the readings obtained with that of 0.001% pyrimethamine SQR solution (w/v) prepared in the same solvent.

Tolerance: not less than 75% (Q) of the amount stated of $C_{12}H_{13}ClN_4$ dissolves within 45 minutes.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. To weigh and pulverize 20 tablets. To transfer an amount of powder equivalent to 25 mg of pyrimethamine to a 100 mL volumetric flask, add 50 mL of 0.1 M hydrochloric acid, put it in water bath for 10 minutes, followed by ultrasound for 30 minutes. To cool down and top up the volume with the same solvent. To filter it. To transfer 5 mL of the filtrate to a 100 mL volumetric flask and top up with 0.1 M hydrochloric acid, thus obtaining 12,5 µg/mL solution. To prepare standard pyrimethamine solution under the same conditions. To measure the absorbances of the solutions at 272 nm, by using 0.1 M hydrochloric acid to adjust zero. To calculate the content of $C_{12}H_{13}ClN_4$ in the tablets, from the readings obtained. Alternatively, make the calculations by taking into account that $A(1\%, 1\text{ cm}) = 316$, at 272 nm, in 0.1 M hydrochloric acid.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

PIROXICAM CAPSULES

It contains at least 95.0 % and at most 105.0 % of the amount stated of $C_{15}H_{13}N_3O_4S$.

IDENTIFICATION

A. The principal spot in the chromatogram of *Solution (2)* in the *Related substances* corresponds in position, color, and intensity to that obtained with *Solution (3)*.

B. The ultraviolet absorption spectrum (5.2.14) ranging from 200 nm to 400 nm, of *Sample solution* obtained in *Dosage* method A., exhibits absorption maxima at 354 nm similar to those observed in the *Standard solution* spectrum.

C. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method B. corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test. To proceed according to method B., *Dosage*.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 900 mL

Equipment: baskets, 100 rpm

Time: 45 minutes

Procedure: after testing, remove aliquot of 10 mL from the dissolution medium and dilute in 0.1 M hydrochloric acid, to obtain suitable concentration. To measure the absorbances of the solutions at 242 nm (5.2.14), by using the same solvent to adjust zero. To calculate the amount of $C_{15}H_{13}N_3O_4S$ dissolved in the medium, by using the $A(1\%, 1\text{ cm}) = 352$, at 242 nm, in 0.1 M hydrochloric acid.

Tolerance: not less than 70% (Q) of the amount stated of $C_{15}H_{13}N_3O_4S$ dissolves within 45 minutes.

PURITY TESTS

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel as support and a mixture of toluene and glacial acetic acid (90:10) as mobile phase. To separately apply to the plate 10 µL of each of the solutions freshly prepared, as described below.

Solution (1): to mix an amount of powder containing 80 mg of piroxicam with 25 mL of methylene chloride, filter, and evaporate the filtrate to dryness by using a rotary

evaporator. To dissolve the residue in 2 mL of methylene chloride.

Solution (2): to dilute 1 mL of *Solution (1)* in 20 mL of methylene chloride.

Solution (3): to prepare 2 mg/mL piroxicam SQR solution in methylene chloride.

Solution (4): to dilute 2 mL of *Solution (2)* in 50 mL of methylene chloride.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). Any secondary spot in the chromatogram obtained with *Solution (1)* is not more intense than that obtained in the chromatogram with *Solution (4)*. To disregard any remaining spot in the application line.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 248 nm; 300 mm column and 3.9 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (10 µm), kept at room temperature; flow of *Mobile phase* of 2 mL/minute. To prepare the solutions as described below.

Dibasic sodium phosphate buffer: To dissolve 5.35 g of dibasic sodium phosphate dodecahydrate in 100 mL of water. To dissolve 7.72 g of citric acid in 400 mL of water. To transfer both solutions to 1000 mL volumetric flask and top up with water.

Mobile phase: mixture of methanol and *Dibasic sodium phosphate buffer* (60:40).

Sample solution: to weigh 20 capsules, remove the content, and weigh them again. To make the content of the capsules homogeneous. To transfer an amount of powder equivalent to 10 mg of piroxicam to a 200 mL volumetric flask, add 150 mL of 0.01 M methanolic hydrochloric acid, stir, and put it in ultrasound for 30 minutes. To cool down and top up the volume with the same solvent. To filter the solution through quantitative filter.

Standard solution: to prepare 0.005% piroxicam SQR solution (w/v) in 0.01 M methanolic hydrochloric acid. If necessary, put the solution in ultrasound bath at room temperature.

Procedure: to separately inject 10 µL of *Sample and standard solutions*, record the chromatograms, and measure the area under the peaks.

To calculate the content of $C_{15}H_{13}N_3O_4S$ from the sample, from the responses obtained for the *Sample solution* and *Standard solution*.

B. To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To weigh 20 capsules, remove the content, and weigh them again. To make the content of the capsules homogeneous. To transfer an amount of powder equivalent to 25 mg of piroxicam to a 250 mL volumetric flask and top up with 0.1 M sodium hydroxide. To successively dilute in the same solvent to obtain 10 µg/mL final concentration. To prepare a standard solution at similar concentration by using the same solvent. To measure the absorbances of the solutions at 354 nm, by using 0.1 M sodium hydroxide to adjust zero. To calculate the content of $C_{15}H_{13}N_3O_4S$ from the capsules, from the responses obtained for the *Sample and standard solutions*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light, at room temperature.

LABELLING

To comply with legislation in force.

PIROXICAM GEL

It contains at least 95.0 % and at most 105.0 % of the amount stated of $C_{15}H_{13}N_3O_4S$.

IDENTIFICATION

A. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel as support and a mixture of ethyl acetate, methanol, and glacial acetic acid (80:10:1) as mobile phase. To separately apply to the plate 5 µl of each of the solutions freshly prepared, as described below.

Solution (1): to mix an amount of gel containing 10 mg of piroxicam with 0.1 mL of saturated hydrochloric acid until the solution becomes cloudy. To dilute to 5 mL with 0.01 M methanolic hydrochloric acid. To stir well, centrifuge, and use the clear supernatant solution. To filter the supernatant solution, if necessary.

Solution (2): to prepare solution with a concentration equivalent to 0.2% (w/v) of piroxicam SQR with 0.01 M methanolic hydrochloric acid.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). The principal spot obtained with *Solution (1)* corresponds in position and dimension to that obtained in the chromatogram with *Solution (2)*.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method B. corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

pH (5.2.19). 7.2 to 8.2. To determine in 10 % gel solution (w/v).

Determination of weight (5.1.1). It complies with the test.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 248 nm; 250 mm column and 4.6 mm inner diameter, packed with silica chemically bonded to the octylsilane group (3 µm to 10 µm); and 100 mm pre-column and 4.6 mm inner diameter, chemically bonded to the octylsilane group (5 µm); kept at 40 °C; flow of *Mobile phase* of 1.0 mL/min. To prepare the solutions as described below.

Mobile phase: mixture of 0.05 M dibasic sodium phosphate dihydrate, pH adjusted to 3.5 with phosphoric acid, acetonitrile, and methanol (55:30:15).

Sample solution: to transfer an amount of gel equivalent to 5 mg of piroxicam to a 100 mL volumetric flask, add 5 mL of 0.01 M methanolic hydrochloric acid, and stir for 30 minutes. To add 50 mL of *Mobile phase* and mechanically stir for 30 minutes. To top up with *Mobile phase* and stir. To filter the solution by using 1.0 µm glass microfiber filter.

Standard solution: to prepare 0.10% piroxicam SQR solution (w/v) in 0.01 M methanolic hydrochloric acid. If necessary, put the solution in ultrasound bath at room temperature. To remove an aliquot of 5 mL from this solution, transfer it to a 100 mL volumetric flask, and top up with *Mobile phase*.

Procedure: to separately inject 10 µL of *Sample and standard solutions*, record the chromatograms, and measure the area under the peaks. To calculate the content of $C_{15}H_{13}N_3O_4S$ from the sample, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light, at room temperature.

LABELLING

To comply with the legislation in force.

SURINAM CHERRY TREE

Eugeniae folium

Eugenia uniflora L. - MYRTACEAE

The vegetable drug consists of the dried leaves of the species containing at least 5.0% of tannin, 1.0% of total flavonoids, expressed in quercetin; and 0.8% of volatile oils. The volatile oil consists of at least 27.0% of curzerene (cis and trans).

CHARACTERISTICS

Organoleptic characteristics. The dry leaves have a citrus odor and spicy taste.

MACROSCOPIC DESCRIPTION

Simple, ovate-lanceolate leaves usually with 4.5 cm to 6.2 cm long and 2.0 cm to 2.7 cm wide, glabrous, membranaceous and slightly coriaceous, with acute to acuminate apex, sometimes slightly sickle-shaped, acute to obtuse base, whole margin, "peninérvea-type", with more prominent midrib in the mid-basal region of the abaxial face. Venation is the camplodromous-brochidodromous-type, each secondary vein starting at an acute angle in relation to the principal, anastomosing with its subsequent superior to form a series of arches near the leaf edge; secondary veins and superior determine incomplete areola, with free vascular terminations. Petiole with 0.3 cm to 0.6 cm long. In the dry material, the adaxial face of the blade is dark green and the abaxial face is lighter. Glands, present in the blade, are hardly viewed without the aid of lenses.

MICROSCOPIC DESCRIPTION

Hypostomatic leaves of dorsiventral mesophyll. In cross section, the leaf blade has unistratified epidermis, covered by a thick cuticle layer. The stomata are paracytic-type, occurring at the same height that the fundamental epidermal cells. These, on both sides, show varying dimensions and sinuous anticlinal walls. In guard cells thickening of the inner face is prominent, being viewed as dumbbells. The palisade parenchyma is unistratified and accompanied by collecting cells. The palisade cells occupy 25.0% to 30.0% of the mesophyll, being generally of smaller dimensions on the basal portion of the blade. The spongy parenchyma has seven to nine strata of cells with brachiform projections relatively long, allowing the formation of large intercellular spaces. In the mesophyll it is common crystal idioblasts containing rhombic crystals of calcium oxalate and drusen, which are especially abundant along with vascular bundles. Schizolysigenous secretory cavities, with an average diameter of 60 µm containing oil drops are common underlying to epidermis on both leaf faces,

although more abundant on the adaxial face. The two to four epidermal cells that cover the external secretory cavity have straight inside walls. The epithelium of these cavities, in cross section, is formed by five to eight cells. In the midrib, plano-convex contour, or rarely slightly concave-convex

or biconvex, one to three layers of annular chlorenchyma with tenuous thickenings occur underlying to the epidermis. The main vascular bundle is bicollateral-type, in open arc, surrounded by two to three strata of parenchyma cells with thickened walls, and a sheath of fibers, except at the ends of the arc. The phloem has plenty of small rhombic crystals. Secondary veins and those of smaller caliber are collateral, with fiber callote on both poles of the conducting tissues. The petiole, with concave-convex contour, has small lateral expansions. Epidermis is unistratified, containing brown substances, also present in the fundamental underlying collenchymatous parenchyma cells, which has all its cells with thin cellulose thickening. Secretory cavities, similar to the blade, are also subepidermally present. Starch grains, druses and crystals occur in abundance throughout the fundamental parenchyma. The vascular bundle is configured as an open, bicollateral arc with plenty of rhombic crystals in the phloem, surrounded by four to eight layers of thick-walled parenchyma tissue, forming a perivascular sheath. Fibers, isolated or in groups of two or three elements, are rarely present around the vascular bundle.

MICROSCOPIC DESCRIPTION OF THE POWDER

The powder meets all the requirements established for the species, unless the macroscopic characters. The characteristics are: epidermal fragments of the blade with sinuous anticlinal walls (adaxial face); epidermal fragments with paracytic stomata; blade fragments which, because of the transparency, allow viewing rhombic crystals, abundant drusen and bright secretory cavities due to the presence of oil drops.

IDENTIFICATION

A. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using F_{254} silica gel with a thickness of 250 µm as a stationary phase and a mixture of ethyl acetate, formic acid and water (75:5:5) as a mobile phase. To separately apply to the band-shaped plate 20 µL of *Solution* (1) and 10 µL of *Solutions* (2) and *Solution* (3), recently prepared as described below.

Solution (1): to accurately weigh about 10 g of the milled drug, add 100 mL of water and heat under reflux for 15 minutes. After cooling at room temperature, filter the solution obtained in cotton under reduced pressure. To extract the resulting aqueous phase with three portions of 25 mL of ethyl acetate in a separatory funnel of 125 mL. To let it rest in a freezer at -18°C for 15 minutes for complete separation of the phases. To gather and filter the organic fractions with 5 g of anhydrous sodium sulfate.

To evaporate the organic fraction in a rotary evaporator under reduced pressure to obtain residue. To resuspend the residue with 1 mL of methanol.

Solution (2): to weigh about 1 mg of epicatechin and dissolve in 2 mL of methanol.

Solution (3): to weigh about 1 mg of 4-O-methyl gallo catechin and dissolve in 1 mL of methanol.

To develop the chromatogram. To remove the plate and allow to dry in fume hood. To nebulize the plate with 1% ferric chloride solution (w/v) in methanol. The chromatogram obtained with *Solution (1)* has two bluish gray spots, in the same height that those verified in the chromatograms obtained from *Solution (2)* and *Solution (3)* (R_f about 0.85 and 0.87, respectively) in the central quadrant two bluish brown spots are observed.

B. To identify curzerene, proceed as described in *Gas chromatography (5.2.17.5)*. To use chromatograph equipped with mass spectrometry detector; capillary column 30 m long, 0.25 mm internal diameter, filled with propylene glycol, film thickness 0.25 μm ; column temperature of 60° C to 250° C, at 3° C per minute (total 80 minutes), injector temperature at 220° C and detector temperature at 230° C, by using helium at a pressure of 80 kPa as carrier gas with a flow rate of 1 mL/minute. To use mixture of nitrogen, synthetic air, and hydrogen (1:1:10) as auxiliary gases.

Sample solution: to dilute the volatile oil in ethyl ether (2:100).

Procedure: to inject 1 μL of Sample solution into the gas chromatograph, by using 1:50 flow division.

Curzerene isomers must present a relative retention time of approximately 1845.

To calculate Retention Index (IK), according to the expression:

$$IK = 100 \times n + \frac{100 \times (tr_x - tr_z)}{(tr_{z+1} - tr_z)}$$

where

n = number of carbon atoms of alkane with lower molecular weight;

tr_x = retention time of compound "x" (intermediate between tr_z and tr_{z+1});

tr_z = retention time of the alkane with 'n' carbons;

tr_{z+1} = retention time of the alkane with "n+1" carbons.

C. To heat, under reflux, about 3 g of pulverized drug with 60 mL of distilled water for 15 minutes. To cool down and filter. To add two drops of hydrochloric acid SR to 2 mL of the extract and drip gelatin SR. The appearance of clear precipitate indicates a positive reaction for tannins.

D. To add 10 mL of water and two to four drops of 1% ferric chloride solution (w/v) in ethanol to 2 mL of extract obtained in *Identification*, test **B**. The development of dark-gray color indicates a positive reaction for tannins.

E. To add 0.5 mL of 1% vanillin (w/v) in 1 mL of methanol and 1 mL of hydrochloric acid to 2 mL of extract obtained in *Identification*, test **B**. The development of red color indicates a positive reaction for tannins.

F. To add 10 mL of 2 M acetic acid and 5 mL of lead acetate SR to 5 mL of extract obtained in *Identification*, test **B**. The appearance of whitish precipitate indicates the presence of tannins.

G. To add small pieces of metal magnesium and 1 mL of hydrochloric acid to 5 mL of extract obtained in *Identification*, test **B**. The appearance of red coloration indicates the presence of flavonoid aglycones.

PURITY TESTS

Foreign matter (5.4.2.2). At most 2.0%.

Water (5.4.2.3). At most 10.0%.

Total ashes (5.4.2.4). At most 11.0%.

Sulfated ashes (5.4.2.6). At most 14.0%.

DETERMINATION OF FOAM INDEX (IE)

To accurately transfer about 1 g of the milled vegetable drug (180 μm) to erlenmeyer flask containing 50 mL of boiling water. To keep under moderate boil for 15 minutes. To cool down, filter through cotton to a 100 mL volumetric flask. To top up to volume through the filter up to 100 mL. To distribute the decoction obtained in 10 test tubes with lid (16 mm diameter and 16 cm height), in a successive series of 1 mL, 2 mL, 3 mL, until 10 mL, and adjust the volume of liquid in each tube to 10 mL of water. To cover the tubes and vigorously stir for 15 seconds in vertical movements, by stirring twice per second. To let it rest for 15 minutes and measure the foam height. Then, add 1 mL of 2 M hydrochloric acid to each tube. If the foam height of all tubes is below 1 cm, the foam index is less than 100. If, in any of the tubes, the foam height remains equal to or greater than 1 cm, the dilution of the vegetable material in this tube (A) is the index observed. To calculate the foam index (IE), according to the expression:

$$IE = \frac{1000}{A}$$

where

A = volume (mL) of decoction used for dilution in the tube, where foam was observed.

The IE for decoction should be at least 125.

DOSAGE

Total tannins

To proceed as described in the *Ultraviolet absorption spectrophotometry (5.2.14)*. To prepare solutions described as follows:

Stock solution: to accurately weigh about 0.75 g of the pulverized drug (250 μm) and transfer to a 250 mL erlenmeyer flask with ground mouth. To add 150 mL of distilled water.

To heat in a water bath at 60°C for 30 minutes. To cool down in running water and transfer to 250 mL volumetric flask. To wash the erlenmeyer flask and transfer the washings with all content of the vegetable drug for the same volumetric flask. To top up with distilled water. To decant and filtrate the supernatant by using filter paper. To discard the first 50 mL of the filtrate.

Sample solution for total polyphenols: to dilute 5 mL of filtrate in 25 mL volumetric flask with distilled water. To volumetrically transfer 2 mL of this solution, 1 mL of phosphotungstic molybdate reagent, and 10 mL of distilled water to a 25 mL volumetric flask and top up with a 29% sodium carbonate solution (w/v). To determine the absorbance at 760 nm (A_1) after 30 minutes by using distilled water to adjust zero.

Sample solution for polyphenols not adsorbed by hide powder: to obtain 10 mL of the filtrate, add 0.1 g of hide powder SQR and mechanically stir in 125 mL erlenmeyer flask for 60 minutes. To use filter paper. To dilute 5 mL of this filtrate in 25 mL volumetric flask with distilled water. To volumetrically transfer 2 mL of this solution, 1 mL of phosphotungstic molybdate reagent, and 10 mL of distilled water to a 25 mL volumetric flask and top up with a 29% sodium carbonate solution (w/v). To determine the absorbance at 760 nm (A_2) after 30 minutes by using distilled water to adjust zero.

Standard solution: just before using it, dissolve 50 mg of pyrogallol in a 100 mL volumetric flask with distilled water. To volumetrically transfer 5 mL of the solution to a 100 mL volumetric flask and top up with distilled water. To volumetrically transfer 2 mL of this solution, 1 mL of phosphotungstic molybdate reagent, and 10 mL of distilled water to a 25 mL volumetric flask and top up with a 29% sodium carbonate solution (w/v). To determine the absorbance at 760 nm (A_3) after 30 minutes by using distilled water to adjust zero.

To calculate the content of tannin (dried drug), in %, expressed as pyrogallol, according to the equation:

$$TT = \frac{62,5 \times (A_1 - A_2) \times m_2}{A_3 \times m_1}$$

where

A_1 = absorbance of the *Sample solution for total polyphenols*; not adsorbed by hide powder;

A_3 = absorbance of the *Standard solution*;

m_1 = mass of the sample used in the test (g), considering the determination of water;

m_2 = mass of pyrogallol (g).

Total flavonoids

To proceed as described in the *Ultraviolet absorption spectrophotometry* (5.2.14). To prepare solutions described as follows:

Stock solution: to accurately weigh about 0.4 g of milled drug (240 µm) and transfer to a 100 mL round bottom flask. To add 1 mL of 0.5% methenamine solution (w/v) to water, 20 mL of acetone and 2 mL of hydrochloric acid. To heat it in a heating mantle for 30 minutes under reflux. To use a small amount of cotton to filter the mixture to a 100 mL volumetric flask. To wash the residue of the drug and cotton in the same round bottom flask with 20 mL of acetone. To keep, under reflux, for 10 minutes, and use cotton to filter to the same 10 mL volumetric flask. To repeat this operation once more. To cool down at room temperature, and top up with acetone. To transfer 20 mL of acetonic solution to a separatory funnel (125 mL), 20 mL of distilled water and extract with a 15 mL portion of ethyl acetate, repeat the extraction three times with 10 mL portions of ethyl acetate. To gather the ethyl acetate phases and wash in a separatory funnel with two 50 mL portions of distilled water. To transfer the ethyl acetate phase to 50 mL volumetric flask and top up to volume with ethyl acetate.

Sample solution: to volumetrically transfer 10 mL of *Stock solution* to a 25 mL volumetric flask, add 1 mL of 2% aluminum chloride (w/v) to methanol and top up with a 5% methanolic solution of acetic acid (w/v).

Blank solution: to transfer 10 mL of *Stock solution* to a 25 mL volumetric flask and top up with a 5% methanolic solution of acetic acid (w/v).

To measure the absorbance of the *Sample solution* at 425 nm in a 1 cm thick cuvette, 30 minutes after its preparation, using the *Blank solution* for zero adjustment. To calculate the total flavonoids content, expressed as quercetin, in the sample according to the equation. To consider the specific absorptivity of quercetin as A (1%, 1 cm) = 500.

$$Q = \frac{\text{Abs} \times 62\,500}{500 \times m \times (100 - \text{Pd})}$$

where

Abs = absorbance of the *Sample solution*;

m = mass of the drug (g),

Pd = loss on drying (%; w/w).

Volatile oils

To proceed as described in *Determination of volatile oils in vegetable drugs* (5.4.2.7). To use 1000 mL round bottom flask containing 500 mL of distilled water as distillation liquid and 0.5 mL of xylene which should be inserted through the side opening K. To use dry scratched plant,

not bruised. To determine the volatile oil from 100 g of the scratched drug. To distill for four hours.

PACKAGING AND STORAGE

In tightly closed containers, protected from light and heat.

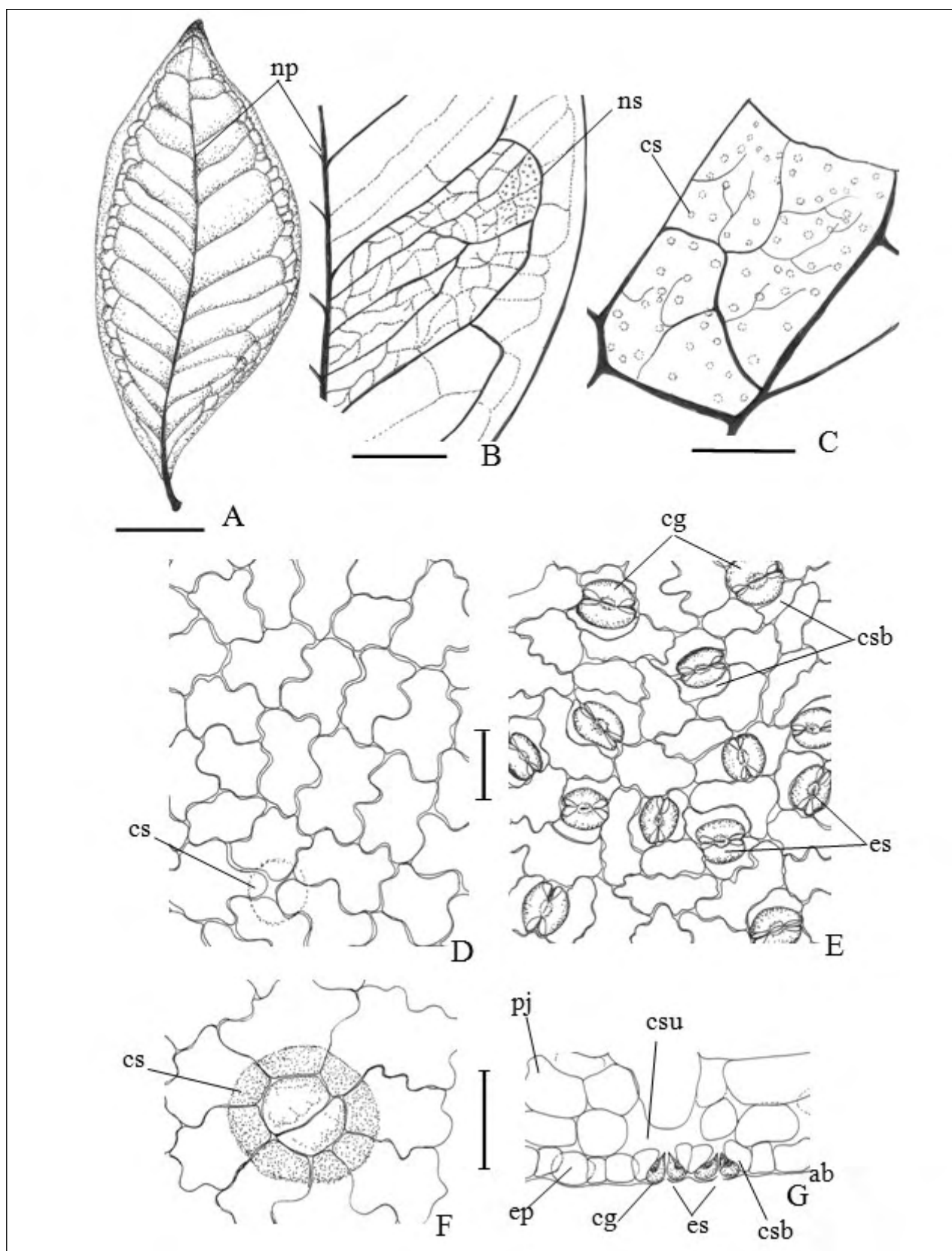


Figure 1 - Macroscopic and microscopic aspects in *Eugenia uniflora* L.

Additional information of **Figure 1**. Scales correspond in **A**, to 1 cm; **B** to 5 mm; and **C** to 1 mm; in **D**, **E**, **F** and **G** to 50 μ m.

A - Scheme of the leaf, in front view: midrib (np). **B** - schematic detail of the portion of the blade showing leaf venation: midrib (np); secondary vein (ns). **C** - scheme detail of areolas and vascular endings: secretory cavity (cs). **D** and **E** - partial details on the adaxial and abaxial leaf blade, respectively, in front view: secretory cavity (cs); guard-cell (cg); subsidiary cell (csb); stomata (es). **F** - partial detail of the adaxial face of leaf blade, in front view, showing a secretory cavity visualized by transparency: stomata (es). **G** - partial detail of the blade, in cross section, showing twin stomatal complex: spongy parenchyma (pj); substomatic chamber (csu); abaxial face (ab); subsidiary cell (csb); stoma (es), guard-cell (cg); epidermis (ep).

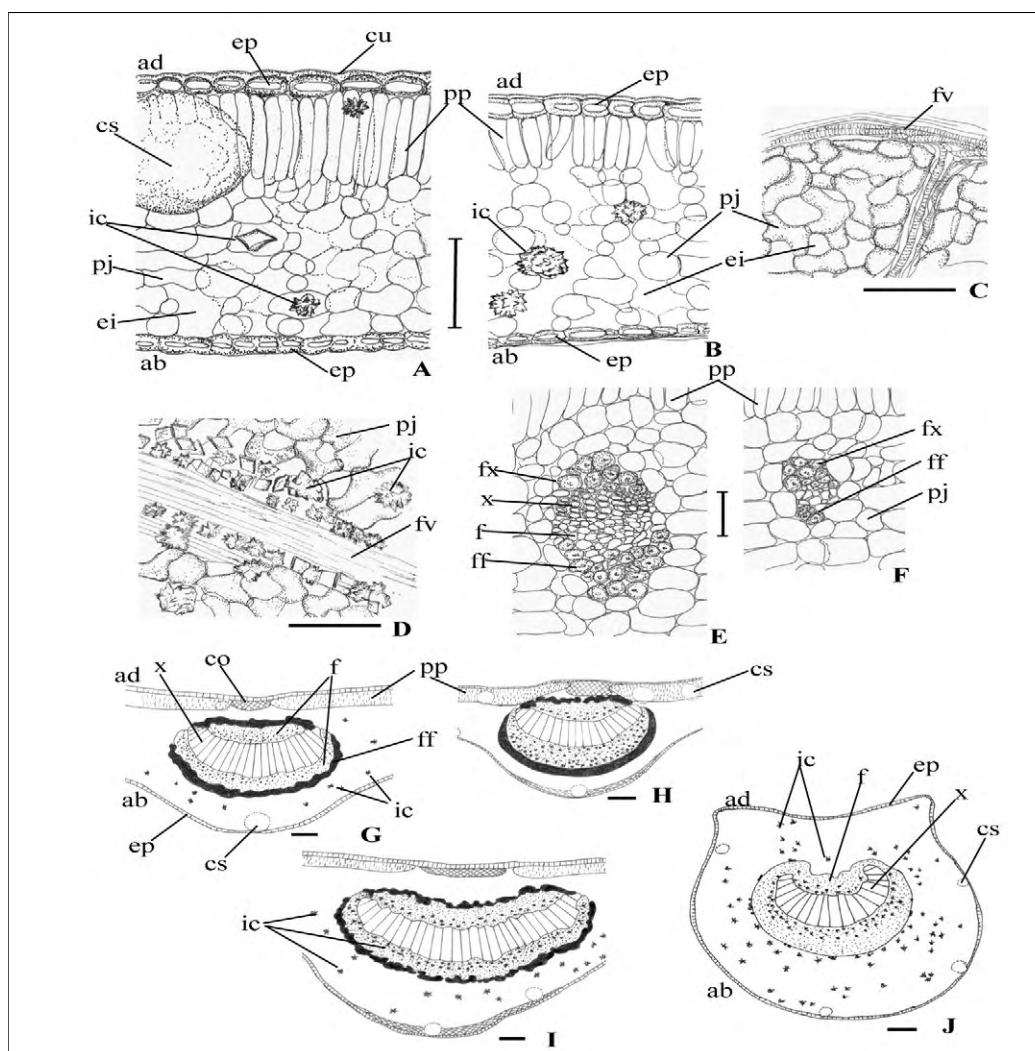


Figure 2 - Microscopic aspects in *Eugenia uniflora* L.

Additional information of Figure 2. Scales correspond in **A** and **B** to 100 µm; in **C**, **D**, **E** and **F** to 50 µm; in **G**, **H** and **I** to 100 µm; in **J** at 200 µm.

A and **B** - partial details of mesophyll of different samples, cross sections: abaxial face (ab); adaxial face (ad); epidermis (ep); cuticle (cu); secretory cavity (cs); crystal idioblasts (ic); spongy parenchyma (pj); palisade parenchyma (pp); intercellular space (ei). **C** and **D** - fragments of powder showing details of the spongy parenchyma: spongy parenchyma (pj); intercellular space (ei); vascular bundle (fv); crystal idioblasts (ic). **E** and **F** - partial details, cross-sections, of a secondary and a tertiary veins, respectively: palisade parenchyma (pp); xylem fibers (fx); xylem (x); phloem (f); phloem fibers (ff); spongy parenchyma (pj). **G**, **H** and **I** - diagrams of midrib, cross-section, in the median region (**G**) and basal region of different samples (**H** and **I**): abaxial face (ab); adaxial face (ad); epidermis (ep); xylem (x); collenchyma (co); phloem (f); palisade parenchyma (pp); phloem fibers (ff); crystal idioblasts (ic); secretory cavity (cs). **J** - diagram, in cross section, of the petiole: abaxial face (ab); adaxial face (d); secretory cavity (cs); epidermis (ep); phloem (f); crystal idioblasts (ic), xylem (x).

HUMAN PLASMA FOR FRACTIONATION

Plasma Humanum ad Separationem

Human plasma for fractionation is the liquid portion of whole blood remaining after separation of blood cell fractions, using appropriate closed system of blood collection that meets the requirements for plastic containers used to collect human blood, containing anticoagulant, conservative, preservative solution or separated by continuous filtration or centrifugation of anticoagulated blood in an apheresis procedure for obtaining products derived from human plasma.

DONORS

Only plasma from a healthy donor and carefully selected that, after medical examination, laboratory blood tests, study of his medical history and free from infectious agents transmitted by the plasma, may be accepted for collection for his plasma fractionation. Refer to the legislation in force for hemotherapeutic products.

Immunization of donors. Plasma from deliberate immunization of donors to obtain hyperimmune globulins may be used for fractionation when sufficient amounts of this material may not be naturally obtained from immunized donors. It is recommended that immunization of donors

is performed in accordance with the procedures adopted by the World Health Organization (WHO).

Records. Data and information about donors and donations should be maintained so that enables the confidentiality of the identity of the donor, the origin of each donation in plasma pool and traceability corresponding to laboratory tests.

Laboratory tests. Properly validated laboratory tests are performed each donation to detect viral markers and other infectious agents, such as those described below.

A. Antibodies against the virus type 1 and type 2 of human immunodeficiency virus (HIV-1 and HIV-2).

B. The surface antigen of Hepatitis B virus (HBsAg).

C. Antibodies against Hepatitis C virus (anti-HCV).

The analytical methods used must have adequate sensitivity and specificity. If a positive repeated result is found in any of the tests the donation should be rejected.

faster in cold chamber at -20°C or below, as soon as possible and not later than 72 hours after collection.

It is not necessary to determine the total protein and factor VIII, described in *DOSAGE* in each unit of plasma. These determinations are parameters of good manufacturing practices, being the relevant test *Factor VIII* for use in the preparations of labile concentrate protein.

The total protein content in each unit of plasma depends on the content of proteins in the serum of the donor and the degree of dilution inherent to the donation procedure.

When plasma is obtained from a selected donor and using an appropriate proportion of anticoagulant conservative and preservative solution, the total protein content obtained is in the limit of 50 g/L. If the volume of blood or plasma collected together with the anticoagulant conservative and preservative solution is less than the established, the resulting plasma is not necessarily suitable for fractionation. The intended goal with good manufacturing practices should be reaching the limit prescribed for all donations

INDIVIDUAL UNITS OF PLASMA

The plasma should be prepared by a method which completely removes, as much as possible, other cell fractions by centrifugation of whole blood. It is obtained from whole blood or by apheresis. The plasma should be separated from its cell by a method designed to prevent the introduction of micro-organisms. No antibacterial or antifungal agent may be added to the plasma. The filling systems for collecting and processing human blood should meet the requirements for closed systems for collection of human blood and should prevent any possibility of contamination.

If two or more units are mixed before freezing, the operation should be performed using sterile connectors or under aseptic conditions, with containers that have not been previously used.

When it is obtained by plasmapheresis or whole blood (after separation of the cellular elements), the plasma may be intended for recovery of labile proteins when frozen within 24 hours after collection, with rapid cooling under conditions validated to ensure that the temperature at -25°C or lower is achieved within each unit of plasma within 12 hours of the start of insertion in the freezer.

When it is obtained by plasmapheresis, plasma intended only for the recovery of non-labile proteins must be frozen by rapid cooling in cold chamber at -20°C or below, as soon as possible and not later than 24 hours after collection.

When it is obtained from whole blood, separated from cellular elements, plasma intended only for the recovery of non-labile proteins should not be frozen by cooling.

The preservation of factor VIII of human coagulation depends on the procedure of collection and subsequently handling the plasma unit. Together with good practices, 0.7 UI/mL may be usually achieved in plasma units, but plasma units with lower factor VIII activity may be still suitable for the production of clotting factor concentrates. The goal intended with good manufacturing practices is to preserve, as much as possible, the labile proteins.



MIXTURES OF PLASMA (PLASMA POOL)

During the manufacture of plasma derivatives, the first mixture of plasma pool (for example, after removal of cryoprecipitate) should be tested for surface antigen of Hepatitis B virus (HBsAg) and antibodies against HIV by using appropriate sensitivity methods and specificity. The results should be negative in all tests.

Also, a test should be performed for RNA of Hepatitis C virus by using a validated technique of nucleic acid amplification. In the test it is included a positive control with 100 UI/mL of RNA of Hepatitis C virus, in order to test for inhibitors, an internal control prepared by adding the appropriate marker to a sample of plasma pool. The test is not valid if the positive control is not reactive or if the result obtained indicates the presence of inhibitors.

The mixture of plasma satisfies the test if it is not reactive to RNA of Hepatitis C virus. The test should be performed compared to an international standard recognized by WHO.

CHARACTERISTICS

Aspect. Before freezing, the plasma for fractionation, a clear or slightly turbid liquid without visible signs of hemolysis, may vary in color from a slightly yellow to greenish.

DOSAGE

Factor VIII: C

To proceed as described in *Determination of Factor VIII of the Human Blood Clotting, Lyophilisate (5.5.1.7)*. To perform the test by using plasma pool with no less than 10 units of plasma sample. If necessary, thaw the samples to be examined at a temperature not exceeding 37°C. To use a calibrated reference plasma against an international Standard of Factor VIII. The activity is not less than 0.7 UI/mL.

Total proteins

To proceed as described in *Determination of nitrogen by the Kjeldahl method (5.3.3.2)*. To perform the test using a mixture with not less than 10 plasma units. To dilute the plasma mixture in a solution of 0.9% sodium chloride (w/v) to obtain a solution containing about 15 mg of protein at 2 mL. To add to a round bottom centrifuge tube, 2 mL of this solution, 2 mL of 7.5% sodium molybdate solution (w/v), and 2 mL of sulfuric acid mixture free of nitrogen and water (1:30). To stir centrifuge for 5 minutes, decant the supernatant and invert the tube, allowing its contents to drain on filter paper. To determine the nitrogen content in the residue after mineralization and calculate the protein content by multiplying the amount of nitrogen by 6.25. The total protein content is not less than 50 g/L.

STORAGE AND TRANSPORTATION

The frozen plasma should be stored and transported under conditions developed to maintain the temperature at -20°C or lower; for accidental reasons, the storage temperature may rise above -20°C on one or more occasions during the storage and transportation, however, plasma is acceptable for fractionation if all the following conditions are met:

- total time during which the temperature exceeds -20°C may not be greater than 72 hours;
- the temperature should not exceed -15°C on more than one occasion;
- on no occasion the temperature may exceed -5°C.

LABELLING

To comply with the legislation in force. The label should enable that each individual unit is traceable to its specific donor.

POLYGALA

Senegae radix

Polygala senega L. - POLYGALACEAE

The vegetable drug consists of roots and short nodosum rhizomes of *Polygala senega* L. and its cultivars containing at least 6% of saponins expressed as oleanolic acid derivatives ($C_{30}H_{48}O_3$, 456.70).

CHARACTERISTICS

Organoleptic characteristics. The root has mild sweet odor, reminding a slightly rancid methyl salicylate. The taste is initially sweet and then acre. The root powder is irritating and causes sneezing; when shaken with water it produces abundant foam.

MACROSCOPIC DESCRIPTION

The root is axial and fusiform, slightly tortuous, sometimes branched or forked, presenting in the apical region a short nodosum, subglobose, and strongly extended rhizome, up to 4 cm wide, warty, reddish-brown, showing numerous traces of aerial stems, whose presence may not exceed 2% of total weight, covered at level of insertion by rudimentary, flaky, oval, obtuse, leafs, 2 mm to 3 mm long, often pink to purplish color, with ciliated edges. Laterally, the root has a keel-shaped appendage, arranged across its length generally distributed in a helical manner. The root, below nodosum apical rhizome, generally has 5 cm to 20 cm in length and 0.5 cm to 1.2 cm width, and it may have a small number of lateral roots. Its surface, with yellowish brown and brownish color in the upper region and yellow in the lower region, is striped, both longitudinally and transversely. In cross section, it is observed that yellow-brown cortex of varying thickness, surrounding a central woody area, with light opaque yellow, more or less circular to irregular. This section shows a structure predominantly ec-

centric generally oval or pear-shaped, due to the presence of the keel. The cross-sectional shape is variable, including at different heights in the same individual. The fracture is plain and clear.

MICROSCOPIC DESCRIPTION

By microscopic examination of the cross section of the root, by using aqueous solution of 3% sodium hypochlorite (w/v), it is evidenced a suber with two to six layers of light yellowish-brown cells, tangentially elongated, with thin walls. The cortical region is formed by about ten or more cell layers, and the outermost parts are colenchimatics and the others are parenchymatous, which have an amorphous, colorless to pale yellow substance, which is separated in the form of large droplets of

oil by adding a drop of potassium hydroxide. The cambium forms a continuous ring, producing abnormal secondary growth tissues, mostly with eccentric disposition. The phloem has parenchyma cells that are radially distributed and in its conductive elements it is also observed the presence of an amorphous substance. The xylem forms a massive secondary wood, usually arranged in a fan shape, consisting of tracheids with a diameter up to 65 μm and vessel elements with wall with reticulate thickening and lateral perforation plates, associated with few lignified parenchyma cells. More internally, it is verified the presence of the primary xylem, which allows the classification of the organ as diarco. There is no medullary parenchyma. The region of the keel whose shape is variable from prominent to almost circular, is originated from an irregular activity of the cambium, which may promote an anomalous development of the xylem and/or phloem, resulting in the formation of one or two, rarely three large cuneiform parenchyma beams, in the region of these tissues. The anomalies observed in cross section correspond to profound changes in the anatomical structure of the bark and wood, making it very obvious when treated with phloroglucinol and hydrochloric acid. In none of the tissues verified the presence of crystals or starch. Remains of the stems, when present, show, in cross section, sub-rectangular and elongated epidermal cells, parenchyma cortex, not lignified pericyclic fiber sheath, phloem with elements with small diameter, xylem formed by tracheids and vessels elements with walls with reticular, helical or marked thickness and parenchyma medulla. Squamous leaves, when they are present, show epidermis with sinuous anticlinal walls, unicellular rounded trichome at the apex and anomocytic stomata.

MICROSCOPIC DESCRIPTION OF THE POWDER

The powder meets all the requirements for the species, unless the macroscopic characters. The characteristics are: light brown color; suber fragments; fragments of yellowish cortical parenchyma, with oil droplets; collenchyma cells with oil drops; fragments of short tracheids; cells of lignified parenchyma beams with large simple pores; eventually occur fragments originating from epidermal squamous leaves of aerial stems, with unicellular trichomes and anomocytic stomata; absence of sclereids, crystals and starch grains.

IDENTIFICATION

To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using silica gel G with a thickness of 250 μm as a support and lower phase of the a mixture of glacial acetic acid, water and 1-butanol (10:40:50) as a mobile phase. To separately apply to the band-shaped plate 10 μL of *Solution* (1) and 10 μL and 40 μL of *Solution* (2), recently prepared as described below.

Solution (1): to weigh 1 g of the powder drug, add 10 mL of 70% ethanol (v/v) and allow it to boil for 15 minutes under reflux. To filter and cool down.

Solution (2): to prepare an escin solution at 1 mg/mL in 70% ethanol (v/v).

To develop the chromatogram. To remove the plate, and allow it to air dry. To nebulize with anisaldehyde SR1 and heat in an oven at 100°C to 105°C, until the appearance of red spots corresponding to saponosides. The region of the chromatogram obtained with *Solution* (1) has between three and five red spots in the central and lower regions, with R_fs similar to the grayish violet spots obtained with *Solution* (2). To nebulize the plate with 20% phosphomolybdic acid (w/v) in ethanol, and heat in an oven at 100°C to 105 °C, until the spots corresponding to saponosides become blue. The intensity and size of the spots obtained in the chromatogram of *Solution* (1) are between the two spots corresponding to escin, obtained by application of 10 μL and 40 μL of *Solution* (2).

PURITY TESTS

Foreign matter (5.4.2.2). At most 2.0%. Referring to traces of aerial stems.

Water (5.4.2.3). At most 10%.

Total ashes (5.4.2.4). At most 6%.

DOSAGE

Saponins

To proceed as described in the *Ultraviolet absorption spectrophotometry* (5.2.14). To prepare solutions described as follows:

Stock solution: to accurately weigh about 1 g of pulverized plant and transfer to a 250 mL round bottom flask. To add 70 g of 50% ethanol (v/v), 0.1 mL of defoamer silicone and some glass beads. To accurately weigh the set and heat it under reflux in a water bath for 60 minutes. To cool down and top up to the initial weight with 50% ethanol (v/v). To centrifuge, separate the residue and the decanted solution, which is heavy and reduced to a residue in a rotary evaporator at a maximum temperature of 60°C. To resuspend the residue in 10 mL of 0.1 M hydrochloric acid, transfer to a 250 mL separatory funnel and wash the flask with two portions of 5 mL of 0.1 M hydrochloric acid. To gather acid

phases and extract with three portions of 70 mL of the upper phase of chloroform mixture, 0.1 M hydrochloric acid and 1-butanol (30:90:180). After stirring, the two phases should remain at rest for 15 minutes, at least before their separation. The organic phases are gathered and washed with two portions of lower phase of the chloroform, 0.1 M hydrochloric acid and 1-butanol (30:90:180) mixture. The lower phase is discarded. To evaporate the organic phase to a residue in a rotary evaporator at a maximum temperature of 60°C. To resuspend the residue with 98% glacial acetic acid (v/v) by transferring the content of saponins, as oleanolic acid derivatives, according to the equation:

50 mL volumetric flask. To top up with glacial acetic acid. To filter the solution, by discarding the first 20 mL of the filtrate.

Sample solution: to transfer 0.5 mL of the *Stock solution* to the test tube, add 4 mL of coloring reagent. To make it homogeneous and heat the test tube in a water bath at 60 °C ± 1°C for 25 minutes. To cool down in an ice bath for 30 seconds and immediately take a reading.

Blank solution: to transfer 0.5 mL of glacial acetic acid to the test tube and add 4 mL of coloring reagent. To make it

homogeneous and heat the test tube in a water bath at 60 °C ± 1°C for 25 minutes. To cool down in an ice bath for 30 seconds and immediately take a reading.

To measure the absorbance of the *Sample solution* at 425 nm, by using the *Blank solution* for zero adjustment. To calculate

$$AO\% = \frac{436,2 \times A}{m_1 \times m_2}$$

where

AO% = content of oleanolic acid derivatives (%);

A = absorbance measured;

m_1 = mass of the solution after centrifugation (g);

m_2 = mass of the drug (g) considering the water content determined.

PACKAGING AND STORAGE

In tightly closed containers, protected from light and humidity.

p

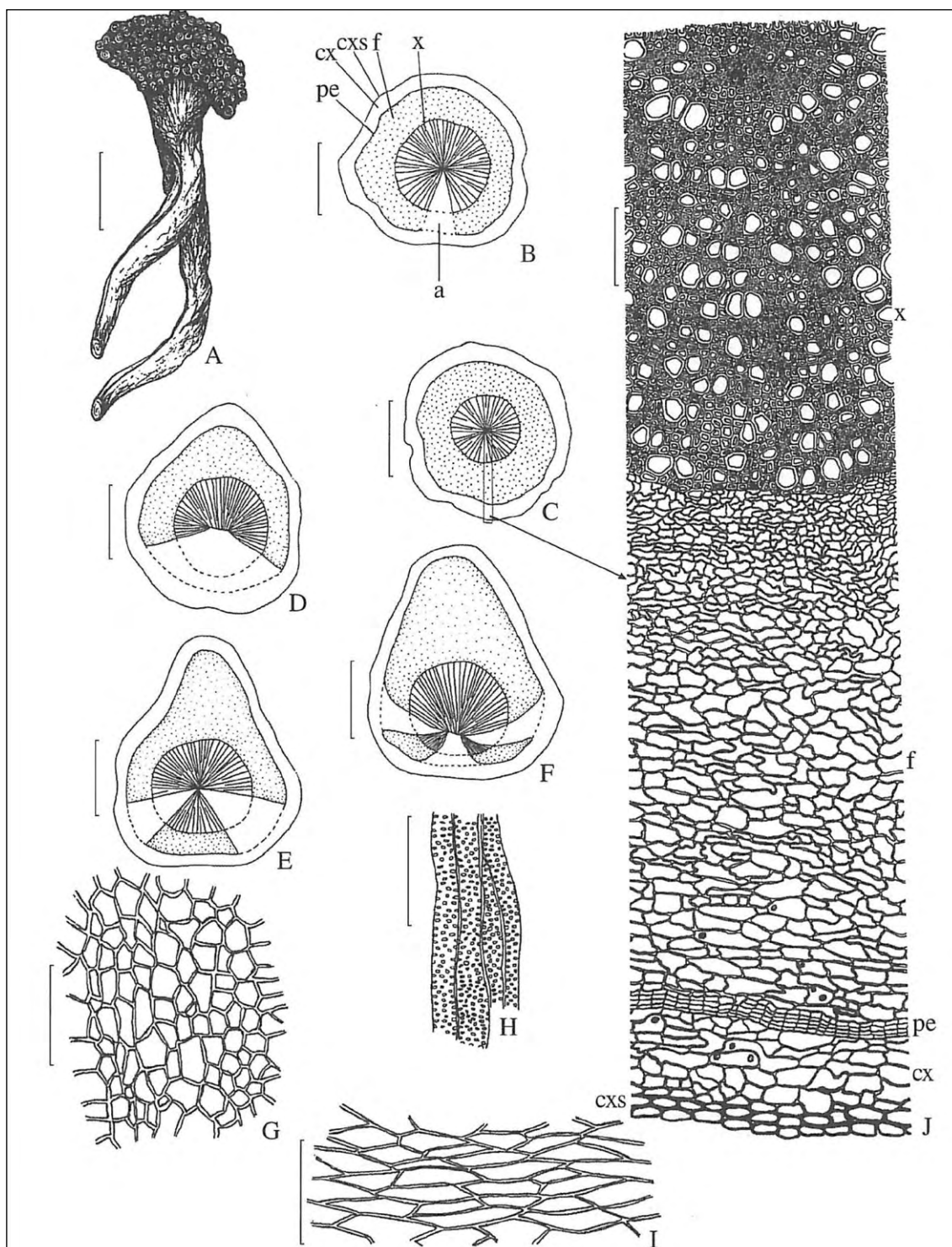


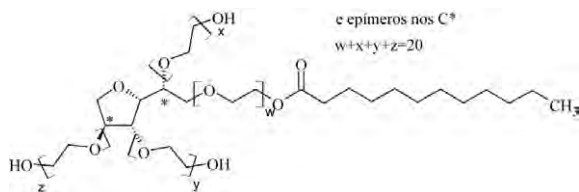
Figure 1 - Macroscopic and microscopic aspects of the root of *Polygala senega* L.

Additional information of **Figure 1**. Scales correspond in A to 1 mm; B, C, D, E, F to 1 mm; G, H, I and J to 100 µm.

A - general appearance of the root with nodosum apical rhizome. B, D, E and F - general appearances of the cross sections of the root: abnormality of parenchyma beams (a), cortex (cx); suberised cortex (cxs); phloem (f); periderm (pe); xylem (x). C - general appearance of the cross section of the root with normal structure. G - cortical parenchyma cells of the innermost region. H - Detail of vessel elements. I - cortical parenchyma cells of the outermost region. J - detail of a portion of the root in cross-section as shown in C: cortex (cx); suberised cortex (cxs); phloem (f); periderm (pe); xylem (x).

POLYSORBATE 20

Polysorbatum 20



polysorbate 20; 07272

Derivatives of sorbitan, monododecanoate, poly (oxy-1,2-ethanediyl)

[9005-64-5]

Mixture of partial lauric esters of sorbitol and its anhydrides copolymerized with approximately 20 moles of ethylene oxide to each mole of sorbitol and its anhydrides. Lauric acid used in the esterification may contain varying amounts of other fatty acids.

DESCRIPTION

Physical and chemical characteristics. Oily liquid, clear or slightly opalescent, yellowish or amber. *Relative density* (5.2.5): about 1.1.

Solubility. Miscible with water, absolute ethanol, ethyl acetate, and methanol. Practically insoluble in fixed oils and liquid paraffin.

IDENTIFICATION

A. To dissolve 0.5 g of the sample in water at 100 mL (about 50 °C) and dilute to 10 mL with the same solvent. The solution produces abundant foam after stirring. To add 0.5 g of sodium chloride and heat to boiling. The turbidity disappears upon cooling down to about 50 °C.

B. To heat under reflux 4 g of sample in a water bath for 30 minutes with 40 mL of 5% potassium hydroxide (w/v). To cool down to about 80 °C, acidify with 20 mL of 2 M nitric acid, and heat under reflux for about 10 minutes, in order to separate the emulsion. The fatty acids remain on the surface as oily liquid. To cool down at room temperature and extract with 50 mL of petroleum ether (distillation range 40-60 °C), by avoiding vigorous stirring. To wash the organic phase with 3 portions (5 mL each) of water and evaporate to dryness in a water bath. The index of acidity (5.2.29.7), determined in 0.3 g of the residue with 50 mL of the solvent, is 245 - 300.

C. To dissolve 0.1 g of the sample in 5 mL of chloroform, add 0.1 g of potassium thiocyanate and 0.1 g of cobalt nitrate (II), and mix by using a glass rod. The blue color develops.

PURITY TESTS

Index of acidity (5.2.29.7). To determine in 5 g of the sample. At most 2.0

Index of hydroxyl (5.2.29.7). 96 to 108. To determine in 2 g of the sample.

Index of iodine (5.2.29.10). At most 5.0.

Index of saponification (5.2.29.8). 40 to 50. To use 15 mL of 0.5 M SV alcoholic potassium hydroxide and dilute in 50 mL of water, before titration.

Reducing impurities. To dissolve 2 g of the sample in 25 mL of hot water, add 25 mL of M sulfuric acid and 0.1 mL of ferroin SI. To titrate with 0.01 M SV cerium ammonium nitrate, by continuously stirring to red turns greenish-blue for 30 seconds. To perform sample blank correction and adjust it as required. At most 2 mL of 0.01 M SV cerium ammonium nitrate are spent.

Heavy metals (5.3.2.3). To use *Method III*. At most 0.001% (10 ppm).

Water (5.2.20.1). At most 3.0% determined in 1 g.

Sulfated ashes (5.2.10). To transfer 2 g of the sample to a platinum or silica crucible, add 0.5 mL of sulfuric acid, and heat it in a water bath for 2 hours. To carefully heat in burner to obtain total carbonization. To add 2 mL of nitric acid and 0.25 mL of sulfuric acid to carbonized mass, carefully heat it to obtain white smoke, and incinerate at 600 °C to obtain constant weight. At most 0.2%.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

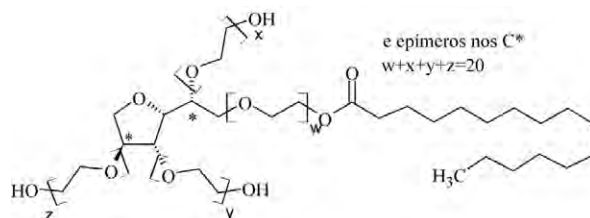
To comply with the legislation in force.

CATEGORY

Nonionic surfactant. Emulsifier, solubilizer, and wetting.

POLYSORBATE 40

Polysorbatum 40



polysorbate 40; 07273

Derivatives of sorbitan, monohexadecanoate, poly (oxy-1,2-ethanediyl)

[9005-66-7]

Palmitic ester of sorbitol and its anhydrides copolymerized with approximately 20 moles of ethylene oxide to each mole of sorbitol and its anhydrides.

DESCRIPTION

Physical characteristics. Yellow liquid with a strong typical odor.

Solubility. Soluble in water and ethanol. Insoluble in mineral oil and vegetable oils.

IDENTIFICATION

A. To add 5 mL of *M* sodium hydroxide to 5 mL of solution of the sample (1:20), heat to boiling for a few minutes, cool down, and acidify with 3 *M* hydrochloric acid. The preparation becomes strongly opalescent.

B. To add dropwise 0.5 mL of bromine water SR to 2 mL of solution of the sample (1:20). Bromine does not discolor (unlike polysorbate 80).

PURITY TESTS

Index of hydroxyl (5.2.29.7). 89 to 105. To determine in 2 g of the sample.

Index of saponification (5.2.29.8). 41 to 52. To use 15 mL of 0.5 *M* SV ethanolic potassium hydroxide and dilute in 50 mL of water, before titration.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

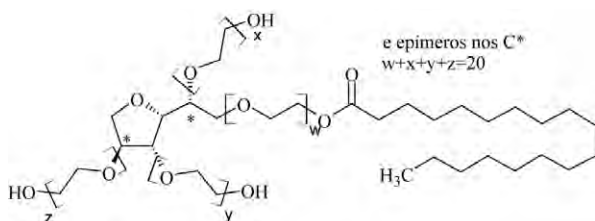
LABELLING

To comply with the legislation in force.

CATEGORY

Nonionic surfactant. Emulsifier, solubilizer, and wetting.

POLYSORBATE 60 Polysorbatum 60



polysorbate 60; 07274

Derivatives of sorbitan, mono octadecanoate, poly (oxy-1,2-ethanediyl)
[9005-67-8]

Mixture of partial stearic esters of sorbitol and its anhydrides copolymerized with approximately 20 moles of ethylene oxide to each mole of sorbitol and its anhydrides. Stearic acid used for the esterification may contain other fatty acids, particularly palmitic acid.

DESCRIPTION

Physical and chemical characteristics. Yellowish-brown gelatinous mass. It is a clear liquid at temperatures above 25 °C. *Relative density (5.2.5):* about 1.1.

Solubility. Miscible with water, absolute ethanol, ethyl acetate, and methanol. Practically insoluble in fixed oils and liquid paraffin.

Heavy metals (5.3.2.3). To use *Method III*. At most 0.001% (10 ppm).

IDENTIFICATION

Water (5.2.20). To determine in 1 g. At most 3.0%.

Sulfated ashes (5.2.10). To transfer 2 g of the sample to a platinum or silica crucible, add 0.5 mL of sulfuric acid, and heat it in a water bath for 2 hours. To carefully heat in burner to obtain total carbonization. To add 2 mL of nitric acid and 0.25 mL of sulfuric acid to carbonized mass, carefully heat it to obtain white smoke, and incinerate at 600 °C to obtain constant weight. At most 0.2%.

A. To dissolve 0.5 g of the sample in water at about 50 °C and top up to 10 mL with the same solvent. The solution produces abundant foam after stirring. To add 0.5 g of sodium chloride and heat to boiling. The turbidity disappears upon cooling down to about 50 °C.

B. To heat under reflux 4 g of sample in a water bath for 30 minutes with 40 mL of 5% potassium hydroxide (w/v). To cool down to about 80 °C, acidify with 20 mL of 2 *M* nitric acid, and heat under reflux for about 10 minutes, in order to separate the emulsion.

The fatty acids remain on the surface as oily liquid. To cool down at room temperature and extract with 50 mL of petroleum ether (distillation range 40-60 °C), by avoiding vigorous stirring. To wash the organic phase with 3 portions (5 mL each) of water and evaporate to dryness in a water bath. The index of acidity (5.2.29.7), determined in 0.5 g of the residue with 50 mL of the solvent, is 190 - 220.

C. To dissolve 0.1 g of the sample in 5 mL of chloroform, add 0.1 g of potassium thiocyanate and 0.1 g of cobalt nitrate (II), and mix by using a glass rod. The blue color develops.

D. To add 5 mL of *M* sodium hydroxide to 5 mL of solution of the sample (1:20), boil it for a few minutes, cool down, and acidify with 3 *M* hydrochloric acid. The preparation becomes strongly opalescent.

PURITY TESTS

Index of acidity (5.2.29.7). To determine in 5 g of the sample. At most 2.0%.

Index of hydroxyl (5.2.29.7). 81 to 96. To determine in 2 g of the sample.

Index of iodine (5.2.29.10). At most 5.0.

Index of saponification (5.2.29.8). 45 to 55. To use 15 mL of 0.5 M SV ethanolic potassium hydroxide and dilute in 50 mL of water, before titration.

Heavy metals (5.3.2.3). To use *Method III*. At most 0.001% (10 ppm).

Water (5.2.20). To determine in 1 g of the sample. At most 3.0%.

Sulfated ashes (5.2.10). To transfer 2 g of the sample to a platinum or silica crucible, add 0.5 mL of sulfuric acid, and heat it in a water bath for 2 hours. To carefully heat in burner to obtain total carbonization. To add 2 mL of nitric acid and 0.25 mL of sulfuric acid to carbonized mass, carefully heat it to obtain white smoke, and incinerate at 600 °C to obtain constant weight. At most 0.2%.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

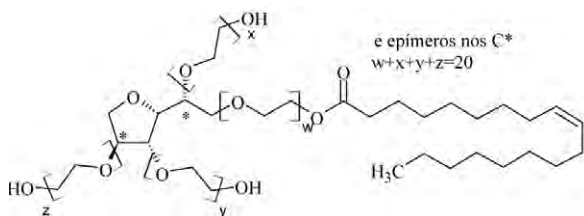
LABELLING

To comply with the legislation in force.

CATEGORY

Nonionic surfactant. Emulsifier, solubilizer, and wetting.

POLYSORBATE 80 Polysorbatum 80



polysorbate 80; 07275

Derivatives of sorbitan, mono-(9Z)-9-octadecenoate, poly(oxy-1,2-ethanediyl)

[9005-65-6]

Mixture of partial oleic esters of sorbitol and its anhydrides copolymerized with approximately 20 moles of ethylene oxide to each mole of sorbitol and its anhydrides.

DESCRIPTION

Physical and chemical characteristics. Oily liquid, clear, yellow or light brown, with typical odor and slightly bitter taste. *Relative density (5.2.5):* about 1.1. *Viscosity (5.2.7):* about 400 mPa.

Solubility. Miscible with water, absolute ethanol, ethyl acetate, and methanol. Practically insoluble in fixed oils and liquid paraffin.

IDENTIFICATION

A. To dissolve 0.5 g of the sample in water at about 50 °C and top up to 10 mL with the same solvent. The solution produces abundant foam after stirring. To add 0.5 g of sodium chloride and heat to boiling. The turbidity disappears upon cooling down to about 50 °C.

B. To heat under reflux 4 g of sample in a water bath for 30 minutes with 40 mL of 5% potassium hydroxide (w/v). To cool down to about 80 °C, acidify with 20 mL of 2 M nitric acid, and heat to boiling under reflux for about 10 minutes, in order to separate the emulsion. The fatty acids remain on the surface as oily liquid. To cool down at room temperature and extract with 50 mL of petroleum ether (distillation range 40-60 °C), by avoiding vigorous stirring. To wash the organic phase with 3 portions (5 mL each) of water and evaporate to dryness in a water bath. To resuspend the resulting residue by mixing 2 mL of nitric acid with 3 mL of water. To carefully add, in small portions, 0.5 g of sodium nitrite and let it rest at room temperature. The layer of fatty acid solidifies in 4 hours.

C. To dissolve 0.1 g of the sample in 5 mL of chloroform, add 0.1 g of potassium thiocyanate and 0.1 g of cobalt nitrate (II), and mix by using a glass rod. The blue color develops.

D. To add 5 mL of M sodium hydroxide to 5 mL of solution of the sample (1:20), heat to boiling for a few minutes, cool down, and acidify with 3 M hydrochloric acid. The preparation becomes strongly opalescent.

E. To add dropwise 0.5 mL of bromine water SR to 2 mL of solution of the sample (1:20). Bromine discolors (unlike polysorbate 40).

PURITY TESTS

Index of acidity (5.2.29.7). At most 2.0.

Index of hydroxyl (5.2.29.12). 65 to 80. To determine in 2 g of the sample.

Index of iodine (5.2.29.10). 18 to 24.

Index of saponification (5.2.29.8). 45 to 55. To use 15 mL of 0.5 M SV ethanolic potassium hydroxide and dilute in 50 mL of water, before titration.

Reducing impurities. To dissolve 2 g of the sample in 25 mL of hot water, add 25 mL of M sulfuric acid and 0.1 mL of ferroin SI. To titrate with 0.01 M SV cerium ammonium nitrate, by continuously stirring to red turns greenish-blue for 30 seconds. To perform sample blank correction and adjust it as required. At most 5 mL of 0.01 M SV cerium ammonium nitrate are spent.

Heavy metals (5.3.2.3). To use *Method III*. At most 0.001% (10 ppm).

Water (5.2.20). To determine in 1 g of the sample. At most 3.0%.

Sulfated ashes (5.2.10). To transfer 2 g of the sample to a platinum or silica crucible, add 0.5 mL of sulfuric acid, and heat it in a water bath for 2 hours. To carefully heat in burner to obtain total carbonization. To add 2 mL of nitric acid and 0.25 mL of sulfuric acid to carbonized mass, carefully heat it to obtain white smoke, and incinerate at 600 °C to obtain constant weight. At most 0.2%.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

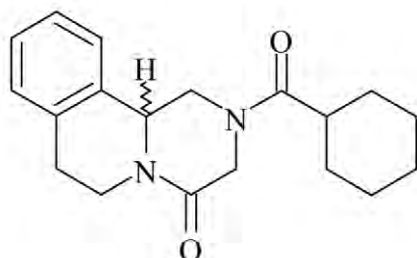
LABELLING

To comply with the legislation in force.

CATEGORY

Nonionic surfactant. Emulsifier, solubilizer, and wetting.

PRAZIQUANTEL Praziquantelum



$C_{19}H_{24}N_2O_2$; 312.41

praziquantel; 07321

2 - (cyclohexyl carbonyl) - 1,2,3,6,7,11b-hexahydro-4H-pyrazino [2,1-α] isoquinolin-4-one
[55268-74-1]

It contains at least 98.5% and at most 101.0% of $C_{19}H_{24}N_2O_2$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white or almost white powder. It shows polymorphism.

Solubility. Very slightly soluble in water, freely soluble in ethanol and methylene chloride.

Physical and chemical constants. *Melting range (5.2.2):* 136°C to 142°C.

IDENTIFICATION

The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the praziquantel SQR spectrum similarly prepared.

PURITY TESTS

Related substances. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 210 nm; 250 mm column and 4 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μm to 10 μm), flow of *Mobile phase* of 1.03 mL/min.

Mobile phase: mixture of water and acetonitrile (55:45).

Solution (1): to dissolve about 40 mg of the sample in *Mobile phase* and dilute to 10 mL with the same solvent, thus obtaining 4 mg/mL solution.

Solution (2): to dilute 1 mL of *Solution (1)* to 100 mL with *Mobile phase*. To dilute 5 mL of this solution to 10 mL with *Mobile phase*, thus obtaining 20 μg/mL solution.

Solution (3): 0.2 mg/mL praziquantel SQR solution in *Mobile phase*.

Solution (4): to dissolve 5 mg of 2 - benzoyl-1,2,3,6,7,11b-hexahydro-4H-pyrazino [2,1-α] isoquinolin-4-one (*Impurity A*) SQR in *Solution (3)* and dilute to 5 mL with the same solvent. To dilute 1 mL of this solution to 10 mL with *Mobile phase*, thus obtaining 20 μg/mL *Impurity A* and praziquantel solutions.

To inject 20 μL of *Solution (4)*. The resolution between *Impurity A* and praziquantel peaks is greater than 3.0.

Procedure: to separately inject 20 μL of *Solutions (1)* and (2), record the chromatograms at least 5 times the retention time of praziquantel, and measure the areas under the peaks. The sum of any peak obtained in the chromatogram with *Solution (1)*, except the main peak's one, is not greater than the area under the main peak obtained with *Solution (2)* (0.5%). The area of up to a secondary peak obtained

in the chromatogram with *Solution (1)*, except the main peak's one, is greater than 4 times the area under the main peak obtained with *Solution (2)* (0.2%). The sum of the areas of all the peaks obtained in the chromatogram with *Solution (1)*, except the main peak's one, is not greater than the area obtained with *Solution (2)* (0.5%). To disregard the peaks with an area less than 0.1 times the area under the main peak obtained with *Solution (2)* (0.05%).

IDENTIFICATION

Heavy metals (5.3.2.3). To use *Method III*. At most 0.002% (20 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 50°C under reduced pressure for 2 hours. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 210 nm; 250 mm column and 4 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm to 10 µm), flow of *Mobile phase* of 1.5 mL/min.

Mobile phase: mixture of water and acetonitrile (40:60).

Sample solution: to accurately transfer about 45 mg of the sample to a 25 mL volumetric flask. To top up to volume with *Mobile phase* and make it homogeneous. To transfer 5 mL of this solution to 50 mL volumetric flask and top up with *Mobile phase*.

Standard solution: to dissolve an amount accurately weighed of praziquantel SQR in *Mobile phase* and properly dilute with the same solvent, to obtain 0.18 mg/mL solution.

To inject replicates of 10 µL of the *Standard solution*. The tailing factor is not greater than 1.5. The relative standard deviation for replicate areas of the peaks recorded is not greater than 1.0 %.

Procedure: to separately inject 10 µL of *Sample and standard solutions*, record the chromatograms, and measure the area under the peaks. To calculate the content of $C_{19}H_{24}N_2O_2$ from the sample, from the responses obtained for the *Sample and standard solutions*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Anthelmintic.

PRAZIQUANTEL TABLETS

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_{19}H_{24}N_2O_2$.

To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using G silica gel as support and ethyl acetate as mobile phase. To separately apply to the plate 10 µL of each of the solutions freshly prepared, as described below.

Solution (1): to weigh and pulverize the tablets. To transfer an amount of powder equivalent to 30 mg praziquantel to a centrifuge tube and add 5 mL of methanol. To stir for 5 minutes and centrifuge. To use the clear supernatant.

Solution (2): 6 mg/mL praziquantel SQR solution in methanol.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test. Within 15 minutes.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.2% sodium lauryl sulfate (w/v) in 0.1 M hydrochloric acid, 900 mL

Equipment: baskets, 50 rpm

Time: 60 minutes

Procedure: after the test, remove aliquot from dissolution medium and filter. To measure the absorbances at 263 nm (5.2.14), by using the *Dissolution medium* to adjust zero. To calculate the amount of $C_{19}H_{24}N_2O_2$ dissolved in the medium, by comparing the readings obtained with those of the *Standard solution* prepared as described below.

Standard solution: to dissolve accurately weighed amount of praziquantel SQR, to obtain a solution whose concentration is (L/900) mg/mL where L is the stated amount in

milligrams of praziquantel per tablet. To transfer 5 mL of the resulting solution to 50 mL volumetric flask, dilute, and top up with *Dissolution medium*.

Tolerance: not less than 75% (Q) of the amount stated of $C_{19}H_{24}N_2O_2$ dissolves within 60 minutes.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To proceed as described in *Dosage*, in the monograph *Praziquantel*. To prepare *Sample solution* and *Standard solution* as described below.

Sample solution: To weigh and pulverize the tablets. To transfer an amount of powder equivalent to 150 mg of praziquantel to a 100 mL volumetric flask. To add 70 mL of *Mobile phase* and put it in ultrasound for 15 minutes. To top up with the same solvent, make it homogeneous, and filter it. To transfer 3 mL to 25 mL volumetric flask, top up with *Mobile phase*, and make it homogeneous.

Standard solution: 0.18 mg/ mL praziquantel SQR solution in *Mobile phase*.

Procedure: to separately inject 10 μ L of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of $C_{19}H_{24}N_2O_2$ in the tablets, from the responses obtained for the *Sample solution* and *Standard solution*.

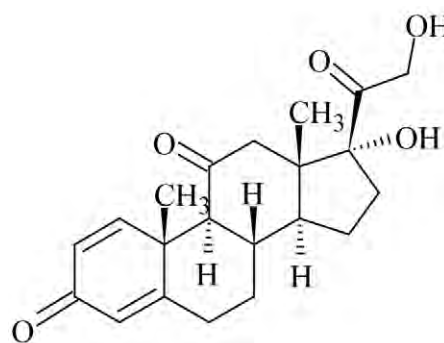
PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

PREDNISONE Prednisonum



$C_{21}H_{26}O_5$; 358.43

prednisone; 07341

17,21-dihydroxy pregna-1,4-diene-3,11,20-trione
[55-05-2]

It contains at least 97.0% and at most 103.0% of $C_{21}H_{26}O_5$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white or almost white, and odorless powder. It shows polymorphism.

Solubility. Very slightly soluble in water, sparingly soluble in chloroform, ethanol, dioxane, and methanol.

Physical and chemical constants

Specific rotation (5.2.8): +167° to +175°. To determine in 0.5% solution (w/v) in dioxane.

IDENTIFICATION

The identification tests B. and D. may be omitted if the tests A. and C. are conducted. The identification test A. can be omitted if the tests B., C., and D. are carried out.

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the prednisone SQR spectrum similarly prepared. To separately dissolve prednisone SQR and sample in acetone, if the spectra are not identical, and evaporate them to dryness. To obtain new spectra from these residues.

B. The ultraviolet absorption spectrum (5.2.14) ranging from 200 nm to 400 nm, of 0.001% solution of the sample (w/v) in ethanol, exhibits absorption maximum at 239 nm, similar to that observed in the spectrum of a similar prednisone SQR solution. The absorbance at 239 nm is between 0.405 and 0.435.

C. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF₂₅₄ silica gel as support and a mixture of methylene chloride, ethyl ether, methanol, and wa-

ter (77:15:8:1.2) as mobile phase. To apply separately to the plate 5 µl of each of the solutions freshly prepared, as described below.

Solution (1): 1 mg/mL solution of the sample in a mixture of chloroform and methanol (9:1).

Solution (2): 1 mg/mL prednisone SQR solution in a mixture of chloroform and methanol (9:1).

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). To nebulize with 20% sulfuric acid (v/v) in ethanol. To heat the plate at 120 °C for 10 minutes. To cool down. To examine under ultraviolet light (365 nm). The spot obtained in the chromatogram with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

D. To dissolve about 6 mg of the sample in 2 mL of concentrated sulfuric acid. Let it rest for 5 minutes. Orangish color develops. By stirring it, pour dropwise the solution was in 10 mL of water. The color turns yellow and gradually becomes bluish-green.

PURITY TESTS

Related substances. To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 254 nm; 250 mm chromatograph column and 4.0 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at 45 °C; flow of *Mobile phase* of 2.5 mL/min.

Solution (1): to dissolve 25 mg of the sample in methanol and dilute to 10 mL with the same solvent.

Solution (2): to dissolve 2 mg of prednisone SQR and 2 mg of prednisolone in methanol and dilute to 100 mL with the same solvent.

Solution (3): to dilute 1 mL of *Solution (1)* to 100 mL with methanol.

Eluent A: To add 100 mL of acetonitrile with 650 mL of water to 1000 mL volumetric flask. To make it homogeneous. To adjust the volume to 1000 mL with water and mix again.

Eluent B: acetonitrile.

Mobile phase gradient: to adopt the gradient system described in the following table:

Time (minutes)	Eluent A (% v/v)	Eluent B (% v/v)	Elution
0	100	0	stabilize
0-25	100	0	isocratic
25-40	100 → 40	0 → 60	linear gradient
40-41	40 → 0	60 → 100	linear gradient
41-46	0	100	isocratic
46-47	0 → 100	100 → 0	linear gradient
47-52	100	0	stabilize

To balance the column for 30 minutes with *Eluent B* at flow of 2.5 mL/minute, then with *Eluent A* for 5 minutes. To proceed under the conditions described for 40-52 minutes. To adjust the sensitivity of the system, to obtain at least 50% of full scale for the height of the main peak of the chromatogram obtained with 20 µL of *Solution (3)*. To inject 20 µL of *Solution (2)*. The retention times are about 19 minutes to prednisone and 23 minutes to prednisolone. The resolution between prednisone and prednisolone is greater than 2.7. If necessary, adjust the concentration of acetonitrile in the *Eluent A*.

Procedure: To separately inject 20 µL of methanol as blank, 20 µL of *Solution (1)*, and 20 µL of *Solution (3)*. In the chromatogram obtained with *Solution (1)*, the area of no other peak, other than the area under the main peak, is not greater than 0.25 times the area under the main peak of the chromatogram obtained with *Solution (3)* (0.25%); the sum of the areas of all peaks, other than the main peak's one, is not greater than 0.75 times the area under the main peak in the chromatogram obtained with *Solution (3)* (0.75%). To discard any peak in the sample blank correction and any peak whose area is smaller than 0.05 times the area under the main peak of the chromatogram obtained with *Solution (3)*.

Water (5.2.20.1). At most 1.0% for the anhydrous substance and 5% for the monohydrated substance.

Loss on drying (5.2.9). To determine in 0.5 g of the sample. To desiccate in an oven at 105 °C. At most 1.0%.

Sulfated ashes (5.2.10). To determine in 0.1 g of the sample. At most 0.5%.

DOSAGE

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 240 nm; 250 mm column and 4.0 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at room temperature; flow of *Mobile phase* of 1.0 mL/minute.

Mobile phase: mixture of water, tetrahydrofuran, and methanol (69:25:6.2).

Internal standard solution: to dissolve accurately weighed amount of acetanilide in 33 mL of methanol. To top up to 100 mL with purified water, to obtain 110 µg/mL solution.

Sample solution: to dissolve accurately weighed amount of sample in 33 mL of methanol. To top up to 100 mL with purified water, to obtain 0.2 mg/mL solution. To transfer 5 mL of the solution and 5 mL of *Internal standard solution* to a 50 mL volumetric flask. To top up to volume with a mixture of methanol and water (1:2) and make it homogeneous, to obtain 20 µg/mL prednisone solution.

Standard solution: to dissolve accurately weighed amount of prednisone SQR in 33 mL of methanol. To top up to 100 mL with purified water, to obtain 0.2 mg/mL solution. To transfer 5 mL of the solution and 5 mL of *Internal standard solution* to a 50 mL volumetric flask. To top up to volume with a mixture of methanol and water (1:2) and make it homogeneous, to obtain 20 µg/mL prednisone solution.

To inject replicates of 20 µL of the *Internal standard solution*. The resolution between prednisone and acetanilide is greater than 3.0. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2.0 %.

Procedure: to separately inject 20 µL of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_{21}H_{26}O_5$ from the sample, from the responses obtained for the *Sample solution* and *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Anti-inflammatory, steroid.

PREDNISONE TABLETS

BIOLOGICAL SAFETY TEST

It contains at least 95.0 % and at most 105.0 % of the amount stated of $C_{13}H_{16}N_3NaO_4S.H_2O$.

IDENTIFICATION

A. To weigh and pulverize the tablets. To stir an amount of powder equivalent to 20 mg of prednisone with about 100 mL of chloroform. To filter and evaporate to dryness. To dry the residue in an oven at 105 °C for 3 hours. The infrared absorption spectrum (5.2.14) of the residue, dispersed in potassium bromide, has absorption maxima only at the same wavelength, with the same relative intensities to those observed in the prednisone SQR spectrum similarly prepared.

B. To add 2 mL of sulfuric acid to about 6 mg of the residue obtained in test **A**. **Identification.** Let it rest for 5 minutes. Orangish color develops. By stirring it, pour dropwise the solution was in 10 mL of water. The orangish color firstly becomes yellow and then gradually turns bluish-green.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test. To proceed as described in *Dosage* method B.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 500 mL (tablets containing prednisone: 10 mg or less) or 900 mL (tablets containing prednisone: more than 10 mg).

Equipment: blades, 50 rpm

Time: 30 minutes

Procedure: after testing, remove aliquot from the dissolution medium, filter, and dilute with water to obtain suitable concentration. To measure the absorbances at 242 nm, by using the same solvent to adjust zero. To calculate the amount of $C_{12}H_{26}O_5$ dissolved in the medium, by comparing the readings obtained with that of 0.001% prednisone SQR solution (w/v) prepared in the same solvent, but previously adding ethanol to obtain solubilization. The amount of ethanol should not exceed 5% of the total volume.

Tolerance: not less than 80% (Q) of the stated amount of $C_{12}H_{26}O_5$ dissolves within 30 minutes.

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To weigh and pulverize 20 tablets. To transfer an amount of powder equivalent to 5 mg of prednisone to 50 mL volumetric flask and add 30 mL of ethanol. To mechanically stir for 15 minutes and top up with the same solvent. To make it homogenous and filter it. To successively dilute in ethanol to obtain 0.001% concentration (w/v). To prepare standard solution under the same conditions.

To measure the absorbance of the solutions at 239 nm, by using ethanol to adjust zero. To calculate the amount of $C_{12}H_{26}O_5$ in the tablets, from the readings obtained. Alternatively, make the calculations by taking into account that $A(1\%, 1\text{ cm}) = 420$ at 239 nm, in ethanol.

B. To proceed as described in *High performance liquid chromatography* (5.2.17.4). To proceed as described in *Dosage*, in the monograph *Prednisone*. To prepare the Sample solution as described below.

Sample solution: To weigh and pulverize 20 tablets. To transfer an amount of powder equivalent to 20 mg of prednisone to 100 mL volumetric flask and add 5 mL of water. To put it in ultrasound for 1 minute. To add 50 mL of ethanol and put it in ultrasound for 1 minute. To top up with purified water and make it homogeneous. To transfer 5 mL of this solution and 5 mL of *Internal standard solution* to a 50 mL volumetric flask and top up with a mixture of ethanol and water (1:2), to obtain 20 µg/mL prednisone solution. To make it homogenous and filter it.

Procedure: to separately inject 20 µL of *Sample and standard solutions*, record the chromatograms, and measure the area under the peaks. To calculate the amount of $C_{12}H_{26}O_5$ in the tablets, from the responses obtained for the *Sample and standard solutions*.

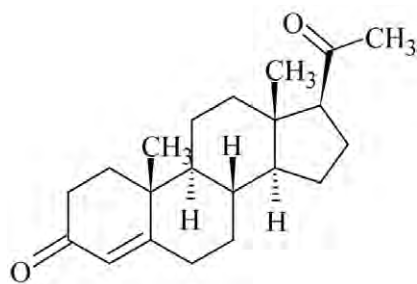
PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

PROGESTERONE Progesteronum



$C_{21}H_{30}O_2$; 314,46
progesterone; 07413
Pregn-4-ene-3,20-dione
[57-83-0]

It contains at least 97.0% and at most 103.0% of $C_{21}H_{30}O_2$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white or slightly yellowish, odorless, tasteless powder. Stable to air.

Solubility. Practically insoluble in water, soluble in ethanol, acetone, and dioxane; sparingly soluble in vegetable oils.

Physical and chemical constants.

Melting range (5.2.2): 126 °C to 131 °C. It has a polymorph with a melting range around 121 °C.

Specific rotation (5.2.8): +175° a +183°, in relation to the desiccated substance. To determine in 2,0% solution (w/v) in dioxane.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the progesterone SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14) ranging from 200 nm to 400 nm, of 0.001% solution (w/v) in ethanol, exhibits maxima and minima only in the same wavelength of a similar progesterone SQR solution.

PURITY TESTS

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel as support and a mixture of chloroform and ethyl acetate (2:1) as mobile phase. To separately apply to the plate 10 µL of each of the solutions freshly prepared, as described below.

Solution (1): to dissolve 0.1 g of the sample in ethanol and top up to 10 mL with the same solvent.

Solution (2): to dilute 1 mL of *Solution (1)* to 100 mL with ethanol.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (2)* (1%).

Loss on drying (5.2.9). To determine in 0.5 g of the sample. To desiccate in an oven at 105 °C for 2 hours. At most 0.5%.

Sulfated ashes (5.2.10). At most 0.1 %.

DOSAGE

To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14).

To accurately transfer about 20 mg of the sample to a 100 mL volumetric flask, dissolve, and top up the volume with ethanol. To successively dilute with the same solvent to obtain 0.001% concentration (w/v). To prepare a standard solution at the same concentration by using the same solvent. To measure the absorbances of the resulting solutions at 240

nm, by using ethanol to adjust zero. To calculate the content of $C_{21}H_{30}O_2$ in the sample from the readings obtained.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

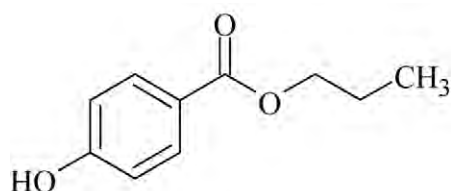
LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Progestogen.

PROPYLPARABEN Propylis parahydroxybenzoas



$C_{10}H_{12}O_3$, 180.20
propylparaben; 07461
4-hydroxybenzoic acid propyl ester
[94-13-3]

It contains at least 98.0% and at most 102.0% of $C_{10}H_{12}O_3$.

DESCRIPTION

Physical characteristics. White, crystalline powder.

Solubility. Very slightly soluble in water, freely soluble in methanol, ethanol, and ethyl ether.

Physical and chemical constants. *Melting range (5.2.2):* 96.0 °C to 99.0 °C.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the propylparaben SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14) ranging from 230 nm to 280 nm, of 0.0005% solution (w/v) in ethanol, exhibits maxima at 258 nm. The absorbance at 258 nm is between 0.440 and 0.475.

PURITY TESTS

Appearance of solution. The 10% solution (w/v) in ethanol is clear (5.2.25) and colorless (5.2.12).

Acidity. To add 3 mL of ethanol, 5 mL of carbon dioxide-free water, and 0.1 mL of bromocresol green SI to 2 mL of solution described in *Appearance of solution*. At most 0.1 mL of 0.1 M sodium hydroxide is spent to change the indicator.

Related substances. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF_{254} silica gel as support and formic acid anhydrous, ethyl acetate, and methylene chloride (2:10:88) as mobile phase. To separately apply to the plate 5 µL of each of the solutions freshly prepared, as described below.

Solution (1): to dissolve 0.1 mg of the sample in methanol and dilute to 5 mL with the same solvent.

Solution (2): to dilute 1 mL of *Solution (1)* to 100 mL with methanol.

To develop the chromatogram. To remove the plate, allow it to dry in hot air, and examine it under ultraviolet light (254 nm). Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (2)* (1.0%).

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To accurately weigh about 1 g of the sample, transfer to erlenmeyer flask fitted with ground stopper, and add 20 mL of *M* SV sodium hydroxide. To adapt reflux condenser and heat at 70 °C for 1 hour. To cool down at room temperature. To titrate the *M* SV sodium hydroxide in excess with 0.5 M SV sulfuric acid. To potentiometrically determine the endpoint, and proceed titration to the second inflection point. To perform sample blank correction and adjust it as required. Each mL of *M* SV sodium hydroxide is equal to 180.200 mg of $C_{10}H_{12}O_3$.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

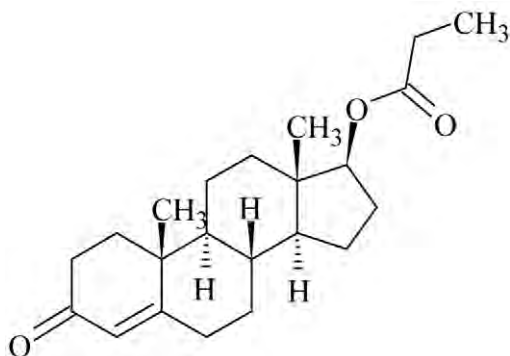
To comply with the legislation in force.

CATEGORY

Preservative.

PROPIONATE, TESTOSTERONE

Testosteroni propionas



$C_{22}H_{32}O_3$; 344.49
testosterone propionate; 08458
(17 β)-17 - (1-oxopropoxy) androst-4-en-3-one
[57-85-2]

It contains at least 97.0% and at most 103.0% of $C_{22}H_{32}O_3$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or almost white powder, or colorless crystals.

Solubility. Practically insoluble in water, freely soluble in acetone and ethanol, soluble in vegetable oil.

Physical and chemical constants.

Melting range (5.2.2): 118 °C to 123 °C.

Specific rotation (5.2.8): +83° to +90°, in relation to the desiccated substance. To determine in 1% solution (w/v) in absolute ethanol.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the testosterone propionate SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14) ranging from 200 nm to 400 nm, of 0.001% solution (w/v) in ethanol, exhibits maxima and minima only in the same wavelength of a similar testosterone propionate SQR solution.

The absorbance values measured at 241 nm does not exceed 3.0%.

PURITY TESTS

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using HF_{254} silica gel as support and a mixture of chloroform and diethylamine (19:1)

as mobile phase. To separately apply to the plate 10 μ L of each of the solutions freshly prepared, as described below.

Solution (1): to dissolve 40 mg of the sample in 2 mL of ethanol.

Solution (2): to dilute 1 mL of *Solution (1)* to 100 mL with ethanol.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (2)* (1%).

Loss on drying (5.2.9). To determine in 0.5 g of the sample. To desiccate in an oven at 105 °C for 2 hours. At most 0.5%.

DOSAGE

To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To accurately weigh about 25 mg of the sample and dissolve in absolute ethanol. To top up the volume to 250 mL with the same solvent. To dilute 10 mL of the solution to 100 mL by using absolute ethanol, to obtain 0.001% solution (w/v). To prepare a standard solution at the same concentration by using the same solvent. To measure the absorbances of the resulting solutions at 240 nm, by using absolute ethanol to adjust zero. To calculate the content of $C_{22}H_{32}O_3$ in the sample from the readings obtained. Alternatively, make the calculations by taking into account that A (1%, 1 cm) = 490 at 240 nm, in absolute ethanol.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Androgenic hormone.

SEED-UNDER-LEAF

Phyllanthus niruri herbae

Phyllanthus niruri L. - PHYLLANTHACEAE

The drug consists of the dried aerial parts of *Phyllanthus niruri* and its subspecies, *Phyllanthus niruri* ssp. *niruri* L. and *Phyllanthus niruri* ssp. *lathyroides* (Kunth) G.L. Webster, containing at least 6.5% of total tannins and 0.15% gallic acid.

CHARACTERISTICS

Organoleptic characteristics. Odorless drug; bitter taste at the beginning of chewing, subsequently it becomes pleasant.

MACROSCOPIC DESCRIPTION

Herbaceous plant, glabrous, up to 80 cm height, simple or branched stems, the main ones are thin and without leaves, with lateral filiform branches, bearing leaves. Alternate, distichous, simple, membranous, glabrous, oblong- elliptic leaves, with attenuated apex, sometimes mucronate and with an asymmetric base, plain margin; discolor blades, olive-green adaxial faces and pale-green to pale-gray abaxial face. The leaves, when viewed together, have the appearance of being composed by small legumes. Blades with 0.5 cm to 1.4 cm long and 0.3 cm to 0.6 cm wide. Ribs are visible, not prominent, except midrib on the abaxial surface. Brochidodromous venation. Petiole of 0.05 cm to 0.1 cm length. Interpetiolar, triangular-lanceolate stipule, of 0.1 cm to 0.2 cm long, with long apex, narrowly acute to acicular, with whole base and plain edge. Female flowers up to 0.4 cm in diameter, isolated and axillary in the apical nodes, with five elliptic tepals and a whole fleshy disk; tricarpetate ovarian, trilocular, each bispermic locule, three styli, bifid, globose stigmas; pedicel with 0.1 cm to 0.4 cm in length. Male flowers up to 0.3 cm in diameter, in fascicles of one to two flowers, arranged in basal nodes, with five broad-ovate tepals, pentalobed disk, fleshy papillose lobes, and three stamens with connate fillets on the base; pedicels with about 0.2 cm in length. Schizocarpic fruits, tricola-type with 0.1 cm to 0.25 cm in diameter, globose-depression, exposed to the abaxial region of branches, separating into carpidium (Cokes); olive-green membranous exocarp and warty endocarp; two seeds per locule, triangular with two straight ventral faces and dorsal rounded face, warty, prominent warts, with acute to rounded Apex; cylindrical pedicels, about 0.4 cm to 0.5 cm in length at maturity; persistent membranous developed calyx, reaching 2/3 of the height of the fruit. The macroscopic characteristics of the two species, *Phyllanthus niruri* and *Phyllanthus tenellus* are crucial to distinguish them, since they are very similar in terms of anatomical characteristics for some vegetative organs.

MICROSCOPIC DESCRIPTION

In cross section, the secondary developing stem has uniseriate epidermis, with stomata. Subepidermally, one or more

layers of collenchymatic cells with angular thickening interrupted only in the regions of substomatic chambers. Chlorenchyma formed by two to four layers of isodiametric cells with intercellular spaces, evident or not and with starch grains. More internally, there are one or more layers of cortical parenchyma, whose cells have greater axis in the periclinal direction. The phloem externally consists by groups of fibers with very thick walls and small lumen. Druses of calcium oxalate are present in chlorenchyma, cortical parenchyma, medullary parenchyma and in greater abundance in phloem, always in cells larger than the others, covering almost the entire cell volume. Vessel elements with radial disposition are alternated with one or more rows of xylem fibers and sclerified parenchyma cells. The medullary parenchyma consists of isodiametric cells, with large volume, with large intercellular spaces. In stems of larger diameter, periderm may occur, followed by two or more chlorenchyma layers with large amount of starch grains. The cortical parenchyma retains the same characteristics found in younger stems. The phloem has small amount of starch grains, and no significant differences in terms of development, comparing to young stems and more developed stems are observed. The biggest development of the xylem, compared to the younger stems, is very evident, with consequent reduction of the area occupied by the medullary parenchyma. In the parenchyma beam, there is the presence of starch grains and phenolic compounds. The leaf is hypostomatic. Stomata are rarely found also in the adaxial face, where they occur on the veins of lower order. The stomata are of paracytic-type and, less often, anomocytic. In front view, the epidermal cells of the adaxial face show irregular contours and wavy walls, and the wave may be more expressive in this face than that on the abaxial face. The epicuticular waxes have granulations. The cuticle is thin, making it thicker in the midrib. The epidermis is uniseriate on both sides and has fundamental flat cells and some papillary cells. The fundamental cells of the adaxial face are larger than those of the abaxial face. Crystals occur in this cell layer. The mesophyll symmetry is dorsiventral. The palisade parenchyma is unistratified occupying about 2/3 the thickness of the mesophyll, presenting crystal idioblasts drusen-type, mostly. Small crystals of different shapes are common and rhombohedral crystals are rarely found. The spongy parenchyma consists of two or three layers of cells with irregular contour whose greatest length corresponds to the periclinal direction. Small crystals aggregate and/or isolate are common. In paradermic section, it is evident the brachiform character of these cells. At the midrib, the palisade parenchyma may be continuously distributed or interrupted by a small number of chlorenchymatic cells or even by chlorenchymatic isodiametric cells. In this region, near the abaxial epidermis, a layer of angular collenchyma, with slightly thick-walled occurs, and it may contain chloroplasts, followed of chlorenchymatic tissue of isodiametric cells with few chloroplasts. The vascular system is of the collateral type and is composed of two to six beams. The phloem has small number of cells. The venation pattern is brochidodromous.

q

MICROSCOPIC DESCRIPTION OF THE POWDER

The powder meets all the requirements for the species, unless the macroscopic characters. The characteristics are: presence of globular-depression fruits, isolated carpidium (cokes) and seeds as described; flowers as described; drusen crystals of calcium oxalate; fragments of epidermal tissue as described; fragments of stem and leaf tissues as described; fragments of vascular tissue with vessel elements showing ringed, spiral and pitting thickening and fibers.

MICROSCOPIC DESCRIPTION OF IMPURITIES

The root in secondary growth has periderm formed by two to four suberized layers, phellogen and phelloderm. Below this tissue there is the cortical parenchyma, composed of three to six cell layers. Phloem shows fibers peripherally arranged. The xylem has two to four rows of fibers radially arranged. The parenchyma beams are rich in starch grains. The medullary region is often occupied by xylem tissue, or more rarely by parenchyma. Crystals are common in all tissues, being more abundant in the cortical and medullary parenchyma; they are commonly small, isolated and/or aggregate, occurring under different types, generally under drusen-type.

To develop the chromatogram. Allow the plate to dry in an oven at a temperature between 100°C and 105°C, still warm, nebulize with 1% aminoethanol diphenyl borate SR (w/v) in methanol, followed by a solution of 5% macrogol 400 (w/v) in methanol. Allow the plate to air dry for 30 minutes and examine it with under ultraviolet light (365 nm). The chromatogram obtained from *Solution (1)* should present in the lower third of the plate a fluorescence greenish-yellow stain corresponding to vitexin-2-rhamnoside and immediately below it, a fluorescent yellow stain.

B. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF₂₅₄ silica gel with a thickness of 250 µm as support and a mixture of hexane and ethyl acetate (70:30) as a mobile phase. To separately apply to band-shaped plate, 25 µL of *Solution (1)* and 5 µL of *Solution (2)*, recently prepared as described below:

Solution (1): to transfer about 1 g of the milled drug to a round bottom flask, add 10 mL of methanol and heat under reflux for 30 minutes in a water bath. To cool down at room temperature and filter under reduced pressure. To re-extract with more 10 mL of methanol for 30 minutes. To filter the extract by washing the residue with 5 mL of methanol grouping it to the first. To top up with 25 mL with methanol and filter through a membrane of 0.45 µm.

Solution (2): to separately dissolve 10 mg of phyllanthin SQR and niranthy SQR in 2 mL of methanol.

To develop the chromatogram. To remove the plate, allow it to air dry and examine it under ultraviolet light (254 nm). To nebulize the plate with anisaldehyde solution, sulfuric acid and acetic acid (1:2:97) followed by heating in an oven. The chromatogram obtained from *Solution (1)* must

not show the stains corresponding to phyllanthin and niranthy obtained from *Solution (2)*.

IDENTIFICATION

A. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF₂₅₄ silica gel chromoplate with a thickness of 250 µm as support and a mixture of ethyl acetate, formic acid, acetic acid and water (100:11:11:26) as a mobile phase. To separately apply to band-shaped plate, 25 µL of *Solution (1)* and 5 µL of *Solution (2)*, recently prepared as described below:

Solution (1): to transfer about 1 g of the milled drug to a round bottom flask, add 10 mL of methanol and heat under reflux for 30 minutes in a water bath. To cool down at room temperature and filter under reduced pressure. To re-extract with more 10 mL of methanol for 30 minutes. To filter the extract by washing the residue with 5 mL of methanol grouping it to the first. To top up with 25 mL with methanol and filter through a membrane of 0.45 µm.

Solution (2): to dissolve about 10 mg of vitexin-2-rhamnoside SQR in 2 mL of methanol.

PURITY TESTS

Foreign matter (5.4.2.2). At most 2.0%, corresponding to the roots.

Water (5.4.2.3). At most 10.0%.

Total ashes (5.4.2.4). At most 6.0%.

DOSAGE

Total tannins

To prepare the solutions as described below.

Stock solution: to accurately weigh about 0.75 g of the milled drug, transfer to a round bottom flask and add 150 mL of water. To heat in water bath, under reflux, for 30 minutes at room temperature between 80°C and 90°C. To cool down with running water, transfer the mixture to 250 mL volumetric flask and top up with water. To decant the sediment and filter, discarding the first 50 mL of the filtrate.

Sample solution for total polyphenols: to transfer 5 mL of the *Stock solution* to 25 mL volumetric flask and top up with distilled water. To add to 5 mL of this solution, 2 mL of Folin-Denis reagent and dilute it to 50 mL with sodium carbonate SR. To measure the absorbance of the solution (A_λ) at 715 nm (5.2.14) 3 minutes after adding the last reagent, by using water to adjust zero.

Sample solution for polyphenols not adsorbed by hide powder: to add 0.2 g of hide powder SQR to 20 mL of *Stock solution* and mechanically stir for 60 minutes. To filter. To dilute 5 mL of this solution in 25 mL of water. To add to 5

mL of this solution, 2 mL of Folin-Denis reagent and dilute it to 50 mL with sodium carbonate SR. To measure the absorbance of the solution (A_2) at 715 nm (5.2.14) 3 minutes after adding the last reagent, by using water to adjust zero.

Standard solution: to dissolve 50 mg of pyrogallol in water and dilute 100 mL with the same solvent. To dilute 5 mL of this solution in 100 mL of water. To add 2 mL of Folin-Denis reagent to 5 mL of this solution and dilute it to 50 mL with sodium carbonate SR. To measure the absorbance of the solution (A_3) at 715 nm (5.2.14) 3 minutes after adding the last reagent, and within 15 minutes from the dissolution of pyrogallol, by using water as a blank. To calculate the total tannins content according to the equation:

$$TT = \frac{13,12 \times (A_1 - A_2)}{A_3 \times m}$$

where

TT = total tannins;

A_1 = absorbance measured from the *Sample solution* for total polyphenols;

A_2 = absorbance measured from the *Sample solution* for polyphenols not adsorbed by hide powder; A_3 = absorbance measured from the *Standard solution*; m = mass of the vegetable drug considering the determination of water.

Gallic acid

To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 275 nm; pre-column containing hydrophilic reverse phase C-18 and column of 2.1 mm inner diameter, packed with with hydrophilic C-18 (3 µm); flow of the *Mobile Phase* of 0.25 mL/min.

Eluent A: water containing 0.05% of trifluoroacetic acid.

Eluent B: methanol containing 0.05% of trifluoroacetic acid.

Gradient of the mobile phase: adopt the gradient system described in the following table:

Time (minutes)	Eluent A (%)	Eluent B (%)	Elution
0-7	95	5	isocratic
7-25	95-0	5-100	linear gradient

Sample solution: to analytically weigh about 0.75 g of dried and milled drug (800 µm) and transfer to a round bottom flask. To add 10 mL of water, adapt to reflux condenser and heat it in a mantle to boiling for 15 minutes. To cool down with running water and filter the extract under reduced pressure. To wash the residue with water. To volumetrically transfer the filtrate to 250 mL volumetric flask and top up with water. To filter through a membrane of 0.45 µm and inject into the chromatograph.

Standard solution: to dissolve amount accurately weighed of gallic acid SQR in the *Eluent A* to obtain solution at 1 mg/mL.

Solutions for analytical curve: to dilute 1 mL of the *Standard solution* in a 50 mL volumetric flask by topping up with *Eluent A*. To dilute aliquots of 1 mL, 2 mL, 3 mL, 5 mL and 7 mL of this solution by using 10 mL of *Eluent A* obtaining solutions with concentrations of 2.0 µg/mL; 4.0 µg/mL; 6.0 µg/mL; 10.0 µg/mL and 14.0 µg/mL. To filter the solutions through a membrane of 0.45 µm and inject into the chromatograph.

Procedure: to separately inject 5 µL of the *Standard solution* and the *Sample solution* and obtain the areas corresponding to the peak of gallic acid. The relative retention time is about 4.6 minutes for gallic acid. To calculate the content of gallic acid in the sample from the equation of the line obtained from the analytical curve of gallic acid. The result is expressed as the mean of the determinations in grams of gallic acid per 100 grams of the drug considering the determination of water (%).

PACKAGING AND STORAGE

In tightly closed containers, protected from light and heat.

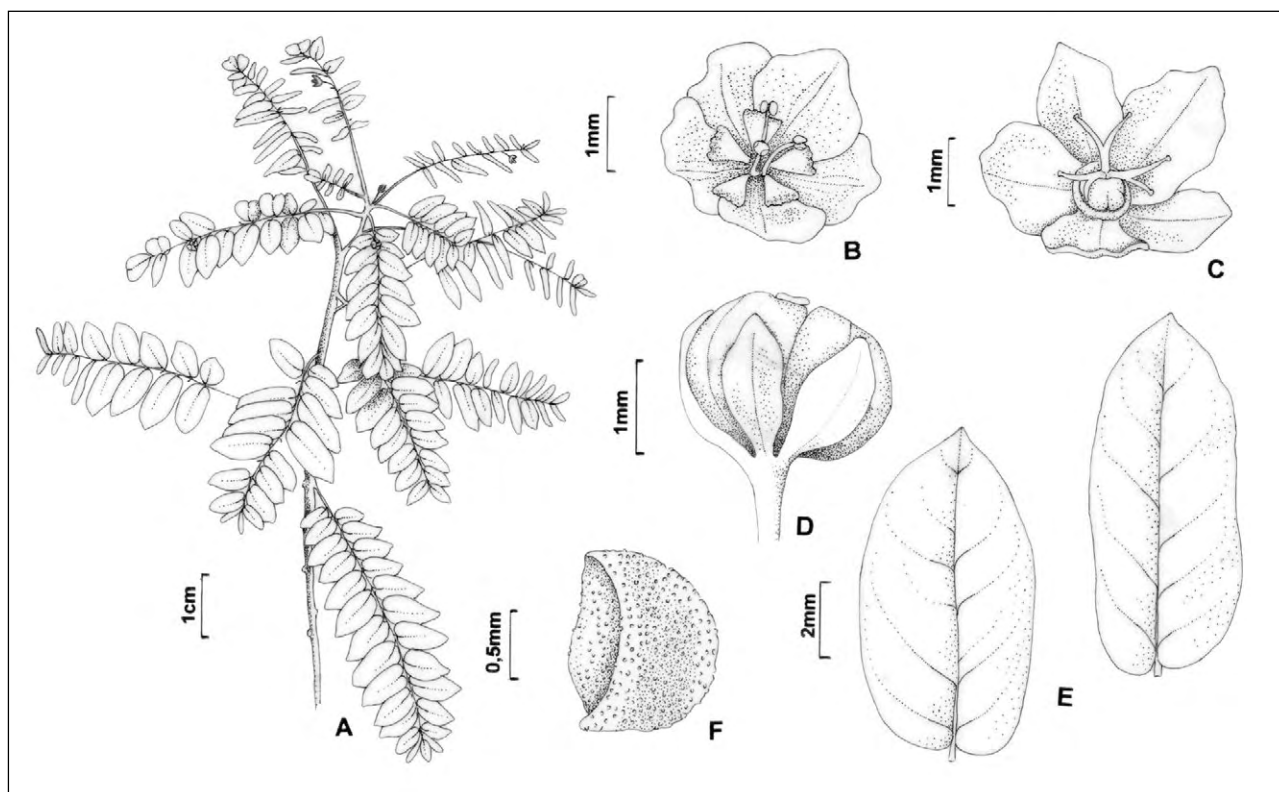


Figure 1a - Macroscopic aspects in *Phyllanthus niruri* L.

Additional information of **Figure 1a**.

A — habit. **B** — male flower with 5 nectaries and three stamens. **C** — female flower with ovary and nectary disc. **D** — fruit, persistent tepals. **E** — leaves with asymmetrical base. **F** — general appearance of the seed.

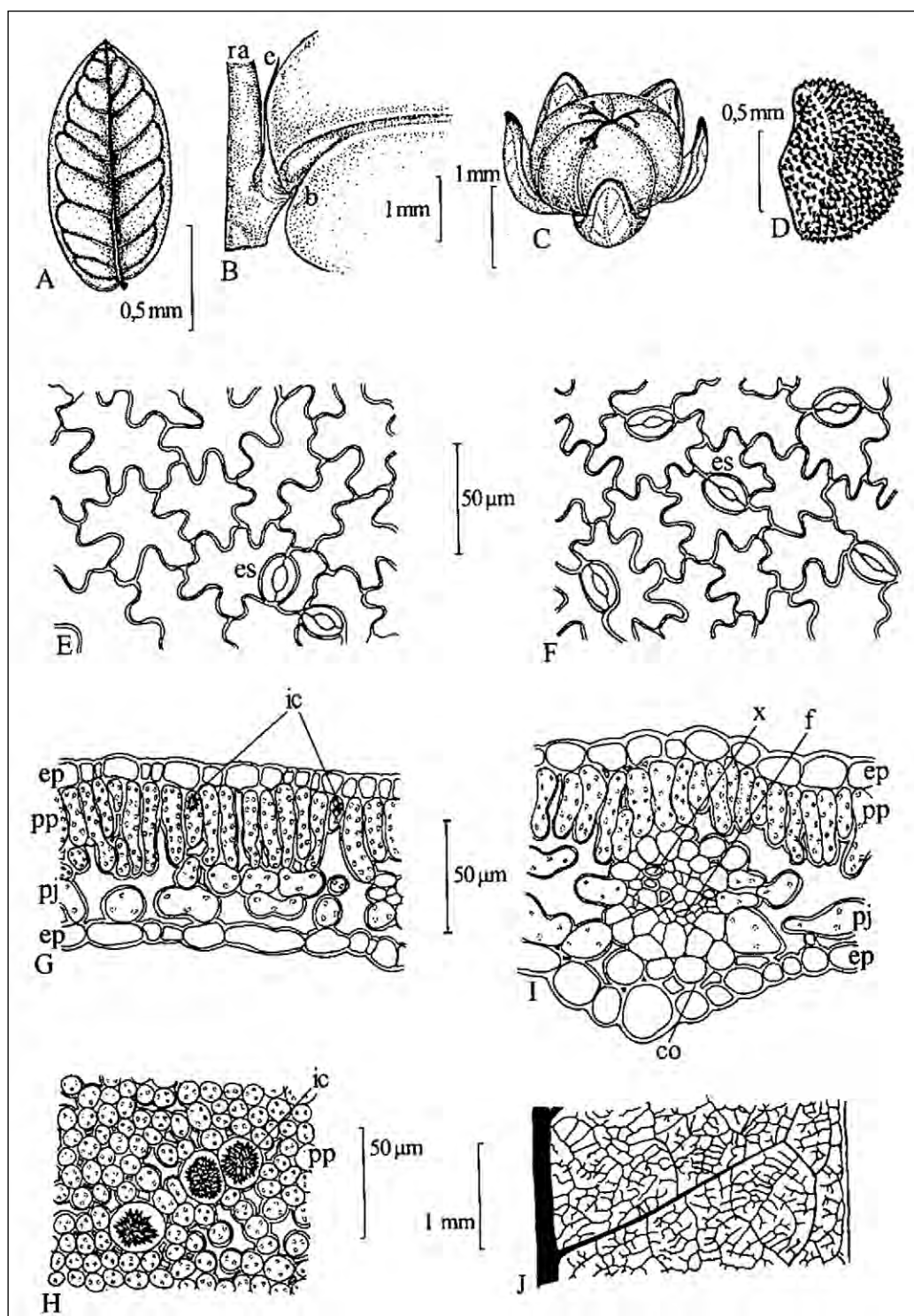


Figure 1b - Macroscopic and microscopic aspects in *Phyllanthus niruri* L.

Additional information of **Figure 1b**. The scales correspond in **A** and **D** to 0.05 cm; **B**, **C** and **J** to 0.1 cm; **E**, **F**, **G**, **H**, and **I** to 50 µm

A — general appearance of the leaf. **B** — general appearance of stipule in the node region: branch (ra); stipule (e); leaf base (b). **C** — general appearance of the fruit. **D** — general appearance of the seed. **E** — front view of the epidermis of the adaxial face: stomata (es). **F** — front view of the epidermis of abaxial face: stomata (es). **G** — leaf blade in the mesophyll region, in cross-section: epidermis (ep); palisade parenchyma (pp); spongy parenchyma (pj); crystal idioblast (ic). **H** — mesophyll region at palisade parenchyma level in paradermic section, showing idioblasts with drusen: crystal idioblast (ic); stomata (es). **I** — leaf blade at midrib in cross section: epidermis (ep); palisade parenchyma (pp); spongy parenchyma (pj); xylem (x); phloem (f); collenchyma (co). **J** — detail of the brochidodromous venation of a segment of the leaf blade in front view.

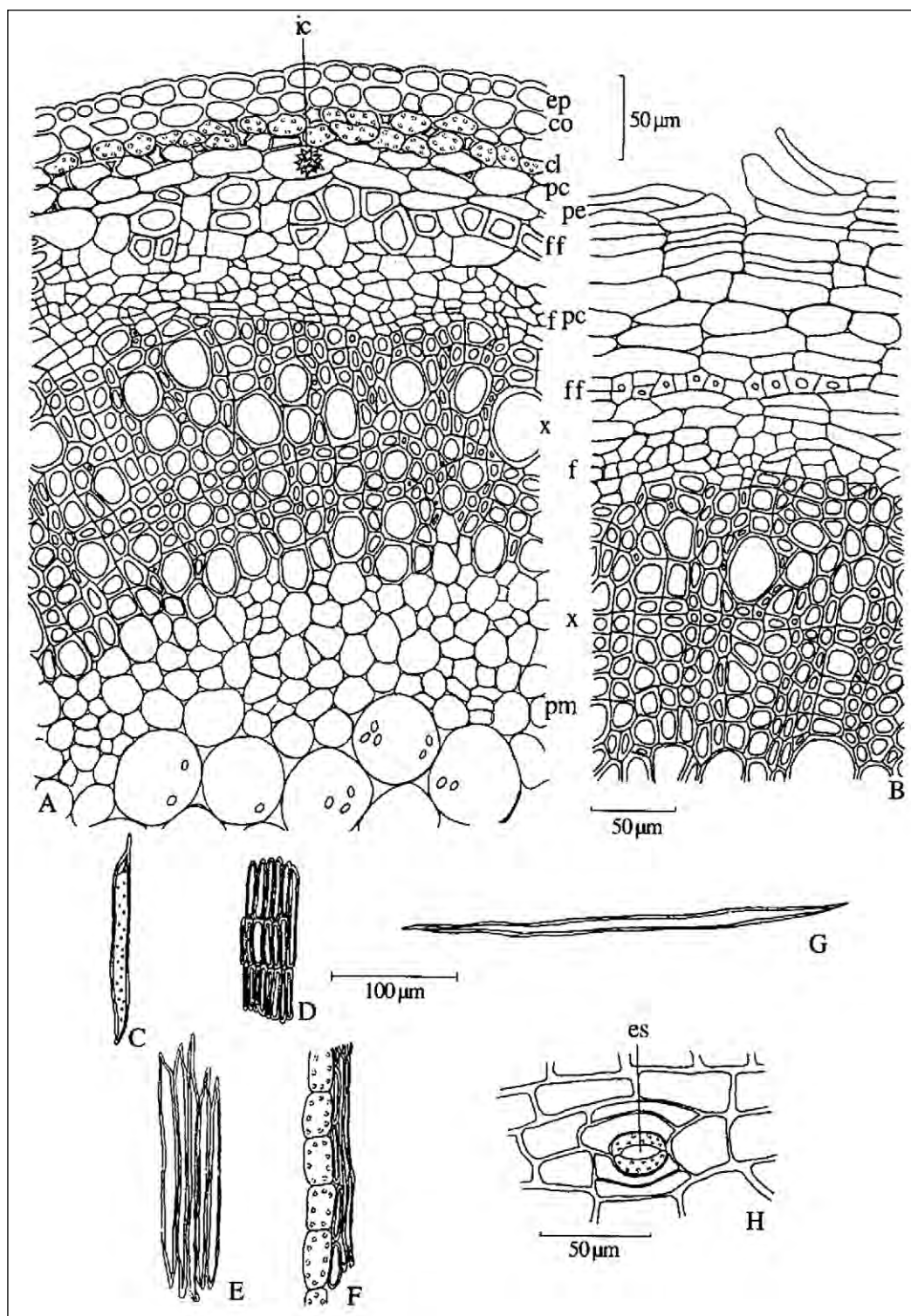


Figure 2 - Macroscopic and microscopic aspects in *Phyllanthus niruri* L.

Additional information of **Figure 2**. The scales correspond to **A**, **B** and **H** to 50 µm; **C**, **D**, **E**, **F** and **G** to 100 µm.

A — detail of stem in cross section: epiderm (ep); collenchyma (co); chlorenchyma (cl); cortical parenchyma (pc); phloem fibers (ff); phloem (f); xylem (x); medullary parenchyma (pm); crystal idioblast (ic). **B** — detail of the root in cross-section: periderm (pe); cortical parenchyma (cp); phloem fibers (ff); phloem (f); xylem (x). **C** — vessel element with pitting thickening in longitudinal view. **D** — sclerified parenchyma cells. **E** — phloem fibers in longitudinal view. **F** — chlorenchyma cells next to the phloem fibers. **G** — fiber in longitudinal view. **H** — detail of a fragment of stem epidermis in front view showing stomata (es).

SEED-UNDER-LEAF

Phyllanthus tenellus herbae

Phyllanthus tenellus Roxb. - PHYLLANTHACEAE

The drug consists of the dried aerial parts containing at least 9.0% of total tannins and 0.12% of gallic acid.

CHARACTERISTICS

Organoleptic characteristics. Odorless drug; bitter taste at the beginning of chewing, subsequently it becomes pleasant.

MACROSCOPIC DESCRIPTION

Herbaceous plant, glabrous, up to 60 cm height, simple or branched stems, the main ones are thin and without leaves, with lateral filiform branches, bearing leaves. Alternate, distichous, simple, membranous, glabrous, oblong- elliptic leaves, with obtuse apex and obtuse to acute base, plain margin; discolor blades, olive-green adaxial faces and pale-green abaxial face. The leaves, when viewed together, have the appearance of being composed by small legumes. Blades with 0.8 cm to 2.5 cm long and 0.5 cm to 1.2 cm wide. Ribs are visible, not prominent, except midrib on the abaxial surface. Brochidodromous venation. Short-cylindrical petiole of up to 0.1 cm in length. Interpetiolar, membranous to scarious, narrow-triangular stipule, with acute apex and whole base, up to 0.15 cm in length. Unisexual flowers, clustered in pauciflorous axillary fascicles, the female flowers are developed first, with pedicels much longer than those of male flowers. In the distal region of the branches it may occur isolated female flowers. Female flowers up to 0.3 cm in diameter, with five oval tepals, with scarious and whitish margin and whole disk; tricarpeolate trilobular ovary, each bispermic locule; three fillets, each bifid on the apical portion; pedicel 0.1 cm to 0.8 cm long. Male flowers of five suborbicular tepals, with scarious margin and pentalobed disk, each reniform lobe; five stamens with fillets free from each other; pedicels 0.05 cm to 0.15 cm long. Schizocarpic fruits, tricola-type with 0.1 cm to 0.2 cm in diameter, globose-depression, exposed to the abaxial region of branches, separating into carpidium (Cokes); olive-green membranous exocarp and warty endocarp; two seeds per locule, triangular with two straight ventral faces and dorsal rounded face, warty, prominent warts, with acute to rounded Apex; cylindrical pedicels, about 0.9 length at maturity; persistent membranous developed calyx, reaching half the height of the fruit. The macroscopic characteristics of the two species, *Phyllanthus tenellus* and *Phyllanthus niruri* are crucial to distinguish them, since they are very similar in terms of anatomical characteristics for some vegetative organs.

MICROSCOPIC DESCRIPTION

In cross section, the secondary developing stem has uniseriate epidermis, with stomata. Subepidermally, one or more layers of collenchymatic cells with angular thickening may have chloroplasts, interrupted only in the regions of sub-

stomatic chambers. Chlorenchyma formed by two to four layers of isodiametric cells with intercellular spaces, evident or not and with starch grains. More internally, there are one or more layers of cortical parenchyma, whose cells have greater axis in the periclinal direction. The phloem consists externally by dense clusters of fibers of very thick walls and reduced lumen, often isolated or grouped with sclereids scattered and clusters interspersed with parenchyma cells. Rhombohedral crystals of calcium oxalate occur in chlorenchyma, cortical parenchyma and phloem which are always present in the larger cells than in the other. Vessel elements with radial disposition are alternated with one or more rows of xylem fibers and sclerified parenchyma cells. The medullary parenchyma consists of isodiametric cells, with large volume, with large intercellular spaces and many rhombohedral and drusen crystals. In stems of larger diameter, periderm may occur, followed by two or more chlorenchyma layers with large amount of starch grains and crystals. The cortical parenchyma retains the same characteristics found in younger stems. The phloem has a small amount of starch grains with a higher degree of development in relation to younger stems and the fibers are peripherally distributed forming a ring. The biggest development of the xylem, compared to the younger stems, is very evident, with consequent reduction of the area occupied by the medullary parenchyma. In parenchyma beams, the starch grains are also present. The leaf is hypostomatic. The stomata are of paracitic-type and, less often, anomocytic. In front view, the epidermal cells facing the adaxial face show irregular contours and wavy walls. The epicuticular waxes have granulations. The cuticle is thin, making it thicker in the midrib. The epidermis is uniseriate on both sides and has fundamental flat cells and some papillary cells. The fundamental cells of the adaxial face are larger than those of the abaxial face. The mesophyll symmetry is dorsiventral. The palisade parenchyma is unistratified occupying about half the thickness of the mesophyll, showing idioblasts with rhombohedral crystals of calcium oxalate. The spongy parenchyma consists of two or three layers of cells with irregular contour whose greatest length corresponds to the periclinal direction. In paradermic section, it is evident the brachiform character of these cells. At the midrib, the palisade parenchyma is interrupted by a small number of cells collenchymatic cells with angular thickening. In adaxial region below the collenchyma one or two layers of chlorophyllous isodiametric cells are observed. In this region, near the abaxial epidermis, a layer of angular collenchyma, with slightly thick-walled occurs, with no

chloroplast, followed by a chlorenchymatic tissue with isodiametric cells with few chloroplasts or a fundamental parenchyma. The vascular system is of the collateral type and is composed of three to six beams. The phloem has small number of cells. The venation pattern is brochidodromous.

MICROSCOPIC DESCRIPTION OF THE POWDER

The powder meets all the requirements for the species, unless the macroscopic characters. The characteristics are: presence of globular-depression fruits, isolated carpidium (cokes) and seeds as described; flowers as described; dru-

sen pyramidal crystals of calcium oxalate; fiber fragments; fragments of epidermal tissue as described; fragments of stem and leaf tissues as described; fragments of vascular tissue with vessel elements showing ringed, spiral and more often pitting thickening and fibers.

MICROSCOPIC DESCRIPTION OF IMPURITIES

The root in secondary growth has periderm formed by two to three suberized layers, phellogen and phelloderm. Below this tissue there is the cortical parenchyma, composed of three to four cell layers. Phloem shows fibers peripherally arranged. The xylem shows fibers between vessel elements or two to four rows of fibers radially arranged beyond parenchyma beams composed of one or two rows of thick-walled cells containing starch granules. The crystals are common in the parenchyma tissue, including the vascular system, and present in different types, singly or grouped together.

B. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel with a thickness of 250 µm as support and a mixture of hexane and ethyl acetate (70:30) as a mobile phase. To separately apply to band-shaped plate, 25 µL of *Solution (1)* and 5 µL of *Solution (2)*, recently prepared as described below:

Solution (1): to transfer about 1 g of the milled drug to a round bottom flask, add 10 mL of methanol and heat under reflux for 30 minutes in a water bath. To cool down at room temperature and filter under reduced pressure. Re-extract with more 10 mL of methanol for 30 minutes. To filter the extract by washing the residue with 5 mL of methanol grouping it to the first. To top up with 25 mL with methanol and filter through a membrane of 0.45 µm.

Solution (2): to separately dissolve 10 mg of phyllanthin SQR and nirantiny SQR in 2 mL of methanol.

To develop the chromatogram. To remove the plate, allow it to air dry. To examine under ultraviolet light (254 nm). To nebulize with a mixture of anisaldehyde solution, sulfuric acid and acetic acid (1:2:97) followed by heating in an oven. The chromatogram obtained from *Solution (1)* must not show the stains corresponding to phyllanthin and nirantiny obtained from *Solution (2)*.

IDENTIFICATION

A. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel chromoplate with a thickness of 250 µm as support and a mixture of ethyl acetate, formic acid, acetic acid and water (100:11:11:26) as a mobile phase. To separately apply to band-shaped plate, 25 µL of *Solution (1)* and 5 µL of *Solution (2)*, recently prepared as described below:

Solution (1): to transfer about 1 g of the milled drug to a round bottom flask, add 10 mL of methanol and heat under reflux for 30 minutes in a water bath. To cool down at room temperature and filter under reduced pressure. Re-extract

with more 10 mL of methanol for 30 minutes. To filter the extract by washing the residue with 5 mL of methanol grouping it to the first. To top up with 25 mL with methanol and filter through a membrane of 0.45 µm.

Solution (2): to dissolve about 10 mg of rutin in 2 mL of methanol.

To develop the chromatogram. To allow the plate to dry in an oven at a temperature between 100°C and 105°C, still warm, nebulize with aminoethanol diphenyl borate SR, followed by a solution of 5% macrogol 400 (w/v) in methanol. Allow the plate to air dry for 30 minutes and examine it under ultraviolet light (365 nm). The chromatogram obtained with *Solution (1)* should present in the upper third of the plate of a fluorescence yellow-green stain. In the lower third of the plate, the species may exhibit rutin traces, characterized by a fluorescent orange stain corresponding in position to the stain obtained with the rutin pattern obtained from *Solution (2)*.

PURITY TESTS

Foreign matter (5.4.2.2). At most 2.0%, corresponding to the roots.

Water (5.4.2.3). At most 9.5%.

Total ashes (5.4.2.4). At most 8.0%.

DOSAGE

Total tannins

To prepare the solutions as described below.

Stock solution: to accurately weigh about 0.75 g of the milled drug, transfer to a round bottom flask and add 150 mL of water. To heat in water bath, under reflux, for 30 minutes at room temperature between 80°C and 90°C. To cool down with running water, transfer the mixture to 250 mL volumetric flask and top up with water. To decant the sediment and filter, discarding the first 50 mL of the filtrate.

Sample solution for total polyphenols: to transfer 5 mL of the *Stock solution* to 25 mL volumetric flask and top up with distilled water. To add to 5 mL of this solution, 2 mL of Folin-Denis reagent and dilute it to 50 mL with sodium carbonate SR. To measure the absorbance of the solution (A₁) at 715 nm (5.2.14) 3 minutes after adding the last reagent, by using water to adjust zero.

Sample solution for polyphenols not adsorbed by hide powder: to add 0.2 g of hide powder SQR to 20 mL of *Stock solution* and mechanically stir for 60 minutes. To filter. To dilute 5 mL of this solution in 25 mL of water. To add to 5 mL of this solution, 2 mL of Folin-Denis reagent and dilute it to 50 mL with sodium carbonate SR. To measure the absorbance of the solution (A₂) at 715 nm (5.2.14) 3 minutes after adding the last reagent, by using water to adjust zero.

Standard solution: To dissolve 50 mg of pyrogallol in water and dilute 100 mL with the same solvent. To dilute 5 mL of this solution in 100 mL of water. To add to 5 mL of this solution, 2 mL of Folin-Denis reagent and dilute it to 50 mL with sodium carbonate SR. To measure the absorbance of the solution (A_3) at 715 nm (5.2.14) 3 minutes after adding the last reagent, and within 15 minutes from the dissolution of pyrogallol, by using water as a blank. To calculate the total tannins content according to the equation:

$$TT = \frac{13,12 \times (A_1 - A_2)}{A_3 \times m}$$

where

TT = total tannins;

A_1 = absorbance measured from the Sample solution for total polyphenols;

A_2 = absorbance measured from the Sample solution for polyphenols not adsorbed by hide powder;

A_3 = absorbance measured from the *Standard solution*;

m = mass of the vegetable drug considering the determination of water.

Gallic acid

To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 275 nm; pre-column containing hydrophilic reverse phase C-18 and column of 10 cm long and 2.1 mm inner diameter, packed with hydrophilic C-18 (3 μ m); flow of the *Mobile Phase* of 0.25 mL/minute.

Eluent A: water containing 0.05% of trifluoroacetic acid.

Eluent B: methanol containing 0.05% of trifluoroacetic acid.

Gradient of the mobile phase: adopt the gradient system described in the following table:

Time (minutes)	Eluent A (%)	Eluent B (%)	Elution
0 - 8	100	0	isocratic
8 - 15	100 ^ 50	0 ^ 50	linear gradient
15 - 20	50	50	isocratic

Sample solution: to analytically weigh about 0.75 g of dried and milled drug (800 μ m) and transfer to a round bottom flask. To add 30 mL of water, adapt to reflux condenser and heat it in a mantle to boiling for 15 minutes under magnetic stirring. To cool down with running water and filter the extract under reduced pressure. To wash the residue with water. To volumetrically transfer the filtrate to 250 mL volumetric flask and top up with water. To filter through a membrane of 0.45 μ m and inject into the chromatograph.

Standard solution: to dissolve amount accurately weighed of gallic acid SQR in the Eluent A to obtain solution at 400 mg/mL.

Solution for analytical curve: to dilute 1 mL of the *Standard solution* in a 25 mL volumetric flask by topping up with *Eluent A*. To dilute aliquots of 2 mL, and 5 mL of this solution by using 25 mL of *Eluent A* obtaining solutions with concentrations of 2.6 μ g/mL and 6.4 μ g/mL. To additionally dilute aliquots of 3 mL, 5 mL and 7 mL to 10 mL by using *Eluent A*, obtaining solutions with concentrations of 9.6 μ g/mL, 16.0 μ g/mL and 22.4 μ g/mL. To use the five prepared solutions (2.6 μ g/mL; 6.4 μ g/mL, 9.6 μ g/mL, 16.0 μ g/mL and 22.4 μ g/mL) in the construction of the analytical curve, after filtration through membrane of 0.45 μ m and injection into the chromatograph.

Procedure: To separately inject 5 μ L of the *Standard solution* and the *Sample solution* and obtain the areas corresponding to the peak of gallic acid. The relative retention time is about 5.3 minutes for gallic acid. To calculate the content of gallic acid in the sample from the equation of the line obtained from the analytical curve of gallic acid. The result is expressed as the mean of the determinations in grams of gallic acid per 100 grams of the drug considering the determination of water (%).

PACKAGING AND STORAGE

In tightly closed containers, protected from light and heat.

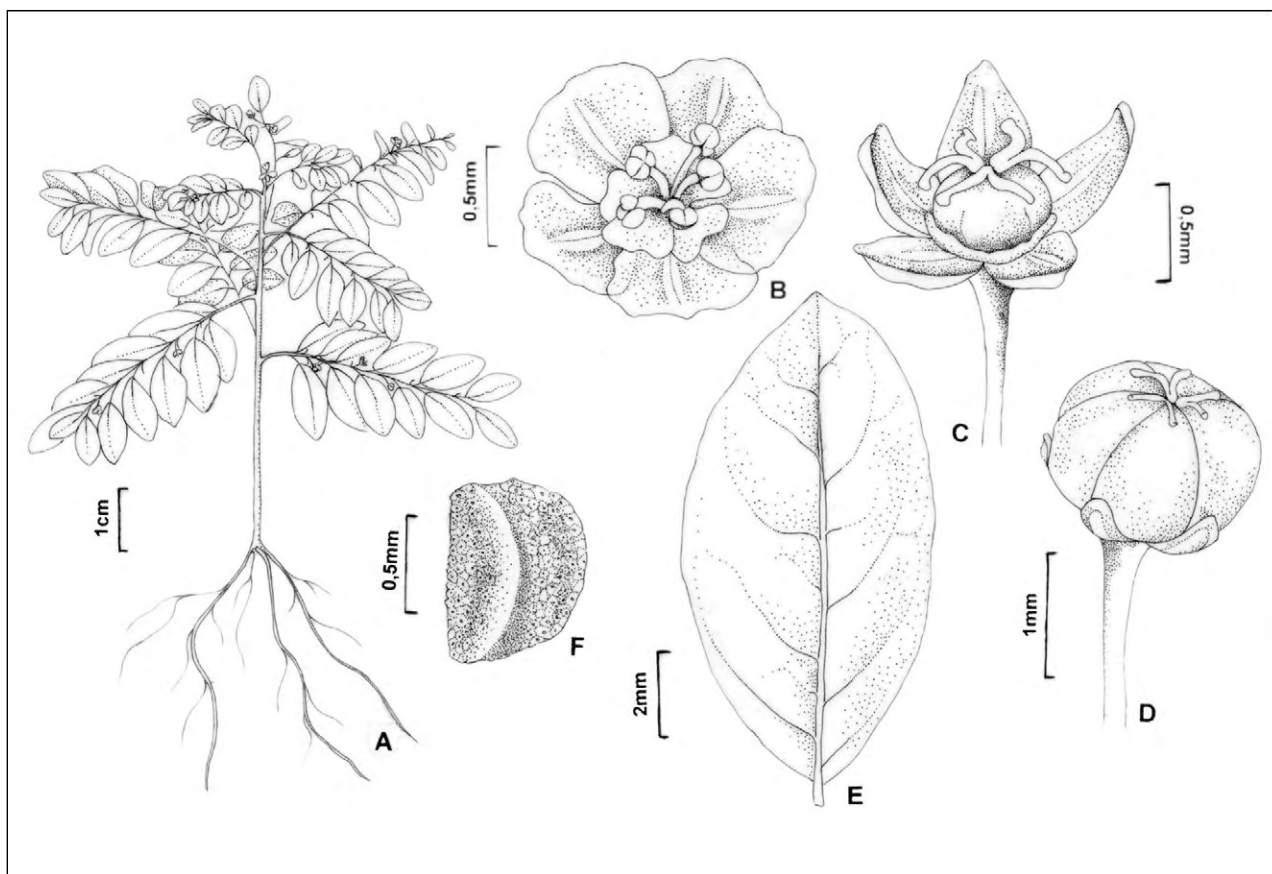


Figure 1a - Macroscopic aspects in *Phyllanthus tenellus* Roxb.

Additional information of **Figure 1a**.

A — habit. **B** — male flower with five nectaries and five stamens. **C** — female flower with ovary and nectary disc. **D** — fruit, persistent tepals. **E** — symmetrical base leaf. **F** — general appearance of the seed.

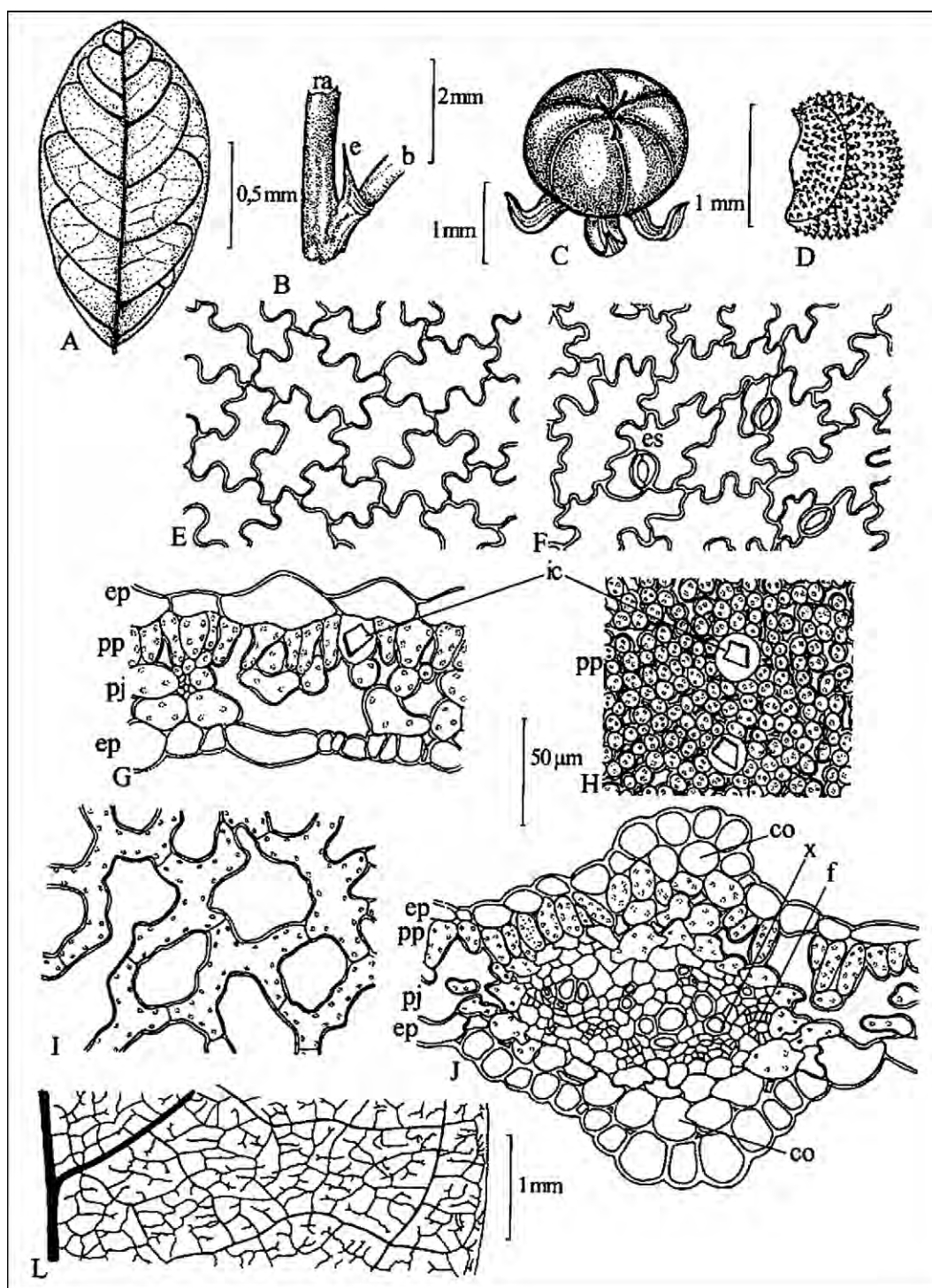


Figure 1b - Macroscopic and microscopic aspects in *Phyllanthus tenellus* Roxb.

Additional information of **Figure 1b**. The scales correspond to A to 0.5 mm; B to 2 mm; C, D and L to 1 mm; E, F, G, H, I and J to 50 µm.

A — general appearance of the leaf. B — detail of stipule in the region of the node: branch (ra); stipule (e); leaf base (b). C — general appearance of the fruit. D — general appearance of the seed. E — front view of the epidermis of the adaxial face: F — front view of the epidermis of abaxial face: stomata (es). G — leaf blade in the mesophyll region, in cross-section: epidermis (ep); palisade parenchyma (pp); spongy parenchyma (pj); crystal idioblast (ic). H — mesophyll region at palisade parenchyma level in paradermic section, showing crystal idioblasts: crystal idioblast (ic); palisade parenchyma (pp). I — the mesophyll region at the level of spongy parenchyma in paradermic section, showing brachiform cells. J — leaf blade at midrib in cross section: epidermis (ep); palisade parenchyma (pp); spongy parenchyma (pj); collenchyma (co); xylem (x); phloem (f). L — detail of the brochidodromous venation of a segment of the leaf blade in front view.

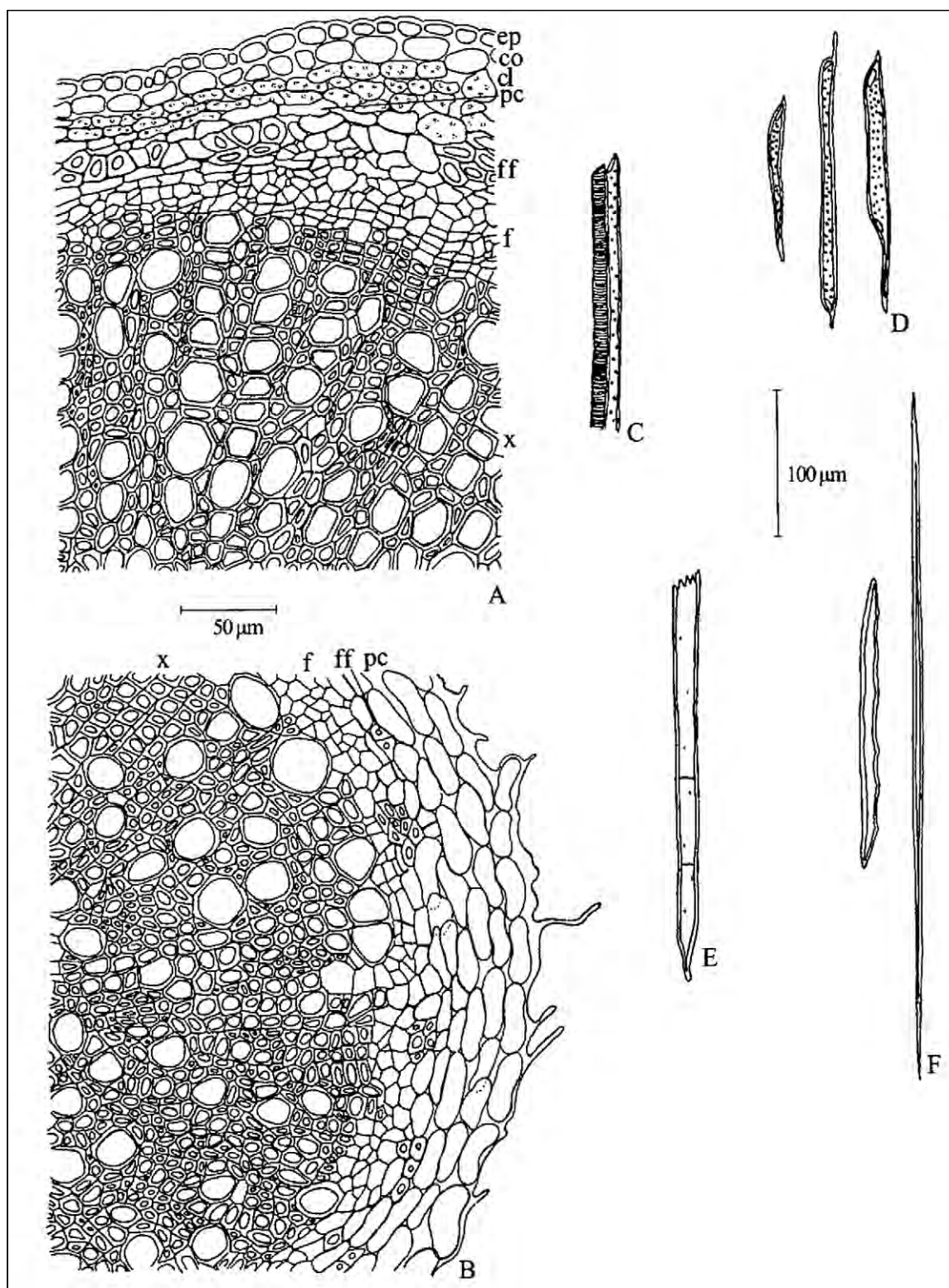


Figure 2 - Macroscopic and microscopic aspects in *Phyllanthus tenellus* Roxb.

Additional information of **Figure 2**. The scales correspond to **A** and **B** to 50 µm; **C**, **D**, **E** and **F** to 50 µm

A — detail of stem in cross section: epiderm (ep); angular collenchyma (co); chlorenchyma (cl); cortical parenchyma (pc); phloem fibers (ff); phloem (f); xylem (x). **B** — detail of the root in cross-section: xylem (x), phloem (f); phloem fibers (ff); cortical parenchyma (pc). **C** — vessel elements with helical pitting thickening in longitudinal view. **D** — vessel elements with pitting thickening in longitudinal view. **E** - partial view of a septate fiber. **F** — fiber in longitudinal view.

QUILLAY

Quillaiae cortex

Quillaja saponaria Mol. - QUILLAJACEAE

The drug consists of barks of branches without periderm, desiccated and fragmented.

CHARACTERISTICS

Organoleptic characteristics. The drug is virtually odorless, it causes sneezing effect and has a sour to astringent taste.

MACROSCOPIC DESCRIPTION

The drug is marketed in flat or slightly curved parts forming plaques of approximately 1 cm long, 10 cm wide and 1 mm to 5 mm thick. The outer surface has a whitish color, with small brown stains, usually plain or finely striated longitudinally. Parts of rhytidome with brownish to dark-brown color are often found attached to this bark. The inner surface is yellowish-white and plain. The fracture is plain to slightly fibrous. In cross section, the fracture presents a structure regularly checkered by tangential dark bands and light radial lines.

MICROSCOPIC DESCRIPTION

The liber constitutes by itself the thickness of the commercial barks and characteristically exhibits a grid aspect, which is due to the successive intersection of parenchyma beams with parenchyma zones, alternating with fiber bundles. Throughout this region, the cells are juxtaposed, characterizing intercellular spaces of meatus type. The parenchyma beams contain two to six layers of cells of 60 µm to 100 µm long by approximately 20 µm wide. The liberian fibers are tortuous and often are accompanied by small groups of sclereids. The parenchyma contains cells of 20 µm to 40 µm long and 60 µm to 200 µm, typically 90 µm wide. It has numerous mucilage cells, starch cells with starch grains of 5 µm to 20 µm in diameter and many cells containing single crystals of calcium oxalate which may reach 50 µm to 170 µm long and up to 30 µm wide.

MICROSCOPIC DESCRIPTION OF THE POWDER

The powder meets all the requirements for the species, unless the macroscopic characters. The characteristics are: very fine powder with straw-yellow color, with fragments of the structures described above; sclerenchyma fibers; lot of prismatic

crystals of calcium oxalate in parenchyma fragments or free; parenchyma portions with starch grains; some stone

cells elongated with oblique pores; sieve tubes fragments; occasionally suberosos fragments.

IDENTIFICATION

To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel with a thickness of 250 µm as support and a mixture of chloroform, ethanol and water (30:40:5) as a mobile phase. To separately apply to band-shaped plate, 15 µL to 20 µL of *Solution (1)* and 5 µL to 10 µL of *Solution (2)*, prepared as described below:

Solution (1): 1 g of pulverized drug, add 20 ml of 50% ethanol (v/v) and stir for 20 minutes. To filter and concentrate the filtrate to dryness in water bath at temperature not exceeding 50°C. To dissolve the residue in 5 ml of methanol.

Solution (2): to weigh 0.1 g of purified saponin SQR and dissolve in 5 mL of methanol to obtain 2.0% solution. To filter.

To develop the chromatogram. To remove the plate, allow it to air dry for 15 minutes. To nebulize the plate with anisaldehyde SR and put it in an oven between 100°C to 105°C for 5 minutes. To view it under visible light. Quillay saponins have greenish blue stain with sequential R_f around 0.23 and 0.33.

PURITY TESTS

Water (5.4.2.3). At most 8.0%.

Total ashes (5.4.2.4). At most 6.0%.

Ashes insoluble in acid (5.4.2.5). At most 1.0%.

Foam index (5.4.2.10). To weigh 0.1 g of quillay powder and add 100 mL of distilled water. To boil for 5 minutes. To filtrate and top up with 100 mL of distilled water. At least 1000.

Substances extractable by alcohol (5.4.2.11). To macerate 5 g of the powdered drug in 100 ml of 45% ethanol (v/v) in a tightly closed container for 24 hours while maintaining constant stirring during the first 6 hours and allowed to rest for 18 hours. To filter and top up with 100 mL with 45% ethanol (v/v). To evaporate 20 ml of the filtrate to dryness, weighting bottle previously tared at 105 °C, to constant weight. To calculate the percentage of extractive soluble in ethanol with reference to dry drug. At least 22.0%.

PACKAGING AND STORAGE

In tightly closed container, protected from light and heat.

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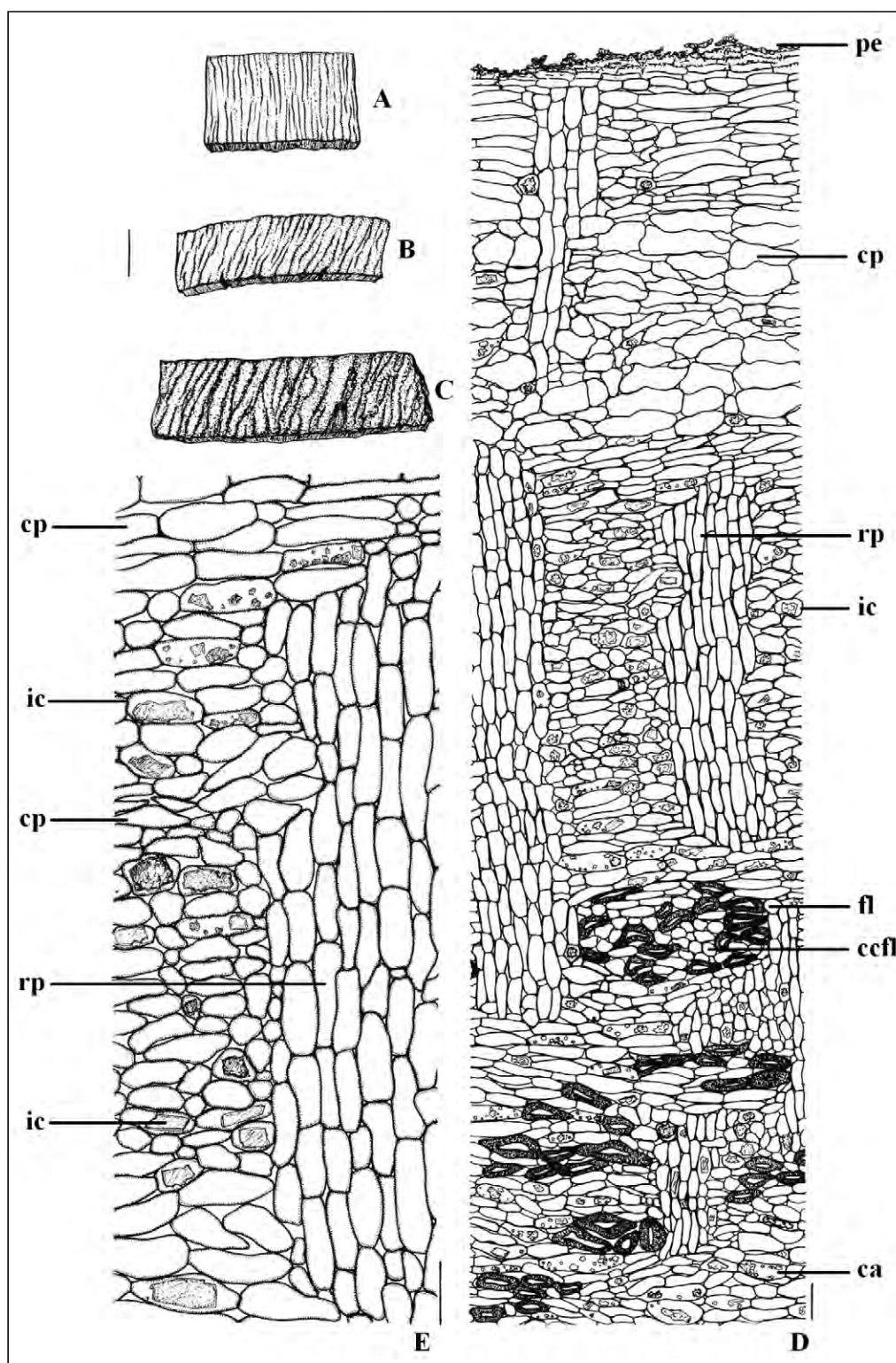


Figure 1 - Macroscopic and microscopic aspects of *Quillaja saponaria* Mol.

Additional information of **Figure 1**. Scales correspond in A to 1 cm; B and C to 50 μ m; D to 150 μ m; E to 50 μ m.

A - general appearance in front view of the stem bark showing the inner face. B and C - general appearance in front view of the stem bark showing the outer face. D - detail of a portion of the stem bark in cross section: starch cell (ca); conducting cells of the phloem (ccfl); parenchyma cell (cp); liberian fiber (fl); crystal idioblast (ic); periderm (pe); parenchyma beam (rp). E - partial detail of the phloem region with parenchyma cells and parenchyma beam showing the intersection between these cells and the presence of crystal idioblasts: parenchyma cell (cp); crystal idioblasts (ic); parenchyma beam (rp).

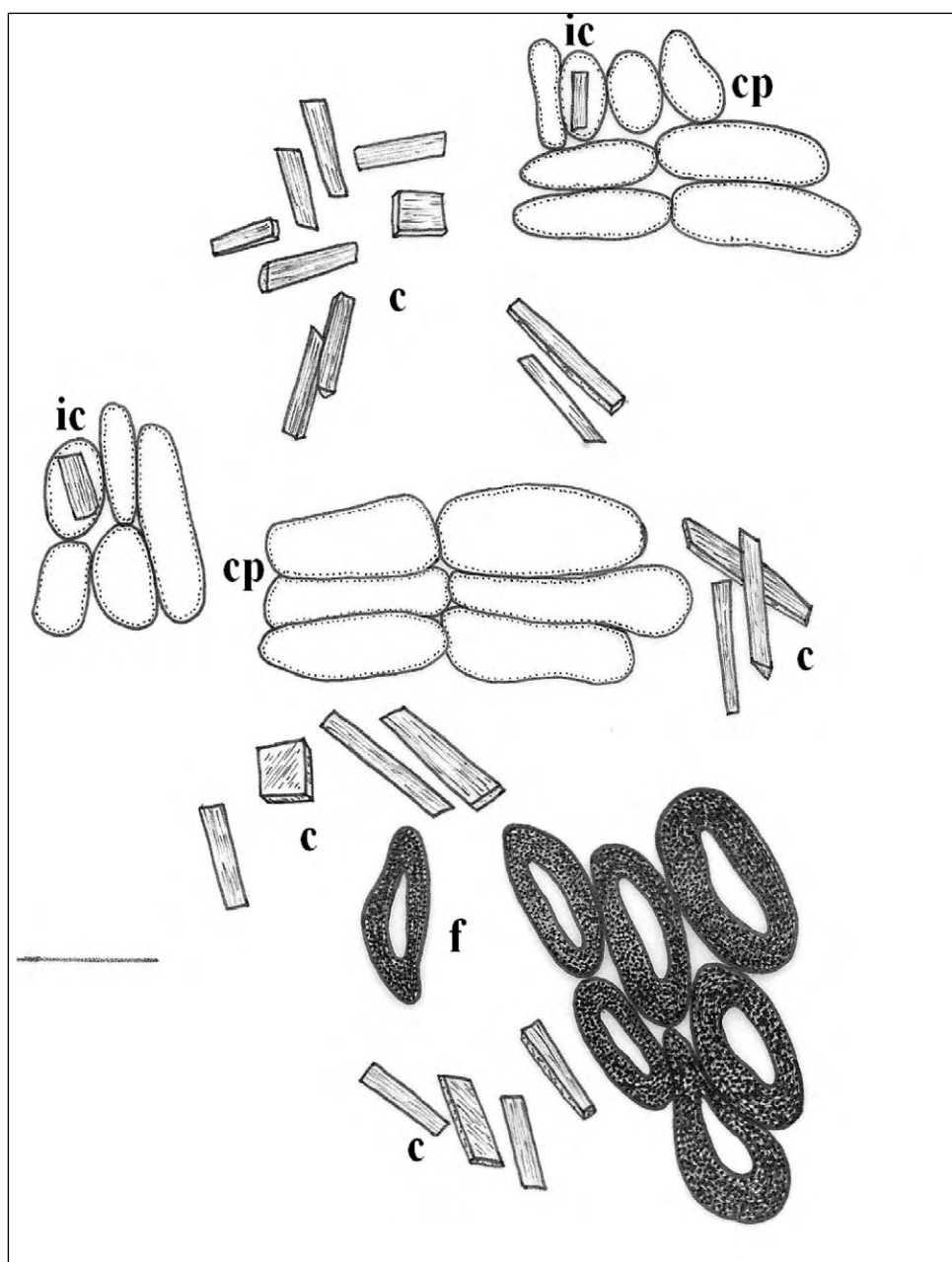


Figure 2 - Microscopic aspects of *Quillaja saponaria* Mol powder.

Additional information of **Figure 2**. Scales correspond to 50µm; **c** - prismatic crystals. **cp** - parenchyma cell. **ic** - crystal idioblasts. **f** – fibers.

QUINA-AMARELA

Cinchonae cortex

Cinchona calisaya Weddell - RUBIACEAE

The drug consists of the barks of the branches of the species and its varieties, containing at least 6.0% of total alkaloids, of which 60% are quinine type.

CHARACTERISTICS

Organoleptic characteristics. The drug has faintly aromatic odor and bitter taste.

MACROSCOPIC DESCRIPTION

The bark is presented in tubes or curved pieces of variable length and width and 3.0 mm to 7.0 mm thick. The outer surface is gray-brown, often with lichens, and presents numerous transverse and longitudinal cracks and sometimes transverse cracks of few millimeters stand out. The inner face is yellow-brown and finely grooved presenting ovoid depressions marked in a form more or less intense. In cross section three distinct regions are highlighted: the outer region is thin and has grayish-brown color, the middle region displays rounded and yellowish stains and inner region is radially furrowed by numerous yellow lines.

MICROSCOPIC DESCRIPTION

The suber, in cross section, consists of approximately 15 cell layers rich with brown-color content that has a regular shape in radial rows. The phelloderm presents numerous layers of regular cells with dark cell walls. The cortical parenchyma consists of thin-walled cells, highlighting idioblasts containing crystalline sand distributed sparsely throughout the cortical parenchyma. Internally and also more sparsely, in the same cortical region, oval cells, which reach a large diameter in relation to other cells occur. The phloem is greatly developed, highlighting narrow sieve tube elements and parenchyma beams that are distributed in a parenchyma developed. The width of the parenchyma beams generally corresponds to three cell rows. The remaining phloematic parenchyma cells contain spherical or plano-convex starch grains singly arranged or in triad. In the phloem fibers that resemble stone cells stand out and they have very thick wall and are clearly striated crossed by infundibuliform pits. Phloematic fibers are radially arranged in isolation form, gathered in small groups or forming short and irregular rows throughout the phloem region.

MICROSCOPIC DESCRIPTION OF THE POWDER

The powder meets all the requirements for the species, unless the macroscopic characters. The characteristics are: larger brown fractures and smaller reddish-brown fractures; fragments of yellowish to reddish-brown suber; fragments of cortical parenchyma containing starch grains and spherical idioblasts with calcium oxalate crystals in the form of crystal sand; phloematic fiber fragments with thick wall, lignified, with infundibuliform pits; fragments

of phloematic parenchyma and parenchyma beams, associated with fibers containing spherical starch grains; big and oval cells; spherical or simple plano-convex starch grains or associations with two or three grains.

IDENTIFICATION

A. Grahe Reaction: To add 500 mg of Quina's yellow pulverized bark in a test tube and directly heat into the flame. To observe the detachment of purple vapors and its condensation on the tube walls. This distillate is soluble in ethanol.

B. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF₂₅₄ silica gel with a thickness of 250 µm as a support and mixture of chloroform and diethylamine (90:10) as a mobile phase. To separately apply, band-shaped, 15 µL to 20 µL of *Solution (1)* and 3 µL to 5 µL of the *Solution (2)*, prepared as described below.

Solution (1): to add 0.1 mL of 25% ammonium hydroxide (w/v) and 5 mL of methylene chloride at 0.1 g of pulverized drug. To allow it to rest for 30 minutes, occasionally stirring. To filter and evaporate the filtrate to dryness in a water bath. To dissolve the residue in 1 mL of absolute ethanol.

Solution (2): to separately dissolve 17.5 mg of quinine, 0.5 mg of quinidine and 10 mg of cinchonine in 5 mL of absolute ethanol.

To develop the chromatogram. To remove plate and allow to dry in an oven at 100°C to 105°C for about 10 minutes. To cool the plate down and nebulize with ethanolic solution of 50% sulfuric acid (w/v). To examine it under ultraviolet light (365 nm). The stains obtained with *Solution (1)* correspond in position, color, and intensity to that obtained with *Solution (2)*. These spots are corresponding to quinine (R_f about 0.15), quinidine (R_f about 0.30) and cinchonine (R_f about 0.45).

PURITY TESTS

Foreign matter (5.4.2.2). At most 2%.

Water (5.4.2.3). At most 8%.

Total ashes (5.4.2.4). At most 12.5%.

DOSAGE

Alkaloids

To proceed as described in the *Ultraviolet absorption spectrophotometry (5.2.14)*. To accurately weigh 1 g of pulverized drug, transfer to 250 mL erlenmeyer flask and add 10 mL of water and 7 mL of 2M hydrochloric acid. To heat in water bath for 30 minutes. To cool down and add 25 mL of methylene chloride, 50 mL of ethyl ether and 5 mL of 20% sodium hydroxide (w/v). To stir the mixture for 30 minutes. To add 3 g of powder tragacanth gum and stir

until the preparation becomes clear. To filter through filter paper, and wash erlenmeyer flask and filter paper with 100 mL of mixture of methylene chloride and ethyl ether (1:2). To gather the washings, evaporate to dryness and dissolve the residue in 10 mL of absolute ethanol. To evaporate 5.0 mL of the solution to dryness. To dissolve the residue in 0.1 M hydrochloric acid, transfer to a volumetric flask and top up with 1000 mL of the same solvent. To prepare two reference solutions by dissolving 30 mg of quinine or 30 mg of cinchonine in 0.1 M hydrochloric acid, by topping up with 100 mL. To measure the absorbance of the three solutions at 316 nm and 348 nm by using 0.1 M hydrochloric acid as a blank. To calculate the percentage of alkaloids of quinine group (x) and alkaloids of cinchonine group (y) by the equations:

$$y = \frac{[A_{316} \times A_{c348}] - [A_{c346} \times A_{348}] \times 100 \times 2}{[A_{q316} \times A_{c348}] - [A_{c316} \times A_{q348}] \times m \times 1000}$$

$$x = \frac{[A_{316} \times A_{q348}] - [A_{q346} \times A_{348}] \times 100 \times 2}{[A_{c316} \times A_{q348}] - [A_{q316} \times A_{c348}] \times m \times 1000}$$

where

m = weight of sample in g;

x = percentage of quinine-type alkaloids;

y = percentage of cinchonine-type alkaloids;

A_{316} = absorbance of sample solution at 316 nm,

A_{348} = absorbance of the sample at 348 nm; A_{q316} = absorbance of the reference solution containing quinine at 316 nm, corrected to concentration of 1 mg in 1000 mL;

A_{q348} = absorbance of the reference solution containing quinine at 348 nm, corrected to concentration of 1 mg in 1000 mL;

A_{c316} = absorbance of the reference solution containing cinchonine at 316 nm, corrected to concentration of 1 mg in 1000 mL;

A_{c348} = absorbance of the reference solution containing cinchonine at 348 nm, corrected to concentration of 1 mg in 1000 mL;

To calculate the content of total alkaloids ($x + y$) and determine the relative content of quinine-type alkaloids, from the following equation: $(100x) + (x + y)$.

PACKAGING AND STORAGE

In tightly closed container made of glass or metal, protected from light and heat.

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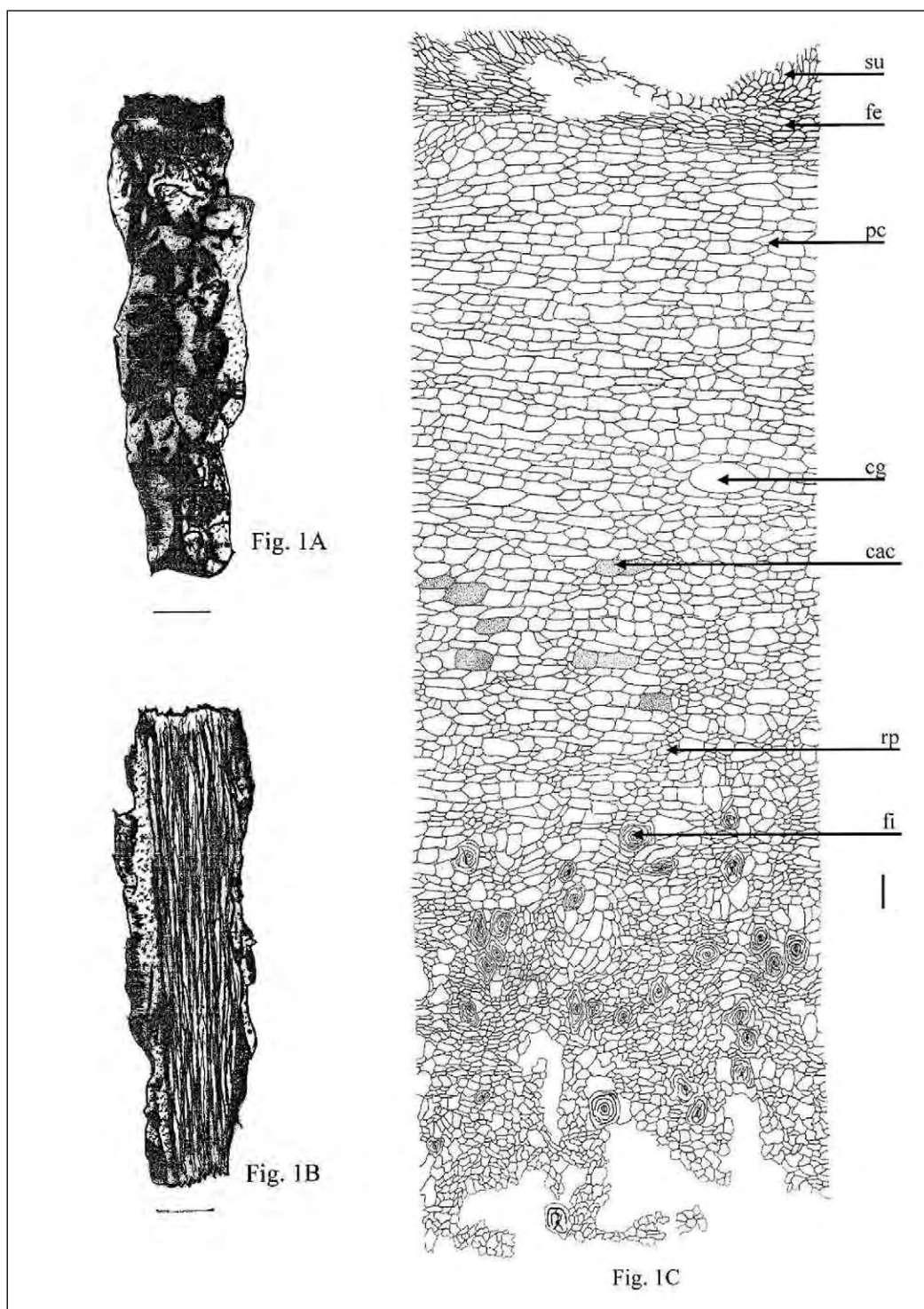


Figure 1 - Macroscopic and microscopic aspects in *Cinchona calisaya* Weddell

Additional information of **Figure 1**. The Scales correspond to: **A** and **B** to 1 cm, **C** to 500 μm .

A - general appearance in front view of the stem bark showing the outer face; **B** - general appearance in front view of the stem bark showing the inner face; **C** - Cross section showing the general appearance of the branches' bark; cac: cells with crystal sand; cg: giant cells, fe: phelloderm; fi: fiber; pc: cortical parenchyma cell; rp: parenchyma beam in the phloematic region; su: suber.

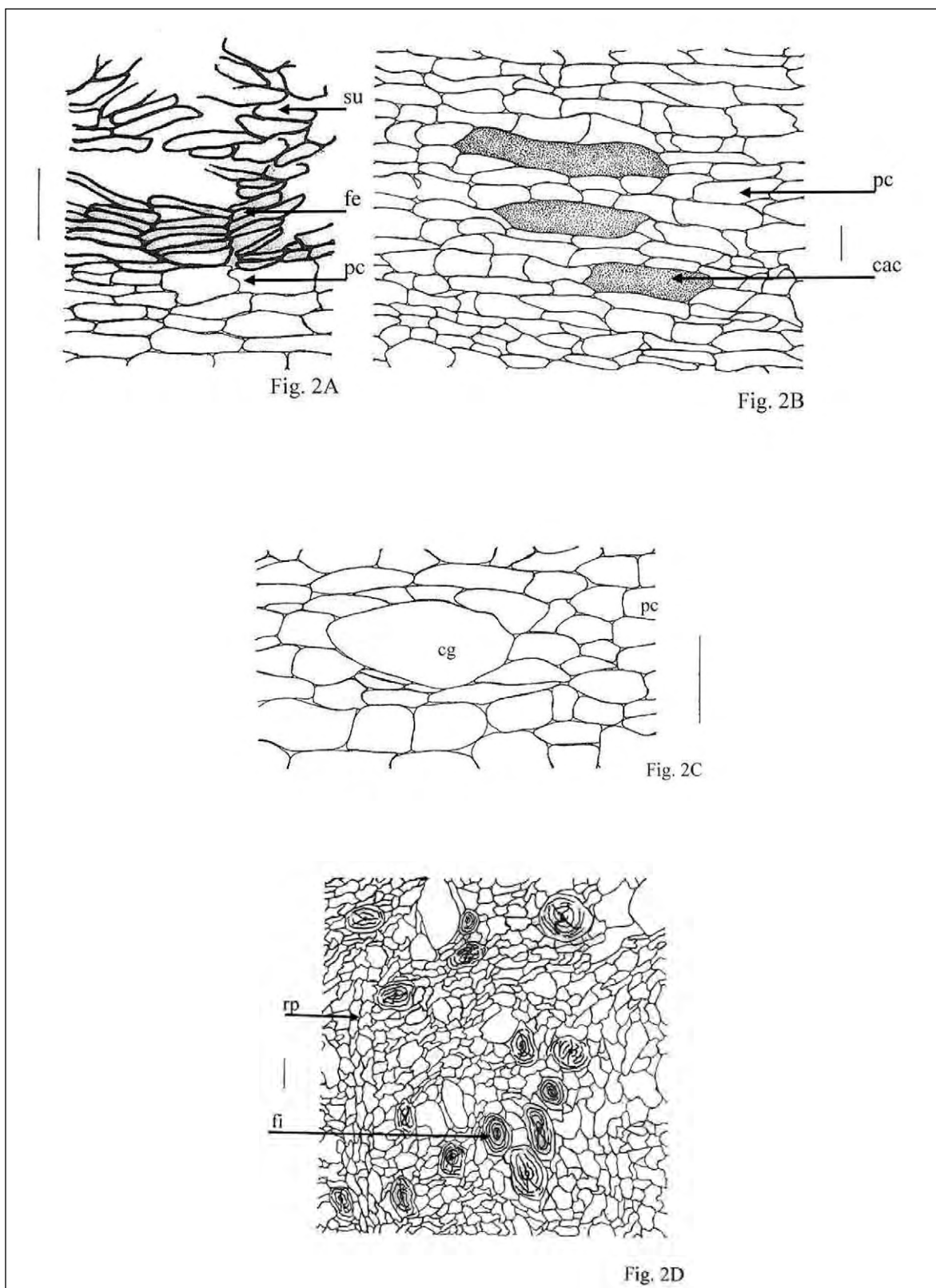


Figure 2 - Macroscopic and microscopic aspects in *Cinchona calisaya* Weddell

Additional information of **Figure 2**. Scales correspond in A and C to 500 μm ; B to 200 μm and D to 350 μm .

A - cross section showing a detail of the outer region of the bark near to lenticels; B - cross section showing a detail at cortical parenchyma level showing cells with crystal sand; C - cross section showing a detail of the cortical parenchyma region with giant cells. D - cross section showing a detail of the phloem region; cac: cells with crystal sand; cg: giant cells, fe: phelloderm; fi: fiber; pc: cortical parenchyma cell; rp: parenchyma beam in the phloem region; su: suber

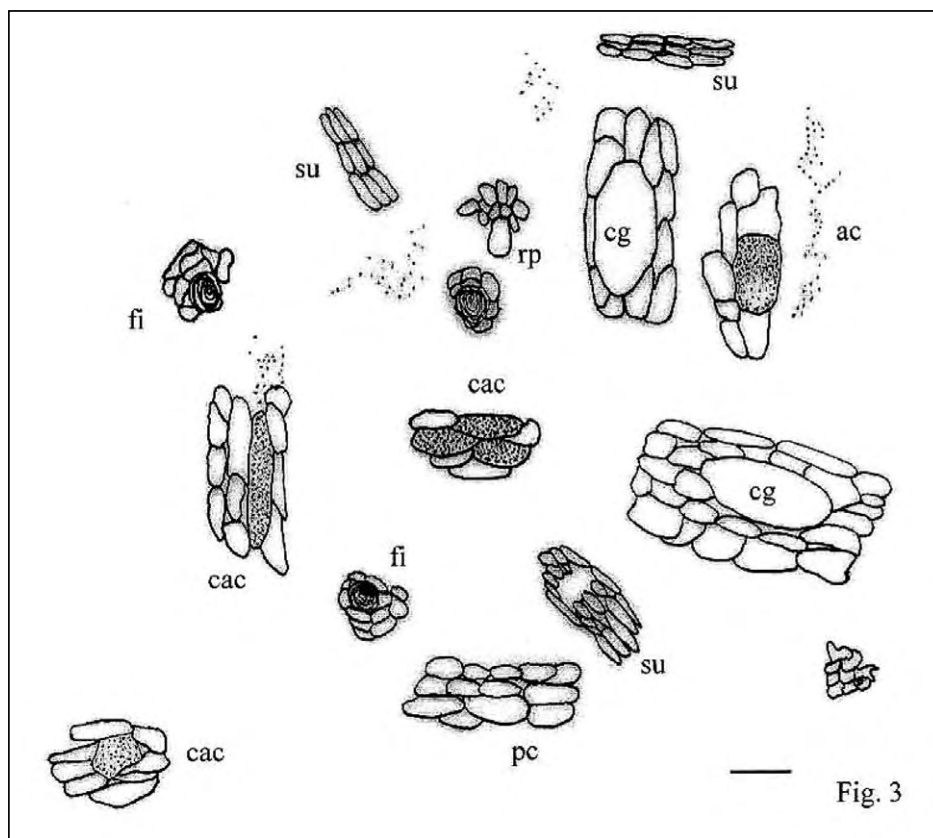


Figure 3 - Macroscopic and microscopic aspects in *Cinchona calisaya* Weddell

Additional information of **Figure 3**. Scales correspond to 500 μm .

General appearance of the powder drug; ac: crystal sand; cac: cells with crystal sand; cg: giant cells; fi: fiber; pc: cortical parenchyma cell; su; suber axial parenchyma, paratracheal.

RHATANY

Ratanhiae radix

Krameria triandra Ruiz & Pav. - KRAMERIACEAE

The vegetable drug consists of the dried root portions containing at least 5% of total tannins, expressed as pyrogallol ($C_6H_6O_3$; M 126.1 M) in relation to the dry drug.

CHARACTERISTICS

Organoleptic characteristics. The drug is odorless, almost tasteless, and the bark has a strong astringent flavor.

MICROSCOPIC DESCRIPTION OF THE POWDER

The powder meets all the requirements for the species, unless the macroscopic characters. The characteristics are: tasteless powder; suber fragments with reddish-brown color, with typical uniform and tabular cells; fragments of cortical tissue with spherical starch grains, simple or compound, large, associated with fibers arranged in a branched way; fragments of wood with cells with bordered pits. The perforation plate is simple. The xylem beams are uniseriate and frequent.

MACROSCOPIC DESCRIPTION

The roots, both dried and fixed in ethyl alcohol, are covered by very dark suber, reddish-brown, irregularly streaked, which enables the formation of small plates of irregular shape and which are relatively easily detached from the rhytidome. Starch cortex and phloem are underlying, forming a layer of clear brown color. The bark is formed by strata above and it is easily detached from the central woody cylinder, which is externally light brown and reddish-brown in the central portion. Fragmented and dehydrated samples have curved shapes with different lengths, twisted, sometimes fibrous.

MICROSCOPIC DESCRIPTION

In the roots with secondary growing established, suber is formed by several strata of cells of uniform size, with slightly thickened walls, tabular and rowed, and that positively react to polyphenol in the presence of 10% ferric chloride. More internally it is formed a new periderm, whose cells are deformed or broken by mechanical action exerted by developing tissues internally. In the cortical region, the cells have varying dimensions and thickened walls, always elongated along the portions closest to the phloem and are filled with starch grains with large dimensions and spherical shape, simple or compound by 2-3 portions. The phloem presents uniseriate parenchyma beams formed by voluminous cells, plenty of idioblasts with prismatic crystals of varying shapes and sizes and reserve parenchyma with starch grains, such as the cortex. Both in the starch cortex as in the phloem fibers isolated or clusters of 5-15 elements occur, which walls are relatively thin, suffering deformations by mechanical compression in their outer portions, configuring a branched arrangement in relation to underlying cells. The xylem is formed by a large number of fibers

relatively wide, and lignified plenty of bordered pits. This type of pits is also present in the vessel elements, which are generally isolated, rarely in pairs, always associated with

IDENTIFICATION

A. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using F_{254} silica gel chromoplate with a thickness of 250 μ m as support and a mixture of ethyl acetate, toluene, formic acid and water (100:10:10:1) as a mobile phase. To separately apply to band-shaped plate, 20 μ L of *Solution (1)* and 10 μ L of *Solutions (2)* and (3), previously prepared as described below.

Solution (1): to accurately weigh about 10 g of the milled drug, add 100 mL of 70% ethanol (v/v), and heat under reflux for 10 minutes. After cooling to room temperature, filter, remove the ethanol in rotary evaporator under reduced pressure. To extract the resulting aqueous phase with three portions of 25 mL of ethyl acetate in a separatory funnel (125 mL). Let it rest in a freezer at (-18°C) for 15 minutes for complete separation of the phases. To gather organic fractions and filter through filter paper with 2 g of anhydrous sodium sulfate. To evaporate the organic fraction obtained in a rotary evaporator under reduced pressure to obtain residue. To take up the residue with 5 mL of methanol.

Solution (2): to weigh about 1 mg of catechin and dissolve in 2 mL of methanol.

To develop the chromatogram. To remove the plate and allow to dry in fume hood. To examine it under ultraviolet light (254 nm). The chromatogram obtained with *Solution (1)* presents an attenuated fluorescence stain at the same height of that obtained with *Solution (2)* (R_f about 0.75). Then, nebulize plate with 1% ferric chloride (w/v) in methanol. After nebulize it, the band corresponds to catechin should present the same grayish-brown color. A second greyish brown spot R_f 0.60 corresponds to epiafzelechin-(4 β →8)-epicatechin. Above the range of R_f 0.75 an intense blue stain should appear.

B. To heat, under reflux, about 3 g of milled vegetable drug with 60 mL of water for 15 minutes. To cool down and filter. To add two drops of hydrochloric acid SR to 2 mL of the extract and drip gelatin SR to precipitation. The appearance of clear precipitate indicates a positive reaction for total tannins.

C. To add 10 mL of water and 2 to 4 drops of 1% ferric chloride solution (w/v) in ethanol to 2 mL of extract obtained in *Identification*, test **B**. The development of dark-gray color indicates a positive reaction for total tannins.

D. To add 0.5 mL of 1% vanillin (w/v) in methanol and 1 mL of hydrochloric acid to 2 mL of extract obtained in *Identification*, test **B**. The development of red color indicates a positive reaction for condensed tannins.

E. To add 10 mL of 2 M acetic acid and 5 mL of lead acetate SR to 5 mL of extract obtained in *Identification*, test

B The appearance of whitish precipitate indicates the presence of tannins.

PURITY TESTS

Foreign matter (5.4.2.2). At most 2%.

Water (5.4.2.3). At most 12%.

Total ashes (5.4.2.4). At most 5.5%.

Sulfated ashes (5.2.10). At most 7%.

Ashes insoluble in acid (5.4.2.5). At most 2%.

DOSAGE

Total tannins

To perform all operations of extraction and dilution protected from light.

Stock solution: to accurately weigh about 0.75 g of the pulverized drug (180 µm) and transfer to a 250 mL erlenmeyer flask with ground mouth. To add 150 mL of distilled water. To heat in a water bath at 60°C for 30 minutes. To cool down in running water and transfer to 250 mL volumetric flask. To wash the erlenmeyer flask and transfer the washings with all content of the vegetable drug for the same volumetric flask. To make up to volume with distilled water. To decant and filtrate the supernatant by using filter paper. To discard the first 50 mL of the filtrate.

Sample solution for total polyphenols: to dilute 5 mL of filtrate in 25 mL volumetric flask with distilled water. To volumetrically transfer 2 mL of this solution, 1 mL of phosphotungstic molybdate reagent, and 10 mL of distilled water to a 25 mL volumetric flask and top up with

29% sodium carbonate solution (w/v). To determine the absorbance at 760 nm (A_1) after 30 minutes by using distilled water to adjust zero.

Sample solution for polyphenols not adsorbed by hide powder: to obtain 10 mL of the filtrate, add 0.1 g of hide powder SQR and mechanically stir in 125 mL erlenmeyer flask for 60 minutes. To use filter paper. To dilute 5 mL of this filtrate in 25 mL volumetric flask with distilled water. To volumetrically transfer 2 mL of this solution, 1 mL of phosphotungstic molybdate reagent, and 10 mL of distilled water to a 25 mL volumetric flask and top up with a 29% sodium carbonate solution (w/v). To determine the absorbance at 760 nm (A_2) after 30 minutes by using distilled water to adjust zero.

Standard solution: just before using it, dissolve 50.0 mg of pyrogallol in a 100 mL volumetric flask with distilled water. To volumetrically transfer 5 mL of the solution to a 100 mL volumetric flask and top up with distilled water. To volumetrically transfer 2 mL of this solution, 1 mL of phosphotungstic molybdate reagent, and 10 mL of distilled water to a 25 mL volumetric flask and top up with a 29% sodium carbonate solution (w/v). To determine the absorbance at 760 nm (A_3) after 30 minutes by using distilled water to adjust zero.

To calculate the content of tannin (dried drug), in %, expressed as pyrogallol, according to the equation:

$$TT = \frac{65,5 \times (A_1 - A_2) \times m_2}{A_3 \times m_1}$$

where

A_1 = absorbance of the Sample solution for total polyphenols;

A_2 = absorbance of the Sample solution for polyphenols not adsorbed by hide powder;

A_3 = absorbance of the Standard solution;

m_1 = mass of the sample used in the test in grams considering the determination of water;

m_2 = mass of pyrogallol, in grams.

PACKAGING AND STORAGE

In tightly closed containers, protected from light and heat.

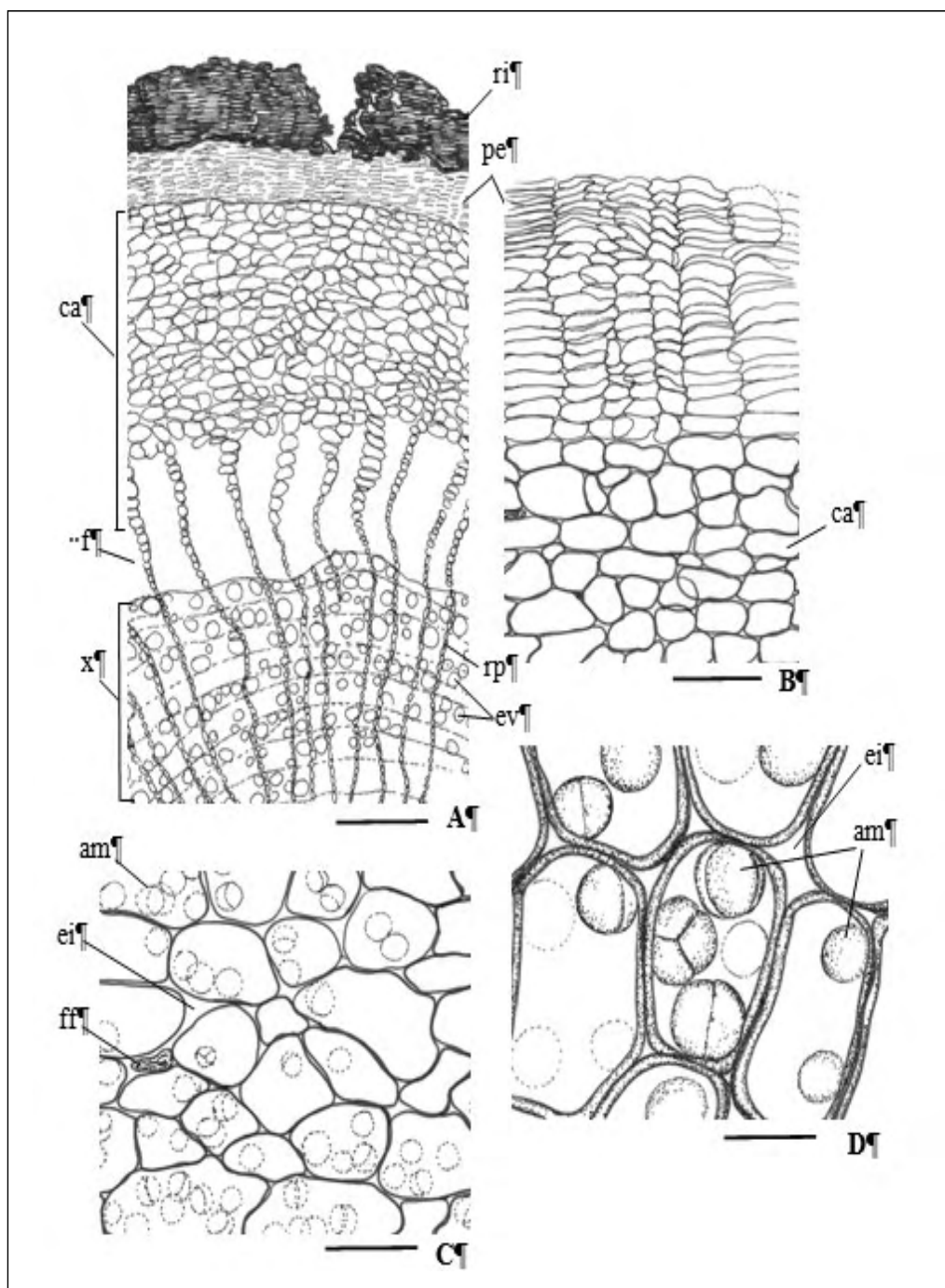


Figure 1 - Microscopic aspects in *Krameria triandra* Ruiz & Pav.

Additional information of **Figure 1**. The Scales correspond to: 250 μm (A), 100 μm (B), 50 μm (C), 25 μm (D).

A - general aspect of the distribution of root tissues in cross-section: starch cortex (ca); vessel element (ev); phloem (f); periderm (pe); rhytidome (ri); parenchyma beam, xylem (x). B - partial detail of the bark with newly formed periderm (pe) and starch cortex (am), in cross section; C and D - partial detail of starch cortex in cross and radial longitudinal section, respectively: starch grains (am), intercellular space (ei); phloem fiber (ff).

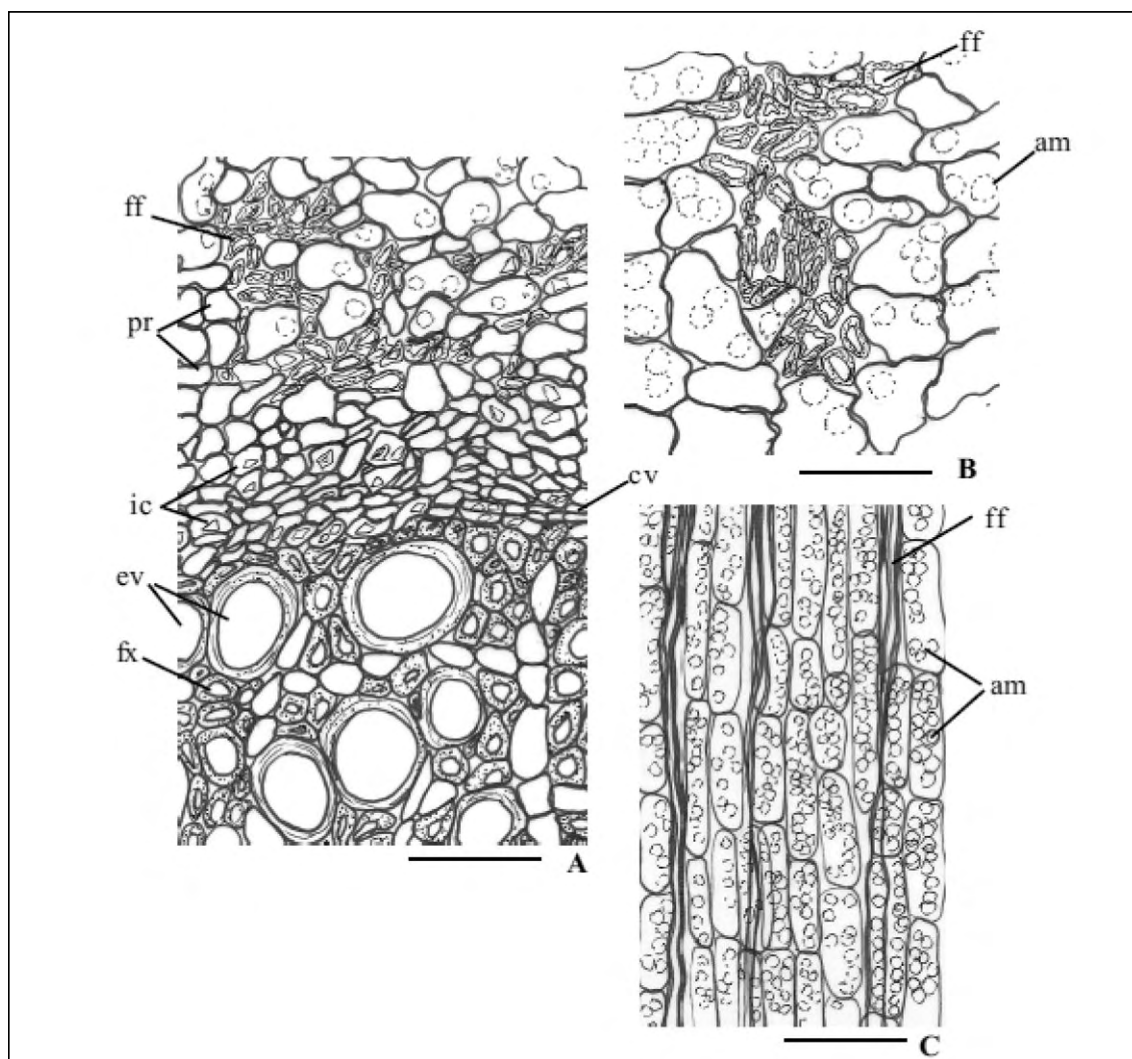


Figure 2 - Microscopic aspects in *Krameria triandra* Ruiz & Pav.

Additional information of **Figure 2**. The Scales correspond to: 50 μm (A e B), 100 μm (C).

A - Partial detail of cambial region and conducting tissues in cross-section: vascular cambium (cv); vessel element (ev); crystal idioblast (ic); phloem fibers (ff); xylem fiber (fx); reserve parenchyma. **B** - partial detail of phloem, in cross section, showing reserve parenchyma and phloem fibers: starch grains (am); phloem fibers (ff). **C** - partial detail of phloem in radial longitudinal section: starch grains (am); phloem fibers (ff).

RHATANY, TINCTURE

Ratanhiae tinctura

The tincture is prepared from the roots of *Krameria triandra* Ruiz & Pav. - KRAMERIACEAE, 10% (w/v) by maceration or percolation by using 70% ethanol (v/v) as the extraction liquid. It contains at least 0.5% of tannins, expressed as pyrogallol ($C_6H_6O_3$; 126.1 M).

CHARACTERISTICS

Organoleptic characteristics. The tincture is red-dish-brown color.

Physical and chemical constants.

Relative density (5.2.5): 0.891 to 0.906.

IDENTIFICATION

To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using F_{254} silica gel chromoplate with a thickness of 250 μ m as support and a mixture of ethyl acetate, toluene, formic acid and water (60:20:15:15) as a mobile phase. To separately apply to band-shaped plate, 20 μ L of *Solution (1)* and 10 μ L of *Solution (2)*, prepared before use as described below:

Solution (1): to heat 5.0 mL of tincture to dry residue in a water bath. To take up the residue in 10.0 mL of water. To extract the resulting aqueous phase with three portions of 10.0 mL of ethyl acetate in a separatory funnel. To allow to rest in a freezer (-18°C) for 15 minutes for complete separation of phases. To gather the organic fractions and wash with 20.0 mL of water.

Solution (2): to weigh about 1 mg of catechin and dissolve in 2 mL of methanol.

To develop the chromatogram. To remove the plate and allow to dry in fume hood. Then, nebulize plate with 1% ferric chloride (w/v) in methanol. After viewing they should be observed in the region of the chromatogram of *Solution (1)* four bands of red-brown coloration in the upper quadrant, the band corresponding to the catechin has a bluish brown color (Rf) 0.71.

PURITY TESTS

Determination of alcohol (5.3.3.8). 63.0% to 67.0%

Dry residue (5.4.3.2.2). At least 1.9%.

DOSAGE

Total tannins

Note: To perform all operations of extraction and dilution protected from light.

To proceed as described in the *Ultraviolet absorption spectrophotometry* (5.2.14). To use the newly prepared solutions described below.

Stock solution: to transfer just about 1.5 g of tincture to a 250 mL volumetric flask and top up with distilled water. To filter the mixture through filter paper. To discard the first 50.0 mL of the filtrate.

Sample solution for total polyphenols: to volumetrically transfer 5 mL of the filtrate of the *Stock solution* to 25 mL volumetric flask and top up with distilled water. To volumetrically transfer 2 mL of this solution, 1 mL of phosphotungstic molybdate reagent, and 10 mL of distilled water to a 25 mL volumetric flask and top up with a 29% sodium carbonate solution (w/v).

Sample solution for polyphenols not adsorbed by hide powder: to obtain 10 mL of the filtrate of the *Stock solution*, add 0.1 g of hide powder and mechanically stir in 125 mL erlenmeyer flask for 60 minutes. To filter on paper filter. To dilute 5 mL of this filtrate in 25 mL volumetric flask with distilled water. To volumetrically transfer 2 mL of this solution, 1 mL of phosphotungstic molybdate reagent, and 10 mL of distilled water to a 25 mL volumetric flask and top up with a 29% sodium carbonate solution (w/v).

Standard solution: to transfer 50.0 mg of pyrogallol to a 100 mL volumetric flask and top up with distilled water. To volumetrically transfer 5 mL of this solution to 100 mL volumetric flask and top up with distilled water. To volumetrically transfer 2 mL of this solution,

1 3 mL of phosphotungstic molybdate reagent, and 10 mL of distilled water to a 25 mL volumetric flask and top up with a 29% sodium carbonate solution (w/v).

To measure the absorbance of the Sample solutions for total polyphenol, sample for polyphenol not adsorbed by hide powder and standard at 760 nm (5.2.14) 30 minutes after preparation using distilled water for zero adjustment. To calculate the content of tannin in %, expressed as pyrogallol, according to the equation:

$$TT = \frac{13,12 \times (A_1 - A_2)}{A_3 \times m}$$

where

A_j = absorbance of the Sample solution for total polyphenols;

A_2 = absorbance of the Sample solution for polyphenols not adsorbed by hide powder;

A_3 = absorbance of the Standard solution;

m_1 = mass of the sample used in the test (g);

m_2 = mass of pyrogallol (g).

PACKAGING AND STORAGE

In tightly closed containers, protected from light and heat.

RAUVOLFIA

Rauvolfiae radix

Rauvolfia serpentina (L.) Benth. ex Kurz - APOCYNACEAE

The drug consists of the root and must contain at least 0.15% of the reserpine-rescinamine alkaloids, in relation to dried material.

CHARACTERISTICS

Organoleptic characteristics. It is almost odorless and has a very bitter taste.

MACROSCOPIC DESCRIPTION

Cylindrical root, often tapered at the distal end, winding; whole root or its portions of 1 cm to 10 cm in length and 3 mm to 22 mm in diameter; the outer surface longitudinally wrinkled to irregularly grooved, light grayish-brown; it may occur remains of secondary roots or mainly rounded scars from their fall, with 0.5 mm to 1.0 mm in diameter; the bark may partially miss and it may be observed failures in these inner layers of yellowish-brown color. Lenticels are often observed. The cross section shows three distinct regions, the cortex, the cambium band and central cylinder. The cortex is yellowish-brown and central cylinder is light yellow, with 2 to 8 concentric rings, showing a thin radial striations; the central cylinder occupies approximately four fifths of the diameter of the root. Remains of the rhizome, characterized by presenting medulla may be rarely present.

Falsifications and confusion are possible, primarily with roots of other species of *Rauvolfia* originated from India, for example, *Rauvolfia heterophylla* Wild. ex Roem. & Schult. Unlike these other species, however, in *Rauvolfia serpentina* root fibers and stone cells on the outer part of the cambium are insufficient. The distribution of starch in the cross section of the roots is Useful for differentiation: Unlike other species, *R. serpentina* root shows an almost homogeneous distribution of starch throughout the cross section (except in the suber and the primary xylem). Falsifications of *R. serpentina* drugs are also made with roots of *Withania somnifera* (L.) Dunal (Solanaceae). Useful characters for identifying this forgery include: *Rauvolfia* wood is light yellow and shows thin radial strips (it is microscopically verified the presence of parenchyma beams and vessel elements with radial disposition) being blank and forming a closed loop in *Withania* (microscopically showing dispersed vessel elements in parenchyma).

MICROSCOPIC DESCRIPTION

The periderm has up to 20 layers of tangentially flattened cells with radial arrangement. The suber is homogeneous and consists of about 15 layers of thin-walled suberous cells. The pheloderm has up to four layers of thin-wall cells composed of cellulose, hemicellulose and pectic compounds. The cortical parenchyma has starch, with non-lignified multilayered cell; the starch grains, as evidenced by

Lugol reagent, may be small and numerous or voluminous, of round or ovoid shape. Branched laticifers, with intrusive growth, permeate the cortical parenchyma. The phloem consists of only sieve-tube elements and parenchyma cells; fibers and sclereids are absent. The parenchyma beams are multiseriate and may be narrow or wide; their cells have starch grains and/or crystals of varied formats. The secondary xylem also has radial arrangement. The tracheal elements and fibers are arranged in radial series uniserial or biserial and they alternate to multiseriate parenchyma. The tracheal elements are narrow (about 40 µm in diameter) with simple or scalariform perforation plates; fibers are libriform and have thickened lignified walls. Parenchyma beams are multiseriate, their cells have lignified walls and starch grains are bulkier than those found in the phloem and cortical parenchyma. The primary xylem, with six to eight poles of protoxylem, occupies medullary position; tracheal elements are also narrow have a caliber similar to the underlying parenchyma cells.

DESCRIPTION OF POWDER

The powder meets all the requirements for the species, unless the macroscopic characters. The characteristics are: light gray or light yellowish-brown; suber fragments, yellow, with thin suberous walls; fragments of vessel elements of thick walls with bordered pits; fragments of xylem parenchyma cells with thick walls and simple pits; fragments of parenchyma cells of the cortex with thin walls; numerous rounded starch grains, sometimes aggregated with the central region in the y-shape or star-shape.

IDENTIFICATION

To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using F₂₅₄ silica gel chromoplate as stationary phase and a mixture of butanol, acetic acid and water (40:10:10) as a mobile phase. To separately apply the chromoplate, band-shaped, 10 µL of *Sample solution* and 5 µL of *Reference solution* prepared as follows.

Sample solution: to boil under reflux 1 g of the dried and pulverized drug with 5 mL of methanol of a solution of 10% sodium carbonate (w/v) for 10 minutes, cool down and filter.

Reference solution: to prepare a reserpine solution at 10 mg/mL in methanol.

To develop the chromatogram. To allow the plate to dry in an oven between 100°C and 105°C and then nebulize with potassium iodide and bismuth subnitrate SR. To allow the plate to dry for 10 minutes and examine it with the naked eye and then under ultraviolet light (365 nm). To the naked eye, the chromatogram region obtained with *Reference solution*, should present in the middle third of the plate, almost superior, an orange stain (reserpine). The chromatogram region of the *Sample solution* should present orange stain corresponding in position to the stain obtained with reserpine in the chromatogram of the *Reference solution*. Other orange stains, below reserpine, still in the middle

third may be present. The reserpine stain after exposure to ultraviolet light (365 nm) should be fluorescence blue. The chromatogram of the *Sample solution* should present a fluorescence blue stain corresponding in position to the stain obtained with reserpine in the chromatogram of the *Reference solution*. Other fluorescence blue stains, below reserpine, still in the middle third may be present.

PURITY TESTS

Foreign matter (5.4.2.2). At most 5%.

Water (5.4.2.3). At most 12%.

Total ashes (5.4.2.4). At most 10%.

DOSAGE

To proceed as described in the *Ultraviolet absorption spectrophotometry* (5.2.14). Accurately weigh 2.5 g of milled dried plant and perform extraction with 100 mL of ethanol under reflux for 4 hours, always protecting from light. After extraction, top up with ethanol, in a 100 mL volumetric flask. To transfer a volumetric

aliquot of 20 mL to a separatory funnel. To add with a beaker, 200 mL of 0.25 M sulfuric acid and extract four times with 60 mL of chloroform, by discarding the phase containing sulfuric acid and reserving the phase containing the chloroform. To extract four times the phase containing chloroform with 60 mL of sodium bicarbonate at 2% (w/v) and filter the organic phase in a 250 mL volumetric flask. After filtration, top up with ethanol. To transfer, in duplicate, a volumetric aliquot of 25 mL of the solution to round bottom flask and allow it to dry in a rotary evaporator (bath at about 40°C). The two dry solutions are called *Sample solution (1)* and *(2)*.

Sample solution (1): to volumetrically add 5 mL of ethanol and 2 mL of 0.25 M sulfuric acid.

Sample solution (2): to volumetrically add 5 mL of ethanol, 1 mL of 0.25 M sulfuric acid, and 1 mL 0.3% sodium nitrite (w/v).

Standard solution of reserpine: to analytically weigh and transfer 20 mg of reserpine SQR to 50 mL volumetric flask. To add 25 mL of ethanol and take it to ultrasound. To heat if needed. To wait the cooling of the solution and take up with ethanol. To pipette an aliquot of 5 mL of this solu-

tion to 100 mL volumetric flask and top up with ethanol, resulting in a concentration of 20 µg/mL.

Sample solution (1): to volumetrically add 5 mL of *Standard solution of reserpine* and 2 mL of 0.25 M sulfuric acid.

Standard solution (2): to volumetrically add 5 mL of *Standard solution of reserpine*, 1 mL of 0.25 M sulfuric acid and 1 mL of 0.3% sodium nitrite (w/v).

Note: Do not use Standard solutions (1) and (2) from previous days.

To heat the four solutions, *Sample solutions (1)* and *(2)* and *Standard solutions (1)* and *(2)* simultaneously in water bath at 50°C to 60°C for 20 minutes. To cool down the solution to room temperature and volumetrically add 0.5 mL of 5% sulfamic acid (w/v) in each one of them and wait for 20 minutes. After the waiting time, measure the absorbance of the solutions at 390 nm, by using a mixture of ethanol and water (2:1) to adjust zero. To calculate the amount in mg of alkaloids of reserpine-rescinamine group such as reserpine, by using the following equation.

$$MAL = 5x \frac{(A_1 - A_2)}{(S_1 - S_2)}$$

where

MAL = mass of alkaloids (mg);

*A*₁ = reading of the *Sample solution (1)*;

*A*₂ = reading of the *Sample solution (2)*;

*S*₁ = reading with the *Standard solution (1)*;

*S*₂ = reading with the *Standard solution (2)*;

To calculate the content of alkaloids such as reserpine-rescinamine, on a dry basis, by the equation.

$$AL = \frac{MAL}{M} \times 100$$

where

AL = alkaloid content (% w/w);

MAL = mass of alkaloids (mg);

M = mass of dry sample (mg).

PACKAGING AND STORAGE

In tightly closed containers, protected from light and heat.

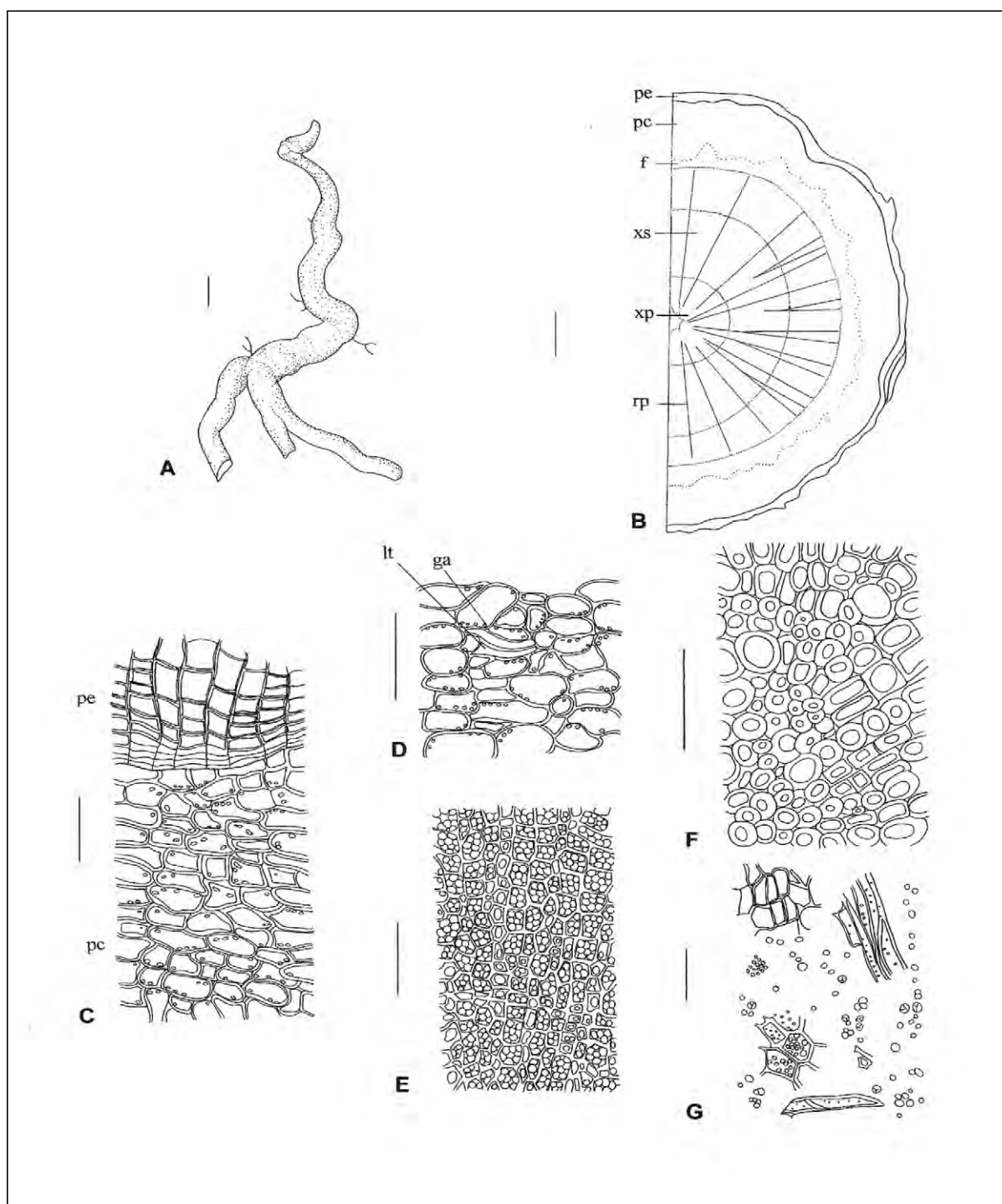


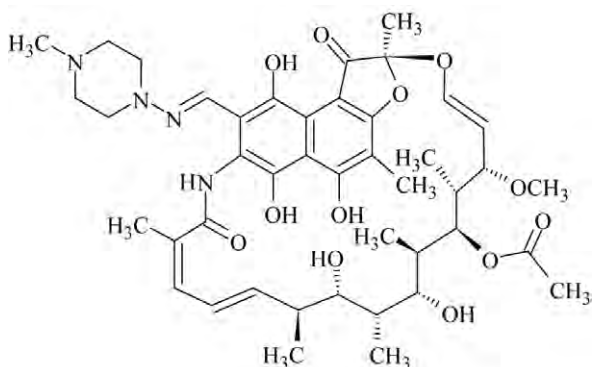
Figure 1 - Macroscopic and microscopic aspects in *Rauvolfia serpentina* (L.) Benth. ex Kurz

Additional information of Figure 1. Scales correspond: in A to 100 mm, B and G to 100 µm, and C to F to 50 µm.

A - general appearance of the root; B - scheme of cross section of the root; C - detail of the periderm portion and cortical parenchyma, in cross section; D - detail of a portion of the cortical parenchyma in cross section; E - detail of the portion of the secondary xylem multiseriate parenchyma beams with abundant starch grains, fibers and vessels arranged in radial series, in cross section; F - detail of the portion of the primary xylem in cross section; G - general appearance of the root powder with fragments of suber (above left), fibers and vessels (above, right and below, in the central region) parenchyma cells of the secondary xylem (below left) and numerous starch grains, isolated or aggregates; region of primary and secondary phloem (f); starch grain (ga); branched laticifer of intrusive growth (lt); cortical parenchyma (pc); periderm (pe); parenchyma beams (rp); primary xylem (xp); secondary xylem (xs).

RIFAMPICIN

Rifampicinum



$C_{43}H_{58}N_4O_{12}$: 822,94
rifampicin; 07719

3 - [[(4-Methyl-1-piperazinyl) imino] methyl] rifamycin
[13292-46-1]

It contains at least 97% and a maximum of 102% of
 $C_{43}H_{58}N_4O_{12}$

DESCRIPTION

Physical characteristics. Crystalline powder, red-dish-brown to red-brown.

Solubility. Slightly soluble in water, soluble in methanol, slightly soluble in acetone and ethanol.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed among paste based on liquid paraffin, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the rifampicin SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14) ranging from 220 nm to 500 nm, of sample solution obtained in *Dosage*, exhibits maximum in 237 nm, 254 nm, and 3344 nm and 475 nm. The ratio between the absorbances determined at 334 nm and 475 nm is approximately 1.75.

C. Suspend about 25 mg of sample in 25 mL of purified water. To stir for 5 minutes and filter it. The 5 mL of the filtrate add 1 mL of ammonium persulfate to 10% (w/v) in solution of phosphate buffer, pH 7.4 and stir it for a few minutes. The solution changes from yellow-orange to red-violet without precipitate formation.

PURITY TESTS

pH (5.2.19). 4,5 a 6,5. To determine at 0.1% (w/v) from the sample suspension in water free from carbon dioxide.

Related substances. To proceed as described in High performance liquid chromatography (5.2.17.4). To use chromatograph equipped with an ultraviolet detector at 254 nm;

100 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octylsilane group (5 μ m); flow of *Mobile phase* of 1.5 mL/min.

Phosphate Buffer: to dissolve 136.1 g of monobasic potassium phosphate in 500 ml of water, add 6.3 ml of phosphoric acid, dilute with water to 1000 ml and mix thoroughly.

Mobile phase: to preparer the mixture of water, acetonitrile, *phosphate buffer*, citric acid M, sodium perchlorate and 0.5 M (510:350:100:20:20), filter it on membrane filter 0, 7 μ m or less, and degas it. To make the adjustments as required.

Diluent: to preparer mixture of water, acetonitrile, dibasic potassium phosphate M, monobasic potassium phosphate M, and citric acid M (640:250:77:23:10).

Solution (1): to accurately transfer about 0.2 g of the sample to a 100 mL volumetric flask and dissolve it with acetonitrile, dilute and top up the volume. To let it in ultrasound bath for about 30 seconds if necessary to ensure complete dissolution. To use this solution within 2 hours.

Solution (2): to transfer 5 mL of the solution (1) to 50 mL volumetric flask, dilute it with the *Diluent* top up and make it homogeneous. To prepare this solution immediately before use it.

Solution (3): to transfer 10 mL of the Solution (1) to 100 mL volumetric flask, dilute it with acetonitrile, top up and make it homogeneous. To transfer 5 mL of this solution to 50 mL volumetric flask, dilute it with the *Diluent*, top up and make it homogeneous. To prepare the final dilution immediately before use it.

Resolution Solution: to dissolve accurately weighed quantity of rifampicin SQR and quinone rifampicin SQR in acetonitrile to obtain a solution containing about 0.1 mg/mL. To transfer 1 mL of this solution to 10 mL volumetric flask, dilute it with the *Diluent*, top up and make it homogeneous.

To inject replicates of 50 μ L of the Resolution solution. The relative retention times are about 0.6 for quinone rifampicin and 1,0 for rifampicin. The resolution between the peaks of quinone rifampicin and rifampicin is not less than 4.0.

Procedure: to inject 50 μ L of the Solutions (2) and (3), record the chromatograms, and measure the areas under the peaks. To calculate the percentage of each substance related by the formula:

$$r_{Ti}/(r_D + 0,01 \sum r_{Ti})$$

wherein r_{Ti} is the area under the peak of each related substance in the chromatogram obtained with *Solution (2)*, r_D is the area under the peak of rifampicin in the chromatogram obtained with *Solution (3)* and $\sum r_T$ is the sum of the areas of all peaks

DOSAGE

To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To dissolve 0.1 g of the sample in methanol and top up the volume to 100 mL with the same solvent. To dilute 2 mL of the solution to 100 mL with phosphate buffer pH 7.4. To determine the maximum absorbance at 475 nm, using as the compensation liquid the phosphate buffer pH 7.4. To calculate the concentration in $C_{43}H_{58}N_4O_{12}$ taking 187 as the value of specific absorbance.

1258 Brazilian Pharmacopoeia 5th edition

of the related substances obtained in the chromatogram of *solution* (2) no more than 1.5% of quinone rifampicin is found, no more than 1% of any other related substance is found, and a total of no more than 3.5% of total of the individual related substances, in addition of quinone rifampicin, having a retention time above of 3 in relation to the retention time of the rifampicin that is found.

Heavy metals (5.3.2.3). To use *the Method III*. To determine in 1 g of the sample. At most 0.002% (20 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 80° C at a maximum pressure of 670 Pa, during 4 hours. At most 1.0%.

Sulfated ashes (5.2.10). To determine in 2 g of the sample. At most 0.1%.

PACKAGING AND STORAGE

In airtight closed containers, protected from light, at maximum temperature of 25 °C.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS ANTIBACTERIAL; TUBERCULOSTATIC.

RIFAMPICIN CAPSULES

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_{43}H_{58}N_4O_{12}$.

IDENTIFICATION

A. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using G silica gel as support and a mixture of chloroform and methanol (90:10) as mobile phase. To apply separately to the plate 3 µL of each of the solutions as described below.

Solution (1): dissolving a quantity of powder, equivalent to about 50 mg of rifampin with 5 mL of chloroform and filter it.

Solution (2): 0.01% solution (w/v) of the sample in chloroform.

To develop the chromatogram. To remove the plate, and let it drying. The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Weight determination (5.1.1). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage unit (5.1.6) It complies with the test.

Procedure for content uniformity. To transfer the content of a capsule to a 100 mL volumetric flask. To wash the body and the cap of the capsule with a mixture of acetonitrile and methanol (1:1) and to transfer to the volumetric flask. To dilute in order to obtain a solution with a concentration of 1.5 mg/mL. To let in ultrasound bath for 5 minutes and cool to room temperature. To transfer 10 mL of this solution to 50 mL volumetric flask, top up with *Diluent*, and stir it. To proceed as described in *Dosage*.

DISSOLUTION TEST (5.1.5)

Dissolution medium: hydrochloric acid 0.1 M, 900 mL
Equipment: baskets, 100 rpm *Time:* 45 minutes

Procedure: after testing, remove aliquot from the dissolution medium, filter, and dilute it in 0.1 M hydrochloric acid, to obtain suitable concentration. To measure the absorbances at 475 nm (5.2.14), by using the same solvent to adjust zero. To calculate the amount of $C_{43}H_{58}N_4O_{12}$ dissolved in the medium, by comparing the readings obtained with that of 0.0032% rifampicin SQR solution concentration (w/v) prepared in the same solvent.

Tolerance: not less than 75% (Q) of the amount stated of $C_{43}H_{58}N_4O_{12}$ dissolves within 45 minutes.

PURITY TESTS

Loss on drying (5.2.9). To determine in 100 mg of sampling. To desiccate in an oven at 60 °C under reduced pressure for 3 hours. At most 3.0%.

BIOLOGICAL SAFETY TESTS

Counting of the total number of mesophyll microorganisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic microorganisms (5.5.3.1.3). It complies with the test.

DOSAGE

To proceed as described in *High performance liquid chromatography (5.2.17.4)*. To use chromatograph equipped with an ultraviolet detector at 254 nm; 250 mm chromatograph column and 4 mm inner diameter, packed with silica bonded to the octylsilane group (5 µm), kept at room temperature; flow of *Mobile phase* of 1.5 mL/min.

Phosphate Buffer: to dissolve 136.1 g of monobasic potassium phosphate in 500 ml of water, add 6.3 ml of phosphoric acid, dilute with water to 1000 ml and stir it. To adjust pH to 3.1 ± 0.1 .

Mobile phase: mixture of water, acetonitrile, phosphate buffer, citric acid *M* and 0.5 *M* sodium perchlorate (510:350:100:20:20). To degas and filter it.

Diluent: to prepare mixture of water, acetonitrile, dibasic sodium phosphate *M*, monobasic potassium phosphate *M*, (640:250:77:23:10).

Standard solution: to transfer about 37.5 mg of rifampicin SQR to 25 mL volumetric flask and top up the volume with a mixture of acetonitrile and methanol (1:1). To transfer 10 mL of this solution to 50 mL volumetric flask, top up with acetonitrile, and stir it. To transfer 5 mL of this solution to 50 mL volumetric flask, top up with *Diluent*, and stir it. Each mL of the *Standard solution* contains approximately 0.03 mg of rifampicin SQR.

Sample solution: to weigh 20 capsules, remove the content, and weigh them again. To make the content of the capsules homogeneous. To transfer amount of powder equivalent to 300 mg of rifampicin to a 200 mL volumetric flask, add about 180 mL of a mixture of acetonitrile and methanol (1:1), and put the flask in ultrasound for 5 minutes. To transfer 10 mL of this solution to 50 mL volumetric flask, top up with acetonitrile, and stir it. To transfer 5 mL of this solution to 50 mL volumetric flask, top up with *Diluent*, and stir it.

Resolution solution: to dissolve accurately weighed amount of quinone rifampicin SQR in a mixture of acetonitrile and methanol (1:1), to obtain a solution with 0.1 mg/mL concentration. To transfer 1.5 mL of this solution and 5 mL of rifampicin SQR solution to 0.3 mg/mL to 50 mL volumetric flask, top up with *Diluent*, and stir it.

To inject 50pL of *Resolution solution*. The retention time of quinone rifampicin is about 0.6 times that of rifampicin. The resolution between the peaks of quinone rifampicin and rifampicin is not less than 4.0. To inject replicates of

50pL of the *Standard solution*. The relative standard deviation for the replicate areas for the peaks recorded should not be greater than 1.0%.

Procedure: to separately inject 50pL of *Standard solution* and *Sample solution*, record the chromatograms, and measure the areas under the peaks. Calculate the amount of

$C_{21}H_{23}FCINO_2$ in the tablets, from the responses obtained for the *Sample* and *Standard solutions*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

RIFAMPICIN ORAL SUSPENSION

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_{43}H_{58}N_4O_{12}$.

IDENTIFICATION

For an amount of 0.1 g of rifampicin, add 30 mL of water and stir it with two amounts of 50 mL of chloroform. To dry the combined extracts with anhydrous sodium sulfate, filtering and evaporating the dried filtrate at a temperature not exceeding 70 °C. The residue, after washing with 1 mL of ethyl ether and drying at 70 °C complies with the following tests.

A. The infrared absorption spectrum (5.2.14) of the sample has absorption maximum at the same wavelength, with the same relative intensities to those observed in the rifampicin SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14) ranging from 220 nm to 500 nm, of *Sample solution* obtained in Dosage method **B.**, exhibits absorption maximum at 237 nm, 254 nm, 334 nm and 475 nm similar to those observed in the *standard solution spectrum*.

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

pH (5.2.19). 4.2 to 4.8. To determine in the reconstituted suspension as informed on the label.

PURITY TESTS

Related substances. To proceed as described in *High performance liquid chromatography (5.2.17.4)*. To use chromatograph equipped with an ultraviolet detector at 254 nm; 120 mm chromatograph column and 4.6 mm inner diame-

ter, packed with silica chemically bonded to the octylsilane group (5 µm); flow of *Mobile phase* of 1.5 mL/min.

Mobile phase: mixture of 35 volumes of acetonitrile and 65 volumes of a solution containing 0.1% phosphoric acid (v/v) of sodium perchlorate with 1.9 mg/mL, citric acid 5.9 mg/mL and monobasic potassium phosphate to 20.9 mg/mL.

Diluents: mixture of citric acid solution to 210.1 mg/mL, solution of the monobasic potassium phosphate to 136.1 mg/mL solution of dibasic potassium phosphate to 174.2 mg/mL, acetonitrile and water (10:23:77:250:640).

To prepare the Solutions (1), (2), (3), (4), (5) and (6) immediately before use it.

Solution (1): to add 5 mL of water in an amount of oral suspension containing 20 mg of rifampin and extract with four successive portions of 10 mL of methylene chloride. To filter the combined extracts and evaporating until dry at a temperature not exceeding 40 °C. To dissolve the residue in 10 mL of acetonitrile and dilute 5 mL of this solution to 50 mL with Diluent. To make it homogeneous.

Solution (2): to transfer 1 mL of *Solution (1)* to 100 mL volumetric flask, top up with *Diluent*, and make it homogeneous.

Solution (3): to dissolve accurately weighed amount of quinone rifampicin SQR in *Diluent* thus obtaining 0.3 mg/mL solution. To transfer 1 mL of this solution to 100 mL volumetric flask and top up the volume with *Diluent*, thus obtaining 0.0003% (w/v) solution.

Solution (4): to dissolve accurately weighed amount of N-oxide rifampicin SQR in *Diluent* thus obtaining 0.2 mg/mL solution. To transfer 1 mL of this solution to 100 mL volumetric flask and top up the volume with *Diluent*, thus obtaining 0.0002% (w/v) solution.

Solution (5): to dissolve accurately weighed amount of 3-formyl rifamycin SQR in *Diluent* to obtain a solution of 0.1 mg/mL. To transfer 10 mL of this solution to 100 mL volumetric flask and top up the volume with *Diluent*, thus obtaining 0.001% (p/v) concentration.

Solution (6): to transfer 10 mL of the *Solution (3)* for erlenmeyer, thus adding 5 mL of *Diluent* and mix thoroughly. To transfer 5 mL of this solution for the erlenmeyer, thus adding 5 mL of *Solution (2)* and mix thoroughly.

To inject 20 mL of the *Solution (6)*. To adjust the sensitivity of the detector so that the height of the two principal peaks is not less than half of the scale. The resolution between the two principal peaks is greater than 4.0. If necessary, adjusting the concentration of acetonitrile in the *Mobile phase*.

Procedure: to separately inject 20 µL of *Solutions (2)* and (5), record the chromatograms, and measure the areas under the peaks. To inject the *Solution (1)* and developing

chromatography for at least 3 times the retention time of the peak relative to rifampicin. The area under the peak corresponding to quinone rifampicin is not greater than the area under the peak of the chromatogram obtained with *Solution (3)* (1.5%). The area under the peak corresponding to N-oxide rifampicin is not greater than the area under the peak of the chromatogram obtained with *Solution (4)* (1%), the area under the peak corresponding to 3-formyl rifamycin is not greater than the area under the peak of the chromatogram obtained with *Solution (5)* (5%), and the area of any secondary peak is not greater than the area under the peak of the chromatogram obtained with *Solution (2)* (1%). Disregarding any peaks with retention times shorter than the characteristic peak of quinone rifampicin.

BIOLOGICAL SAFETY TESTS

Counting of the total number of mesophyll microorganisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic microorganisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. To dilute the volume of the injection solution equivalent to 0.4 g of rifampin in 500 mL of methanol and mix thoroughly. To dilute 2 mL of this solution into 100 mL of phosphate buffer at pH 7.0 and measuring the absorbance at 475 nm. To calculate the content of $C_{43}H_{58}N_4O_{12}$ in the sample considering $A(1\%, 1\text{ cm}) = 187$, at 475 nm. To determine the *Relative density (5.2.5)* of the oral suspension and calculate the content of $C_{43}H_{58}N_4O_{12}$ in the sample from the obtained readings.

B. To proceed as described in *High performance liquid chromatography (5.2.17.4)*. To use chromatograph equipped with an ultraviolet detector at 254 nm and 100 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octylsilane group (5 Mm).

Phosphate Buffer: to dissolve 136.1 g of monobasic potassium phosphate in 500 mL of water, add 6.3 mL of phosphoric acid and mix thoroughly. Top up the volume with water to 1000 mL and make it homogeneous.

Mobile phase: mixture of water, acetonitrile, *Phosphate Buffer*, citric acid *M* and 0.5 *M* sodium perchlorate (510:350:100:20:20). To filter in 0.7 µm membrane or less and degas it.

Mixture of solvents: to prepare mixture of water, acetonitrile, dibasic potassium phosphate *M*, monobasic potassium phosphate *M*, and citric acid *M* (640:250:77:23:10).

Diluent: to prepare mixture of acetonitrile and water (1:1).

Sample solution: to stir the flask containing the sample and immediately transfer 5 mL of oral suspension, free from bubbles to a 100 mL volumetric flask. To dissolve, top up with *Diluent* and make it homogeneous. To transfer 5 mL of the resulting solution to 50 mL volumetric flask, top up the volume with *Diluent* and make it homogeneous.

Standard solution: to dissolve accurately weighed amount of quinone rifampicin SQR in *Diluent* thus obtaining 0.5 mg/mL solution. To let it in ultrasound for about 30 seconds if necessary to dissolve. To transfer 5 mL of this solution to the 50 mL volumetric flask, top up the volume with *Diluent* and make it homogeneous. To use this preparation at most 1 hour.

Resolution Solution: to dissolve accurately amounts of rifampicin SQR and quinone rifampicin SQR in acetonitrile to obtain a solution containing about 0.1 mg/mL. To transfer 1 mL of this solution to 10 mL volumetric flask, top up the volume with the *mixture of solvents*. To make it homogeneous.

To inject replicates of 20 µL of the *Resolution solution*. The relative retention times are about 0.6 for quinone rifampicin and 1.0 for rifampicin. The resolution between the peaks of quinone rifampicin and rifampicin is not less than 4.0. The relative standard deviation for the replicate areas for the peaks recorded is not greater than 1.0%.

Procedure: to separately inject 20 µL of the *Sample and standard solutions*, record the chromatograms, and measure the areas under the peaks. To calculate the content of $C_{43}H_{58}N_4O_{12}$ in the sample from the responses obtained for the *Sample and Standard solutions*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

RITONAVIR CAPSULES

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_{37}H_{48}N_6O_5S_2$.

IDENTIFICATION

The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Weight determination (5.1.1). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage unit (5.1.6) It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.7% (w/v) sodium lauryl, 900 mL

Equipment: blades, 25 rpm. To use inert coated blades

Time: 120 minutes

Procedure: after testing, remove aliquot from the dissolution medium, filter and dilute it to obtain the suitable concentration. To proceed as described in *Dosage*. To separately inject 20 µL of *Sample and standard solutions*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of $C_{37}H_{48}N_6O_5S_2$ dissolved in the medium from the responses obtained from *Sample and standard solutions*.

Tolerance: not less than 40% (Q) of the amount stated of $C_{37}H_{48}N_6O_5S_2$ dissolves within 60 minutes.

and not less than 75% (Q) of the amount stated of $C_{37}H_{48}N_6O_5S_2$ dissolves within 120 minutes.

BIOLOGICAL SAFETY TESTS

Counting of the total number of mesophyll microorganisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic microorganisms (5.5.3.1.3). It complies with the test.

DOSAGE

To proceed as described in *High performance liquid chromatography (5.2.17.4)*. To use chromatograph equipped with an ultraviolet detector at 210 nm; 125 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at room temperature; flow of Mobile phase of 1.0 mL/min.

Mobile phase: mixture of methanol and water (67:23).

Sample solution: to weigh 20 capsules, remove the content, and weigh them again. To accurately transfer an amount equal to 20 mg of ritonavir to a 100 mL volumetric flask and add 70 mL of *Mobile phase*; stirring it and top up the volume with the same solvent. Make it homogeneous and filter, obtaining 0.2 mg/mL solution.

Standard solution: to dissolve accurately weighed amount of ritonavir SQR in *Mobile phase* in order to obtain a 0.2 mg/mL solution. To top up with the same solvent and make it homogeneous.

Procedure: to separately inject 20 µL of the *Sample and standard solutions*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of

$C_{37}H_{48}N_6O_5S_2$ in the capsules from the responses obtained from *Sample* and standard solutions.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

RHUBARB

Rhei rhizoma et radix

Rheum palmatum L. and/or *Rheum officinale* Baill. - POLYGONACEAE

The vegetable drug consists of dried, fragmented rhizomes and roots. The rhizomes must be stripped of leaf petiole base as well as the roots of almost the entire cortex. The vegetable drug must belong to the species above or their interspecific hybrids, or even mix them, except for parts or mixtures with *Rheum rhaponticum* L., containing at least 2.2% hydroxy anthracene derivatives, expressed in reigns.

CHARACTERISTICS

Organoleptic characteristics. The drug has an odor and aromatic characteristic, bitter and astringent taste.

MACROSCOPIC DESCRIPTION

Irregular rhizome fragments, cylindrical or discoid, round, flat or plano-convex, up to 15.0 cm diameter and 1.0 cm to 5.0 cm thick, generally devoid of the cortical region and/or part of the vascular region, usually close to or beyond the communication zone. The outer surside is generally smooth and coated with a layer of yellow-brown powder. If this layer is removed, showing pink color, which, when wet, has dark and light lines that intersect themselves, showing numerous lattice-shaped diamonds interrupted by the scars from the roots. In cross section, there is a dark ring, corresponding to the communication, followed by another narrow ring regularly crossed by radial streaks orange, parallel to each other. The inside of the central cylinder is filled by a pink tissue, which include many star-shaped structures corresponding to anomalous vascular bundles. These vascular bundles have a diameter of 2.0 mm until 4.1 mm each one and are irregularly arranged and/or also on one or two rings, giving to the drug a marbled appearance. The rhizomes of *Rheum palmatum* are characterized by exhibiting anomalous small vascular bundles an average diameter of 2.5 mm and a set of bundles forming a continuous ring, sometimes two, while the *Rheum officinale* have greater bundles up to 4.1 mm in diameter irregularly distributed in the cross section. The root fragments are cylindrical or conical devoid of cortex, measuring 3.0 cm up to 6.0 cm in diameter and 4.0 cm up to 17.0 cm long, similar in color to the rhizome. In cross section, they are crisp parenchyma-

tous rays of radial layout, from the central portion to the periphery. The fracture of the rhizomes and roots is grainy.

MICROSCOPIC DESCRIPTION

In cross-section, the rhizome, when accompanied by the cortical region or its debris, it presents little developed storied cork and outer cortical parenchyma. The storied cork cells have thin walls and radial disposition. The outer cortical parenchyma, which tracks the storied cork, as well as other parenchyma, has occasionally rounded or polygonal cells, thin-walled, with numerous starch grains and crystals of Druze type. The parenchyma cells, which contain large drusen, have greater volume. These calcium oxalate crystals have a diameter of 100 µm up to 200 µm. The starch grains measuring from 2 µm to 35 µm, usually from 10 µm to 20 µm are simple or compounds of two until five units, a central and radiated hilum. The vascular system is presented in two different shapes. The outermost derived from the normal communication is continuous and more or less circular and the inner one has anomalous vascular bundles, which are star point and are distributed irregularly on the medullary parenchyma or some of them form one or two rings. The external vascular system has secondary phloem little developed and its elements are often obliterated. The phloem is devoid of fiber. The secondary xylem has radial disposition and consists of a few layers of vessel elements that have polygonal or irregular thickening usually crosslinked, whose diameter can reach 100 µm. The parenchymatous ray are formed each by one until four row of cell, containing yellow-brown or yellow intense amorphous masses corresponding to hydroxy anthracene derivatives. These masses take intense red color when subjected to a solution of potassium hydroxide when 10% (w/v). The medullary parenchyma fills almost the entirety central cylinder, being interrupted by anomalous vascular bundles. Each of these bundles has stellar appearance, its phloem is inside and xylem is outside. Featuring this vascular bundle occur parenchymatous rays, which depart from the center of the bundle. Its phloem has whitish appearance and the parenchyma cells of this tissue are filled with starch grains and some of them have crystals of druses type. The communication zone is continuous and comprises three to four layers of cells. The xylem has few vessel elements, arranged in two to five rows, showing thickening usually cross linked and absence of lignin. The parenchymatous ray are formed by one to four rows of cells presenting the same characteristics as those occurring in the external vascular system. These expand themselves into the xylem, often mingling with the medullary parenchymatous tissue or the parenchymatous ray of the vascular bundles (star) next to it, crisscrossing in a way that is difficult to define its trajectory. The root, in cross section, has the same characteristics of the rhizome, except the anomalous vascular bundles and the medullary parenchyma. The yellowish amorphous masses containing hydroxy anthracene derivatives occur more abundantly compared to those found in the rhizome parenchymatous rays.

MICROSCOPIC DESCRIPTION OF POWDER

The powder meets all the requirements for those species, unless the macroscopic characters. To use aqueous solution of sodium hypochlorite and 3% (w/v) for microscopic examination. They are typical: orange coloration to yellow-brown, when having 10% (w/v) potassium hydroxide assumes the red color; parenchymatous ray cells with yellow amorphous substance; fragments of vessel elements crosslinked not lignified, which can reach up to 175 μm in length; numerous groups of parenchyma cells, round or polygonal, thin-walled, with starch grains; fragments of parenchymatous ray in radial longitudinal view or in its tangential view; large number of spherical starch grains with central and radiated hilum, simple or compound, with two to five units; druses of calcium oxalate or their fragments. Fibers and sclerids are absent.

IDENTIFICATION

A. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF_{254} silica gel, with 250 μm thickness as stationary phase and a mixture of petroleum ether and ethyl acetate and anhydrous formic acid (75:25:1) as mobile phase. To separately apply to the plate, as band, 20 μL of the *Solution (1)* and 10 μL of the *Solution (2)* freshly prepared, as described below.

Solution (1): to weigh about 50 mg of the powdered drug (250 μm), adding 1 mL of hydrochloric acid and 30 mL of water, heating under reflux in water for 15 minutes. To cool down in room temperature and extract with 25 mL of ethyl ether. To filter over anhydrous sodium sulfate. To evaporate the filtrate until residue. To dissolve the residue in 0.5 mL of ethyl ether.

Solution (2): emodin with 0.1% (w/v) in ethyl ether.

To develop the chromatogram. To remove the plate, and let it air drying. To observe under ultraviolet light (365 nm). The principal spot obtained in the chromatogram with *Solution (1)* corresponds in position and intensity to that obtained with *Solution (2)* (R_f around 0.50). The spot corresponding to emodin presents orange fluorescence. The chromatogram obtained with *Solution (1)* has other similar spots with fluorescence corresponding to aloe-emodin (R_f about 0.05) reigen (R_f about 0.12), fisiona (R_f about 0.80) and chrysophanol (R_f about 0.85). To nebulise the plate with potassium hydroxide with 10% (w/v) in methanol. All spots have reddish color.

B. To weigh 50 mg of powdered drug (250 μm), adding 25 mL of hydrochloric acid 2 *M*. To heat in water for 15 minutes. After to cool down, transfer the acidic solution to a separatory funnel and extract with 10 mL of ethyl ether. To decant the ether layer and stir it with 10 mL of ammonium hydroxide 6 *M*. It develops red color in the ammonia aqueous layer.

PURITY TESTS

Rhaponticin. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF_{254} silica gel with 250 μm thick as support, as stationary phase and a mixture of methanol and methylene chloride (20:80) as mobile phase. To separately apply to the plate, as band, 20 μL of the *Solution (1)* and 5 μL of the *Solution (2)* freshly prepared, as described below.

Solution (1): to weigh 0.2 g of the powdered drug and add 2 mL of methanol. To heat under reflux in water bath for 15 minutes. To cool down and filter it.

Solution (2): 1 mg/mL rhaponticin solution in methanol.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (365 nm). The chromatogram region obtained with *Solution (1)* should not exhibit dark blue spot near to the application point, indicating the presence of rhaponticin. To nebulise the plate with phosphomolybdic acid SR. The chromatogram region obtained with *Solution (1)* should not exhibit dark blue spot near to the application point, indicating the presence of rhaponticin.

Foreign material (5.4.2.2). At most 1.0%.

Water (5.4.2.3). At most 12.0 %.

Total ash (5.4.2.4). At most 13.0 %.

DOSAGE

Hydroxy anthracene derivatives

To proceed as described in *Visible absorption spectrophotometry (5.2.14)*. To prepare the solutions as described below.

Stock solution: in 50 mL round bottom flask to accurately weigh about 0.1 g of pulverized and desiccated drug. To add 30 mL of water, mixing and weighing it together. To heat under reflux in water bath for 15 minutes. To let it cool down and add 50 mg of sodium bicarbonate. To weigh and restore the original weight with water. To centrifuge and transfer 10 mL of the supernatant liquid to a 50 mL round bottom flask. To add 20 mL of ferric chloride SR and stir it. To heat the mixture under reflux for 20 minutes. Make it frequently homogeneous. To add 1 mL of hydrochloric acid and heating for 20 more minutes. To cool down and transfer to a separatory funnel. To extract with three successive 25 mL portions of diethyl ether, previously used to rinse the round bottom flask. To gather the ethereal extracts and washing with two portions of 20 mL of water, filter into a 100 mL volumetric flask and top up the volume with ethyl ether.

Sample solution: to evaporate 10 mL of *Stock solution* until residue. To resuspend the residue in 10 mL of 0.5% magnesium acetate (w/v) in methanol.

Blank solution: to use methanol.

To measure the absorbance of the *Sample solution* at 515 nm (5.2.14), immediately after preparation, by using Blank solution to adjust zero. To take into account for rhein $A(1\%, 1\text{ cm}) = 440$ at 515 nm in methanol. To calculate the content of hydroxy anthracene derivatives expressed in rhein, according to the equation:

$$\text{DHC} = \frac{A \times 0,68}{m}$$

where

DHC = hydroxy anthracene derivatives in %; A = measured absorbance;

m = mass of drug by considering the content of water determined (g).

PACKAGING AND STORAGE

In tightly closed container, protected from light and heat.

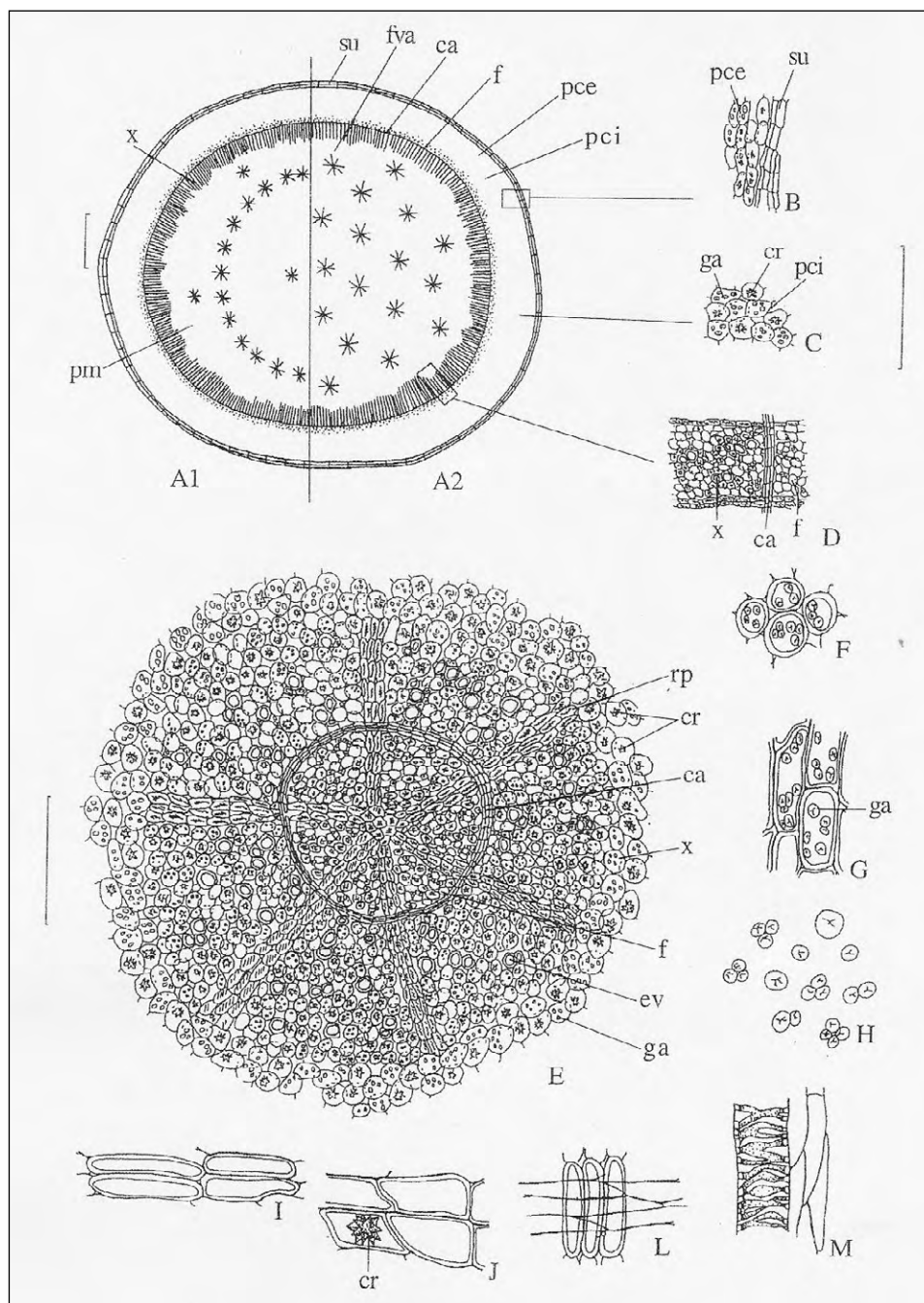


Figure 1 - Macroscopic and microscopic aspects of the powder in *Rheupalmatum* L. (A1) and *Rheum officinale* Baill. (A2)

Additional information of **Figure 1**. The scales correspond to **A, B, C, D and E** to 500 mm, in **F, G, H, I, J, L and M** to 100 mm.

A1 e A2 - partial schemes of rhizomes in cross section. Communication (ca), phloem (f), anomalous vascular bundle (fva), external cortical parenchyma (pce), internal cortical parenchyma (pci), medullary parenchyma (pm), storied cork (su). **B** - cross-section detail of the external cortex of the rhizome. External cortical parenchyma (pce), storied cork (su). **C** - cross-section detail of the cortical region. Crystal (cr), starch grain (ga), internal cortical parenchyma (pci). **D** - detail of the vascular region. Communication (ca), phloem (f), xylem (x). **E** - detail of the anomalous vascular system in cross section. Communication (ca), crystal (cr), vessel element (ev), phloem (f), starch grain (ga), parenchyma ray (rp), xylem (x). **F** - detail of parenchyma cells in cross section containing starch grains. **G** - detail of parenchyma cells in longitudinal section. Starch grain (ga). **H** - details of starch grains. **I** - detail of parenchyma ray cell in cross section. **J** - detail of parenchyma cells in cross section. crystal (cr). **L** - detail of radial parenchyma cells in cross section and associated with other parenchymal cells in radial longitudinal section. **M** - detail of vessel element with crosslinked thickening and parenchyma cells in longitudinal section.

ELDERBERRY

Sambucus nigra flos

Sambucus nigra L. - CAPRIFOLIACEAE

The vegetable drug consists of the dried flowers containing at least 1.5% of total flavonoid, it expressed in quercetin and in at least 1% of rutin.

CHARACTERISTICS

Organoleptic characteristics. The dried flowers have faint odor and characteristic aroma, faintly bitter taste.

MACROSCOPIC DESCRIPTION

Dried flowers, yellowed by desiccation, pentamerous or tetramerous, diclamideas, gamopetalas, actinomorphic, hermaphrodite, measuring 3.0 mm to 5.0 mm in diameter, each featuring up to three tiny green bracts, distributed in the pedicel, receptacle and/or base of the calice, at different heights, visible with magnifying glass. Bracts slightly papillose, with tector and glandular trichomes on the adaxial surside, with unicellular marginal teeth. Flower buds globose, whitish or brownish tints, measuring 1.5 mm to 3.0 mm in diameter. Calice with sepals whitish-yellow, brownish or greenish, triangular, measuring 0.5 mm to 1.2 mm in length and 0.5 mm to 0.7 mm wide at the basal portion, slightly welded together at the base and having unicellular marginal teeth. Rotated corolla, yellowish-white to light yellow, pre-flowering imbricated with petals welded together at the base into a short tube. Elliptical and oval petals, rounded apex, retrorso, measuring 2.0 mm to 3.5 mm in length and 2.0 mm to 3.0 mm wide. In fresh material the corolla easily falls off, showing appearance of five-pointed star. Androecium consisting of four or five stamens, the petals arranged alternately, with fillets attached to the corolla tube. Anthers dithecous, extrorsas, dorsifixed, oblong, dehiscent, yellow color, with 1.0 mm in length. Glabrous and cylindrical fillets, of 1.0 mm to 1.5 mm in length. Nether ovary, tube welded to the calicino, tricarpele, tetracarpele, rare trilocular, rare tetralocular with carpels well demarcated in dried flowers, with a wheat rudiment seminal per locule of axial placentation. Globular and papillose gynoecium with a short stylet and trilobed stigma. A ringed and prominent disk surrounds the base of the gynoecium.

MICROSCOPIC DESCRIPTION

Hypostomatic bracts, stomata of anomocytic type, homogenous mesophyll; in frontal view, the cuticle presents grooves that accompany the major axis of the epidermal cells, which contain some spherical lipid droplets; tectors and glandular trichomes occur throughout the blade and mainly at the base of the adaxial side;

rare idioblasts with blackened appearance containing crystals of calcium oxalate in the form of crystal sand are visible; in cross section, the striated cuticle is thick, the epidermis is uniseriate, the mesophyll has up to four

chlorenchyma layers of isodiametric cells and the vascular system usually consists of a single grouping xylem, which may be surrounded by endoderm, with no or few chloroplastids; spherical lipid droplets occur in all tissues except in the xylem. Receptacle in front view, with striated cuticle; cross-sectional presents uniseriate epidermis, parenchymatous tissue composed of up to twelve layers of isodiametric cells, vascular bundles of collateral type, distributed in the form of a ring for parenchymatous tissue; spherical lipid droplets occur in all tissues. Amphistomatic sepals with anomocytic type stomata, with three parallel veins, the cuticle, in front view, is heavily striated and epidermal cells have straight walls or nearly it; glandular and tectors trichomes are visible, plus idioblasts of blackish appearance, containing crystals of calcium oxalate in the form of crystal sand, in cross section, the epidermis is unistratified, the mesophyll is homogeneous, consisting of up to five layers of isodiametric cells, the vascular system is represented by one to three xylematic groups with up to five tracheary elements of helical thickening; spherical lipid droplets occur in all tissues. Amphihypostomatic petals with anomocytic type stomata, and with three, rarely four parallel veins, the secondaries starting from the primary, linear or branched, glandular and tectors trichomes occur mainly on the adaxial surside; idioblasts of blackish appearance containing oxalate crystals of calcium in form of crystalline sand are visible on both sides; cuticle in front view is more striated on the abaxial side and less striated on the adaxial side, the epidermis is unistratified, with papillary cells, papillae less prominent in the regions of the edges, the mesophyll is homogeneous, composed of up to ten layers of loose parenchyma, the vascular system is represented by three to six collateral vascular bundles; lipid droplets are present in all tissues; ellipsoidal starch grains are present in the parenchyma. The fillet in front view, has striated cuticle; cross-section has a circular shape, the epidermis is unistratified, and without stomata, parenchyma is loose, devoid of chloroplastids and few lipid droplets and the vascular system is formed by tracheal elements of helical thickness. The anther, in cross section, has very papillae epidermis, the carpet is unistratified and the endothecium is formed by two or three layers of fibrous cells with evident punctuations. The pollen grain is prolate, tricolporate with 15µm to 25µm in diameter, with a reticulate surside in rounded polar view and ellipsoidal in equatorial view. The gynoecium is formed by three carpels, rarely four and each cavity has a seminal rudimentary; in cross section, the parenchymatous tissue of the carpel wall is compact formed by cells rich in chloroplastids and lipid droplets and the vascular bundles are distributed in the ring; the innermost parenchyma is devoid of chloroplastids and presents thickening evident on all walls; epidermal cells of the stigma are extremely papillose.

MACROSCOPIC DESCRIPTION OF IMPURITIES

The pedicels of their own species are considered strangers; they are whitish by desiccation, longitudinally grooved, measuring 1.0 mm to 7.0 mm in length, with tectors and glandular trichomes.

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MICROSCOPIC DESCRIPTION OF IMPURITIES

The pedicel in front view, presents striated cuticle, rectangular epidermal cells, anomocytic stomata and in the basal portion, glandular and tector trichomes, in cross section, it presents sharp protrusions and recesses, striated cuticle uniseriate epidermis with tabular cells and thick inner periclinal walls; in the cortical region occurs one to six layers of tabular collenchyma followed by an intercellular spaces parenchyma, vascular system is formed up to sixteen collateral bundles arranged in a ring shape, medullary region is filled with parenchyma with thin walls cells; chloroplasts occur in the parenchyma; lipid droplets occur in the epidermis and cortical parenchyma; starch grains are observed in the endodermis and phloem.

MICROSCOPIC DESCRIPTION OF POWDER

The powder meets all the requirements for the flowers of this species, unless the macroscopic characters. Characteristics are: greenish-yellow; sepals fragments with marginal teeth unicellular isolated; fragments of sepals epidermis and papillary petals and striated cuticle; fragments of epidermis with anomocytic stomata; isolated guard cells; fragments of epidermis with tector trichomes from different types; rare isolated glandular and tectors trichomes or parts thereof; fragments of parenchyma; portions of tissues with lipid droplets, part of tracheary elements of helical thickness, fragments of anther epidermis extremely papillose; fragments of fibrous layer of anther; numerous grains of pollen as described, pollen grains isolated or in groups or associated with anther fragments and the epidermis of several parts, stigma portions with papillae epidermis; bracts portions, sepals edge portions of the petals and bracts.

IDENTIFICATION

A. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel, with 250 µm thickness as stationary phase and a mixture of ethyl acetate, formic acid, acetic acid and water (100:11:11:27) as mobile phase. To separately apply, as band, 10 µL of *Solution* (1), 10 µL of

Solution (2), recently prepared, as described below.

Solution (1): to transfer about 0.5 g of the ground drug to 100 mL round bottom flask, adding 5 mL of methanol. To heat under reflux in water bath for 30 minutes. To filter it by using filter paper.

Solution (2): to dissolve the amount of 5 mg of rutin SQR, hyperoside RSS, isoquercitrin SSR, chlorogenic acid in methanol to obtain 1 mg/mL solution.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (365 nm). In the chromatogram obtained with *Solution* (1) near the front appears a bluish fluorescent spot for the chlorogenic acid. Then, nebulise the plate with anisaldehyde SR and

put it in an oven at 100 °C - 105 °C for 5 to 10 minutes. The chromatogram obtained with *Solution* (2) has violet color spots corresponding to rutin (Rf about 0.49), hyperoside (Rf about 0.68) and isoquercitrin (Rf about 0.72). The chromatogram obtained with *Solution* (1) shows similar spots in the position and color to the spots obtained in the chromatogram of *Solution* (2). Other spots of lower intensity can be observed.

B. To proceed as described in *High performance liquid chromatography* (5.2.17.4). To use the system described in *Dosage for Rutin*. The major chromatogram peak corresponding to rutin; it is observed a peak at lower retention time with characteristics of caffeoylquinic acid and four peaks after rutin, both of which are promptly after the ultraviolet absorption spectrum similar to that rutin and other two in the sequence, with absorption spectrum and caffeoylquinic acid characteristic.

PURITY TESTS

Foreign material (5.4.2.2). At most of 8% of gross pedicels and other foreign material, and at most 15% of the sample with changed color (blackened).

Water (5.4.2.3). At most 11 %.

Total ash (5.4.2.4). At most 9%.

DOSAGE

Total flavonoid

To proceed as described in *Visible absorption spectrophotometry* (5.2.14). To prepare the solutions as described below.

Stock solution: to accurately weigh about 0.1 g of pulverized drug (800 µm) and put into a 100 mL round bottom flask. To add 0.25 mL of 0.5% aqueous methenamine solution, 10 mL of acetone, and 0.5 mL of hydrochloric acid. To heat under reflux in water bath for 30 minutes. To filter the mixture to a 25 mL volumetric flask. To take up the residue of the drug and the cotton in the same round bottom flask, adding 7 mL of acetone. To heat under

reflux for 10 minutes. To use cotton again to filter to the same 25 mL volumetric flask. To repeat the operation, returning to the residue of the drug and cotton to a round bottom flask, add 7 mL of acetone and heat it under reflux for 10 minutes. To filter into the same flask of 25 mL. After cooling to room temperature adjust the volume to 25 mL with acetone. In a separatory funnel, add 10 mL of this solution and 10 mL of water, and after extracting with 10 mL of ethyl acetate, repeat two times with portions of 6 mL of ethyl acetate. To gather the ethyl acetate phases in separatory funnel and wash them with two portions of 15 mL of water. To transfer the organic phase in the sequence to 25 mL volumetric flask and top up the volume with ethyl acetate.

Sample solution: to transfer 10 mL of Stock solution to 25 mL volumetric flask, add 1 mL of aluminum chloride, 2% (w/v) in methanol and top up the volume with 5% acetic acid solution (w/v) in methanol.

Blank solution: to transfer 10 mL of Stock solution to 25 mL volumetric flask and top up with 5% acetic acid solution (w/v) in methanol.

To measure the absorbance of the *Sample solution* at 425 nm (5.2.14), 30 minutes after preparation, by using *Blank solution* to adjust zero. To calculate the content of total flavonoids, calculated as quercetin, according to the expression:

$$Q = \frac{A \times 15625}{500 \times m \times (100 - Pd)}$$

where

Q = total flavonoid content, expressed as quercetin (%); A = absorbance of the sample solution; m = mass of the vegetable drug; Pd = determination of water (%).

Rutin

To proceed as described in *High performance liquid chromatography* (5.2.17.4). To use the chromatograph equipped with an ultraviolet detector at 356 nm; pre-column packed with silica chemically bonded to the octadecylsilane group (3 to 10 μ m); 150 mm column and 3.9 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (4 μ m); kept at room temperature; flow of *Mobile phase* of 0.7 mL/min.

Eluent A: mixture of acetonitrile, water and trifluoroacetic acid (5:95:0.01).

Eluent B: mixture of acetonitrile and trifluoroacetic acid (100:0.01).

Mobile phase gradient: to adopt gradient system described in the following table.

Time (minutes)	Eluent A (%)	Eluent B (%)	Elution
0 – 7	90 → 70	10 → 30	linear gradient
7 – 8	70 → 0	30 → 100	linear gradient
8 – 11	0	100	isocratic
11 – 12	0 → 90	100 → 10	linear gradient
12 – 18	90	10	isocratic

Sample solution: to accurately weigh, about 0.25 g of dried and milled drug (800 μ m) and put in a glass bottle, stir per turbolise, speed 3 for 5 minutes with 5 mL of ethanol with 80 % (v/v). To filter through filter paper under vacuum to 5 mL volumetric flask and top up the volume with the same solvent. To filter through a membrane and dilute 50 μ L in 950 μ L of acetonitrile: water (1:9).

Standard stock solution: to dissolve 5 mg of rutin SQR in 10 mL of methanol.

Solutions for analytical curve: to dilute an aliquot of 2.5 mL of *standard stock solution*, in 25 mL volumetric flask to obtain a 50 pg/mL. To dilute aliquots of 1 mL, 1.5 mL, 2 mL, 2.5 mL, 3 mL, 3.5 mL, 4 mL and 4.5 mL in 5 mL volumetric flask with methanol to obtain concentrations of 10 μ g/mL, 15 μ g/mL, 20 pg/mL, 25 pg/mL, 30 pg/mL, 35 μ g/mL, 40 pg/mL and 45 pg/mL.

Procedure: to separately inject 10 μ L of Solutions for Analytical curve and Sample solution. To record the chromatograms and measure the areas under the peaks. The retention time is approximately 5 minutes to stevioside. To calculate the rutin content in the sample, from the linear equation obtained with analytical curve. The result is expressed as the mean of the determinations in grams for rutin per 100 g of drug (%), by taking into account the water content.

PACKAGING AND STORAGE

In tightly closed glass container, protected from light, heat and humidity.

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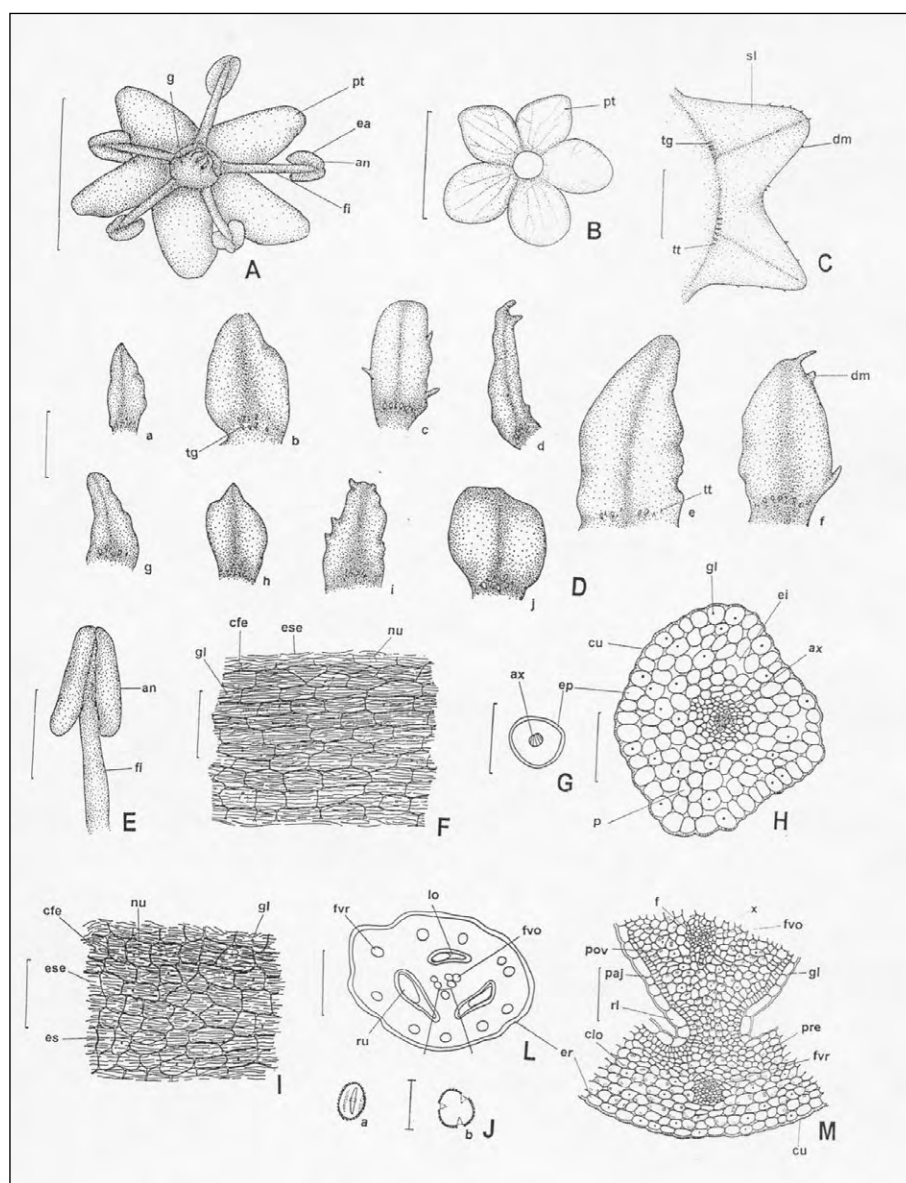


Figure 1 - Macroscopic and microscopic aspects of *Sambucus nigra* L.

Additional information of **Figure 1**. The rules correspond in **A** at 3,0 mm; in **B e E** at 5,0 mm; in **C** at 1,0 mm; in **D e G** at 0,4 mm; in **F, H, I** and **M** at 100 µm; in **J** at 30 µm; in **L** with 400 µm.

A - general appearance of the flower in front view; anther (an); stamen (ea); fillet (fi); gynoecium (g); petal (en). **B** - general appearance of the detached corolla in front view; petal (pt). **C** - general appearance of the calyx, in front view; marginal tooth (dm); sepal (sl); glandular trichomes (tg); tector trichomes (tt). **D** - general appearance of the bract adaxial side, in front view, showing its different forms: (a, b, e, f, i) elliptic bracts, (c) oblong bract, (d) laminar bract (g) triangular bract, (h, j) elliptic-obovate bracts; (dm) marginal tooth; (tg) glandular trichomes; (tt) tector trichomes. **E** - general appearance of the stamen in a lateral position, (na) anther, (fi) fillet. **F** - detail of the fillet's epidermis portion, in front view, the fundamental cell of the epidermis (cfe); epicuticular striations (esse); lipid droplet (gl); nucleus (nu). **G** - general scheme of the fillet, in cross section, grouping xylem (ax); epidermis (ep). **H** - fillet detail in cross section; grouping xylem (ax); striated cuticle (cu); intercellular space (ei); epidermis (ep), lipid droplet (gl); parenchyma (p). **I** - detail of the receptacle's epidermis portion, in front view, the fundamental cell of the epidermis (cfe); stomata (es); epicuticular striations (esse); lipid droplet (gl); nucleus (nu). **J** - general scheme of pollen grain; a: polar view, b: equatorial view. **L** - general scheme of the receptacle and ovarian in cross section; epidermis of the receptacle (er); vascular bundle of the ovary (fvo); vascular bundle of the receptacle (fvr); locule (lo); seminal rudiment (ru). **M** - detail of the receptacle and ovarian portion, in cross section, as outlined in **L**; chloroplast (clo); striated cuticle (cu); epidermis of the receptacle (er); phloem (f); vascular bundle of the ovary (fvo); vascular bundle of the receptacle (fvr); lipid droplet (gl); parenchyma of juxtaposed cells (paj), ovarian parenchyma (pvo); parenchyma of the receptacle (pre); coating of the locule (rl), xylem (x).

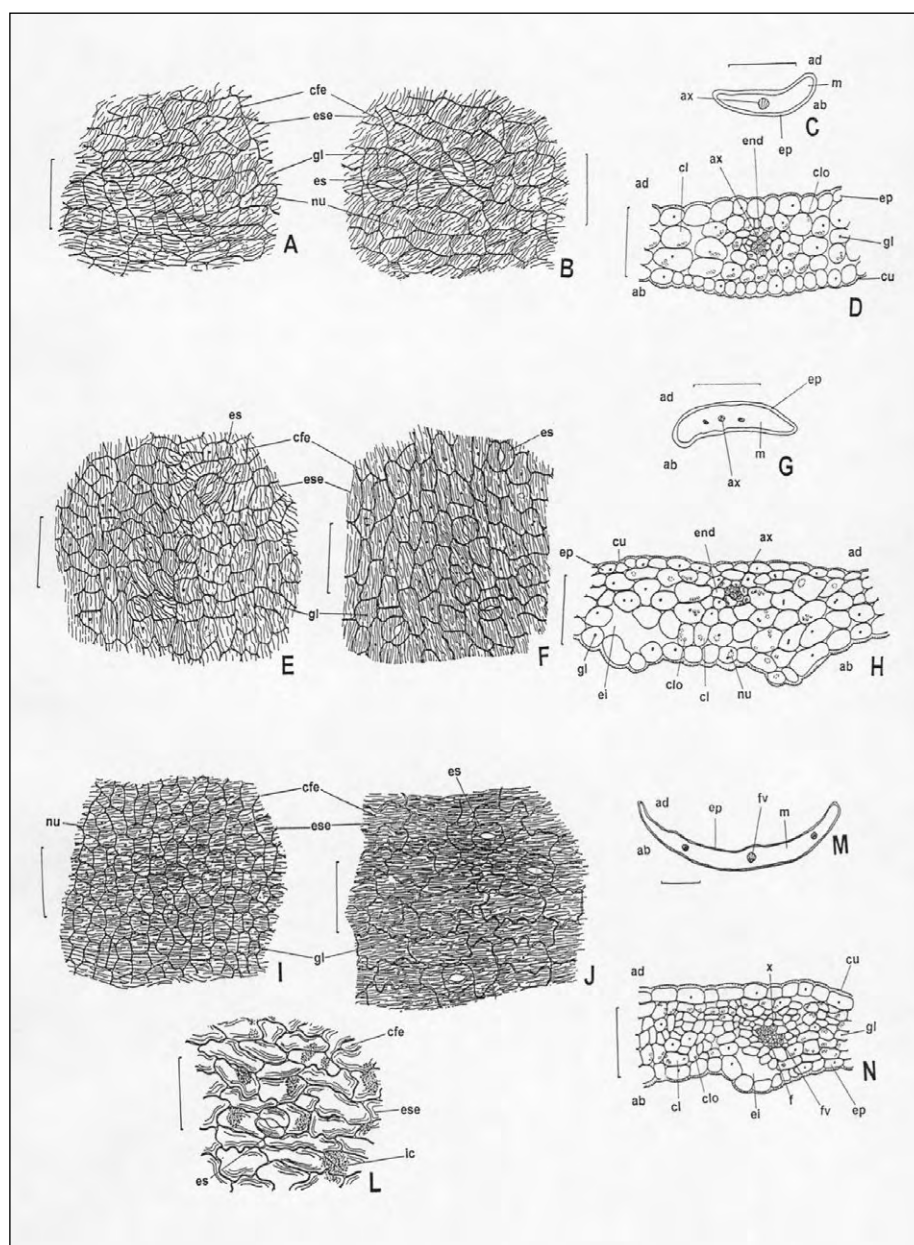
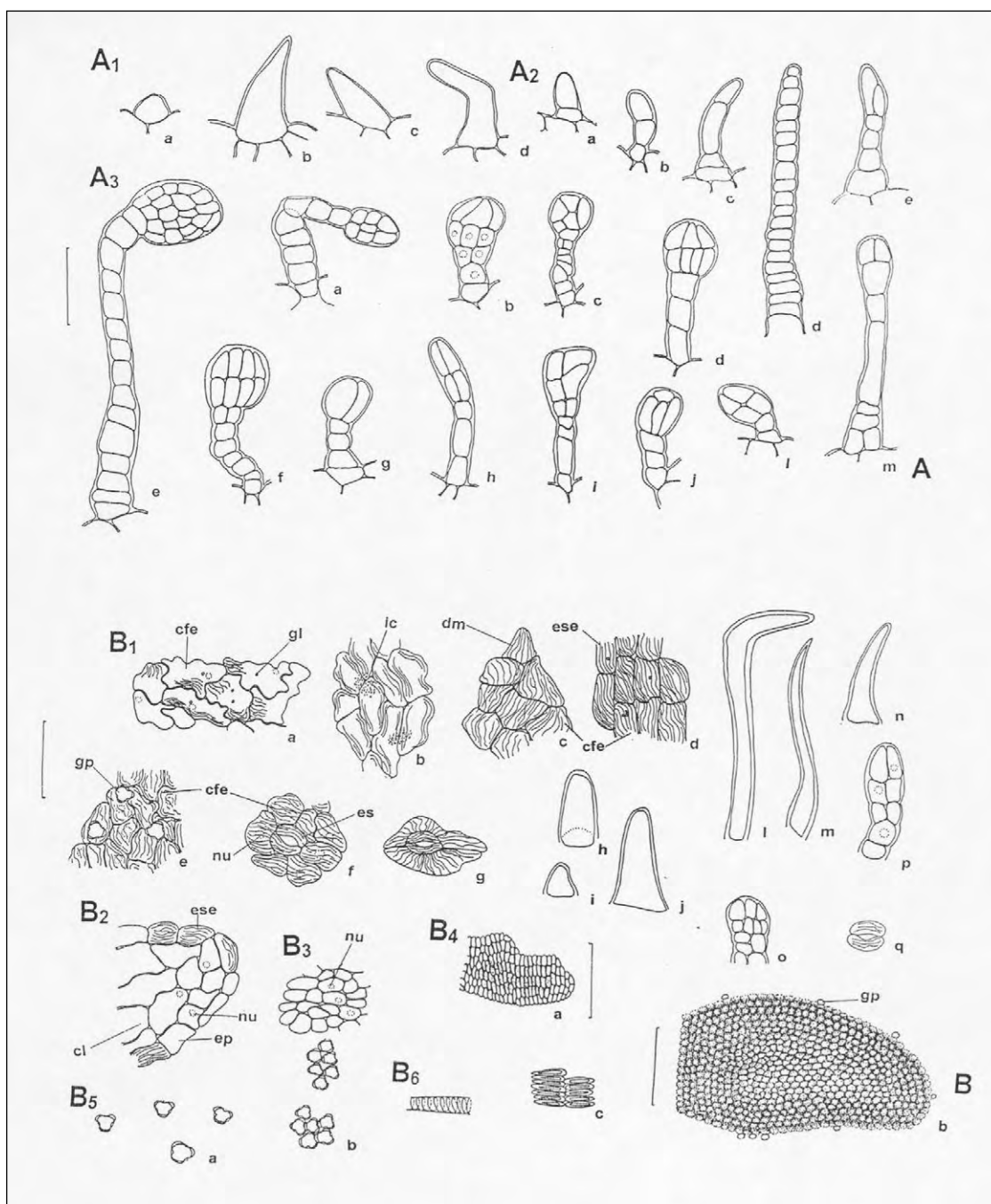


Figure 2 - Macroscopic and microscopic aspects of *Sambucus nigra* L.

Additional information of **Figure 2**. The rules correspond to **A, B, D, E, F, H, and I**- and **N** a 100 µm; in **C, G** and **M** 0,4 mm.

A - detail of the portion of the adaxial epidermis of the bract, in front view, the fundamental cell of the epidermis (cfe); epicuticular striations (esse); lipid droplet (gl); nucleus (nu). **B** - detail of the portion of the abaxial epidermis of the bract, in front view, the fundamental cell of the epidermis (cfe); stomata (es); epicuticular striations (esse); lipid droplet (gl); nucleus (nu). **C** - general scheme of bract, in cross section; abaxial side (ab); adaxial side (ad); grouping xylem (ax); epidermis (ep), mesophyll (m). **D** - detail of the main nerve region of the bract in cross-section; abaxial side (ab); adaxial side (ad); grouping xylem (ax); chlorenchyma (cl); chloroplast (clo); cuticle (cu); endoderm (end); epidermis (ep); lipid droplet (gl). **E** - detail of the portion of the adaxial epidermis of the sepal, in front view, the fundamental cell of the epidermis (cfe); stomata (es); epicuticular striations (ese); lipid droplet (gl); nucleus (nu). **F** - detail of the portion of the abaxial epidermis of the sepal, in front view, the fundamental cell of the epidermis (cfe); stomata (es); epicuticular striations (ese); lipid droplet (gl). **G** - general scheme of sepal, in cross section; abaxial side (ab); adaxial side (ad); grouping xylem (ax); chlorenchyma (cl); chloroplast (clo); cuticle (cu); intercellular space (ei); endoderm (end); epidermis (ep); lipid droplet (gl), nucleus (nu). **H** - detail of the sepal portion in the main nerve region, in cross-section; abaxial side (ab); adaxial side (ad); grouping xylem (ax); chlorenchyma (cl); chloroplast (clo); cuticle (cu); intercellular space (ei); endoderm (end); epidermis (ep); lipid droplet (gl), nucleus (nu). **I** - detail of the portion of the adaxial epidermis of the petal, in front view, the fundamental cell of the epidermis (cfe); epicuticular striations (esse); lipid droplet (gl); nucleus (nu). **J** - detail of the portion of the abaxial epidermis of the petal, in front view, the fundamental cell of the epidermis (cfe); stomata (es); epicuticular striations (ese); lipid droplet (gl). **L** - detail of the portion of the abaxial epidermis of the petal, in front view, the fundamental cell of the epidermis (cfe); stomata (es); epicuticular striations (ese); crystalliferous idioblasto (ic). **M** - general scheme of the petal, in cross section; abaxial side (ab); adaxial side (ad); epidermis (ep), vascular bundle (fv); mesophyll (m). **N** - detail of the petal portion in the main nerve region, in cross-section; abaxial side (ab); adaxial side (ad); chlorenchyma (cl); chloroplast (clo); striated cuticle (cu); intercellular space (ei); epidermis (ep); phloem (f); vascular bundle (fv); lipid droplet (gl), xylem (x).



Additional information of **Figure 3**. The rules correspond in **A and B (B1 - B3, B4c-B6)** 100 μm ; in **B (B4a and b)** to 400 μm .

A - detail of trichomes occurring in bracts, sepals and petals; **A1**. Unicellular tector trichomes; **A2**. Multicellular tector trichomes; **A3**. Glandular trichomes. **B** - powder details. **B1**. (a-q): portions of the epidermis, (a-g): fragments of epidermis in front view; fundamental cell of the epidermis (cfe); marginal tooth (dm); stomata (es); epicuticular striations (ese), lipid droplet (gl), pollen grain (gp); crystalliferous idioblasto (ic); nucleus (nu), (h-j) portions of unicellular tector trichomes; (l-n) portions of marginal teeth; (o-p) portions of glandular trichomes with multicellular head; (q) isolated guard cells; **B2**. edge portion of the petal; epidermis (ep); epicuticular striations (ese); chlorenchyma (cl); nucleus (nu); **B3**. fragment of parenchyma; nucleus (nu); **B4**. fragments of anther, (a) concave portion, (b) the convex portion (c) fragment of the anther's fibrous layer, pollen grain (gp); **B5**. Pollen grains, (a) isolated, (b) grouped; **B6**. Portion of tracheal element with helical thickening.

ELDERBERRY, BRAZILIAN

Sambucus australis flos

Sambucus australis Cham. & Schltdl. - CAPRIFOLIACEAE

The vegetable drug consisted by the dried flowers containing at least 2.0% of total flavonoid, it expressed in quercetin and in at least 0.80% of rutin.

CHARACTERISTICS

Organoleptic characteristics. Dried flowers have light and characteristic aromatic odor; slightly bitter flavor.

MACROSCOPIC DESCRIPTION

Dried flowers, yellowed by desiccation, pentamerous or tetramerous, diclamideas, gamopetalas, actinomorphic, staminate with long stamens or pistillate, with short stamens, measuring 7.0 mm to 10.0 mm in diameter, each featuring up to three tiny green bracts, distributed in the pedicel, receptacle and/or base of the calice, at different heights, visible with magnifying glass. Papillary bracts with glandular and tector trichomes on the basal portion of the adaxial side. Flower buds globose, whitish or brownish tints, measuring 1.0 mm to 3.0 mm in diameter. Calice with greenish yellow sepals, triangular - oval, measuring 1.0 mm to 1.5 mm long and 1.0 mm wide in the basal portion, lightly welded together at the base, with glandular and tector trichomes abundant in the basal portion the adaxial side and without marginal teeth. Rotated corolla, yellowish-white to white, pre-flowering imbricated with petals welded together at the base into a short tube. Elliptical and oval petals, rounded apex, retrorso, measuring 2.5 mm to 5.0 mm in length and 1.5 mm to 3.0 mm wide. In fresh material the corolla easily falls off, showing appearance of five-pointed star. Androeceum consisting of four or five stamens, short and long, the petals arranged alternately, with fillets attached to the corolla tube. Anthers dithecous, extrorsas, dorsifixed, oblong, dehiscent in the staminate flowers and indehiscent in the pistillate flowers, yellow, with 1.0 mm in length. Hairless and cylindrical fillets, short in pistillate flowers, with 1.0 mm to 2.0 mm in length and long in the staminate flowers with 3.0 mm to 4.0 mm in length. Nether ovary, tube welded to the calicino, pentacarpelar or tetracarpelar, rare trilocular, pentalocular or tetralocular, rare trilocular with carpels well demarcated in dried flowers, with a wheat rudiment seminal per locule of axial placentation. Globular and papillose gynoecium with a short stylet and pentalobed stigma. A ringed and prominent disk surrounds the base of the gynoecium.

MICROSCOPIC DESCRIPTION

Amphistomatic and stomata bracts from anomocytic type, homogeneous mesophyll, in front view, the cuticle has striations that accompany the major axis of the epidermal cells, which contain abundant lipid droplets

Spherical, glandular and tector trichomes occur on the basal adaxial side, in cross section, the cuticle is thickened and

striated, the epidermis is uniseriate, the mesophyll has up to four layers of chlorenchyma of isodiametric cells and the vascular system is composed of one to four vascular bundles or xylem groups, with or without endoderm, without or presenting few chloroplast; spherical lipid droplets occur in all tissues except in the xylem. Receptacle in front view, with slightly striated cuticle and glandular trichomes in the insertion area of the bract, in cross section, and having uniseriate epidermis, parenchymatous tissue composed of up to twelve layers of isodiametric cells, vascular bundles of collateral type, distributed in the form of a ring for parenchymatous tissue; spherical lipid droplets occur in all tissues. Hypostomatic sepals with anomocytic type stomata, with three parallel veins, the cuticle, in front view, is heavily striated and epidermal cells have sinuous walls; glandular and tectors trichomes are visible, lack of calcium oxalate idioblasts, in cross section, the epidermis is unistratified, the mesophyll is homogeneous, consisting of two up to seven layers of isodiametric cells, the vascular system is represented by one to three xylematic groups with up to five tracheary elements of helical thickening; spherical lipid droplets occur in all tissues. Amphihypostomatic petals with anomocytic type stomata, and with five, rarely four parallel veins, the secondaries starting from the primary, linear or branched, glandular and tectors trichomes occur mainly on the adaxial surside; idioblasts of blackish appearance containing oxalate crystals of calcium in form of crystalline sand are visible on both sides; cuticle in front view is very striated on the abaxial side and less striated on the adaxial side, the epidermis is unistratified, with papillary cells, papillae less prominent in the regions of the edges, the mesophyll is homogeneous, composed of up to twelve layers of loose parenchyma, the vascular system is represented by three to six collateral vascular bundles; lipid droplets are present in all tissues; ellipsoidal starch grains are present in the parenchyma of the conduction tissues. The fillet, in front view, presents striated cuticle; cross-section has a circular shape, the epidermis is unistratified, and without stomata, parenchyma is loose, devoid of chloroplastids and few lipid droplets and the vascular system is formed by tracheal elements of helical thickness. The anther, in cross section, has papillae epidermis, the carpet is unistratified and the endothecium is formed by two or three layers of fibrous cells with evident punctuations. The pollen grain is prolate, tricolporate with 18 pm to 34 pm in diameter, with a reticulate surside in rounded polar view and ellipsoidal in equatorial view. The gynoecium is formed by five, four or rare three carpels and each cavity has a seminal rudimentary; in cross section, the parenchymatous tissue of the carpel wall is compact formed by cells rich in chloroplastids and lipid droplets and the vascular bundles are distributed in the ring; the innermost parenchyma is devoid of chloroplastids and presents thickening evident on all walls; epidermal cells of the stigma are extremely papillose.

MICROSCOPIC DESCRIPTION OF POWDER

The powder meets all the requirements for the flowers of this species, unless the macroscopic characters. Characteristics are: greenish-yellow; sepals fragments of epidermis

with striated cuticle of sepals or papillary petals; fragments of anomocytic stomata; isolated guard cells; fragments of epidermis with tector trichomes from different types; rare isolated glandular and tectors trichomes or parts thereof; fragments of parenchyma; portions of tissues with lipid droplets, part of tracheary elements of helical thickness; fragments of anther epidermis extremely papillose; fragments of fibrous layer of the anther; numerous grains of pollen as described, pollen grains isolated or in groups or associated with anther fragments and the epidermis of several parts, stigma portions with papillae epidermis; bracts portions, sepals edge portions of the petals and bracts.

MACROSCOPIC DESCRIPTION OF IMPURITIES

The pedicels of their own species are considered strangers; they are whitish by desiccation, longitudinally grooved, measuring 1.0 mm to 6.0 mm in length, with tectors and glandular trichomes.

MICROSCOPIC DESCRIPTION OF IMPURITIES

The pedicel in front view, presents striated cuticle, rectangular epidermal cells, anomocytic stomata and in the basal portion, glandular and tector trichomes, in cross section, it presents sharp protrusions and recesses, striated cuticle uniseriate epidermis with tabular cells and thick inner periclinal walls; in the cortical region may occur one layer of tabular collenchyma followed by an intercellular spaces parenchyma, vascular system is formed up to twelve collateral bundles arranged in a ring shape, medullary region is filled with parenchyma with thin walls cells; chloroplasts occur in the parenchyma; lipid droplets occur in the epidermis and cortical parenchyma; starch grains and lipid droplets occur in all tissues.

IDENTIFICATION

A. To proceed as described in Thin-layer chromatography (5.2.17.1) by using GF254 silica gel, with 250 µm thickness as support and a mixture of ethyl acetate, formic acid, acetic acid and water (100:11:11:27) as mobile phase. To separately apply to the plate, as band, 10 µl of each of the solutions freshly prepared, as described below.

Solution (1): to transfer about 0.5 g of the ground drug to 100 mL round bottom flask and add 5 mL of methanol. To heat under reflux in water bath for 30 minutes. To filter it by using filter paper.

Solution (2): to dissolve the amount of 5 mg of rutin SQR, hyperoside SQR, isoquercitrin SQR, and the chlorogenic acid in methanol to obtain 1 mg/mL solution.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (366 nm). The blue color fluorescent spot obtained with Solution (1) near the forehead, refers to chlorogenic acid. Then, to nebulise the plate with sulphuric anisaldehyde solution and put it in an oven at 100 °C - 105 °C for 5 to 10 minutes. The violet spots obtained with *Solution (2)* corresponding to rutin (Rf

about 0.49), the hyperoside (Rf about 0.68) and isoquercitrin (Rf about 0.72) corresponds in position and color to those obtained with Solution (1). Other spots of lower intensity can be observed.

B. To proceed as described in *Dosage* for *Rutin*. The major chromatogram peak corresponding to rutin; it is observed a peak with lower retention time with characteristics of caffeoylquinic acid and three peaks after rutin, and all of them presenting absorption spectrum in ultraviolet similar to rutin.

PURITY TESTS

Foreign material (5.4.2.2). At most 8% of gross pedicels and other foreign materials. At most 15% of the sample with changed color (blackened).

Water (5.4.2.3). At most 10%.

Total ash (5.4.2.4). At most 9.4%.

DOSAGE

Total flavonoid

To proceed as described in *Visible absorption spectrophotometry (5.2.14)*. To prepare the solutions as described below.

Stock solution: to accurately weigh about 0.1 g of pulverized drug (800 µm) and put into a 100 mL round bottom flask. To add 0.25 mL of 0.5% aqueous methenamine solution (w/v), 10 mL of acetone, and 0.5 mL of hydrochloric acid. To heat under reflux in water bath for 30 minutes. To filter the mixture to a 25 mL volumetric flask. To take up the residue of the drug and the cotton in the same round bottom flask, adding 7 mL of acetone. To heat to boiling under reflux for 10 minutes. To use cotton again to filter to the same 25 mL volumetric flask. To repeat the operation, returning to the residue of the drug and cotton to a round bottom flask, add 7 mL of acetone and heat it under reflux for 10 minutes. To cool down to the same 25 mL volumetric flask. After cooling to room temperature adjust the volume to 25 mL with acetone. In a separatory funnel, add 10 mL of this solution and 10 mL of water and 10 mL of ethyl acetate, extracting and repeating the process two more times, but using 6 mL of ethyl acetate. To gather the ethyl acetate phases and wash them in separatory funnel with two portions of 15 mL of water and transferring it to 25 mL volumetric flask. To top up the volume with ethyl acetate.

Sample solution: to transfer 10 mL of Stock solution to 25 mL volumetric flask, add 1 mL of aluminum chloride solution, 2% (w/v) and top up the volume with 5% acetic acid solution (w/v) in methanol.

Blank solution: to transfer 10 mL of *Stock solution* to 25 mL volumetric flask and top up with 5% acetic acid solution (w/v) in methanol.

To measure the absorbance of the Sample solution at 425 nm, 30 minutes after preparation, by using Blank solution to adjust zero. To calculate the content of total flavonoids, calculated as quercetin, according to the expression:

$$Q = \frac{A \times 15625}{500 \times m \times (100 - Pd)}$$

where

Q = total flavonoid content, expressed as quercetin (%);

A = absorbance of the *Sample solution*

m = mass of the vegetable drug,

Pd = water content (%).

Rutin

To proceed as described in *High performance liquid chromatography (5.2.17.4)*. To use the chromatograph equipped with an ultraviolet detector at 356 nm; pre-column packed with silica chemically bonded to the octadecylsilane; 150 mm column and 3.9 mm inner diameter, packed with silica chemically to the octadecylsilane group (4 µm); kept at room temperature; flow of *Mobile phase* of 0.7 mL/minute.

Eluent A: mixture of acetonitrile, water and trifluoroacetic acid (5:95:0.01).

Eluent B: mixture of acetonitrile and trifluoroacetic acid (100:0.01).

Mobile phase gradient: to adopt gradient system described in the following table.

<i>Time (minutes)</i>	<i>Eluent A (%)</i>	<i>Eluent B (%)</i>	<i>Elution</i>
0 – 7	90 → 70	10 → 30	linear gradient
7 – 8	70 → 0	30 → 100	linear gradient
8 – 11	0	100	isocratic
11 – 12	0 → 90	100 → 10	linear gradient
12 – 18	90	10	isocratic

Sample solution: to accurately weigh, about 0.25 g of dried and milled drug (800 µm) and put in a glass bottle and stir per turbolise, speed 3 for 5 minutes with 5 ml of ethanol with 80 % (v/v). To filter through filter paper under vacuum to 5 mL volumetric flask and top up the volume with the same solvent. To filter through a thin membrane and dilute 50 µL in 0.95 mL of acetonitrile and water mixture (1:9).

Standard stock solution: to dissolve 5 mg of rutin SQR in 10 mL of methanol.

Solutions for analytical curve: to dilute an aliquot of 2.5 mL of *standard stock solution*, in 25 mL volumetric flask to obtain a 50 µg/mL. To dilute aliquots of 1 mL, 1.5 mL, 2 mL, 2.5 mL, 3 mL, 3.5 mL, 4 mL and 4.5 mL in 5 mL volumetric flask with methanol in order to obtain concentrations of 10 µg/mL, 15 µg/mL, 20 µg/mL, 25 µg/mL, 30 µg/mL, 35 µg/mL, 40 µg/mL and 45 µg/mL.

Procedure: to separately inject 10 µL of *Solutions for analytical curve* and *Sample solution*. To record the chromatograms and measure the areas under the peaks. The retention time is approximately 5 minutes to rutin. To calculate the rutin content in the sample, from the linear equation obtained with analytical curve. The result is expressed as the mean of the determinations in grams for rutin per 100 g of drug (%), by taking into account the water content.

PACKAGING AND STORAGE

In tightly closed glass container, protected from light, heat and humidity.

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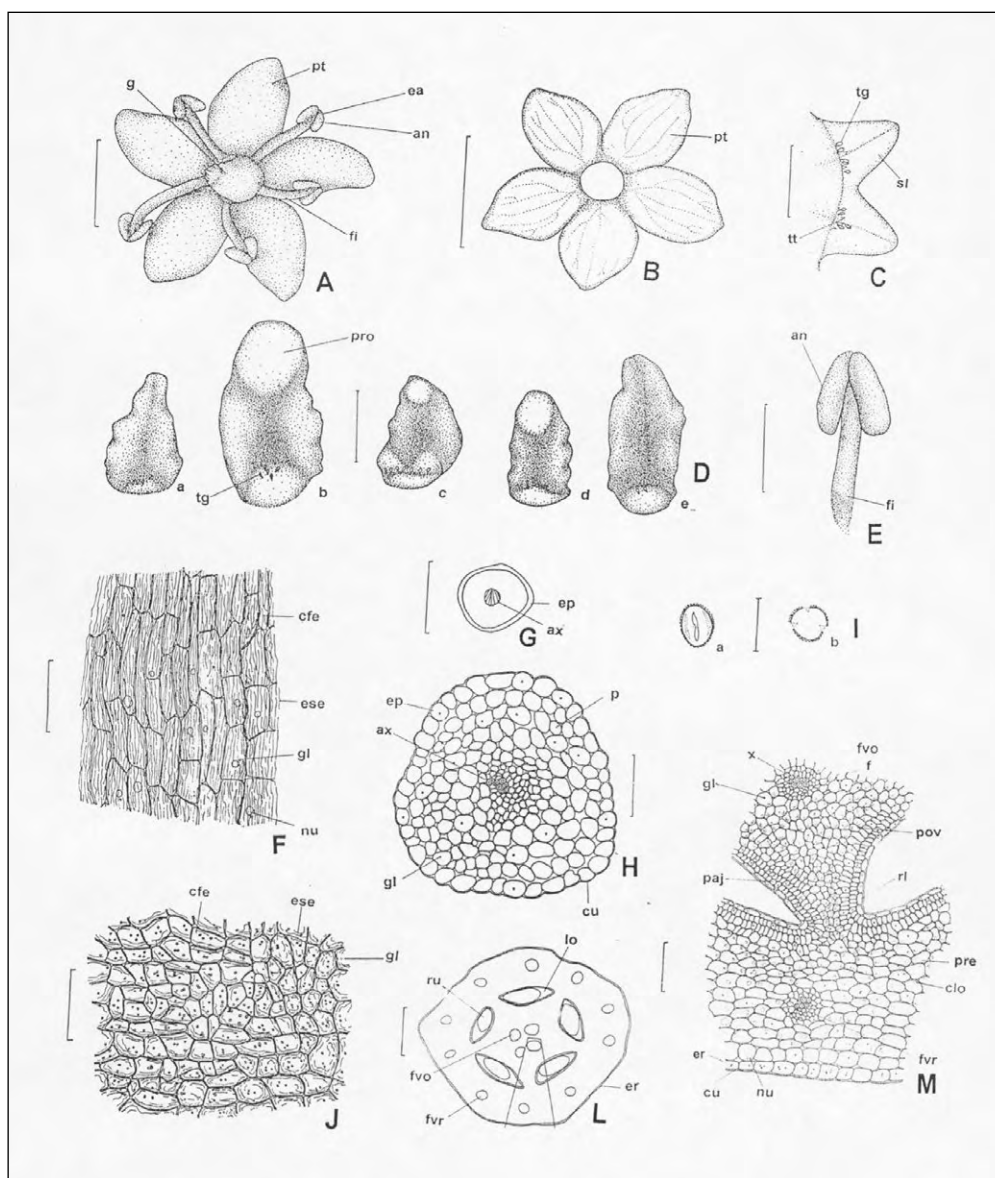


Figure 1 - Macroscopic and microscopic aspects of *Sambucus australis* Cham. & Schltdl.

Additional information of **Figure 1**. The rules correspond in **A** and **E** to 2,5 mm; in **B** to 5 mm; in **C** and **D** to 1,0 mm; in **F**, **H**, **J** and **M** to 100 µm; in **G** and **L** to 400 µm; in **I** to 30 µm.

A - general appearance of the staminate flower in front view; anther (an); stamen (ea); fillet (fi); gynoecium (g), petal (pt). **B** - general appearance of the detached corolla in front view; petal (pt). **C** - general appearance of the calyx, showing the adaxial side of its sepals in front view; sepal (sl); glandular trichomes (tg); tector trichomes (tt). **D** - general appearance of the bract adaxial side, in front view, showing its different forms: triangular bract (a); elliptical bracts (b, c); oblong bracts (d, e); apical prominence (pro); glandular trichomes (tg). **E** - general appearance of the stamen in a lateral position, anther (an), fillet (fi). **F** - detail of the fillet's epidermis portion, in front view; the fundamental cell of the epidermis (cfe); epicuticular striations (ese); lipid droplet (gl); nucleus (nu). **G** - general scheme of the fillet, in cross section: epidermis (ep); grouping xylem (ax). **H** - fillet detail in cross section; grouping xylem (ax); striated cuticle (cu); epidermis (ep), lipid droplet (gl); parenchyma (p). **I** - general scheme of pollen grain; polar view (a), equatorial view (b). **J** - detail of the receptacle epidermis portion, in front view; the fundamental cell of the epidermis (cfe); epicuticular striations (ese); lipid droplet (gl). **L** - general scheme of the ovary and ovarian in cross section; epidermis of the receptacle (er); vascular bundle of the ovary (fvo); vascular bundle of the receptacle (fvr); locule (lo); seminal rudiment (ru). **M** - detail of the receptacle and ovarian portion, in cross section, as outlined in **L**; striated cuticle (cu); chloroplast (clo); epidermis of the receptacle (er); vascular bundle of the ovary (fvo); vascular bundle of the receptacle (fvr); lipid droplet (gl); nucleus (nu); parenchyma of juxtaposed cells (paj), ovary parenchyma (pvo); parenchyma of the receptacle (pre); coating of the locule (rl), xylem (x).

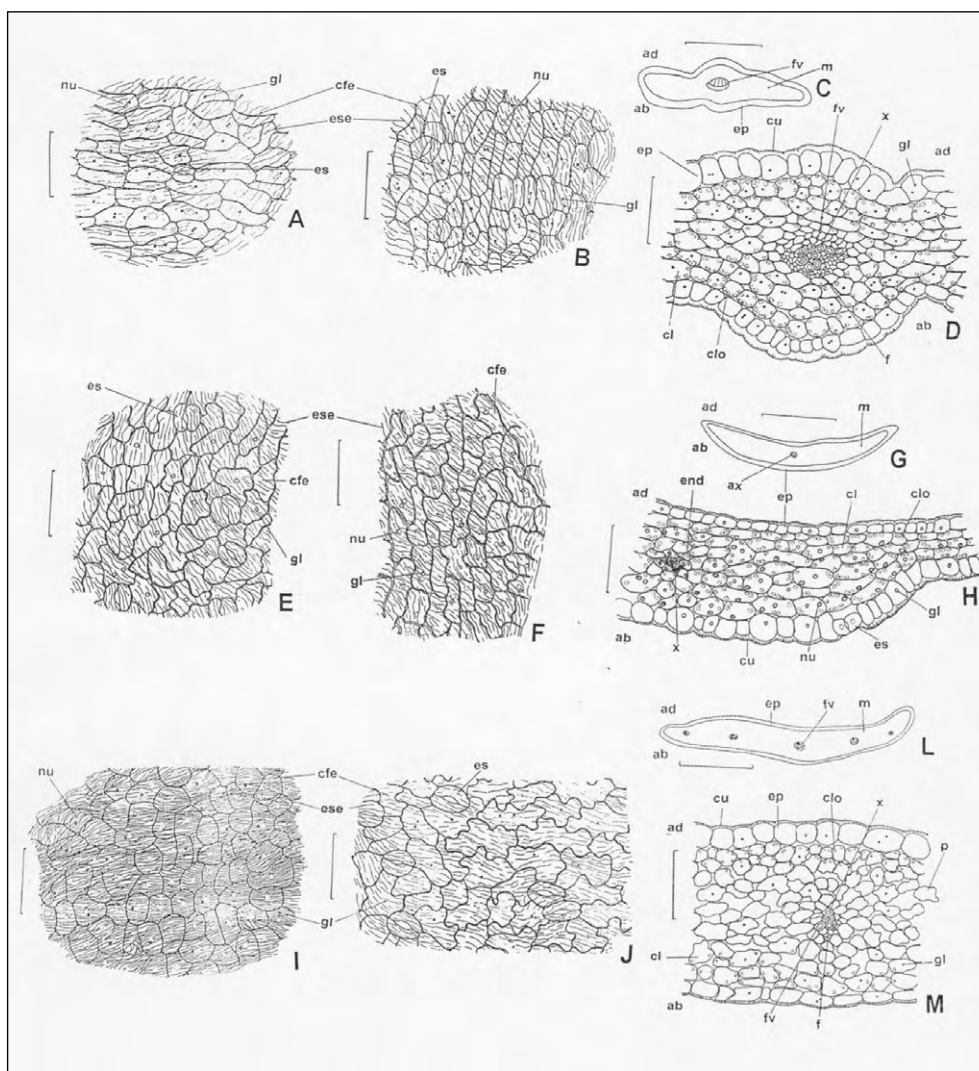


Figure 2 - Macroscopic and microscopic aspects of *Sambucus australis* Cham. & Schltdl.

Additional information of **Figure 2**. The rules correspond to **A, B, D, E, F, H, I, J** and **M** to 100 pm; in **C** and **G** to 400 pm; in **L** to 800 pm.

A - detail of the portion of the adaxial epidermis of the bract, in front view, the fundamental cell of the epidermis (cfe); stomata (es); epicuticular striations (ese); lipid droplet (gl); nucleus (nu). **B** - detail of the portion of the abaxial epidermis of the bract, in front view, the fundamental cell of the epidermis (cfe); stomata (es); epicuticular striations (ese); lipid droplet (gl); nucleus (nu). **C** - general scheme of the bract, in cross section; abaxial side (ab); adaxial side (ad); epidermis (ep), vascular bundle (fv); mesophyll (m). **D** - detail of the main nerve region of the bract, in cross-section; abaxial side (ab); adaxial side (ad); chlorenchyma (cl); chloroplast (clo); striated cuticle (cu); epidermis (ep); phloem (f); vascular bundle (fv); lipid droplet (gl), xylem (x). **E** - detail of the portion of the adaxial side of the sepal epidermis, in front view, fundamental cell of the epidermis (cfe); epicuticular striations (ese); lipid droplet (gl); nucleus (nu). **F** - detail of the abaxial portion of the sepal epidermis, in front view, fundamental cell of the epidermis (cfe); stomata (es); epicuticular striations (ese); lipid droplet (gl); nucleus (nu). **G** - general scheme of the sepal, in cross section; abaxial side (ab); adaxial side (ad), grouping xylem (ax); epidermis (ep), mesophyll (m). **H** - detail of the sepal portion, in cross-section; abaxial side (ab); adaxial side (ad); chlorenchyma (cl); chloroplast (clo); cuticle (cu); endoderm (end); epidermis (ep); stomata (es) lipid droplet (gl), nucleus (nu); xylem (x). **I** - detail of portion of the adaxial side of the petal epidermis, in front view: fundamental cell of the epidermis (cfe); epicuticular striations (ese), lipid droplet (gl); nucleus (nu). **J** - detail of portion of the abaxial side of the petal epidermis, in front view, the fundamental cell of the epidermis (cfe); stomata (es); epicuticular striations (ese); lipid droplet (gl). **L** - general scheme of the petal, in cross section; abaxial side (ab); adaxial side (ad); epidermis (ep), vascular bundle (fv); mesophyll (m). **M** - detail of petal portion in the main nerve region, in cross-section; abaxial side (ab); adaxial side (ad); chlorenchyma (cl); chloroplast (clo); striated cuticle (cu); epidermis (ep); phloem (f); vascular bundle (fv); lipid droplet (gl), parenchyma (p); xylem (x).

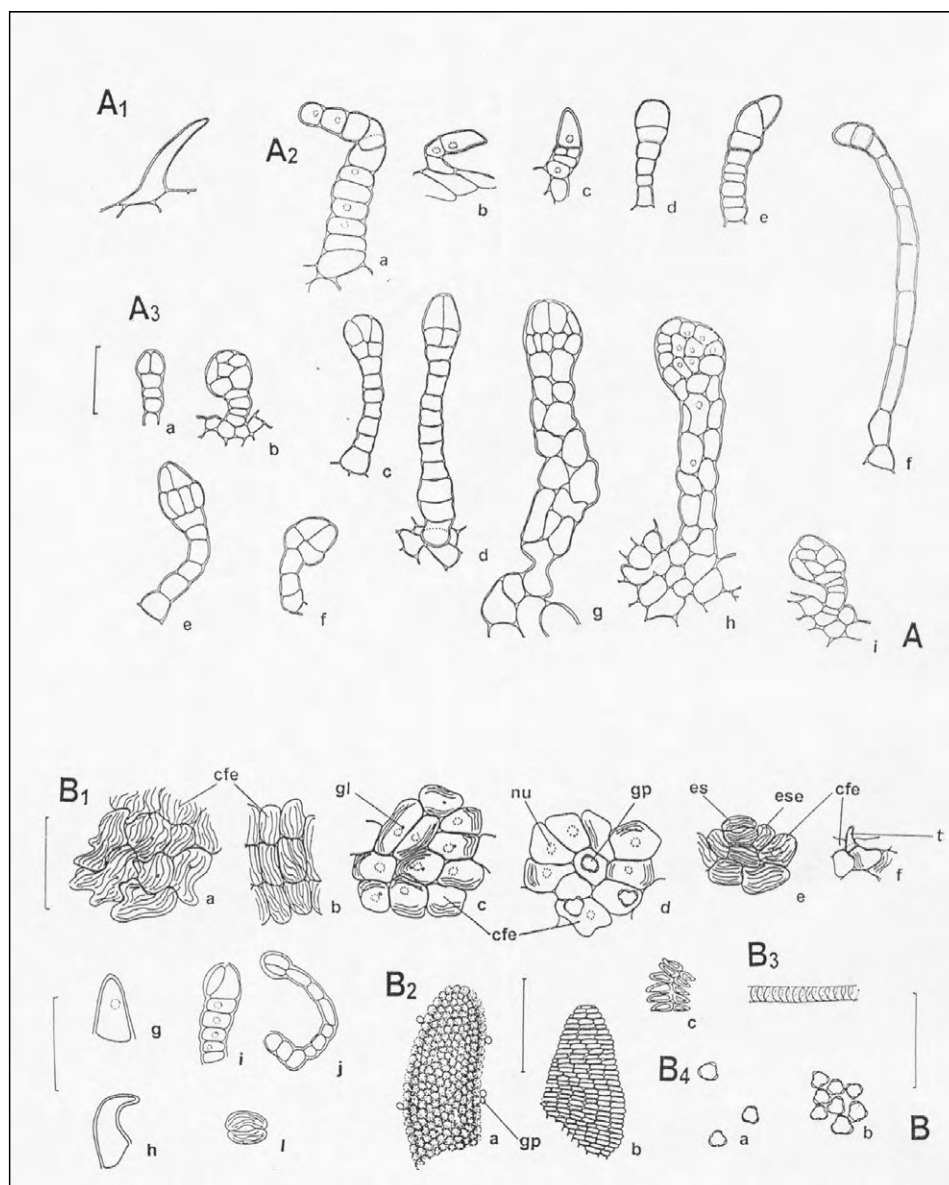


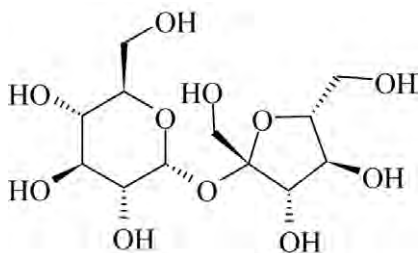
Figure 3 - Macroscopic and microscopic aspects of *Sambucus australis* Cham. & Schltdl.

Additional information of **Figure 3**. The rules correspond in **A**, **B**, **B2 c** and in **B3 a B5** to 100 µm; in **B2 a e b** to 400 µm.

A - detail of trichomes occurring in bracts, sepals and petals: **A1** = unicellular tector trichomes; **A2** = multicellular tectors trichomes; **A3** = glandular trichomes. **B** - powder details. **B₁** = epidermis portions (a, b, c, d, e, f, g, h, i, j, l), epidermis fragments in front view (a, b, c, d, e) and in lateral view (f), trichomes portions (g, h), portions of glandular trichomes with multicellular head (i, j), isolated guard cells (l); fundamental cell of the epidermis (cfe); stomata (es), epicuticular striations (ese), lipid droplet (gl); pollen grain (gp); nucleus (nu); tector trichomes (tt). **B₂** = anther fragments: convex portion (a), concave portion (b) fragment of the anther fibrous layer (c); pollen grain (CT). **B₃** = portion of tracheal element with helical thickening. **B₄** = pollen grains: isolated (a) grouped (b).

SUCROSE

Saccharum



C₁₂H₂₂O₁₁; 342,30

sucrose; 07854

β-D-fructofuranosyl-α-D-glucopyranoside
[57-50-1]

Sugar obtained from *Saccharum officinarum* L. (POACEAE) *Beta vulgaris* L. (CHENOPODIACEAE) and other sources.

DESCRIPTION

Physical characteristics. Crystalline powder or white or colorless crystalline mass, odorless and sweet.

Solubility. Very soluble in water, freely soluble in ethanol - 70% (v/v), insoluble in chloroform and in ethyl ether.

Physical and chemical constants

Specific rotation (5.2.8): Not less than + 65.9°, relative to the desiccated substance at 105 °C for 2 hours. To determine in 26% aqueous solution (w/v).

IDENTIFICATION

A. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using G silica gel as support and a mixture of water, methanol, glacial acetic acid and ethylene chloride (10:15:25:50) as mobile phase. To apply separately to the plate 2 µL of each of the solutions as described below, drying each point of application.

Solution (1): to dissolve 10 mg/mL of the sample in mixture of water and methanol (2:3). To top up the volume for 20 ml with the same mixture of solvents.

Solution (2): to dissolve 10 mg of sucrose SQR as a mixture of water and methanol (2:3). To top up the volume for 20 ml with the same mixture of solvents.

Solution (3): to dissolve 10 mg of glucose SQR, lactose SQR, fructose SQR and sucrose SQR in a mixture of water and methanol (2:3) and topping up for 20 mL with the same mixture of solvents.

to develop the chromatogram until the front of the mobile phase rise 17 cm above the line of application. To remove the plate, and dry in hot air. To nebulise with

thymol solution at 0.5% (w/v) in mixture of ethanol and sulfuric acid (95:5). To heat the plate at 130 °C for 10 minutes. The principal spot obtained with *Solution (1)* corresponds in position, color, and dimension to that obtained with *Solution (2)*. The chromatogram of *Solution (3)* must have these four spots clearly separated from each other.

B. To dilute 1 ml of the solution obtained under *Appearance of the solution* to 100 mL with water. Along with 5 mL of this solution, add 2 mL of sodium hydroxide solution 2 M freshly prepared and 0.15 mL of cupric sulfate solution at 10% (w/v) in water. The resulting solution is blue and clear remaining unchanged upon heating. Along with the hot solution, add 4 mL of hydrochloric acid solution SR, heat to the boiling point and add 4 mL of sodium hydroxide solution 2 M. It is immediately formed a precipitated orange coloring.

PURITY TESTS

Appearance of solution. To dissolve 150 g of the sample in distilled water free from carbon dioxide and top up the volume to 300 mL. The obtained solution is clear, (5.2.25) odorless and is not darker than the *Standard solution color SC F* diluted in water with proportion 1:4.

Alkalinity. To add 10 mL of the solution obtained in *Appearance of solution*, add 0,3 mL of phenolphthalein SI. The solution is colorless. At most 0.3 mL of sodium hydroxide 0.01 M are needed for turning the indicator in pink color.

Dextrins. Along with 2 mL of the solution obtained in *Appearance of solution*, adding 8 mL of water, 0.05 mL of hydrochloric acid 2 M and 0.05 mL of 0.1 iodine solution M. The solution remains yellow.

Glucose and inverted sugar. To dissolve 20 g of the sample in water and top up the volume to 100 mL and filter it, if necessary. To transfer 50 mL of the clear liquid to a 250 mL beaker, add 50 mL of alkaline cupric tartrate SR, cover the beaker with a watch glass and heat the mixture so that boil in about 4 minutes. To continue the boiling for exactly 2 minutes. To add 100 mL once freshly boiled cold water and immediately collecting the precipitate of cuprous oxide in tared funnel with medium pore filter plate. To wash the filter residue with hot water, followed by 10 mL of ethanol and finally 10 mL of ethyl ether. To dry at 105 °C for 1 hour. The weight of cuprous oxide is at most 0.112 g.

Colored substances. to filter 100 mL of the solution obtained in *Appearance of solution* using glass plate with medium pore of 1 µm and 24 mm in diameter. The filter does not stain blue. Along with 100 mL of the solution obtained in *Appearance of solution*, contained in a test tube, adding 1 mL of diluted hypophosphorous acid. to cover the tube. No unpleasant odor is perceived within 1 hour. When examined under ultraviolet light (365 nm), the solution obtained in *Appearance of solution* does not present more intense fluoresce than the reference solution containing 0.4 ppm of quinine sulfate in sulfuric acid 0.005 M.

Barium. Along with 10 mL of the solution obtained in *Appearance of solution*, add 1 mL of sulfuric acid 4 M. After 1 hour, any developed opalescence in the solution is not more intense than that one from the solution obtained in *Appearance of solution* diluted in distilled water at a ratio of 10:1.

Sulfites. To proceed as described in *Visible absorption spectrophotometry* (5.2.14). To dissolve 5 g of sample in 40 mL of water, add 2 mL of sodium hydroxide *M* and top up to 50 mL with water, use it as a *Sample solution*. Separately, to dissolve 76 mg of sodium metabisulfite and top up to 50 mL with water, pipette 5 mL of the solution and top up with water to 100 mL. To pipette 3 mL of this solution and top up with water to 100 mL, and use this solution as the standard. Separately, to pipet 10 mL of *Sample solution* and *Standard solution*, add 1 mL of hydrochloric acid 3 *M*, 2 mL of bleached fuchsin SR and 2 mL of formaldehyde solution, let stand for 30 minutes. To prepare white in parallel using 10 mL of water and the same reagents in the same amounts. To measure the absorbances of the solutions at 583 nm. The absorbance of the *Sample solution* is not greater than that of the *Standard solution*. At most 0,0015% (20 ppm) of SO_2 . If the *Standard solution* does not display red staining purple to purple-blue, the test result is invalid.

Heavy metals (5.3.2.3). At most 0,0005% (5 ppm).

Sulfated ashes (5.2.10). to dissolve 5 g of the sample in 5 mL of water, add 2 mL of sulfuric acid, evaporate to dryness and ash to constant weight. At most 0,02%

PACKAGING AND STORAGE

In airtight containers.

LABELLING

To comply with the legislation in force.

CATEGORY

Coating agent; diluent for capsules and tablets; excipient for syrups.

ORAL REHYDRATION SALTS

Oral rehydration salts constitute themselves in a dry mixture of sodium chloride, sodium bicarbonate and anhydrous dextrose. Alternatively, the sodium bicarbonate can be substituted for sodium citrate (anhydrous or dihydrate). It may contain monohydrate dextrose instead of anhydrous dextrose, since the sodium bicarbonate or sodium citrate are packaged separately with the main content. It contains, at least, 90.0% and not more than 110.0% of sodium (Na^+), potassium (K^+), chloride (Cl^-) and bicarbonate (HCO_3^-) or citrate ($\text{C}_6\text{H}_5\text{O}_7^{3-}$) in relation to the indicated amount of sodium chloride, potassium chloride and sodium bicarbonate [or sodium citrate (anhydrous or

dihydrate)]. It contains, at least, 90.0% and not more than 110.0% of the stated amount of anhydrous dextrose ($\text{C}_6\text{H}_{12}\text{O}_6$) or monohydrate dextrose ($\text{C}_6\text{H}_{12}\text{O}_6 \cdot \text{H}_2\text{O}$). It may contain distinctive flavor.

IDENTIFICATION

A. It responds to the reactions of sodium ion (5.3.1.1).

B. It responds to the reactions of potassium ion (5.3.1.1).

C. It responds to the reactions of chloride ion (5.3.1.1).

D. It responds to the reactions of bicarbonate ion, if present (5.3.1.1).

E. It responds to reactions of citrate ion, if present (5.3.1.1). To use three to five drops of the reconstituted solution as labeled, and 20 mL of acetic-pyridine anhydride SR.

F. To add several drops of the reconstituted solution as labeled in 5 mL of hot alkaline cupric tartrate SR. It produces large amount of red precipitate of cuprous oxide.

G. When heated, the mixture melts, expands and carbonize, yielding odor of burnt sugar.

PURITY TESTS

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 50 °C to obtain constant weight. At most 1 %.

DOSAGE DEXTROSE

To exactly weigh portion of sample equivalent to about 20 g of dextrose, transfer to a 100 mL volumetric flask and dilute with water. To transfer 50 mL of this solution to 100 mL volumetric flask. To add 0,2 mL of 6 *M* ammonium hydroxide and top up the volume with water. If the solution is cloudy, filter it on filter paper. If not enough, to add activated charcoal mesh ASTM (20-35) 0.5-0.75 mm filter again on filter paper and finally filter through membrane of 0.45 μm . To determine the rotation angle (5.2.8) 25 °C. To calculate the amount, in grams, of anhydrous dextrose ($\text{C}_6\text{H}_{12}\text{O}_6$) in the sample, whereas 52.7 ° as specific rotatory power at 25 °C. When the label contains monohydrate dextrose ($\text{C}_6\text{H}_{12}\text{O}_6 \cdot \text{H}_2\text{O}$), 47.9 ° and consider specific rotatory power at 25 °C.

Sodium.

To proceed as described in **atomic absorption spectrometry (5.2.13.1.1)**, using *Method I*. To employ the following conditions: air-acetylene flame, 589.6 nm wavelength. To dissolve accurately weighed amount of sample equivalent to approximately 25 mg of sodium in 50 mL of water. To dilute, then by a factor of 500 times with water. To prepare the

sodium standard stock solution to 1 g/L in water using analytical grade sodium chloride (NaCl). To construct the analytical curve with standard solutions of sodium according to the following concentrations: 0,25 mg/L, 0,5 mg/L, 1,0 mg/L, 2,0 mg/L and 2,5 mg/L. To prepare standard solutions by sequential dilution with water. to add along with the reference solutions and sample solutions the amount equivalent to 0.5% (v/v) of a cesium chloride solution, with 10 g/L (CsCl).

Potassium

To proceed as described in **Atomic absorption spectrometry (5.2.13.1.1)**, using *Method I*. To employ the following conditions: air-acetylene flame, 769.9 nm wavelength. To dissolve accurately weighed amount of sample equivalent to approximately 25 mg of potassium in 50 mL of water. To dilute, then by a factor of 500 times with water. to prepare the potassium standard stock solution with 1 g/L in water using analytical grade potassium chloride (KCl). To construct the analytical curve with standard solutions of potassium according to the following concentrations: 0,25 mg/L, 0,5 mg/L, 1,0 mg/L, 2,0 mg/L and 2,5 mg/L. To prepare standard solutions by sequential dilution with water. To add along with the standard solutions and sample solutions the amount equivalent to 0.5% (v/v) of a cesium chloride solution, with 10 g/L (CsCl).

Bicarbonate (if present)

to dissolve accurately weighed amount of sample equivalent to approximately 0.1 g of bicarbonate (HCO_3^-) (considering that each milligram of sodium bicarbonate is equivalent to 0.726 mg of HCO_3^-) in 100 mL of water. To add three drops of methyl orange SI and titrate with 0.1 M SV hydrochloric acid. Each mL of hydrochloric acid 0.1 M SV is equal to 6,101 mg of bicarbonate (HCO_3^-).

Chloride

to dissolve accurately weighed amount of sample equivalent to approximately 55 mg of chloride (Cl^-) (considering that each milligram of potassium chloride and sodium chloride is equal, respectively, to 0.476 mg and 0.607 mg Cl^-) transfer to a 250 mL beaker, add 100 mL of water and 10 mL of nitric acid M. Stir it until solubilization and titrate with 0.1M silver nitrate, determining the final point potentiometrically using silver electrode and silver chloride. Each mL of 0.1 M silver nitrate is equivalent to 3.545 mg of chloride.

Citrate (if present)

to dissolve accurately weighed amount of sample equivalent to 0.1 g of citrate ($\text{C}_6\text{H}_5\text{O}_7^{3-}$) in 80 mL of anhydrous acetic acid. To warm up about 50 °C, cool it down, dilute to 100 mL with anhydride acetic acid and then immediately let it stand for 10 minutes. To titrate potentiometrically 20 mL of this solution with perchloric acid 0.1 M SV. Each mL of perchloric acid 0.1 M SV is equivalent to 6.303 mg of

citrate ($\text{C}_6\text{H}_5\text{O}_7^{3-}$). Each mg of sodium citrate is equivalent to 0.643 mg of citrate ($\text{C}_6\text{H}_5\text{O}_7^{3-}$).

PACKAGING AND STORAGE

In tightly closed containers prevent exposure to temperatures above 30 °C. The sodium bicarbonate components or sodium citrate may be omitted from the mixture and separately packed, following the main content.

LABELLING

To comply with the legislation in force.

WILLOW, WHITE

Salicis cortex

Salix alba L. - SALICACEAE

The vegetable drug consists of the peels from young branches, dried, whole or fragmented, containing at least 1.5% of salicin derivatives expressed in salicin.

CHARACTERISTICS

Organoleptic characteristics. The dried peel is odorless, bitter and astringent markedly flavor.

MACROSCOPIC DESCRIPTION

The peel, obtained from the branches with two or three years, comes in leathery irregular fragments, flexible, elongated and slightly fluted, with varied length, width and thickness. The outer surface is gleaming, glossy, smooth or longitudinally striated in the dark brown young peels. The inner surface is longitudinally finely striated, fibrous and brown. The fracture is short in the outer portion and fibrous in the inner portion.

MICROSCOPIC DESCRIPTION

In front view, the peel is colored dark brown and in some portions, yellowish. The cells of the storied cork have different shapes, generally polygonal, with straight walls and often elongated. The collenchyma has flattened cells and thickened walls. In cross section, the cortex has extremely thick cuticle and the outer portion of the peel may have different structural organizations: 1) the first epidermal layer uniserial, with small quadrangular cell, thick-walled, followed by five to six layers of tabular chollenchyma, with elongated cells arranged tangentially with thick walls and chloroplast followed by parenchymal with cells of various shapes and thick-walled, 2) outer epidermal layer with the same characteristics as described above, followed by chlorenchyma formed by small cells, rounded and thick walls, followed by parenchyma also having rounded cells and thick-walled, 3) outer coating formed by periderm, comprising different number of layers followed by parenchyma with rounded cells of thick-walls. The outer cortical parenchyma is always formed by several layers of cells,

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the most commonly quadrangular to rectangular or polygonal to rounded, thick-walled, with few intercellular spaces, and many chloroplasts and crystals of calcium oxalate from drusen type, rare single crystals and prismatic crystals. Groupings stone cells in this tissue are uncommon, rare are these isolated cells and those containing phenolic compounds. The inner cortical parenchyma have cells from different shapes, larger intercellular spaces and smaller amount of crystal and chloroplast. More internally, the cells show greater irregularity of shape, larger intercellular spaces, walls very thick, marked with points and more rectilinear. Groupings usually circular, lignified fibers are abundant and are randomly distributed in this parenchyma, which very rarely occur groupings of stone cells. The communication is formed by few cell layers with cells of small size and flattened tangentially, both fusiform as the radial. The tissue adjacent to the internal communication, contains large amounts of starch grains. Parenchymatous rays are formed by cells from different shapes, vertically elongated, thick-walled and containing chloroplast. Among these rays, parenchyma cells occur irregularly, thickened walls without chloroplast and higher volume than those distributed by the communication. Small floematic cells, rich in phenolic compounds, are accompanied by groups of lignified fibers, horizontally aligned and by parenchymal cells, thick-walled, with chloroplast and arranged tangentially. Idioblasts containing phenolic compounds are common along the internal communication. This tissue internally delimits the peel and is composed of thin-walled cells and reduced number of layers. It is usually difficult to be observed because of its characteristics and its borderline position. In longitudinal section, the characteristics of the storied cork and the outer cortical region are similar to those described for the cross section. The inner cortical region shows parenchyma rays often extended, fibers and parenchyma cells with thick walls.

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MICROSCOPIC DESCRIPTION OF POWDER

The powder meets all the requirements for the species, unless the macroscopic characters. Examine under a microscope using chloral hydrate with 50% (w/v). Characteristic are: pale brown color; fragments of storied cork, in front view; fragments of parenchyma, cells with thickened walls, in front view, isolated fibers or portions of their groupings in longitudinal section; fragments of cortical parenchyma with polygonal shaped cells and thick-walled, with crystals from drusen type, in cross section; portion of fibers associated with crystal idioblasts in longitudinal section; fragments of parenchyma, cells with thick walls and radial distribution; and communication portions; communication fragments, in cross section; crystals of calcium oxalate kinds of single crystals type, and prismatic crystals and insulated druses.

IDENTIFICATION

To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel, with 250 µm thickness as stationary phase and a mixture of ethyl acetate, methanol and water (77:13:10) as mobile phase. To apply sepa-

ately, as band, 10 µl of each of the *Solutions* (1), (2) and (3), freshly prepared, as described below.

Solution (1): to warm up 0.5 g of pulverized sample (500 µm) in 10 mL of methanol in a water under reflux, approximately 50 °C for 10 minutes. To cool down and filter it.

Solution (2): to add 5 ml of the *Solution* (1), 1 mL of 50 mg/mL anhydrous sodium carbonate solution. To heat under reflux in water at approximately 60 °C, for 10 minutes. To cool down and filter it.

Solution (3): to dissolve 2 mg of salicin SQR and 1 mL of methanol.

To develop the chromatogram. To remove the plate, and let it drying. Then, to nebulise the plate with 5% sulphuric acid solution (v/v) and put it in an oven at 100 °C - 105 °C for 10 to 15 minutes. The chromatogram obtained with *Solution* (3) presents, in the middle third, a reddish-violet spot corresponding to salicin (R_f about 0.40.) In the chromatogram obtained with *Solution* (1), salicin spot appears with a low to medium intensity. In the chromatogram obtained with *Solution* (2) the spot corresponding to salicin appears more severe, and it appears as a spot (salicortina), and, sometimes, two weak spots (tremulacina) of reddish-violet color. In the chromatograms of *Solutions* (1) and (2) other spots may appear as blue, yellow and brown.

PURITY TESTS

Foreign material (5.4.2.2). At most 3% of branches with a diameter greater than 10 mm. At most 2% from other foreign materials.

Water (5.4.2.3). At most 11 %.

Total ash (5.4.2.4). At most 10%.

DOSAGE

To proceed as described in *High performance liquid chromatography* (5.2.17.4). To use the chromatograph equipped with an ultraviolet detector at 270 nm; pre-column packed with silanized octadecyl silica; 150 mm column and 3.9 mm inner diameter, packed with silanized octadecyl silica (4 µm); kept at room temperature; flow of *Mobile phase* of 1 mL/minute.

Mobile phase: mixture of acetonitrile, water and trifluoroacetic acid (3:97:0.05).

Sample Solution: to weigh exactly, about 0.3 g of dry and ground drug (355 µm) add 25 mL of methanol and

heat to boiling under reflux for 30 minutes. To cool down and filter it. To take up the residue with 25 mL of methanol and treat it as described above. To gather the filtered and evaporated in vacuum to dryness. To take up the residue with 2 mL of methanol, add 2 mL of sodium hydroxide 0.1 M and heat under reflux in water for 1 hour at 60 °C,

approximately, with frequent stirring. To cool down, add 0.25 mL of hydrochloric acid *M* and top up to 5 mL with a mixture of methanol and water (50:50).

Standard solution: to dissolve 10 mg of salicin in 10 mL of acetonitrile.

Solutions for analytical curve: to dilute aliquots of 40, 45, 50, 55 and 60 µL of the *Standard solution* to 100 µL with *Mobile*

phase in order to obtain concentrations of 0.40 mg/mL, 0.45 mg/mL 0.50 mg/mL 0.55 mg/mL and 0.60 mg/mL.

Procedure: to separately inject 10 µL of *Solutions for Analytical curve and Sample solution*. To record the chromatograms and measure the areas under the peaks. The retention time is approximately 6 minutes to salicin. To calculate the salicin content in the sample, from the linear equation obtained with analytical curve. The result is expressed as the mean of the determinations in grams for salicin per 100 g of drug (%), by taking into account the water content.

PACKAGING AND STORAGE

In tightly closed glass container, protected from light, heat and humidity.

S

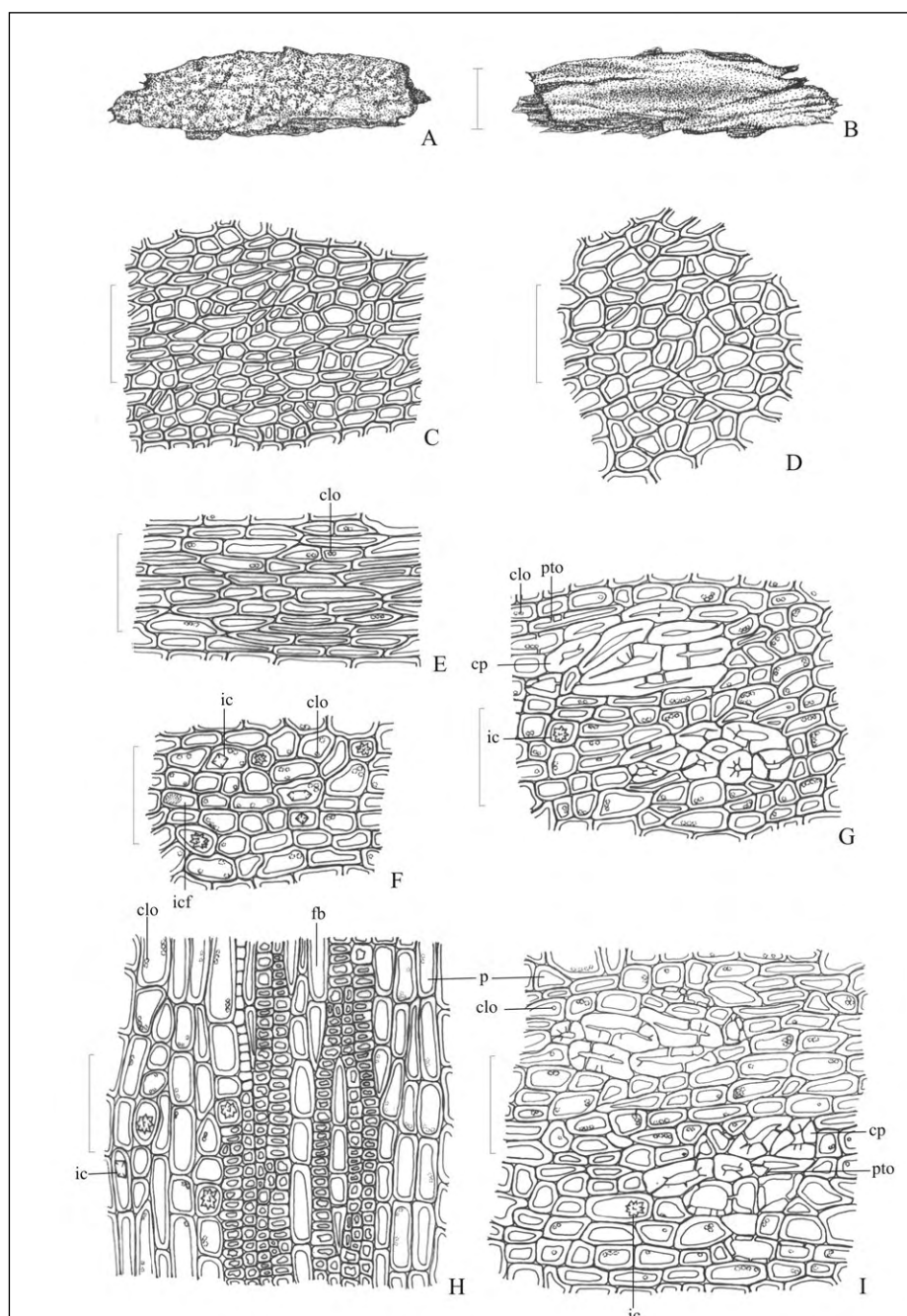


Figure 1 - Macroscopic and microscopic aspects in *Salix alba* L.

Caption under the **Figure 1**. The scales correspond in **A** and **B** to 5 mm, in **C** to **I** to 100 µm.

A - general appearance of outer surface portion of the peel in front view. **B** - general appearance of inner surface portion of the peel in front view. **C** - detail of the storied cork, the brownish region in front view. **D** - detail of the storied cork, the yellowish region, in front view. **E** - portion of chlorenchyma in cross section; chloroplast (clo). **F** - detail of parenchyma portion, showing crystal idioblasts with single crystals, prismatic crystals and druses, and phenolic compounds, in cross section; idioblast containing phenolic compounds (icf); chloroplast (clo); crystalliferous idioblast (ic). **G** - detail of parenchyma portion, showing grouping of stone cells in cross section; chloroplast (clo); stony cell (cp); crystalliferous idioblast (ic); action-point (pto). **H** - detail of the cortex portion in longitudinal section; chloroplast (clo); fiber (fb); crystalliferous idioblast (ic); parenchyma (p). **I** - detail of cortex portion, showing parenchyma of stone cells in cross section; chloroplast (clo); stony cell (cp); crystalliferous idioblast (ic); action-point (pto).

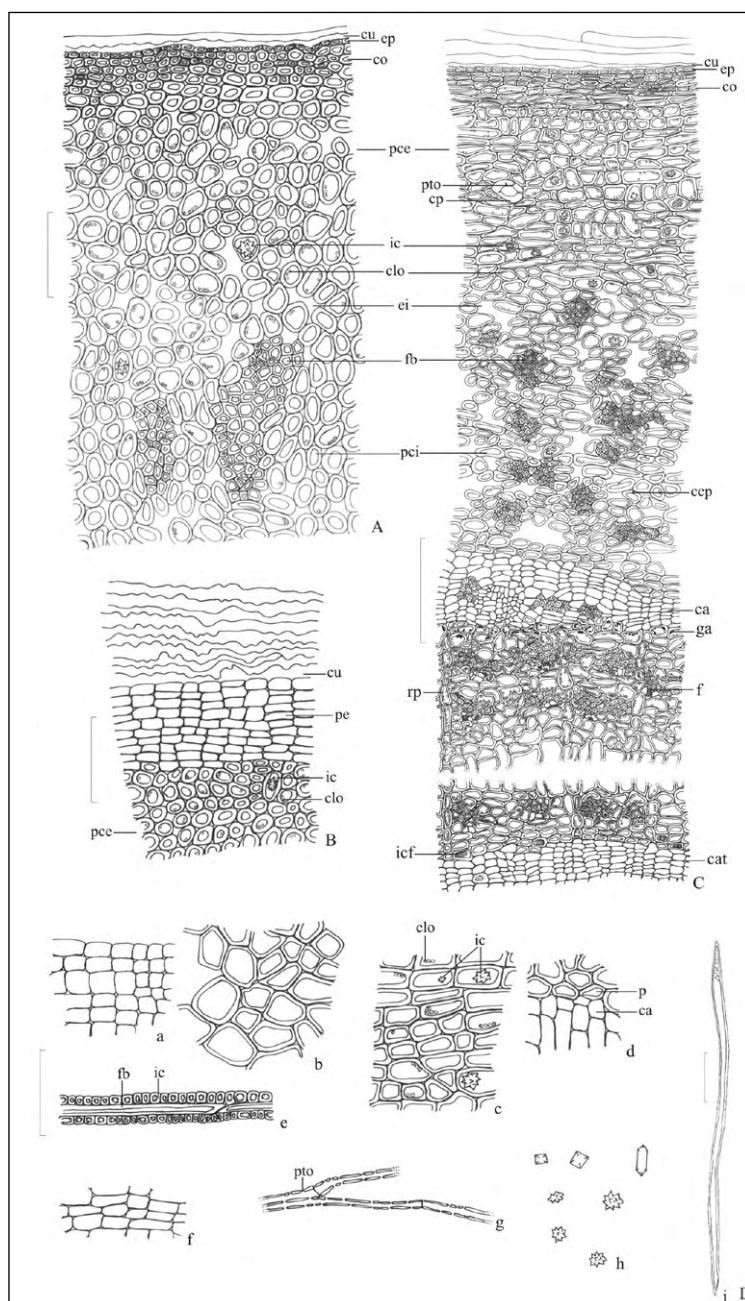


Figure 2 - Microscopic aspects of the peel and powder in *Salix alba* L.

Caption under the **Figure 2**. The scales correspond in **A**, **B** and **D** (a - h) to 100 µm, in **C** and **D** (i) to 200 µm.

A - detail of the outer portion of the cortex in cross section; chloroplast (clo); collenchyma (co); cuticle (cu), epidermis (p); intercellular space (ei); crystalliferous idioblast (ic); fiber (fb); external cortical parenchyma (pce); internal cortical parenchyma (pci). **B** - detail of cortex portion, showing the coating formed by the periderm, in cross section; cuticle (cu); chloroplast (clo); crystalliferous idioblast (ic); external cortical parenchyma (pce); periderm (pe). **C** - detail of the cortex in cross section; communication (ca); internal communication (cat); chloroplast (clo); collenchyma (co); stony cell (cp); cuticle (cu), epidermis (p); intercellular space (ei); phloem (f), fiber (fb), starch grains (ga); crystalliferous idioblast (ic); idioblast containing phenolic compounds (icf); parenchyma (p); external cortical parenchyma (pce); internal cortical parenchyma (pci); action-point (pto); parenchyma ray (rp). **D** - details of the powder, storied cork portion, in front view (a); portion of parenchyma in frontal view (b); portion of parenchyma, showing crystal idioblasts in cross-section (c); portion of parenchyma and the communication in longitudinal section (d); portion of the associated fibers to the crystal idioblasts, in longitudinal section (e); portion of the communication, in cross-section (f); portion of grouped fibers in longitudinal section (g); crystals of isolated calcium oxalate (h); Isolated fiber in longitudinal section. (i); chloroplast (clo); crystalliferous idioblast (ic); communication (ca); parenchyma (p), fiber (fb); crystalliferous idioblast (ic); action-point (pto).

SENE

Sennae folium

Senna alexandrina P.Miller - FABACEAE

The vegetable drug consists of the desiccated leaflets containing at least 2.5% of hydroxy anthracene derivatives expressed in senosideos B, and 0.6% of senosideo B ($C_{42}H_{38}O_{20}$; 862,74) and 0.5% senosideo A

($C_{42}H_{38}O_{20}$; 862,74). It must not be used before one year after harvest.

CHARACTERISTICS

Organoleptic characteristics. The drug has peculiar odor, bitter and astringent flavor.

MACROSCOPIC DESCRIPTION

Integer leaflets, lanceolate, acute apex, obtuse, retuse rare or mucronate-retuse and acute and obtuse base, asymmetric, margin slightly revolute, cartaceos, brittle, grayish-green to brownish green, with clearer abaxial side, 0.6 cm to 5.0 cm long and 0.2 cm to 1.0 cm wide; hairy blade on both sides; conical tector trichomes, geniculate in greater quantity on the abaxial side; venation Camp-todrome-brochidodromous, veins of higher order reaching the margin and the main vein prominent on the abaxial side. Short petiolule, usually curved to the abaxial side, up to 0.1 cm in length and up to 0.1 cm wide, cylindrical or concave adaxial side with two lateral ribs, convex abaxial side; trichomes equal to the blade, antrorse.

MICROSCOPIC DESCRIPTION

Leaflet with a isobilateral symmetric blade, amphistomatic with paracytic stomata, anisocytic or anomocytic. In front view, the cuticle is smooth and epidermis presents polygonal cells with thick and straight anticlinal walls, besides epidermal cells with radial distribution around the base of tector trichomes, which are unicellular, conical, geniculate, with verrucous cuticle. In cross section, the cuticle is thick and uniseriate epidermis with cells in different shapes and thick periclinal walls, with rare crystal idioblasts containing prismatic single crystals and the stomata are located just below the other epidermal cells, the palisade parenchyma is unistratified: and one who is facing the adaxial side is continuous and formed by several elongated cells, rich in chloroplastids and starch grains, some of these cells have mucilage which swell and stain with methylene blue addition, the spongy parenchyma has cells from various shapes, small intercellular spaces, few chloroplasts, many idioblasts containing large drusen and secondary bundles are similar to principal and distributed in this tissue, the palisade parenchyma facing the abaxial side is interrupted in the region of the main vein and is formed by cells shorter than those focusing on the adaxial side. The edge of the blade occurs unistratified subepidermal collenchyma or palisade parenchyma,

followed by several crystal idioblasts containing prismatic single crystals, besides bundles of smaller development with some amounts of fiber. The main bundle is collateral and has well developed phloem and procambium generally discontinuous, consisting of two cell layers; the conductors tissues are externally accompanied by fibers and crystal idioblasts containing prismatic single crystals; near the abaxial side occurs one to four layers of ring collenchyma, followed by an undeveloped chlorenchyma with small cells, walls slightly thick and few chloroplasts, and a parenchyma of polygonal cells with thick walls and small intercellular spaces. In all tissues occur lipid droplets in a small amount. The petiolule in front view, has smooth cuticle, epidermis with cells from different shapes, stomata with smaller density and an amount of trichome greater than those from the blade. In cross section, the cuticle is thick, the epidermis is uniseriate with small polygonal cells, followed by one or two layers of annular collenchyma cortical parenchyma composed of polygonal cells with thick walls and few chloroplasts and large amounts of idioblast containing drusen; endoderm with large amounts of starch grains; vascular system formed by two small collateral bundles in the ribs and usually a single collateral bundle well developed in the central region, with visible procambium and surrounded by sheath closed with fibers, or multiple bundles distributed in the form of open ring to the adaxial side and all surrounded by a sheath of fibers, which presents externally crystal idioblasts containing prismatic single crystals of large volume. Lipid droplets occur in all tissues, a greater quantity in the vascular ones.

MICROSCOPIC DESCRIPTION OF POWDER

The powder meets all the requirements for the species, unless the macroscopic characters. greenish-gray to yellowish-green; portions of tector trichomes, lateral view; fragments of epidermis with stomata in front view; fragments of epidermis with stomata and trichomes in front view; portions of epidermis showing the insertion of the trichomes in front view; fragments of epidermis on the main vein region, with stomata in front view and with crystals from drusen type, visible by transparency; fragments of petiolule epidermis, in front view; epidermal cells in cross section; crystal idioblasts and groups of fibers, in longitudinal section; portions of tracheary elements in longitudinal section; portions of mesophyll, as described in cross section, portion of assimilation parenchyma in cross section and vascular bundle in longitudinal section; portion of vascular bundles in longitudinal section; crystals from prismatic single crystals types and isolated druses. The following structures, if present in the powder, characterize the presence of impurity corresponding to the rachis: fragment of portion of vascular bundles and of parenchyma in longitudinal section; fragments of tracheary elements, with crosslinked thickening in longitudinal section; isolated portion from tracheal element with helicoidal thickness in cross section; portion of xylem, in cross section.

MACROSCOPIC DESCRIPTION OF IMPURITIES

The rachis, if present as an impurity, measures from 2.5 cm to 13.0 cm in length and up to 0.1 cm wide, is cylindrical or concave on the adaxial side with two ribs well developed and convex on the abaxial side; scars from insertion of well defined leaflets.

MICROSCOPIC DESCRIPTION OF IMPURITIES

The rachis, if present as an impurity, present in front view, epidermis with elongated or polygonal cells, straight walls, stomata and trichomes equal to the blade. In cross section, the cuticle is rough and thick, the epidermis is uniseriate, the collenchyma is similar to the petiolule, cortical parenchyma is formed by cells from variable shape and volume, with thick walls, clear intercellular spaces, chloroplasts and Idioblasts containing druses, and their portion toward the adaxial side, features lots of starch grains; endoderm rich in starch grains; central vascular system consists of three to eight collateral bundles and wrapped joint per continuous fibers sheath of small caliber, externally to this, there are idioblasts containing prismatic single crystals equal to the blade, a smaller vascular bundle occurs in each one of the ribs with the outer cap fibers to the phloem, the medullary parenchyma is formed by few cells with polygonal shape and thick walls, it has few starch grains and chloroplasts, besides crystal idioblasts containing large druses. In secondary stage of development, the communication has three to four layers, the phloem and sheath of fibers are well developed and xylem ring may not be continuous. Lipid droplets occur in the phloem, the cortical parenchyma, in the endodermis and xylem.

IDENTIFICATION

A. The 25 mg of pulverized drug (180 µm) add 50 ml of water and 5 ml of nitric acid. To heat in water for 15 minutes, let it cool down and stir with 40 mL of ethyl ether. To desiccate the ether phase with anhydrous sodium sulfate. To transfer 5 mL for a porcelain capsule and evaporate on a water until dryness, cool down and alkaline with ammonium hydroxide. The reddish color is developed.

B. Upon micro-sublimation, one obtains, initially, yellow droplets, which take the crystalline aspect. When added ethanolic potassium hydroxide with 5% (w/v) sublimed in that, it produces reddish pink coloration.

C. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel, with 250 µm thickness as support and a mixture of ethyl acetate, n-propyl alcohol, water and glacial acetic acid

(40:30:30:10) as Mobile phase. To separately apply to the plate, as band, 10 µl of *Solution* (1) and 10 µL of *Solution* (2) freshly prepared, as described below.

Solution (1): to add 0.5 g of milled drug, 5 mL of mixture of ethanol and water (1:1). To heat to boiling. To filter it.

Solution (2): to separately dissolve 2.5 mg of senosideos A and 2,5 mg of senosideos B in 1 mL of methanol and 1 mL of water, heating slightly if necessary.

To develop the chromatogram. To remove the plate, and let it drying. To nebulise with 25% nitric acid and heating for 10 minutes at 120 °C. To allow it to cool down and nebulise the plate with a solution of potassium hydroxide with 5% (w/v) until the appearance of spots. The chromatogram obtained with *Solution* (2) presents two reddish brown color spots for the senosideos B (Rf about 0.2) and senosideos A (Rf about 0.4). The chromatogram obtained with *Solution* (1) shows similar spots in the position and color to the spots obtained in the chromatogram of *Solution* (2). The chromatogram shows two additional spots of pale purplish brown color immediately above the first corresponding to senosideo C (Rf about 0.5) and senosideos D (Rf about 0.45).

PURITY TESTS

Foreign material (5.4.2.2). At most 2.0%, corresponding to the leaf raquis.

Water (5.4.2.3). At most 10.0 %.

Total ash (5.4.2.4). At most 12.0 %.

DOSAGE

Hydroxy-anthracene derivatives

To proceed as described in *Visible absorption spectrophotometry* (5.2.14).

For extraction, weigh, exactly, about 0.15 g of the pulverized drug (180 µm) in a round bottom flask with standard ground mouth, add 30 mL of water, mix and weigh the assembly. To heat it in a heating mantle, under reflux, for 15 minutes. To allow it cool down, weigh and restore the initial weight with water and filter disregarding the initial 10 mL. Transfer 10 ml of filtrate to a separatory funnel, 50 mL, add one drop of hydrochloric acid 2 M and wash with three portions of 5 ml of chloroform. To discard the chloroform phase. To centrifuge the aqueous phase during 10 minutes at 2000 rpm. To transfer 4 mL of the supernatant liquid to a round bottom flask with polished mouth. Adjust the pH to 7.0 - 8.0 with approximately 80 µL of sodium carbonate solution, 5% (w/v). To add 8 mL of ferric chloride solution, 10,5% (w/v). To mix and heat under reflux in water bath for 20 minutes. To add 0.4 mL of concentrated hydrochloric acid and maintaining the heating for 20 minutes, stirring frequently to dissolve the precipitate. To cool down the solution and transfer to theseparatory funnel of 50 mL and extract with 10 mL and twice the 7 mL of diethyl ether, previously used to rinse the round bottom flask. To collect the ethereal extracts and wash with two times the 10 mL of water. To transfer the ether layer to 25 mL volumetric flask and top up the volume with ethyl ether.

S

Sample solution: to evaporate 5 mL of ether solution in a water bath until the residue. To resuspend the residue in 5 mL of 0.5% magnesium acetate (w/v) in methanol. To filter, if necessary.

To measure the absorbance of the *Sample solution* at 515 nm immediately after preparation, by using methanol to adjust zero. To calculate the content of hydroxy anthracene derivatives calculated as senosideos B using 240 nm as specific absorbance value according to the expression:

$$SB = \frac{A \times 0,781}{m}$$

where

SB = hydroxy anthracene derivatives; A = absorbance of the *Sample solution*, m = mass of the sample considering the determination of water.

Senosideos B and A

To proceed as described in *High performance liquid chromatography* (5.2.17.4). To use the chromatograph equipped with an ultraviolet detector at 270 nm; pre-column packed with silanized octadecyl silica; 150 mm column and 3.9 mm inner diameter, packed with silanized octadecyl silica (4 µm); kept at room temperature; flow of *Mobile phase* of 0.9 mL/minute.

Eluent A: mixture of acetonitrile, water and trifluoroacetic acid (10:0.08).

Eluent B: acetonitrile.

Mobile phase gradient: to adopt gradient system described in the following table.

<i>Time (minutes)</i>	<i>Eluent A (%)</i>	<i>Eluent B (%)</i>	<i>Elution</i>
0 – 12	86	14	isocratic
12 – 19	86 → 77	14 → 23	linear gradient
19 – 28	77 → 70	23 → 30	linear gradient
28 – 31	70 → 0	30 → 100	linear gradient
31 – 33	0	100	isocratic

Solution sample: to weigh exactly, about 0.2 g of dried and milled drug (180 µm) and put in a centrifuge tube. To add 5 mL of 0.05% sodium bicarbonate solution (w/v) and put into the ultrasound bath for 10 minutes. To centrifuge for 20 minutes at 2000 rpm. To separate the supernatant transferring it to 5 mL volumetric flask and top up the volume. To filter the supernatant through the membrane. To dilute 50 µL of the resulting solution at 150 µL of water.

Standard stock solution: to dissolve 10 mg of the mixture of senosideo A SQR and senosideo B SQR in 10 mL of methanol.

Solutions for analytical curve: to dilute an aliquot of 2.5 mL of *Standard stock solution*, in 25 mL volumetric flask to obtain a 50 µg/mL. To dilute aliquots of 2 mL, 2.5 mL, 3 mL, 3.5 mL, 4 mL, and 4.5 mL in 5 mL volumetric flask with methanol in order to obtain concentrations of 20 µg/mL, 25 µg/mL, 30 µg/mL, 35 µg/mL, 40 µg/mL, and 45 µg/mL.

Procedure: to separately inject 10 µL of *Solutions for analytical curve* and *Sample solution*. To record the chromatograms and measure the areas under the peaks. The retention time is approximately 18.0 minutes for senosideo B and 20.7 minutes for senosideo A. To calculate the content of senosideo B and A in the sample from the linear equation obtained with the analytical curve. The result is expressed as the mean of the determinations in grams for senosideos B and senosideos A per 100 g of drug (%), by taking into account the water content.

PACKAGING AND STORAGE

In tightly closed glass container, protected from light and heat.

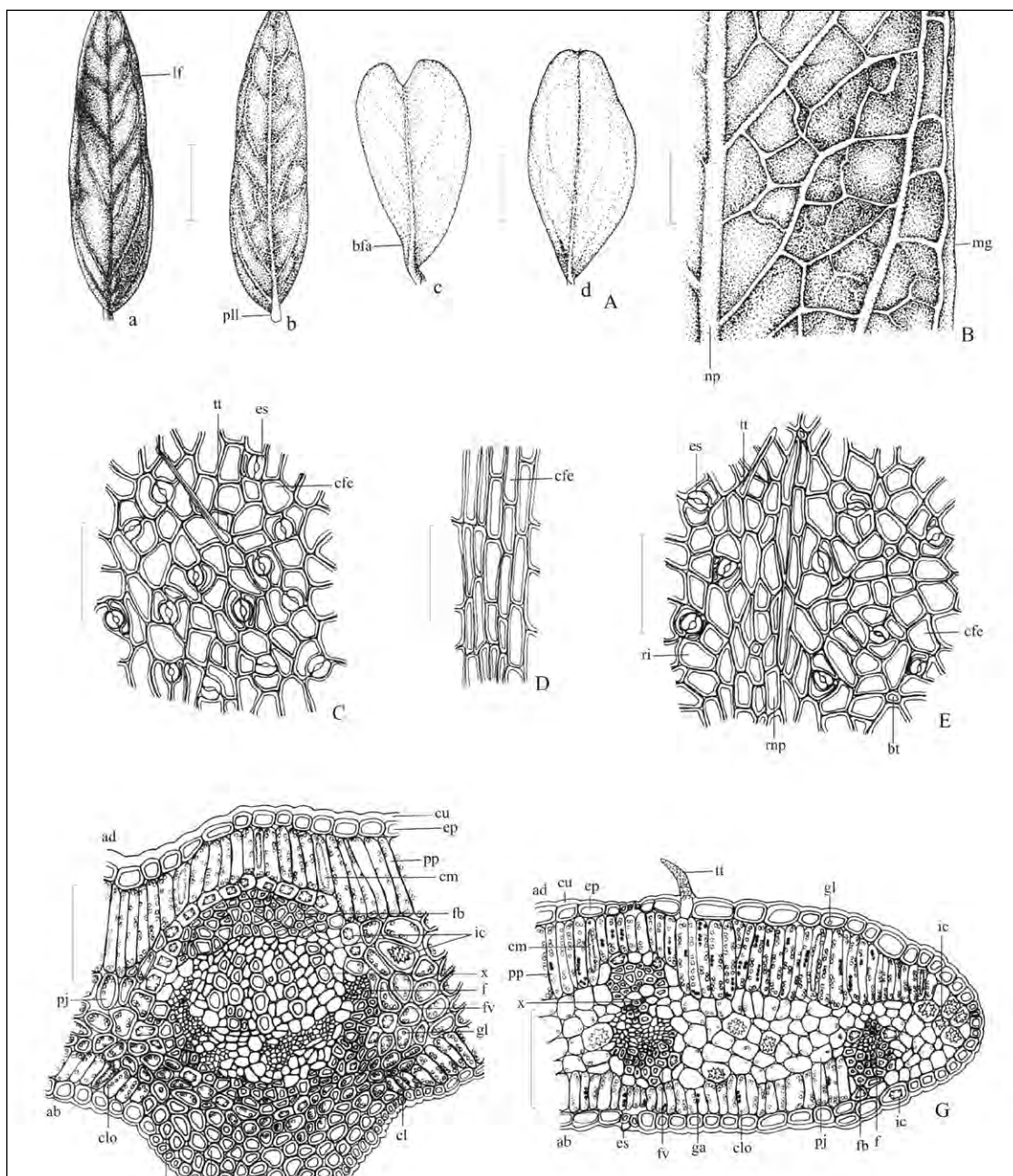


Figure 1 - Macroscopic and microscopic aspects in *Senna alexandrina* P.Miller

Additional information of **Figure 1**. The scales correspond to the **A (a, b and d)** to 5 mm; in **A (c)** to 4 mm; in **B** to 1 mm; in **C, D, E, F and G** to 100 μ m.

A - general appearance of different forms of leaflets, **a** - adaxial side of leaflets with acute apex: leaf blade (f); **b** - abaxial side of the same leaflet: petiolule (pII); **c** - abaxial side of leaflet with retuse apex: asymmetrical leaf base (bfa); **d** - abaxial side of leaflets with mucronate-retuse apex. **B** - partial detail of the leaflets venation in the main nerve region to the margin: margin (mg); main nerve (np). **C** - detail of epidermis facing the upper side, in the intercostal region in front view: tector trichomes (tt); stomata (es); fundamental cell (cfc). **D** - detail of epidermis facing the adaxial side, in the main nerve region in front view: fundamental cell (cfe). **E** - detail of the epidermis facing the abaxial side, in the intercostal and main nerve region, in front view: base of trichomes (bt); fundamental cell (cfe); stomata (es); intercostal region (ri); main nerve region (rnp); tector trichomes (tt). **F** - detail of the main nerve region, in cross-section: adaxial side (ad); cuticle (cu); epidermis (ep); palisade parenchyma (pp); cell containing mucilage (cm); fiber (fb); crystalliferous idioblast (ic); xylem (x), phloem (f); vascular bundle (fv); lipid droplet (gl); chlorenchyma (cl); parenchyma (p), collenchyma (co); chloroplast (clo); abaxial side (ab); spongy parenchyma (pj). **G** - detail of the intercostal region and edge in cross section: adaxial side (ad); cuticle (cu); epidermis (ep); tector trichomes (tt); lipid droplet (gl); crystalliferous idioblast (ic); phloem (f); fiber (fb); spongy parenchyma (pj); chloroplast (clo); starch grain (ga); vascular bundle (fv); stomata (es), abaxial side (ab); xylem (x); parenchyma palisade (pp); cell containing mucilage (cm).

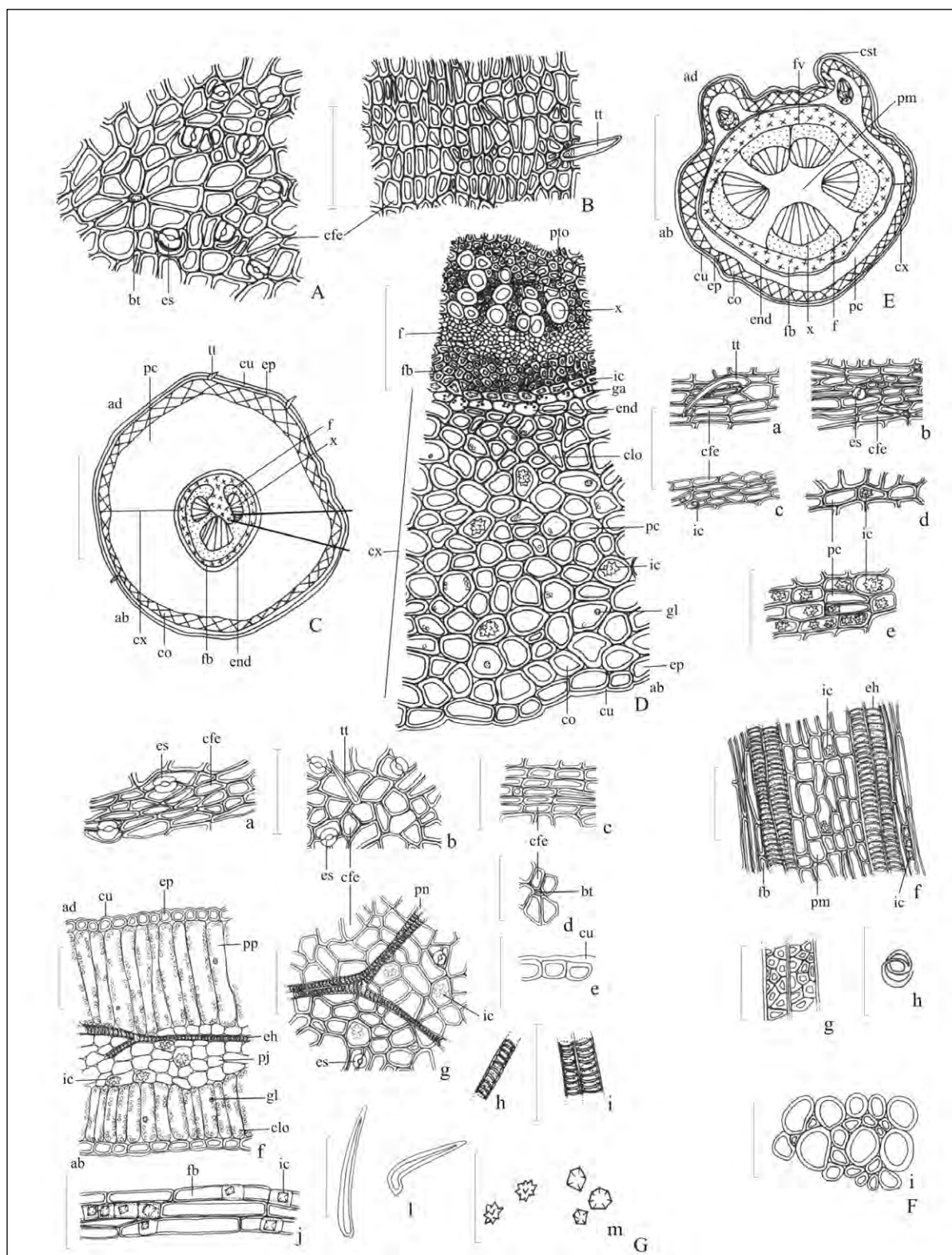


Figure 2 - Macroscopic and microscopic aspects of powder in *Senna alexandrina* P. Miller

Additional information of **Figure 2**. The scales correspond in **A**, **B**, **D**, **F** (**a - i**) and **G** (**a - m**) to 100 mm; in **C** and **E** to 400 mm.

A - detail of the petiolule epidermis facing the adaxial side in front view: the base of trichomes (bt); stomata (es), fundamental cell of the epidermis (cfe). **B** - detail of the petiolule epidermis facing the abaxial side in front view: fundamental cell of the epidermis (cfe); tector trichomes (tt). **C** - schematic representation of the petiolule in cross section: adaxial side (ad); cortical parenchyma (pc); tector trichomes (tt); cuticle (cu); epidermis (ep), phloem (f); xylem (x); endoderm (end); fiber (fb), collenchyma (co), cortex (cx), abaxial side (ab). **D** - detail of the petiolule, in cross section, as highlighted in **C**: action-point (pto); xylem (x), phloem (f), fiber (fb); crystalliferous idioblast (ic); starch grain (ga), endoderm (end); chloroplastic (clo); cortical parenchyma (pc); lipid droplet (gl); epidermis (ep); abaxial side (ab); cuticle (cu), collenchyma (co), cortex (cx). **E** - schematic representation of impurity, corresponding to the rachis in cross section: adaxial side (ad); vascular bundle (fv); rib (CST); medullary parenchyma (pm), cortex (cx), cortical parenchyma (pc); phloem (f); xylem (x); endoderm (end); collenchyma (co); epidermis (ep), cuticle (cu); abaxial side (ab). **F** (**a - f**) - details of the powder from the impurities corresponding to the rachis (**a** - detail of epidermis portion with tector trichomes,

in front view): tector trichomes (tt); fundamental cell of the epidermis (cfe); stomata (es); crystalliferous idioblast (ic); cortical parenchyma (cp); tracheal element with helical thickening, longitudinal section (eh); fiber (fb); medullary parenchyma (pm). **G**- leaflet powder details; **a** - detail of epidermis portion of the blade, under the main nerve region in front view: stomata (es), fundamental cell of epidermis (cfe); **b** detail of epidermis portion of the blade with stomata and trichome tector, in front view: trichome tector (tt), stomata (es), fundamental cell of epidermis (cfe); **c** - detail of portion of the petiolule epidermis, focused on the abaxial side, in front view: fundamental cell of epidermis (cfe); **d** - detail of epidermis portion of the blade, showing base of trichome tector, in front view: fundamental cell of epidermis (cfe), trichome base (bt); **e** - detail of epidermis portion of the blade, in cross section: cuticle (cu); **f** - detail of portion of the intercostal region, in cross section: adaxial face (ad), cuticle (cu), epidermis (ep), palisade parenchyma (pp), tracheal element, with helical thickening, in longitudinal section (eh); spongy parenchyma (pj), crystalliferous idioblast (ic), lipid droplet (gl), chloroplast (clo), abaxial side (ab); **g** - detail of the epidermis fragment showing portion of the nerve, stomata and crystalliferous idioblasts, for transparency, in front view: fundamental cell of epidermis (cfe), nerve portion (pn), crystalliferous idioblast (ic), stomata (s); **h** - detail of tracheal element portion, with helical thickening, in longitudinal section, isolated; **i** - detail of portion of tracheal elements grouped, with helical thickening, in longitudinal section; **j** - detail of portion grouping fibers associated with crystalliferous idioblasts in longitudinal section: fiber (fb), idioblasto crystalliferous (ic); **l** - portions of isolated tectors trichomes in lateral view; **m** - detail of isolated crystals from druses type and prismatic single crystal.

SERUM, ANTIBOTHROPIC, PENTAVALENT

Immunoserum bothropicum

The pentavalent antiothropic serum is a solution that contains specific immunoglobulins purified from plasma obtained from animals hyperimmunized with antigen of the *Bothrops*, genus composed by poisons of snakes *Bothrops jararaca*, *Bothrops jararacussu*, *Bothrops moojeni*, *Bothrops alternatus* and *Bothrops neuwiedi*. It meets the specifications and tests prescribed in the Hyperimmune serums monograph for human use. It contains in each milliliter immunoglobulin sufficient to neutralize 5 mg of reference poison from *B. jararaca*.

IDENTIFICATION

A. to proceed as described in the test **A.** the *Identification of the Hyperimmune serums monograph for human use, using as antigen poison from B. jararaca*.

B. It meets the requirements described in *Dosage*.

CHARACTERISTICS

To proceed as described in the *Characteristics of the hyperimmune serums monograph for human use*.

PHYSICAL AND CHEMICAL TESTS

To proceed as described in the *Physico-chemical tests of the hyperimmune serums monograph for human use*.

BIOLOGICAL SAFETY TESTS

To proceed as described in *Biological safety tests of the hyperimmune serum monograph for human use*.

DOSAGE

The power test of the sample aims to determine the neutralizing dose required (Effective Dose of 50%) to protect susceptible animals against the lethal effects of a fixed dose of reference Poison.

Poison reference: homogeneous mixture of poisons that represent the geographic distribution of the species *B. jararaca*. It should be lyophilized and kept at -20 °C. The poison is standardized by determining the 50% Lethal Dose (DL₅₀).

Determination of the DL₅₀ of poison: to reconstitute the lyophilized preparation of poison for a given weight concentration per volume with saline solution at 0.85% (w/v). To perform dilutions in geometric progression with the same diluent, using the constant dilution factor, not exceeding 1.5, and equating the final volumes. To inoculate, intraperitoneally, 0.5 mL volume per mice of each dilution in groups of, at least 10, Swiss albino mice of 18 g to 22 g. To observe the animals until 48 hours after inoculation

and record the number of the dead ones for each dilution. Calculate the DL₅₀ using proven statistical method. The response range (percentage of deaths) must be between the highest and lowest dilution used forming the regression curve that should present linear relationship. Confidence limits should not be large; they must indicate better test accuracy as smaller their limits. To express the result in poison micrograms per 0.5 ml.

Determination of serum power: to perform progressive dilutions of the sample in saline solution at 0.85% (w/v) using a constant dilution factor no greater than 1.5, so that the final volume after mixing with challenge dose of poison is identical in all the tubes. To reconstitute and dilute the *poison reference* with saline solution at 0.85% (w/v) and adding a constant volume in each tube, so that each dose for each animal to be inoculated contains 5 DL₅₀. To make it homogeneous and incubate the mixture at 37°C for 60 minutes. To inoculate, intraperitoneally, volume of 0.5 mL per mice of each mixture into groups of at least eight Swiss albino mice of 18 g to 22 g. To observe the animals until 48 hours after inoculation and record the number of the living ones for each mixture. To calculate the 50% Effective Dose (DE₅₀) in micro liters, using proven statistical method. The response range (survival rate) must be between the highest and lowest dilution used forming the regression curve that should present linear relationship. Confidence limits should not be large; they must indicate better test accuracy as smaller their limits. To calculate the power in milligrams per milliliter, according to the expression:

$$\text{Potência} \left(\frac{\text{mg}}{\text{mL}} \right) = \frac{T_v - 1}{DE_{50}} \times DL_{50} \text{ do veneno}$$

where

T_v = number of DL₅₀ used per mice in the test dose of poison.

The power title is expressed as milligrams of poison neutralized with 1 ml of the sample. There may be a variation coefficient of 10% because of the inherent variation in the tests with laboratory animals. Thus the minimum power may vary up to 4.5 mg/mL.

PACKAGING AND STORAGE

In accordance with the monograph hyperimmune serums for human use.

LABELLING

To comply with the legislation in force.

PENTAVALENT AND ANTICROTALIC ANTIBOTHROPIC SERUM

Immunoserum bothropicum-crotalicum

The pentavalent and anticrotalic antithropic serum is a solution that contains specific immunoglobulins purified from plasma obtained from animals hyperimmunized with antigen of the Bothrops, genus composed by poisons of *Bothrops jararaca*, *Bothrops jararacussu*, *Bothrops moojeni*, *Bothrops alternatus*, *Bothrops neuwiedi* and *Crotalus durissus*. It meets the specifications and tests prescribed in the Hyperimmune serums monograph for human use. It contains in each milliliter immunoglobulin sufficient to neutralize 5 mg and 1.5 mg of reference poisons from *B. jararaca* and *C. durissus terrificus*, respectively.

IDENTIFICATION

A. To proceed as described in the test **A.** *the Identification of the Hyperimmune serums monograph for human use*, using as poison antigen from *B. jararaca* and *C. durissus terrificus*.

B. It meets the requirements described in *Dosage*.

CHARACTERISTICS

To proceed as described in the Characteristics of the hyperimmune serums monograph for human use.

PHYSICAL AND CHEMICAL TESTS

To proceed as described in the Physico-chemical tests of the hyperimmune serums monograph for human use.

BIOLOGICAL SAFETY TESTS

To proceed as described in Biological safety tests of the hyperimmune serum monograph for human use.

DOSAGE

Botropic fraction

To determine the power as described in *Dosage of the monograph Pentavalent antithropic serum*.

Crotalic fraction

To determine the power as described in *Dosage of the monograph Antithropic serum*.

PACKAGING AND STORAGE

In accordance with the monograph hyperimmune serums for human use.

LABELLING

To comply with the legislation in force.

SERUM, ANTIBOTHROPIC, PENTAVALENT AND ANTILAQUETIC

Immunoserum bothropicum-laqueticum

The pentavalent and laquetic antithropic serum is a solution that contains specific immunoglobulins purified from plasma obtained from animals hyperimmunized with antigen of the Bothrops, genus composed by poisons of *Bothrops jararaca*, *Bothrops jararacussu*, *Bothrops moojeni*, *Bothrops alternatus*, *Bothrops neuwiedi* and *Lachesismuta*. It meets the specifications and tests prescribed in the Hyperimmune serums monograph for human use. It contains in each milliliter immunoglobulin sufficient to neutralize 5 mg and 3 mg of reference poisons from *B. jararaca* and *L. muta*, respectively.

IDENTIFICATION

A. To proceed as described in the test **A.** *the Identification of the Hyperimmune serums monograph for human use*, using as poison antigen from *B. jararaca* and *L. muta*.

B. It meets the requirements described in *Dosage*.

CHARACTERISTICS

To proceed as described in the Characteristics in the hyperimmune serums monograph for human use.

PHYSICAL AND CHEMICAL TESTS

To proceed as described in the Physico-chemical tests in the hyperimmune serums monograph for human use.

BIOLOGICAL SAFETY TESTS

To proceed as described in Biological safety tests of the hyperimmune serum monograph for human use.

DOSAGE

Botropic fraction

To determine the power as described in the Pentavalent antithropic Serum monograph.

Laquetic fraction

The power test of the sample aims to determine the neutralizing dose required (Effective Dose of 50%) to protect susceptible animals against the lethal effects of a fixed dose of reference Poison.

Poison reference: homogeneous mixture of poisons that represent the geographic distribution of the species *L. muta*. It should be lyophilized and kept at -20 °C. The poison is standardized by determining the 50% Lethal Dose (DL₅₀).

S

Determination of DL_{50} of poison: to proceed as described in Determination of DL_{50} of poison in the monograph of pentavalent antipoison serum.

Determining the potency of the serum: to proceed as described in Dosage in the Pentavalent antipoison serum monograph.

There may be a variation coefficient of 10% because of the inherent variation in the tests with laboratory animals. Thus the minimum power for laquetic fraction may vary up to 2.7 mg/mL.

PACKAGING AND STORAGE

In accordance with the *hyperimmune serums monograph for human use*.

LABELLING

To comply with the legislation in force.

SERUM, ANTIBOTHROPIC, PENTAVALENT, ANTICROTALIC AND ANTILAQUETIC

Immunoserum bothropicum-laqueticum-crotalicum

The anticrotalic and antilaquetic pentavalent antithropic serum is a solution that contains specific immunoglobulins purified from plasma obtained from animals hyperimmunized with antigen of the Bothrops, genus composed by poisons of *Bothrops jararaca*, *Bothrops jararacussu*, *Bothrops moojeni*, *Bothrops alternatus*, *Bothrops neuwiedi*, *Lachesis muta* and *Crotalus durissus*. It meets the specifications and controls prescribed in the *Hyperimmune serums monograph for human use*. It contains in each milliliter, immunoglobulins sufficient to neutralize, at least, 5 mg, 3 mg and 1.5 mg of reference poisons from BB. jararaca, L. muta and C. durissus terrificus, respectively.

IDENTIFICATION

A. *To proceed as described in the test A. the Identification of the Hyperimmune serums monograph for human use, using as poison antigen from B. jararaca, L. muta and C. durissus terrificus.*

B. It meets the requirements described in *Dosage*.

CHARACTERISTICS

It complies with the described characteristics in the hyperimmune serums monograph for human use.

PHYSICAL AND CHEMICAL TESTS

It complies with the described Physico-chemical tests in the hyperimmune serums monograph for human use.

BIOLOGICAL SAFETY TESTS

It complies with the described Biological safety tests of the hyperimmune serum monograph for human use.

DOSAGE BOTROPIC FRACTION

To proceed as described in *Dosage* of the monograph *Pentavalent antithropic serum*.

Crotalic fraction

To proceed as described in *Dosage* of the *Antithropic serum monograph*.

Laquetic fraction

To proceed as described in Dosage of the pentavalent antilaquetic and antithropic serum monograph.

PACKAGING AND STORAGE

In accordance with the *hyperimmune serums monograph for human use*.

LABELLING

To comply with the legislation in force.

SERUM, ANTIBOTULINIC, TRIVALENT *Immunoserum botulinicum*

The trivalent anti-botulinum serum is a solution containing purified immunoglobulin from plasma obtained of animals hyperimmunized against toxins type A, type B and type E produced by *Clostridium botulinum*. It meets the specifications and tests prescribed in the *Hyperimmune serums monograph for human use*. It contains in each milliliter at least 375 UI, 275 UI and 425 UI of antitoxin for each of the types A, B and E, respectively.

IDENTIFICATION

A. To proceed as described in the test **A.** of *Identification* the hyperimmune serum *monograph for human use*, using as antigens the toxins types A, B and E produced by *C. botulinum*.

B. It meets the requirements described in *Dosage*.

CHARACTERISTICS

To proceed as described in the Characteristics of the hyperimmune serums monograph for human use.

PHYSICAL AND CHEMICAL TESTS

To proceed as described in the Physico-chemical tests of the hyperimmune serums monograph for human use.

BIOLOGICAL SAFETY TESTS

To proceed as described in *Biological safety tests of the hyperimmune serum monograph for human use*.

DOSAGE

The power test of the sample aims to determine the neutralizing dose required to protect animals used in the test, against the lethal effects of a test dose of each of the types of reference toxins. The serum dose under the test is compared with the dose of the *Botulinum antitoxin of reference* necessary to give the same protection.

Botulinum antitoxins of reference: the international standards of reference of the antitoxins types A, B or E are distributed to laboratories of production and control in ampoules containing lyophilized hyperimmune equine serum, which specifically neutralizes the botulinum toxin from the type that is referred. The equivalence in international units of International Reference Standard is periodically established by World Health Organization.

Determination of the toxin test dose (L +/10): to proceed as described in *Determination of the toxin test dose (L +/10)* of the *Anti-tetanus Serum monograph* or extrapolating the values for L + and L +/100. The mixtures (botulinum + antitoxin) are incubated at room temperature or at 22 °C ± 2 °C for 60 minutes.

Determination of serum power: to dilute the reference toxin in a dose of L +/10 with gelatin phosphate solution (0.2% of gelatin dissolved in phosphate buffer pH 6.5 and autoclaved at 120 °C for 15 minutes). In a series of test tubes, to distribute a constant volume of diluted botulinum toxin. To add variable volumes of the sample. Equal volumes to 5 mL with the same diluent. To make it homogeneous and incubate at room temperature or at 22 °C ± 2 °C for 60 minutes. To inoculate each Swiss albino mice or NIH of 18 g 22 g intraperitoneally, a volume of 0.5 mL in groups of at least 8 mice by mixing it. To observe the animals until 96 hours after inoculation and record the number of the dead ones. Under the same conditions described and parallel, perform the test with botulinum antitoxin of reference, with the objective of verifying the validity of the test and establish correlation in the calculation of the title. To calculate the power of the serum in test, UI/mL, whereas the lowest dilution that determines the death of all animals during the observation period, using the following equation:

$$\text{Power} = \frac{A}{B} \times C$$

where

A = the inverse of the lower dilution (or larger volume) of the serum that kills all animals;

B = the volume used in diluted serum that kills all animals;

C = total number of doses contained in the final volume of each mixture by the title L +/10.

PACKAGING AND STORAGE

In accordance with the *hyperimmune serums monograph for human use*.

LABELLING

To comply with the legislation in force.

ANTICROTALIC SERUM

Immunoserum crotalicum

The anticrotalic serum is a solution containing specific immunoglobulin purified obtained from animals plasma hyperimmunized with poison of *Crotalus durissus*. It meets the specifications and tests prescribed in the *Hyperimmune serums monograph for human use*. It contains in each milliliter immunoglobulin sufficient to neutralize 1.5 mg of reference poisons from *C. durissus terrificus*.

IDENTIFICATION

A. To proceed as described in the test **A.** the *Identification of the Hyperimmune serums monograph for human use*, using as poison antigen from *C. durissus terrificus*.

B. It meets the requirements described in *Dosage*.

CHARACTERISTICS

To proceed as described in the *Characteristics of the hyperimmune serums monograph for human use*.

PHYSICAL AND CHEMICAL TESTS

To proceed as described in the *Physico-chemical tests of the hyperimmune serums monograph for human use*.

BIOLOGICAL SAFETY TESTS

To proceed as described in *Biological safety tests of the hyperimmune serum monograph for human use*.

DOSAGE

To proceed as described in *Dosage of the monograph Pentavalent antithropic serum*. To prepare the *reference poison* as described below.

Reference poison: homogeneous mixture of poisons that represent the geographic distribution of the species *C. durissus terrificus*. It should be lyophilized and kept at -20 °C. The poison is standardized by determining the 50% Lethal Dose (DL₅₀).

There may be a variation coefficient of 10% because of the inherent variation in the tests with laboratory animals. Thus the minimum power for crotalic fraction may vary up to 1.35 mg/mL.

S

PACKAGING AND STORAGE

In accordance with the *hyperimmune serums monograph for human use*.

LABELLING

To comply with the legislation in force.

ANTI-DIPHTHERIA SERUM Immunoserum diphthericum

The anti-diphtheria serum is a solution containing purified immunoglobulin from plasma obtained of animals hyperimmunized against toxins produced by *Corynebacterium diphtheriae*. It meets the specifications and tests prescribed in the *Hyperimmune serums monograph for human use*. In each milliliter it contains at least 1000 UI of antitoxin.

IDENTIFICATION

A. to proceed as described in the test **A.** the *Identification of the Hyperimmune serums monograph for human use*, using as antigen poison from *C. diphtheriae*.

B. It meets the requirements described in *Dosage*.

CHARACTERISTICS

To proceed as described in the *Characteristics of the hyperimmune serums monograph for human use*.

PHYSICAL AND CHEMICAL TESTS

To proceed as described in the *Physico-chemical tests of the hyperimmune serums monograph for human use*.

BIOLOGICAL SAFETY TESTS

To proceed as described in *Biological safety tests of the hyperimmune serum monograph for human use*.

DOSAGE

The power test of the sample aims to determine the neutralizing dose required to protect animals used in the test, against the lethal effects of a test dose of the diphtheria toxin of reference. The serum dose under the test is compared with the dose of the *diphtheria antitoxin* of reference necessary to give the same protection.

Botulinum antitoxin reference: the international standards of reference of the diphtheria antitoxin is distributed to laboratories of production and control in ampoules containing lyophilized hyperimmune equine serum, which specifically neutralizes the diphtheria toxin. The equivalence in international units of International Reference Standard is periodically established by World Health Organization.

Diphtheria Toxin of Reference: it is prepared from sterile filtered of Supernatants from liquid cultures of *C. diphtheriae*.

The filtrate should be concentrated, purified by physical or chemical methods and lyophilized. After reconstitution of the toxin, add saline solution with glycerin and store at -20 °C.

Determination of toxin test dose (L +): to dilute the reference *diphtheria antitoxin* to 10 UI/ml with saline solution and 0.85% (w/v). To dilute the diphtheria toxin of reference to known concentration with saline solution containing peptone at 1% (w/v). In a series of test tubes, add varying amounts of toxin and the constant volume of *diluted diphtheria antitoxin* of reference. To equate the volume with peptonated saline solution, at 1% (w/v). To make it homogeneous and incubate at 37°C for 60 minutes. To inoculate each guinea pig of 230 g - 250 g subcutaneously in a volume containing 1 UI of *diphtheria antitoxin* of reference in groups of at least four guinea pigs by mixing. To observe the animals until 96 hours and record the number of the dead ones for each dilution. The L + (limit of death) or the test dose of toxin is the least amount of toxin, which, when combined with 1 UI of *diphtheria antitoxin of reference*, causes the death of the animals during the stipulated observation period.

Determination of serum power: to dilute the *diphtheria toxin of reference* with buffered saline solution containing peptone at 1% (w/v) at a dose of 10 L +. In a series of test tubes, distributing variable volumes of the sample. Add 1 mL of *diluted diphtheria toxin of reference*. To equal volumes to 10 mL with the same diluent. To make it homogeneous and incubate the mixture at 37°C for 60 minutes. To inoculate a dose of 2 mL in each of the guinea pigs of 230 g - 250 g subcutaneously in groups of, at least, four guinea pigs by mixing. To observe the animals until 96 hours after inoculation and record the number of the dead ones. Under the same conditions described and parallel, perform the test with *diphtheria antitoxin of reference*, with the objective of verifying the validity of the test and establish correlation in the calculation of the title. To calculate the power of the serum in test, UI/mL, whereas the lowest dilution that determines the death of all animals during the observation period, using the following equation:

$$\text{Power} \left(\frac{\text{UI}}{\text{mL}} \right) = \frac{A}{B} \times C$$

where

A = the inverse of the lower dilution (or larger volume) of the serum that kills all animals;

B = the volume used in diluted serum that kills all the animals;

C = total number of doses contained in the final volume of each mixture by the title L +.

PACKAGING AND STORAGE

In accordance with the monograph *hyperimmune serums for human use*.

LABELLING

To comply with the legislation in force.

SERUM, ANTI-ELAPIDIC, BIVALENT

Immunoserum elapidicum

The divalent anti-elapidic serum is a solution containing specific immunoglobulin purified from plasma obtained from animals hyperimmunized with poison of *Micrurus frontalis* and *Micrurus corallinus*. It meets the specifications and tests prescribed in the *Hyperimmune serums monograph for human use*. It contains in each milliliter immunoglobulin sufficient to neutralize 1.5 mg of reference poisons from *M. frontalis*.

IDENTIFICATION

A. To proceed as described in the test **A.** the *Identification of the Hyperimmune serums monograph for human use*, using as poison antigen from *M. frontalis*.

B. It meets the requirements described in *Dosage*.

CHARACTERISTICS

To proceed as described in the *Characteristics of the hyperimmune serums monograph for human use*.

PHYSICAL AND CHEMICAL TESTS

To proceed as described in the *Physico-chemical tests of the hyperimmune serums monograph for human use*.

BIOLOGICAL SAFETY TESTS

To proceed as described in *Biological safety tests of the hyperimmune serum monograph for human use*.

DOSAGE

To proceed as described in *Dosage* of the monograph *Pentavalent antiotherapeutic serum*. To prepare the *reference poison* as described below.

Poison reference: homogeneous mixture of poisons that represent the geographic distribution of the species *Micrurus frontalis*. It should be lyophilized and kept at -20 °C. The poison is standardized by determining the 50% Lethal Dose (DL₅₀).

There may be a variation coefficient of 10% because of the inherent variation in the tests with laboratory animals. Thus the minimum power for elapidic fraction may vary up to 1.35 mg/mL.

PACKAGING AND STORAGE

In accordance with the *hyperimmune serums monograph for human use*.

LABELLING

To comply with the legislation in force.

ANTI-SCORPION SERUM

Immunoserum escorpionicum

The anti-scorpion serum is a solution containing specific immunoglobulin purified from plasma obtained from animals hyperimmunized with antigen solution of the *genre Tityus serrulatus*. It meets the specifications and tests prescribed in the *Hyperimmune serums monograph for human use*. It contains in each milliliter immunoglobulin sufficient to neutralize 1 mg of reference poison from *Tityus serrulatus*.

IDENTIFICATION

A. to proceed as described in the test **A.** the *Identification of the Hyperimmune serums monograph for human use*. To use as poison antigen of *T. serrulatus*.

A. B. It meets the requirements described in *Dosage*.

CHARACTERISTICS

To proceed as described in the *Characteristics of the hyperimmune serums monograph for human use*.

PHYSICAL AND CHEMICAL TESTS

To proceed as described in the *Physico-chemical tests of the hyperimmune serums monograph for human use*.

BIOLOGICAL SAFETY TESTS

To proceed as described in *Biological safety tests of the hyperimmune serum monograph for human use*.

DOSAGE

The power test of the sample aims to determine the neutralizing dose required (Effective Dose of 50%) to protect susceptible animals against the lethal effects of a fixed dose of *reference Poison*.

Poison reference: homogeneous mixture of poisons that represent the geographic distribution of the species *Tityus serrulatus*. It should be lyophilized and kept at -20 °C. The poison is standardized by determining the 50% Lethal Dose (DL₅₀).

Determination of DL₅₀ of poison: to proceed as described in *Determination of DL₅₀ of poison in the monograph of antipoison serum (pentavalent)*.

Determining the potency of the serum: to proceed as described in *Determination of serum power from the antiotherapeutic serum monograph (pentavalent)*.

There may be a variation coefficient of 10% because of the inherent variation in the tests with laboratory animals. Thus the minimum power of the scorpion fraction may vary up to 0.9 mg/mL.

S

PACKAGING AND STORAGE

In accordance with the monograph hyperimmune serums for human use.

LABELLING

To comply with the legislation in force.

SERUM, ANTILONOMIC Immunoserum lonomicum

The antilonomic serum is a solution containing specific immunoglobulin purified from plasma obtained from animals hyperimmunized with extract of *Lonomia obliqua walker*. It meets the specifications and controls prescribed in the *Hyperimmune serums monograph for human use*. It contains in each milliliter,

immunoglobulin sufficient to neutralize 0.35 mg of Reference poison of *L. obliqua*.

IDENTIFICATION

A. To proceed as described in the test **A.** of *identification of the Hyperimmune serum monograph for human use*, using as poison antigen of the extract from bristles of *Lonomia obliqua walker*.

B. It meets the requirements described in *Dosage*.

CHARACTERISTICS

To proceed as described in the Characteristics of the hyperimmune serums monograph for human use.

PHYSICAL AND CHEMICAL TESTS

To proceed as described in the Physico-chemical tests of the hyperimmune serums monograph for human use.

BIOLOGICAL SAFETY TESTS

To proceed as described in Biological safety tests of the hyperimmune serum monograph for human use.

DOSAGE

The power test of the sample aims to determine the neutralizing dose required (Effective Dose of 50%) to protect susceptible animals against the blood incoagulability caused by a fixed dose of poison from *L. obliqua*.

Poison reference: poison extracted from *L. obliqua* through maceration of the bristles with buffered saline solution. After extract centrifugation, the supernatant containing the poison is distributed in bottles and must be maintained at -20° C. The poison is standardized by determining the 50% incoagulability dose (DI_{50}).

Determination of DI_{50} of the poison: to make dilutions of the *Reference poison* with saline solution at 0.85% (w/v), using constant dilution factor of 1:1 to 1:5, and equating the final volumes of the same diluent. To inoculate, intraperitoneally, 0.5 mL volume per mice of each dilution in groups of, at least six, BALB/c mice, male, of 18 g to 22 g. To observe the animals for two hours after inoculation, and collecting, with the aid of a Pasteur pipette, about 300 µL of blood by puncture of the orbital-recto plexus. To transfer to a test tube and determine the clotting time by visual observation. The maximum clotting time is two minutes. Blood samples not forming blood clot in the allotted time interval are considered incoagulable. To record the number of animals in which blood clotting occurs and total of bled animals. To calculate the DI_{50} using proven statistical methods (probit, Spearman & Karber, angular or logits transformation). The response range (percentage of incoagulable) must be between the highest and lowest dilution used in the test sample forming the regression curve that should present linear relationship. Confidence limits should not be large; they must indicate better test accuracy as smaller their limits. To express the result in poison micrograms per 0.5 mL.

Determination of serum power: to perform progressive dilutions of the sample in saline solution at 0.85% (w/v) using a constant dilution factor of 1:1 to 1:5, so that the final volume after mixing with challenge dose of 3 DI_{50} of the *Reference poison* is identical in all the test tubes. To make it homogeneous and incubate the mixture at 37°C for 60 minutes. To inoculate, intraperitoneally, 0.5 mL per mice of each mixture in groups of, at least six, BALB/c mice, male, of 18 g to 22 g. To observe the animals until two hours after inoculation, and with the aid of a Pasteur pipette, collect about 300 µL of blood by puncture of the orbital-recto complex. To transfer to a test tube and determine the clotting time by visual observation. Blood samples not forming blood clot in the allotted time interval up to two minutes are considered coagulable. To record the number of animals in which blood clotting occurs and total of bled animals. To calculate the 50% Effective Dose (DE_{50}) in micro liters, using proven statistical method. The response range (percentage of coagulable) must be between the highest and lowest dilution used in the sample and standard test forming the regression curve that should present linear relationship. Confidence limits should not be large; they must indicate better test accuracy as smaller their limits. To calculate the power in milligrams per milliliter, according to the expression:

$$\text{Potência} \left(\frac{\text{mg}}{\text{mL}} \right) = \frac{T_v - 1}{DE_{50}} \times DI_{50}$$

where

T_v = number of DI_{50} used per mice in the test dose of poison.

The power title is expressed as milligrams of poison neutralized per mL of the sample. There may be a variation coefficient of 10% because of the inherent variation in the

tests with laboratory animals. Thus the minimum power may vary up to 0.32 mg/mL.

PACKAGING AND STORAGE

In accordance with the monograph hyperimmune serums for human use.

LABELLING

To comply with the legislation in force.

SERUM ANTI-LOXOSCELIC TRIVALENT Immunoserum loxoscelicum

The anti-loxoscelic serum is a solution that contains specific immunoglobulins purified from plasma obtained from animals hyperimmunized with antigen solution of the *Loxosceles*, genre composed of *spiders poisons*, *Loxosceles gaucha*, *Loxosceles intermedia* and *Loxosceles laeta*. It meets the specifications and controls prescribed in the *Hyperimmune serums monograph for human use*. Each milliliter contains immunoglobulins sufficient to neutralize 15 minimum necrotizing dose (DMN) of reference poison of *L. intermedia*.

IDENTIFICATION

A. To proceed as described in the test **A.** the *Identification of the Hyperimmune serums monograph for human use*, using as poison antigen from *L. intermedia*.

B. It meets the requirements described in *Dosage*.

CHARACTERISTICS

It complies with the described characteristics in the hyperimmune serums monograph for human use.

PHYSICAL AND CHEMICAL TESTS

It complies with the described Physico-chemical tests in the hyperimmune serums monograph for human use.

BIOLOGICAL SAFETY TESTS

It complies with the described Biological safety tests of the hyperimmune serum monograph for human use.

DOSAGE

The power test of the sample aims to determine the neutralizing dose required to protect susceptible animals against the lethal effects of dermonecrotics effects of a Minimum Necrotizing Dose (DMN) of *Reference poison*.

Poison reference: poison extracted from *L. intermedia*, which must be crystallized or lyophilized and kept at -20 °C. The poison is standardized by determining the DMN, which is the smallest amount capable of causing poison,

within 72 hours, necrosis of approximately one centimeter in diameter by intradermal injection in the internal face of the rabbit ear.

Determination of the DMN of poison: to reconstitute the lyophilized or crystallized preparation of poison for a given concentration (w/v) with saline solution at 0.85% (w/v). To perform dilutions in geometric progression with the same diluent, starting with a dose of 3 mg of poison and using the constant dilution factor, not exceeding 1.5.

to inoculate into two albino rabbits from 1.8 kg to 2.3 kg, intradermally in a volume of 0.1 ml of each dilution on the inner side of both ears of each rabbit. To observe the animals within 72 hours after inoculation, record the appearance of the lesions and measure dermonecrosis. The DMN is calculated according to the expression:

$$DMN = \frac{(A + B)}{2}$$

where

DMN = Minimum Necrotic Dose (cm); *A* = average among the maximum diameters of the four inoculated points;
B = average among the minimum diameters at four inoculated points.

The result is expressed in the least amount in mg of poison capable of causing an dermonecrotic injury of approximately 1 cm in diameter.

Determination of the serum power: to perform dilutions of the sample in saline solution at 0.85% (w/v), to determine the highest dilution that neutralizes 1 DMN of the *Reference poison*, using a constant dilution factor not exceeding to 1.5. To reconstitute and dilute the *Reference poison* with saline solution at 0.85% (w/v), so that each dose of 0,1 mL to be inoculated per animal containing 1 DMN. To inject, intradermally, a dose of 0.1 mL of this dilution of the *Reference poison* on the inner side of one ear of each one of three rabbits. Then deliver 1 mL of diluted serum in the marginal vein of the ear opposite to that in which the poison was inoculated. In parallel, perform a control of the poison by inoculating 1 DMN per ear, at least, plus one rabbit. To observe the animals within 72 hours after inoculation, regarding the appearance of dermonecrosis. To record the highest dilution of serum that does not cause necrosis.

The power title is expressed as DMN of poison neutralized per milliliter of the serum.

PACKAGING AND STORAGE

In accordance with the hyperimmune serums monograph for human use.

LABELLING

To comply with the legislation in force.

ANTIRABIC SERUM

The antirabic serum is a solution containing specific immunoglobulin purified from plasma obtained from animals hyperimmunized against the rabies virus. In the immunization of animals are used strains of fixed virus, inactivated or not, replicated in cultivation of cells other than those used in preparing the rabies vaccine for human use. It meets the specifications and controls

prescribed in the *hyperimmune serums monograph for human use*. In each milliliter it contains at least 200 UI.

IDENTIFICATION

The *Dosagem* methods may be used. CHARACTERISTICS

To proceed as described in the *Characteristics of the hyperimmune serums monograph for human use*.

PHYSICAL AND CHEMICAL TESTS

To proceed as described in the *Physico-chemical tests of the hyperimmune serums monograph for human use*.

BIOLOGICAL SAFETY TESTS

To proceed as described in *Biological safety tests of the hyperimmune serum monograph for human use*.

DOSAGE

Serum neutralization method in mice

The power test of the sample aims to determine the neutralizing dose required (Effective Dose of 50%) to protect mice against the lethal effects of a challenge dose of rabies virus. To comparative evaluation of the power of the sample, it uses lyophilized equine serum of reference, measured in international units, the international standard serum distributed by the World Health Organization

Challenge virus: cepa CVS (challenge virus standard), the known 50% lethal dose (DL_{50}).

Determination of DL_{50} : to make serial decimal dilutions of virus with distilled water solution containing 2% (v/v) of normal serum of animal origin or 0.75% (w/v) of bovine albumin. To mix thoroughly, and inoculate intracerebral, 30 μ L volume of each dilution in groups of, at least, 10 Swiss albino mice of 10 g to 15 g. To observe the animals for 14 days. To calculate the DL_{50} statistically proven method by using the number in each group who die or develop symptoms of anger between 5th and 14th days. The reduced response range (percentage of deaths) must be

between the highest and lowest dilution used forming the regression curve that should present a linear relationship.

Determination of serum power: to perform serial dilutions of the sample, with the same diluent described in the *Determination of DL_{50}* , using constant dilution factor, not exceeding 2, until it reached dilution in which supposedly there is no neutralization. To transfer for the test tubes a constant volume of each one of the last four dilutions. To prepare dilution of *virus challenge* containing 100 DL_{50} to initial 500 DL_{50} , using the same diluent. To add in each one of the four tubes already containing serum, the same volume of the challenge dilution, in order to get folded dilutions of virus containing 50 DL_{50} to 250 DL_{50} of the sample in test. To make the mixtures homogeneous. To proceed in a similar manner for the reference serum. At the same time, to determine the actual number of DL_{50} used as challenge preparing four consecutive decimal dilutions with the same diluent from the dilution used as challenge. To distribute a constant volume of diluent into each one of the four test tubes and transfer to the same, beginning with the challenge dilution, the same volume of each one of the serial dilutions of virus. To mix thoroughly, getting bent dilutions of the challenge virus. To incubate the mixture of serum plus virus and virus plus diluent in a water bath at $37 \pm 0.5^\circ\text{C}$ for 90 minutes. To inoculate, through intracerebral way, volume of 30 μ L of each mixture into groups of, at least, eight Swiss albino mice of 10 g to 15 g. To observe the animals in each group for 14 days and record the numbers of animals that die or show symptoms of rabies in the period 5 - 14 days after the challenge.

To calculate the effective doses 50% (DE_{50}) of the sample and from the reference serum, as well as DL_{50} of the challenge virus by statistically proven method. The response range (survival rate) must be between the highest and lowest dilution used in the sample and standard test forming the regression curve that should present a linear relationship. The power is determined according to the expression:

$$\text{Potência } \frac{\text{UI}}{\text{mL}} = \frac{DE_{50} \text{ da amostra em testex conc. em } \frac{\text{UI}}{\text{mL}} \text{ do soro de referência}}{DE_{50} \text{ do soro de referência}}$$

The estimated power should be at least 200 UI/ml and the confidence limits should not be below 25% or above 400% of the determined activity.

Method of serum neutralization of rabies virus in BHK₂₁ cells

Pre-dilute the reference serum and test sample for the concentration of approximately 1 UI/ml and make serial dilutions in the ratio 2 using Eagle-MEM medium with 2.5% of calf fetal serum. Place 50 μ L of each of these dilutions in polystyrene microplate of 96 holes and add the same volume of a dilution of a CVS-11 fixed virus in BHK₂₁ cells, in order to obtain from 30 to 300 doses forming fluorescent foci 50% (DFF_{50}) after mixing with the serum. At the same plate make a titration of the CVS-11 virus with 4 serial dilutions in base 10, being the first being equal to the dilution added to the reference and test serum. To incubate the microplate with mixtures of serum and virus in the

oven with 5% of CO₂ at 37 °C for 90 minutes. Then, adding to each holes 100 uL of a suspension containing 3.7×10^4 BHK₂₁ cells in Eagle MEM medium with 2.5% of calf fetal serum. Placing just two holes in the culture medium and the cells to control the same. To incubate again the microplate at 37 °C in oven with 5% CO₂ for 22 hours. To wash the cells with buffered saline solution with phosphate pH 8.0 and fasten them with acetone being 80% cooled with -20 °C for 15 minutes. To add a rabies anti-nucleocapsid immunoglobulin conjugated with fluorescein isothiocyanate and maintain at 37 °C for 30 minutes. To wash the plates 2 times in buffered saline solution with phosphate pH 8.0. To observe 8 fields in each hole of the microplate in microscope of inverted fluorescence with an increase of 200 times. To consider the positive field that contains one or more fluorescent foci.

To calculate the effective doses 50% (DE₅₀) of the sample and from the reference serum, as well as DL₅₀ of the challenge virus by statistically proven method. The produced response range (percentage of fluorescent foci) must be between the highest and lowest dilution used in the test and standard sample, forming the regression curve that should display a linear relationship and the statistical analysis proves a significant slope of the dose/response lines and no significant deviations from the linearity and parallelism. The power is determined according to the expression:

$$\text{Potência } \frac{\text{UI}}{\text{mL}} = \frac{\text{DE}_{50} \text{ da amostra em testex conc. em } \frac{\text{UI}}{\text{mL}} \text{ do soro de referência}}{\text{DE}_{50} \text{ do soro de referência}}$$

The estimated power should be at least 200 UI/ml and the confidence limits should not be below 80% or above 125% of the determined activity.

PACKAGING AND STORAGE

In accordance with the monograph hyperimmune serums for human use.

LABELLING

To comply with the legislation in force.

TETANUS ANTITOXIN SERUM

Immunoserum tetanicum ad usum humanum

The tetanus antitoxin serum is a solution containing purified immunoglobulin from plasma obtained of animals hyperimmunized against toxins produced by *Clostridium tetani*. It meets the specifications and tests prescribed in the *Hyperimmune serums monograph for human use*. In each milliliter it contains at least 1000 UI of antitoxin.

IDENTIFICATION

A. To proceed as described in the test **A.** the *Identification* of the *Hyperimmune serums monograph for human use*, using as toxin antigen from *C. tetani*.

B. It meets the requirements described in *Dosage*.

CHARACTERISTICS

To proceed as described in the Characteristics of the hyperimmune serums monograph for human use.

PHYSICAL AND CHEMICAL TESTS

To proceed as described in the Physico-chemical tests of the hyperimmune serums monograph for human use.

BIOLOGICAL SAFETY TESTS

To proceed as described in Biological safety tests of the hyperimmune serum monograph for human use.

DOSAGE

The power test of the sample aims to determine the neutralizing dose required to protect animals used in the test, against the lethal effects of a test dose of the tetanic toxin of reference. The serum dose under the test is compared with the dose of the tetanic antitoxin of reference necessary to give the same protection.

Tetanic antitoxins of reference: the international standards of reference of the tetanic antitoxin is distributed to laboratories of production and control in ampoules containing lyophilized hyperimmune equine serum, which specifically neutralizes the tetanic toxin. The equivalence in international units of International Reference Standard is periodically established by World Health Organization.

Tetanus toxin of reference: it is prepared from sterile filtered of liquid cultures supernatants of *C. tetani* incubated for five to seven days. The filtrate should be concentrated, purified by physical or chemical methods and lyophilized. After reconstitution, add saline solution with glycerin and store at -20 °C.

Determination of toxin test dose (L +/10): to dilute the reference antitoxin to 1 UI/ml with saline solution and 0.85% (w/v). To dilute the toxin to a specific concentration in saline solution containing peptone at 1% (w/v). In a series of test tubes, add varying volumes of toxin and the a constant volume of diluted antitoxin of reference. To equal volumes with the same toxin diluent. To make it homogeneous and incubate at 37°C for 60 minutes. To inoculate in every Swiss albino mice from 17 g to 22 g, subcutaneously, a volume containing 0.1 UI of antitoxin of reference in groups of at least 10 mice for mixture. To observe the animals until 96 hours after inoculation and record the number of the dead ones per mixture. The L +/10 (limit of death per 10) or the test dose of toxin is the least amount of toxin, which, when combined with 0.1 UI of antitoxin of reference, causes the death of the animals during the stipulated observation period.

Determination of serum power: to dilute the tetanic toxin of reference with buffered saline solution containing pep-

tone at 1% (w/v) at a dose of 10 L $\pm$ 10. In a series of test tubes, distributing variable volumes of the sample. Add 1 mL of diluted tetanic toxin of reference. To equal volumes to 2 mL with the same diluent. To make it homogeneous and incubate the mixture at 37°C for 60 minutes. To inoculate in every Swiss albino mice from 17 g to 22 g, subcutaneously, a volume of 0.2 mL in groups of, at least, 10 mice per mixture. To observe the animals until 96 hours after inoculation and record the number of the dead ones. Under the same conditions described and parallel, perform the test with tetanic antitoxin *of reference*, with the objective of verifying the validity of the test and establish correlation in the calculation of the title. To calculate the power of the serum in test, UI/mL, whereas the lowest dilution that determines the death of all animals during the observation period, using the following equation:

$$\text{Power} = \frac{A}{B} \times C$$

where

A = the inverse of the lower dilution (or larger volume) of the serum that kills all animals;

B = the volume used in diluted serum that kills all animals;

C = total number of doses contained in the final volume of each mixture by the title *L* $\pm$ 10.

PACKAGING AND STORAGE

In accordance with the *hyperimmune serums monograph for human use*.

LABELLING

To comply with the legislation in force.

HYPERIMMUNE SERUM FOR HUMAN USE Immunosera ad usum humanum

The hyperimmune serum are preparations containing purified immunoglobulins of animal origin, specifically that neutralize bacterial toxins, bacteria, viruses or toxic components of the poison of one or more species of poisonous animals. A suitable preservative may be added and the final product is presented in liquid or lyophilized form. The liquid product is clear, colorless or slightly yellowish, showing no lumps or particles. The lyophilized serum consists of white or slightly yellowish powder that once reconstituted, presents the same characteristics of the liquid preparations.

The purified immunoglobulins are obtained by enzymatic treatment and fractional precipitation, or other chemical or physical procedures, plasmas of healthy animals immunized with specific antigens. During the process of immunization, the animals should not be treated with penicillin or streptomycin.

To ensure the quality of the product at various stages of processing, tests of sterility, pH, protein, activity or potency by *in vitro* methods or *in vivo* shall be performed.

Before bottling, product samples are subjected to the determinations that follow.

Sodium chloride. 0,70% (w/v) to 0,90% (w/v).

Phenol. At most 0.35% (w/v).

Nitrogen and protein. At most 0.30% (w/v) of non-protein nitrogen. At most 15% (w/v) of protein.

Power. It is determined according to the procedures indicated in the respective monographs.

Total solids. At most 20%.

Ammonium sulfate At most 0.20% (w/v).

The preparation is distributed aseptically in ampoules or vials. Lyophilization of the product when required to ensure water concentration not exceeding 3% of the final product.

IDENTIFICATION

A. Based on the reaction *in vitro* of antigen-antibody by *double radial immunodiffusion (Ouchterlony)*. To prepare agar gel at 1% (w/v) and distribute in blade for microscope, so that results in a thin layer. To place in an oven at 37 °C without drying. To add 4 mL of agar in the plate and put at temperature of 2 °C to 8 °C in a humidified chamber for one hour. To make holes in the gel, maintaining the same distance among the central hole and the peripherals. To fill the central hole with specific antigen solution and peripherals with the test sample in varying dilutions. To fill one of the holes with equine normal serum for negative control. To incubate at 37 °C for 24 hours in a humid chamber and perform a reading in lamp for contrast. To observe the presence of precipitation line and identity reaction between the analyzed components.

B. It meets the requirements described in *Dosage*.

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

pH (5.2.19). 6.0 to 7.0

PHYSICAL AND CHEMICAL TESTS

Sodium chloride. In a 50 mL erlenmeyer, add 10 mL of diluted sample and 10% (v/v) in double distilled water. To add, with stirring, three drops of bromophenol blue difenil-carbazona SR and then a few drops of nitric acid 0.20 *M* SV until the solution is yellow-green. To perform the blank test. To titrate with nitrate of mercury (II) 0.01*M* SV until the turning point, in which a violet coloration indicates the final point. Each mL of 0.1 *M* SV nitrate of mercury (II) is equivalent to 0.585 mg of sodium chloride. Producer is

allowed to use the result from the product before bottling. Between 0.70% (w/v) and 0.90% (w/v).

Phenol. At most 0.35% (w/v). To employ one of the methods described below.

A. To dilute the sample so that the concentration of phenol is between 5 ppm and 30 ppm. To add 5 mL of borate buffer, pH 9.0, 5 mL 4-aminoantipyrine at 0.10% (w/v) and 5 mL of potassium ferricyanide at 5% (w/v). At the same time, developing white and a calibration curve of phenol with concentrations ranging from 5 ppm to 30 ppm. To proceed the reading of the absorbance (5.2.14) of the sample, from the patterns and the white to a wavelength of 546 nm, 10 minutes after the end of the reaction. To use the reading of the patterns to make the analytical curve and determining the concentration of phenol in the sample by graphic interpolation or linear regression. Producer is allowed to use the result from the product before bottling.

B. To add 1 mL of the sample in a volumetric flask and top up the volume to 200 mL with distilled water. From that solution, take 5 mL and transfer it for a 25 mL volumetric flask. To add 3 mL of borate buffer, pH 9.0, 2.5 mL 4-aminoantipyrine at 0.15% (w/v) and 0.5 mL of potassium ferricyanide at 5% (w/v). To stir and top up to volume with distilled water. At the same time, developing the white and a calibration curve of phenol with concentrations ranging from 0.6 µg to 3.9 µg of phenol per milliliter. To proceed the reading of absorbance (5.2.14) of the sample, from the patterns and the white to a wavelength of 495 nm, 20 - 40 minutes after the end of the reaction. To use the reading of the patterns to make an analytical curve and determining the concentration of phenol in the sample by graphic interpolation or linear regression.

Protein nitrogen and protein. To proceed as described in *Determination of nitrogen by the Kjeldahl method* (5.3.3.2) At most 0.3% (w/v) of non-protein nitrogen and 15% (w/v) of proteins. To determine the protein concentration, multiply the result of protein nitrogen by 6.25. Producer is allowed to use the result from the product before bottling.

Total solids. In previously tared filter weighing, weigh exactly 1 g of the sample in duplicate and put it in the chapel of exhaustion on the warming plate until the evaporation of the liquid. To transfer the weights-filter with the sample for the oven at 105 °C and leave it there for 1 hour. To transfer the desiccated sample for the desiccator, leave it there for 30 minutes and weigh. to repeat the procedure of desiccation to constant weight. To calculate the percentage of total solids according to the equation:

$$\% \text{ of total solids} = B \times 100 C$$

where

B = difference between desiccated weights-filter and the empty weights-filter;
C = weight of the sample.

Producer is allowed to use the result from the product before bottling. At most 20%.

Ammonium sulfate to dilute sample with 1% (v/v) with double distilled water and transferred 10 mL of the solution to Nessler tube. To transfer 1 mL of sulfate stock solution of ammonium at 0.6% (w/v) to 100 mL volumetric flask and top up the volume with double distilled water. To dilute the solution in a proportion 1:2, 1:3, 1:4, and transfer 10 mL of each dilution to three Nessler tubes. To add 1 mL of Nessler's reagent to each tube containing the sample and comparing the patterns and color. The color intensity of the sample is equal to or smaller than the diluted standard solution 1:3. Producer is allowed to use the result from the product before bottling. At most 0.2% (w/v).

Residual moisture. It is determined when the product is presented in lyophilized form. To transfer 80 mg of the sample into a weights-filter previously dried and tared. To keep for three hours in an atmosphere of anhydrous phosphorous pentoxide under pressure not exceeding 5 mm of mercury at a temperature of 60 °C. The weights-filter containing the sample is cooled for 20 minutes in a desiccator containing silica gel and immediately weighed. The step of heating and cooling is repeated until a constant weight is obtained. The amount of residual moisture is the average percentage of weight loss, at least, of three evaluations of the sample. The method for determining the volumetric water (5.2.20.1) can also be used. At most 3%.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). It complies with the test. to use only the membrane filtration method, which must have porosity rating no greater than 0.45 µm.

Pyrogens (5.5.2.1). It complies with the test. To inject 1 mL/kg and not reuse the animals used in the test.

DOSAGE

For determination of power proceed as described in the specific monograph. Producer is allowed to use the result from the product before bottling.

PACKAGING AND STORAGE

The temperature and expiry date are indicated by the manufacturer of the serum, based on experimental evidence, approved by the national control authority.

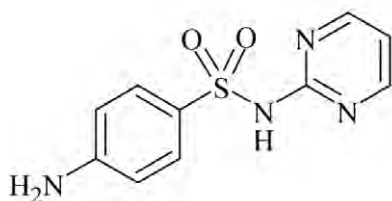
LABELLING

To comply with the legislation in force.

S

SULFADIAZINE

Sulfadiazinum



C₁₀H₁₀N₄O₂S; 250,28

sulfadiazine; 08116

4-Amino-N-2-pyrimidinyl-benzenesulfonamide
[68-35-9]

It contains at least 98.0% and at most 102.0% of C₁₀H₁₀N₄O₂S, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white or yellowish-white powder; odorless. Darkens slowly by exposure to light.

Solubility. Practically insoluble in water, slightly soluble in ethanol and acetone, insoluble in chloroform. Freely soluble in diluted solutions of alkali hydroxides and soluble in hydrochloric acid solutions 3M.

Physical and chemical constants

Fusion range (5.2.2): 252 °C to 256 °C with decomposition. **IDENTIFICATION**

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the sulfadiazine SQR spectrum similarly prepared.

B. The principal spot in the chromatogram of *Solution (1)* in the *Related substances* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

C. To dissolve 50 mg of the sample in 2 mL of hydrochloric acid SR with heating. To cool down in an ice bath and add 2 mL of sodium nitrite SR. To dilute with 2 mL of ice water and add 1 mL of 2-naphthol SR. Orangish precipitate is produced.

D. To heat gently to direct flame or in a sand bath, 50 mg of the sample in a dry test tube. It develops a reddish-brown color, and the vapors they give off does not change the role of moistened lead acetate.

E. to heat 1 g of the sample, gently, in a test tube over a small flame until sublime. With glass rod, gather a few milligrams of sublimated and mix in a test tube with 1 mL of resorcinol at 5% (w/v) in ethanol at 90% (v/v). To add 1 mL of sulfuric acid and stir it. It produces dark red color

immediately. To dilute the mixture thoroughly with 25 mL of ice water and add excess of ammonia 6 M. It develops blue or reddish-blue coloration.

PURITY TESTS

Appearance of solution. To dissolve 1 g of the sample in mixture of 5 mL of sodium hydroxide SR and 20 mL of water. The resulting solution is clear (5.2.25).

Acidity. To heat 1 g of the sample with 50 mL of water free from carbon dioxide at 70 °C for 5 minutes. To cool it down rapidly to 20 °C and filter it. A maximum of 0.2 mL of 0.01 M sodium hydroxide is spent to neutralize 25 mL of the filtrate, by using phenolphthalein SI as an indicator.

Related substances. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF₂₅₄ silica gel as support and a mixture of chloroform, methanol, and ammonium hydroxide (30:12:1) as mobile phase. To separately apply to the plate 20 µL of each of the solutions freshly prepared, as described below.

Solution (1): 100 µg/mL solution of the sample in a mixture of toluene and dimethylformamide (2:1).

Solution (2): 100 µg/mL solution of sulfadiazine SQR in a mixture of toluene and dimethylformamide (2:1).

Solution (3): 2 µg/mL solution of sulfanilamide in a mixture of toluene and dimethylformamide (2:1).

To develop the chromatogram. To remove the plate, and let it air drying. To nebulise with hydrochloric acid 1 M in methanol and then with sodium nitrite at 1% (w/v) in ethanol at 90% (v/v). To wait 3 to 5 minutes to nebulizer with dihydrochloride of W-(1-naphthyl) ethylenediamine in 0,5% (w/v) in ethanol at 90% (v/v). Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (3)* (0,2%).

Arsenic (5.3.2.5). To proceed as described in *Visual method*. At most 0,0002% (2 ppm).

Chlorides (5.3.2.1). To boil mixture of 1 g in 10 mL of nitric acid SR and 5 mL of distilled water. To proceed as described in *Limit test for chlorides*. At most 0.035% (350 ppm).

Heavy metals (5.3.2.3). At most 0.002% (20 ppm).

To use the *Method III*.

Sulfates (5.3.2.2). To dissolve 1 g of the sample, with heating, in the mixture of 10 mL of hydrochloric acid SR and 5 mL of water. To proceed as described in *Limit test for sulfates*. At most 0.12% (1200 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 105 °C for 2 hours. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Titration by diazotization (5.3.3.1), Method 2*. Each mL of 0.1 M SV sodium nitrite is equal to 25.028 mg of $C_{10}H_{10}N_4O_2S$.

B. To proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. To dissolve accurately weighed amount of sample in 0.1 M sodium hydroxide and diluted with the same solvent until the concentration of 0.001% (w/v). To prepare a standard solution at the same concentration by using the same solvent. To measure the absorbances of the solutions at 254 nm, by using 0.1 M sodium hydroxide to adjust zero. To calculate the content of $C_{10}H_{10}N_4O_2S$ in the sample from the readings obtained.

C. To proceed as described in *Visible absorption spectrophotometry (5.2.14)*. To weigh accurately about 0.5 g of the sample, transfer it to a 200 mL volumetric flask with the assistance of 30 mL of sodium hydroxide 0.1 M. To stir until de dissolution, top up it with water and make it homogeneous. To perform serial dilutions in water to concentrations of 0.005% (w/v). To prepare a standard solution of 0.25% (w/v) in a mixture of water and 0.1 M sodium hydroxide (85:15). To perform serial dilutions in water to concentrations of 0.005% (w/v). To transfer 2 mL of the *Standard solution* and the *Sample solution* for 25 mL volumetric flask. To add to each flask, 1 mL of 2 M hydrochloric acid and 1 mL of sodium nitrite at 0.1% (w/v). To stir and let it rest for 2 minutes. To add 1 mL of ammonium sulfamate, 0.5% (w/v), stir and let stand for 2 minutes. To add 1 mL of dihydrochloride Af-(1-naphthyl) ethylenediamine SR, stir and let stand for 10 minutes. To top up the volume with water. To prepare blank at the same time. To measure the absorbances of the solutions at 540 nm, by using the blank to adjust zero. To calculate the content of $C_{10}H_{10}N_4O_2S$ in the sample from the readings obtained.

PACKAGING AND STORAGE

In tightly opaque containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antiseptic.

TABLETS SULFADIAZINE

It contains at least 95.0% and at most, 105.0% of the stated amount of $C_{10}H_{10}N_4O_2S$.

IDENTIFICATION

To weigh and pulverize the tablets. To use amount of powder equivalent to 0.5 g of sulfadiazine and grinding in a mortar with 5 mL of chloroform. To filter and discard the filtrate. To triturate the residue with 10 mL of ammonium hydroxide, 6 M for five minutes, add 10 mL of water and filter it. To heat the filtrate to remove all ammonia and cool it. To add acetic acid 6 M until distinctly acid reaction. It is formed the precipitate of sulfadiazine. To filter and wash the precipitate with cold water. To dry the residue at 105 °C for 1 hour. The infrared absorption spectrum (5.2.14) of the residue, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed with sulfadiazine SQR.

The ultraviolet absorption spectrum (5.2.14) ranging from 200 nm to 400 nm, of 0.001% solution (w/v) of the residue obtained in the test A. of identification in ethanol, exhibits maxima and minima only in the same wavelength of a similar to sulfadiazine SQR solution.

The principal spot obtained with *Solution (1)*, obtained in the *Related substances* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

To dissolve 50 mg of the resulting residue obtained in test A. Identification in 2 mL of hydrochloric acid at 10% (w/v), with heating. To cool down in an ice bath and add 2 mL of sodium nitrite at 10% (w/v). To dilute with 10 mL of ice water and add 2 mL of 2-naphthol SR. Orange precipitate is formed.

CHARACTERISTICS

Weight determination (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage unit (5.1.6) It complies with the test. To proceed as described in Dosage method A. of *Dosage*.

DISSOLUTION TEST (5.1.5)

Dissolution medium: hydrochloric acid 0.1 M, 900 mL
Equipment: shovels, 75 rpm *Time:* 90 minutes

Procedure: after the test, remove the aliquots from dissolution medium and filter it. To dilute in sodium hydroxide solution 0.1 M to the appropriate concentration. To measure the absorbances of the solutions at 254 nm, (5.2.14), by using 0.1 M sodium hydroxide to adjust zero. To calculate the

amount of $C_{10}H_{10}N_4O_2S$ dissolved in the medium, by comparing the readings obtained with that of 0.0005% sulfadiazine SQR solution (w/v) prepared in the same solvent.

Tolerance: not less than 70% (Q) of the amount stated of $C_{10}H_{10}N_4O_2S$ dissolves within 90 minutes.

PURITY TESTS

Related substances. To proceed as described in the related Substances in the sulfadiazine monograph. To prepare the Solution (1) using the obtained residue in test A. of Identification.

Water (5.2.20.1). At most 3%.

BIOLOGICAL SAFETY TESTS

Counting of the total number of mesophyll microorganisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To proceed as described in *High performance liquid chromatography (5.2.17.4)*. To use chromatograph equipped with an ultraviolet detector at 257 nm; 300 mm chromatograph column and 4.0 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group, kept at room temperature; flow of Mobile phase of 1.0 mL/min.

Mobile phase: mixture of water, acetonitrile and glacial acetic acid (87:12:1).

Sample solution: to weigh and pulverize at least 20 tablets. To transfer amount of powder, accurately weighed, equivalent to 0.1 mg of sulfadiazine to 100 mL volumetric flask, add 75 mL of 0.025 M sodium hydroxide, put it in ultrasound for 10 minutes, and top up with the same solvent, mix and filter it.

Standard solution: dissolve the amount, accurately weighed, sulfadiazine SQR in solution of 0.025 M sodium hydroxide so as to obtain a solution of about 1 mg/mL.

To inject replicates of 10 μ L of the *Standard solution*. The tailing factor is less than 1.5. The relative standard deviation for the replicate areas for the peaks recorded should not be greater than 2.0%.

Procedure: to separately inject 10 μ L of *Standard solution* and the *Sample solution*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of $C_{10}H_{10}N_4O_2S$ in the tablets, from the responses obtained for the *Sample solution* and the *Standard solution*.

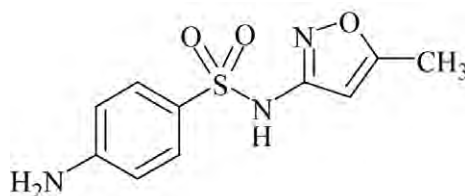
PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

SULFAMETHOXAZOLE Sulfamethoxazolum



$C_{10}H_{11}N_3O_3S$; 253,28 sulfamethoxazole; 08134
4-Amino-N-(5-methyl-3-isoxazolyl) benzenesulfonamide
[723-46-6]

It contains at least 99.0% and at most 101.0% of $C_{10}H_{11}N_3O_3S$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white or almost white powder.

Solubility. Practically insoluble in water, freely soluble in acetone, slightly soluble in ethanol, and sparingly soluble in ethyl ether. Easily soluble in dilute solutions of sodium hydroxide.

Physical and chemical constants

Fusion range (5.2.2): 168 °C to 172 °C.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the sulfamethoxazole SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14), from 200 nm to 400 nm, solution of the sample at 0.001% (w/v) prepared with 0.1 M sodium hydroxide, has absorption maximum and minimum similar to those observed in the spectrum of a similar solution of sulfamethoxazole SQR. The reading sample absorbance at 257 nm does not differ by more than 2% of absorbance of the sulfamethoxazole SSR.

C. The principal spot in the chromatogram of *Solution (2)* in the *Related substances* corresponds in position, color, and intensity to that obtained with *Solution (3)*.

D. To dissolve 0.1 g of the sample in 2 mL of hydrochloric acid 2 M. The solution obtained responds to the primary aromatic amine reaction (5.3.1.1).

PURITY TESTS

Alkalinity. To add 25 mL of water and 1.25 g of finely pulverized sample. To heat at 70 °C for 5 minutes.

To cool it down in ice water for 15 minutes and filter it. With 20 mL of the filtrate, add 0.1 mL of bromothymol blue SI. No more than 0.3 mL of 0.1 M sodium hydroxide is required to change the color of the indicator.

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel as support and a mixture of ammonia, water, nitromethane and dioxane (3:5:40:50) as mobile phase. To separately apply to the plate 5 µL of each of the solutions freshly prepared, as described below.

Solution (1): to accurately transfer about 0.1 g of the sample to a 5 mL volumetric flask. To dissolve in a mixture of ammonia and methanol (2:48) and top up the volume with the same solvent.

Solution (2): to dilute 1 mL of *Solution (1)* to 5 mL in mixture of ammonium and methanol (5:95).

Solution (3): to transfer 20 mg of sulfamethoxazole SQR to 5 mL volumetric flask, dissolve 3 mL of mixture of ammonium and methanol (75:25), and top up with the same solvent.

Solution (4): to dilute 1.25 mL of the *Solution (2)* to 50 mL in a mixture of ammonium and methanol (2:48).

To develop the chromatogram. To remove the plate, dry it at 105 °C. To examine under ultraviolet light (254 nm). Any spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, it should not be more intense than that obtained in the chromatogram with *Solution (4)* (0.5%).

Sulfanilamide and Sulphanilic Acid. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel as support and a mixture of ethanol, n-heptane, chloroform and glacial acetic acid (25:25:25:7) as mobile phase. To separately apply to the plate 10 µL of *Solutions (1) and (3)* and 25 µL of *Solution (2)* freshly prepared, as described below.

Solution (1): to accurately transfer about 0.1 g of sulfamethoxazole SQR to a 10 mL volumetric flask. To dissolve in 0.1 mL of ammonium hydroxide and top up the volume with methanol.

Solution (2): to transfer 20 mg of sulfanilamide SQR to 20 mL volumetric flask, dissolve 10 mL of ammonium hydroxide and top up the volume to 100 mL volumetric flask with methanol. To transfer 2 mL of the resulting solution to

50 mL volumetric flask, add 10 mL of ammonium hydroxide and top up with methanol.

Solution (3): to accurately transfer about 0.1 g of the sample to a 10 mL volumetric flask. To dissolve in 0.1 mL of ammonium hydroxide and top up the volume with methanol.

To develop the chromatogram. To remove the plate, and let it drying. To spray the plate with Erlich reagent modified. The retention factors (Rf) are: 0.7 for sulfamethoxazole, 0.5 for sulfanilamide and 0.1 for the sulfanilic acid. *The Solution (3)* should not present spot greater than 0.2% for sulfanilamide and sulfanilic acid, obtained in the chromatogram of *Solution (2)*.

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 105 °C for 4 hours to obtain constant weight. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To proceed as described in *Titrations by diazotization* (5.3.3.1), *Method 2*. To accurately dissolve about 0.5 g of the sample in mixture of 20 mL of glacial acetic acid and 40 mL of water and add 15 mL of hydrochloric acid. To cool it down to 15 °C. To titrate immediately with 0.1M sodium nitrite SV. To potentiometrically determine the endpoint. Each mL of 0.1 M SV sodium nitrite is equal to 25.330 mg of C₁₀H₁₁N₃O₃S.

PACKAGING AND STORAGE

In airtight containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antibacterial

SULFAMETHOXAZOLE AND TRIMETHOPRIM TABLETS

It contains at least 93.0% and not more than 107.0% of the stated amount of sulfamethoxazole (C₁₀H₁₁N₃O₃S) and trimethoprim (C₁₄H₁₈N₄O₃).

IDENTIFICATION

A. To filter the aqueous layer reserved in method **A.** of *Dosage*. To add, dropwise, sufficient amount of hydrochloric acid 2 M to acidify and extract with 50 mL of ethyl ether. To wash the ethereal layer with 10 mL of water, mix

it with 5 g of anhydrous sodium sulfate, filter and evaporate the filtrate to dryness. To dissolve the residue in minimum volume of anhydrous sodium carbonate at 5% (w/v), add dropwise, hydrochloric acid *M* until precipitation and filter it. To wash the precipitate with water and drying at 105 °C until constant weight. The infrared absorption spectrum (5.2.14) of the residue, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the sulfamethoxazole SQR spectrum.

B. To weigh and pulverize 20 tablets. To transfer quantity of powder equivalent to 50 mg of trimethoprim to a separating funnel, add 30 mL of 0.1 *M* sodium hydroxide and stir it. To extract with four portions of 50 mL of chloroform, washing each extract with two portions of 10 mL, 0.1 *M* sodium hydroxide and 10 mL of water. To stir with 5 mg anhydrous sodium sulfate, filter and dry at 105 °C until constant weight. The infrared absorption spectrum (5.2.14) of the residue, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the trimethoprim SQR spectrum.

C. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using G silica gel as support and a mixture of chloroform and isopropyl alcohol and diethylamine (60:50:10), as mobile phase. To separately apply to the plate 20 µL of each of the solutions freshly prepared, as described below.

Solution (1): to weigh and pulverize the tablets. To accurately transfer amount of powder equivalent to 4 mg of trimethoprim to 10 mL volumetric flask and add 8 mL of methanol. To leave it in ultrasound for 15 minutes and mechanically stir for 15 minutes. To top up with methanol. To make it homogenous and filter it.

Solution (2): to prepare 0.4 mg/mL solution of trimethoprim SQR in methanol.

Solution (3): to prepare 2 mg/mL solution of sulfamethoxazole SQR in methanol.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). The spots relating to sulfamethoxazole and trimethoprim obtained with *Solution (1)* correspond in position, color, and intensity to those obtained with *Solution (2)* and the *Solution (3)*.

D. The retention times of the main peaks in the chromatogram of the *Sample solution* obtained in the method **C.** of *Dosage* corresponds to that of main peaks of *Standard solution*.

CHARACTERISTICS

Weight determination (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage unit (5.1.6) It complies with the test. To proceed as described in the method **C.** of *Dosage*.

DISSOLUTION TEST (5.1.5)

Dissolution medium: hydrochloric acid 0.1 *M*, 900 mL
Equipment: shovels, 75 rpm *Time:* 60 minutes

Procedure: after the test, use aliquot of dissolution medium as *Sample solution* and proceed as described in method **C.** *dilutions, if necessary.*

Tolerance: not less than 70% (Q) of the stated amount of sulfamethoxazole (C₁₀H₁₁N₃O₃S) and trimethoprim (C₁₄H₁₈N₄O₃) dissolve within 60 minutes.

PURITY TESTS

Water (5.2.20.1). At most 3,0%.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll microorganisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic microorganisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To weigh and pulverize 20 tablets. To transfer an amount of powder equivalent to 50 mg of trimethoprim to 25 mL volumetric flask, dissolve in 0.1 *M* sodium hydroxide and top up with the same solvent. To quantitatively transfer, the solution to a separatory funnel, extract with four portions of 50 mL of chloroform, washing it extract with 20 mL of 0.1M sodium hydroxide. Reserve the aqueous layer to the *Identification Test A*. To collect the chloroform extracts and extract with four portions of 60 mL acetic acid *M*. To collect the acid extracts, wash them with 5 mL of chloroform and dilute the aqueous extract to 250 mL with acetic acid *M*. To transfer 10 mL of that solution to 100 mL volumetric flask, To add 10 mL acetic acid *M* and complete the volume with water. To prepare Standard solution of 0.002% trimethoprim SQR (w/v) using 0.1M acetic acid as solvent. To measure the absorbance in of the resulting solutions in 271 nm, using 0.1M acetic acid for zero adjustment. To calculate amount of trimethoprim (C₁₄H₁₈N₄O₃) in tablets from the readings obtained. Alternatively, perform the calculations considering A (1%, 1 cm) = 204, in 271 nm.

B. To weigh and pulverize 20 tablets. To dissolve amount of powder equivalent to 0.5 g of sulfamethoxazole and pro-

S

ceed as described in *DOSAGE* in *Sulfamethoxazole* monograph.

C. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 254 nm; 250 mm length column and 4,6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at room temperature; flow of *Mobile phase* of 2,0 mL/minute.

Mobile phase: mixture 1400 mL of water, 400 mL of acetonitrile and 2 mL triethylamine in a 2000 mL volumetric flask. Adjust the pH to $5,9 \pm 0,1$ with 0.2M sodium hydroxide or glacial acetic acid. Complete the volume with water.

Standard solution: To weigh and pulverize 20 tablets. To transfer an amount of powder equivalent to 0,16g of sulfamethoxazole and 32 mg of trimethoprim to a 100 mL volumetric flask. To add 70 mL of methanol. Leave it on ultrasound for 15 minutes. Top up with the same solvent and filter. To transfer 5 mL of the filtrate to a 50 mL volumetric flask and complete the volume with the *Mobile phase*. Homogenize.

Standard solution: To prepare a solution so as to obtain a concentration of sulfamethoxazole SQR at 1,6 mg/mL and trimethoprim SQR at 0,32 mg/mL in methanol. To transfer 5 mL of that solution to 50 mL volumetric flask and complete the volume with the *Mobile phase*, obtaining solution containing 160 Mg/mL sulfamethoxazole and 32 Mg/mL trimethoprim.

To inject replicates of 20 µL *Standard solution*. The relative retention times are of 1,0 for trimethoprim and 1,8 to sulfamethoxazole. The resolution between peaks of sulfamethoxazole and trimethoprim is not less than 5. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2.0 %.

Procedure: to separately inject, 20 µL of *Standard solution* and of *Standard sample*, record the chromatograms e measure the areas under the peaks. To calculate the amount of sulfamethoxazole ($C_{10}H_{11}N_3O_3S$) and of trimethoprim ($C_{14}H_{18}N_4O_3$) in tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

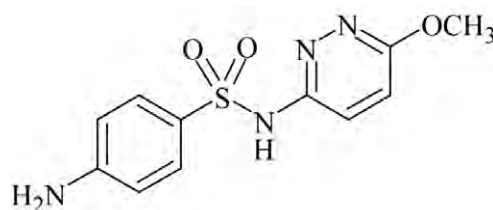
In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

SULFAMETHOXYPYRIDAZINE

Sulfamethoxypyridazinum



$C_{11}H_{12}N_4O_3S$; 280,30 sulfamethoxypyridazine; 08136 4-Amino-4'-(6-methoxy-3-pyridazinyl)-benzenesulfonamide [80-35-3]

It contains, at least, 99,0% and, at most, 101,0% of $C_{11}H_{12}N_4O_3S$, in relation to the desiccated substance.

DESCRIPTION

Physical Characteristics. Crystalline powder, white to yellowish-white, odorless or almost odorless, flavored, at first, tasteless, starting to become bitter.

Solubility. Very soluble in water, soluble in acetone, slightly soluble in ethanol. Easily soluble in dilute solutions of mineral acids and alkali hydroxides.

Physical and chemical constants.

Melting range (5.2.2): 182°C to 183°C.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the sulfamethoxypyridazine SQR spectrum, prepared in a similar manner.

B. Responds to the reaction of primary aromatic amine (5.3.1.1).

PURITY TESTS

Acidity. To heat 1 g of the sample in 50 mL of water free of carbon dioxide around 70 °C for five minutes, cool down at 20 °C and filter. The intake of 25 mL of the filtrate requires for neutralization, at most, 0,35 mL of 0.1M sodium hydroxide.

Loss on drying (5.2.9). Determine in 1 g of the sample. Dry in an oven at 105 °C to constant weight. At most 0,5%.

DOSAGE

To proceed as described in *Diazotization titrations* (5.3.3.1), *Method 2*. Each mL of 0.1 M SV sodium nitrite is equal to 28,03 mg of $C_{11}H_{12}N_4O_3S$.

PACKAGING AND STORAGE

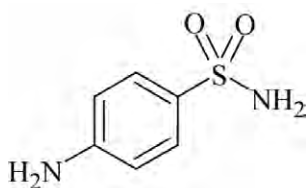
In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS ANTI-BACTERIAL.

SULFANILAMIDE
Sulfanilamidum



$C_6H_8N_2O_2S$; 172,20 sulfanilamide; 08141
4-Aminobenzenesulfonamide
[63-74-1]

It contains, at least, 99,0% and, at most, 101,0% of $C_6H_8N_2O_2S$, in relation to the desiccated substance.

DESCRIPTION

Physical Characteristics. Crystalline powder, white or yellowish white.

Solubility. Slightly soluble in water, easily soluble in acetone, Slightly soluble in water, easily soluble in acetone, slightly soluble in ethanol, practically insoluble in methylene chloride. Soluble in alkalis solutions.

Physical and chemical constants.

Melting range (5.2.2): 164,5 °C to 166 °C.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the spectrum of sulfanilamide SQR, prepared in a similar manner.

B. To dissolve 5 mg of the sample in 10 mL of 0.1 M hydrochloric acid. The solution, without acidification, responds to reactions for primary aromatic amine (5.3.1.1).

PURITY TESTS

Acidity. To heat about 70 °C, for 5 minutes, 1 g of the sample em 50 mL of distilled water, freshly boiled. Cool in an ice bath for 15 minutes and filter. To add 0,1 mL bromo-

thymol blue SI in 20 mL of the filtrate. At most 0,1 mL of 0.02M sodium hydroxide is spent to neutralize the sample.

Heavy metals(5.3.2.3). Use *Method I*. At most 0,001% (10 ppm).

Loss on drying (5.2.9). Determine in 1 g of the sample. Dry in an oven at 105 °C. At most 0,5%.

Sulphated ashes(5.2.10). Determine in 1 g of the sample. At most 0,1%.

DOSAGE

To proceed as described in *Diazotization titrations (5.3.3.1)*, *Method 2*. Each mL of 0.1 M SV sodium nitrite is equal to 17,22 mg de $C_6H_8N_2O_2S$.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

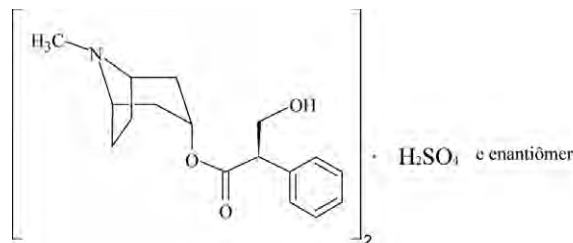
LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Anti-bacterial.

ATROPINE SULPHATE
Atropini sulfas



$(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot 676,82$

$(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O$; 694,83 atropine sulphate; 00935

Sulphate of the ester (3-endo)-8-methyl-8-azabicyclo[3.2.1]oct-3-yl ester of acid α -(hydroxymethyl)benzeneacetic (1:2)
[55-48-1]

Sulphate of the ester (3-endo)-8-methyl-8-azabicyclo[3.2.1]oct-3-yl Ester of acid α -(hydroxymethyl)benzeneacetic hydrated(1:2:1) [5908-99-6]

It contains, at least, 98,5% and, at most, 101,0% of $(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4$, relative to anhydrous substance.

DESCRIPTION

Physical Characteristics. Colourless crystals or White crystalline powder, odorless, efflorescent at dry air, slowly changed by light. Melts at a temperature not less than 187 °C, determined immediately after drying of the sample at 120 °C for 4 hours.

Solubility. Very water soluble, easily soluble in ethanol and in glycerin and practically insoluble in ethyl ether and chloroform.

IDENTIFICATION

The identification tests C., D., e E. podem ser omitidos se forem realizados os testes A., B., e F. The identification test A. can be omitted if the tests are B., C., D., E. and F carried out.

A. To dissolve 50 mg of the sample in 25 mL l, add 2 mL of sodium hydroxide M and extract with two 10 mL portions of ethyl ether. Dry the ether extract with sodium sulphate anhydrous, filter, wash with 5 mL of ethyl ether and evaporate the filtrate in room temperature. Dry the residue on silica gel, using reduced pressure. At the same time, perform the same Procedure using 50 mg of atropine sulphate SQR. The infrared absorption spectrum (5.2.14) of the residue, dispersed in potassium bromide has only absorption maximum at the same the wavelengths and with the same relative intensities to those observed in the spectrum of atropine sulphate SQR.

B. To proceed as described in *Related substances*. The main spot obtained with the *Solution (1)* corresponds in color, size and intensity to that obtained with the *Solution (4)*.

C. To 1 mg of the sample, add 0,2 mL of fuming nitric acid and evaporate to dryness in a water bath. To dissolve the residue in 2 ml of acetone and add 0,1 mL of % Potassium hydroxide solution (w/v) in methanol. Violet color is produced.

D. To dissolve about 25 mg of the sample in 5 mL of water, acidify with 2 M hydrochloric acid and add 1 mL of iodine bismuthate of aqueous-acetic potassium. It is formed, immediately, orange or orange-red precipitate.

E. At 1 mL of 5% aqueous solution (w/v) of the sample, add 1 mL of water and 0,5 mL of 0.1 M solution of iodine. Brown precipitate is formed.

F. The 5% aqueous solution(w/v) responds to the reactions of the sulphate ion (5.3.1.1).

PURITY TESTS

pH (5.2.19). 4,5 to 6,2. Determine in Solution to 2% (w/v).

Related substances. To proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel G, as support, and acetone mixture, water and Concentrated ammonium

solution (90:7:3), as Mobile phase. Apply, separately, to the plate, 10 mL each of the solutions outlined below:

Solution (1): Solution at 2% (w/v) of the sample in methanol.

Solution (2): Solution to 0,02% (w/v) of the sample in methanol.

Solution (3): Solution to 0,01% (w/v) of the sample in methanol.

Solution (4): Solution at 2% (w/v) of atropine sulphate SQR in methanol.

Develop the chromatogram. Remove the plate, dry at temperature from 100 °C to 105 °C, for 15 minutes. Allow to cool down and nebulize with iodine bismuthate of dilute potassium SR until appearing of the spots. None secondary spot obtained in chromatogram with the *Solution (1)* is more intense than the spot obtained with *Solution (2)* and no more than one spot is more intense than that obtained with the *Solution (3)*.

Apoatropine. To prepare Solution to 0,1% (w/v) in 0.01 M hydrochloric acid. Measure the absorbance in 245 nm (5.2.14), using the same solvent for zero adjustment. The absorbance in value is of, at most, 0,4 (0,5%).

Hyoscyamine. To dissolve 1,25 g, exactly weighed, in water, for final volume of 25 mL. Determine the angle of rotation (5.2.8) of Solution, at 25 °C. The rotation observed, in deegres, multiplied by 200 and divided by length (in mm) of the polarimetric tube used, is in between - 0,60° and + 0,05°.

Water (5.2.20.1). At most 4,0%.

Sulphated ashes(5.2.10). Determine in 1 g da substância. At most 0,2%.

DOSAGE

To proceed as described in *Titrations in non-aqueous media (5.3.3.5)*. To dissolve about 1 g of the dried sample, accurately weighed, in 50 mL of glacial acetic acid and titrate with 0.1 M SV perchloric acid, determine the end point potentiometrically. Perform blank test and make the necessary corrections. Each mL of 0.1 M SV perchloric acid equals to 67,682 mg of $(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4$.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Mydriatic and adjuvant of general anesthetics.

ATROPINE SULPHATE SOLUTION INJECTABLE

It contains, at least, 90,0% and, at most, 110,0% of the stated amount of $(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O$.

IDENTIFICATION

A. Use volume of sample equal to 10 mg of atropine sulphate. To add 4 mL of sodium hydroxide M e extract with two 10 mL portions of ethyl ether. Dry the ether extract with sodium sulphate anhydrous, Filter, lavar com 5 mL of ethyl ether and evaporate the filtrate in room temperature. Dry the residue on silica gel, using reduced pressure. At the same time, perform the same Procedure using 50 mg of atropine sulphate SQR. The infrared absorption spectrum (5.2.14) of the residue, dispersed in potassium bromide has only absorption maximum at the same the wavelengths and with the same relative intensities to those observed in the spectrum of atropine sulphate SQR.

B. To proceed as described in *Thin layer chromatography* (5.2.17.1), using silica gel G, as support, and mixture of chloroform, acetone and dietilamine (50:40:10), as Mobile phase. Apply, separately, to the plate, 5 μ L each of the solutions outlined below.

Standard solution: evaporate a volume of Injectable solution containing the equivalent to 5 mg of atropine sulphate, to dryness, in a water bath. Triturate the residue with 1 mL of ethanol, allow to stand and Use supernatant.

Standard solution: Solution of atropine sulphate SQR to 0,5% (w/v) em ethanol.

Develop the chromatogram. Remove plate, secar a 105°C durante 20 minutes. Allow to cool down and nebulize with iodine bismuthate of dilute potassium SR. The spot obtained in chromatogram with the *Standard solution* corresponds in size, color and position of the spot obtained in chromatogram with the *Standard solution*.

C. Evaporate to dryness in volume of Injectable solution equal to 1 mg of atropine sulphate. Add to 0.2 mL residue of fuming nitric acid and evaporate to dryness in a water bath. A yellow residue is formed. After cooling, add 2 mL of acetone and 0,2 mL of % potassium hydroxide solution (w/v) in methanol. Violet color is developed.

D. Responds to the reactions of the sulphate ion (5.3.1.1).

CHARACTERISTICS

Determination of volume(5.1.2). It complies with the test. pH (5.2.19). 3,0 to 6,5.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

Bacterial endotoxins (5.5.2.2). At most 55,6 UE/ mg of atropine sulphate.

DOSAGE

To proceed as described in *High performance liquid chromatography* (5.2.17.4). Use chromatograph equipped with a UV detector with 254 nm; 300 mm length column and 3,9 mm dof inner diameter, packed with silica chemicaly bonded to the octadecylsilane group (5 μ m ou 10 μ m), kept at room temperature; flow of *Mobile phase* of 2 mL/minute.

Acetate buffer: To dissolve the equivalent to 6,8 g of sodium acetate in water, add 2,9 mL of glacial acetic acid and complete the volume with water to 1000 mL.

Mobile phase: To transfer 5,1 g of Tetrabutylammonium hydrogen to a 1000 mL volumetric flask, add 50 mL of acetonitrile and complete the volume com *Acetate buffer*. Adjust the pH to 5,5 + 0,1 with 5 M sodium hydroxide.

Standard solution: To transfer the volume of sample equal to about 2 mg of atropine sulphate to a 25 mL volumetric flask, complete the volume with water and homogenize.

Standard solution: To dissolve amount accurately weighed of atropine sulphate SQR and dilute with water in order to obtain concentration equivalent to 80 μ g/mL of atropine sulphate.

Solution of resolution: dilute a volume of aqueous solution of p-hydroxybenzoic acid 2.5 mg/mL with four volumes of the *Standard solution*.

Inject 100 μ L of *Solution of Resolution*. The retention time of p-hydroxybenzoic acid is about 1,6 times higher than atropine sulphate. The resolution between the peaks of p-hydroxybenzoic acid and of the atropine sulphate is not less than 2,2. Inject 100 μ L replicates of the *Standard solution*. The relative standard deviation of the replicates areas of the peaks obtained not superior than 1,5%

Procedure: Separately inject, 100 μ L of the *Standard solutions* and *sample*, record the chromatograms and measure the areas under the peaks. To calculate the amount of $(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O$ in the Injectable solution from the responses obtained for the *Standard solutions* and *sample*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

S

BARIUM SULPHATE**Barii sulfas**

BaSO₄; 233,39
barium sulphate; 08162
Barium salt of sulfuric acid(1:1)
[7727-43-7]

It contains, at least, 97,5% and, at most, 100,5% of BaSO₄.

DESCRIPTION

Physical Characteristics. Fine powder, white, dense or crystals. Presents polymorphism.

Solubility. Practically insoluble in water and in organic solvents. Slightly soluble in acids and alkalis solutions.

IDENTIFICATION

A. To mix 0,5 g of the sample, 2 g of anhydrous sodium carbonate and 2 g of anhydrous potassium carbonate. To heat the mixture in a furnace until complete fusion. Add hot water and filter. Acidify the filtrate with hydrochloric acid. Responds to reactions of the sulphate ion (**5.3.1.1**).

B. Wash residue obtained in *identification test A*. with water. To dissolve in 5 M acetic acid. Responds to reactions of barium ion (**5.3.1.1**).

PURITY TESTS

pH (5.2.19). 3,5 to 10,0. Determine in slurry of the sample a 10% (p/p).

Heavy metals (5.3.2.3). To heat to boiling point 8,33 g of the sample with 50 mL of 4% acetic acid (v/v) for 10 minutes. Dilute to 50 mL with water and filter. Use 12 mL of the filtrate. To prepare a Standard solution using a Lead solution (2 ppm of Pb). At most 0,001% (10 ppm).

Sulfides. In a 500 mL Erlenmeyer, add 10 g of the sample and 100 mL of 0.5M Hydrochloric acid. Set a filter paper at the top of the Erlenmeyer. Moisten the central area of the filter paper with 0,15 mL lead acetate SR. Boil the mixture gently for 10 minutes. Any darkening produced on the filter paper is not more intense than that produced by the standard containing 5 Mg of sulfide in 100 mL of 0.5M Hydrochloric acid and treated similarly. At most 0,00005% (0,5 ppm).

Acid soluble substances. Cool down the mixture obtained in *Sulfides* and add water to restore the initial volume. Filter in filter paper previously rinsed with a mixture of 10 mL of 0.5M Hydrochloric acid and 90 mL of water. If necessary, re-filter the first portions until a clear filtrate. Evaporate 50 mL of the filtrate to dryness, in a water bath, and add two drops of hydrochloric acid and 10 mL of hot water. Filter again on filter paper, prepared as described above and wash the filter paper with 10 mL of hot water, collecting the filtrate in tared container. Evaporate the fil-

trate along with the washings to dryness, in a water bath. Dry the residue in oven at 105 °C, for 1 hour. At most 0,3% (15 mg).

Soluble barium salts. To add 10 mL of water to the residue obtained in *Acid soluble substances*. Filter in filter paper prewashed with 100 mL of 0.5M Hydrochloric acid and add. Any developed turbidity within 30 minutes is not more intense than that produced by standard containing 50 mg of barium in 10 mL of water and 0.5 mL of M sulfuric acid treated similarly. At most 0,001% (10 ppm).

DOSAGE

Weigh exactly, about 0,6 g of the sample in previously tared platinum crucible. Add 10 g of sodium carbonate anhydrous. Homogenize. Merge to obtain a clear viscous liquid and heat for another 30 minutes. Cool, putting the crucible in a 500 mL beaker, add 250 mL of water, stir and heat the assembly to remove the solid melted. Collect the crucible and wash with water, collecting the washing water of the beaker. Wash the inside of the crucible with 2 mL of 5 M acetic acid and then wash with water, collecting again the portions in the beaker. Continue to heat the beaker, under stirring, until the solid melted disintegrates. Allow to cool in an ice bath. Allow to stand until settling of the solid. Filter the supernatant on filter paper (Whatman No. 40 or equivalent), preventing the precipitate from passing to the filter paper. Wash the precipitate with two 10 mL portions of cold Solution of sodium carbonate anhydrous at 2% (w/v), stirring and wait the decantation of the solid. Filter the supernatant, using the same filter paper without transferring the precipitate. Wash the filter paper with 5 1 mL portions of hydrochloric acid SR and then wash with water, collecting the filtrate in the beaker containing the precipitate of barium carbonate. Add to the 100 mL beaker of water and 5 mL hydrochloric acid, 10 mL of ammonium acetate to 40% (w/v), 25 mL of potassium dichromate at 10% (w/v) and 10 g of urea. Cover the beaker with a watch glass and heat at 85 °C for at least 16 hours. Filter while hot, using a sintered glass funnel of fine porosity and previously tared. Transfer all the precipitate, the aid of a glass rod with the rubber tip. Wash the precipitate with potassium dichromate to 0.5% (w/v) and then wash with 20 1 mL of water. Drying at 105 °C for 2 hours, cool and weigh. The mass of barium chromate obtained, multiplied by 0.9213 equals the mass of BaSO₄.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Diagnostic agent for contrast medium.

S

CALCIUM SULPHATE

Calcii sulfas

CaSO₄; 136,14
 CaSO₄·2H₂O; 172,17
 calcium sulphate; 08164
 dihydrate calcium sulphate; 08165
 Calcium salt of sulfuric acid(1:1)
 [7778-18-9]
 Calcium salt of sulfuric acidhidratado (1:1:2)
 [10101-41-4]

The calcium sulphate is anhydrous or dihydrate. It contains, at least, 98,0% and, at most, 101,0 % of CaSO₄, in relation to the desiccated substance.

DESCRIPTION

Physical Characteristics. Fine powder, white or almost white.

Solubility. Very slightly soluble in water, Practically insoluble in ethanol.

IDENTIFICATION

A. Responds to the reactions of the sulphate ion (5.3.1.1).

B. Responds to the reactions of the calcium ion(5.3.1.1).

PURITY TESTS

Acidity or Alkalinity. Stir for 5 minutes 1.5 g of the sample with 15 ml of water free from carbon dioxide. Allow to stand for 5 minutes and filter. To 10 mL of the filtrate add 0.1 ml of phenolphthalein SI and 0.25 mL of 0.01 M sodium hydroxide. Red color is developed. Add 0.30 mL of 0.01 M hydrochloric acid. The Solution becomes colorless. Add 0.2 mL of methyl red SI. Reddish-orange is developed.

Arsenic (5.3.2.5). To dissolve, heating to 50 °C durante 5 minutes, 1 g of the sample in 50 mL of Hydrochloric acid 10% (v/v). Allow to cool and proceed as described in *Visual Method visual* using 5 mL of that Solution. At most 0,001% (10 ppm).

Iron (5.3.2.4). Use *Method I*. To dissolve 0,1 g of the sample in 8 mL of 3 M hydrochloric acid. Use 1 mL of *Standard solution of iron (10 ppm Fe)*. At most 0,01% (100 ppm).

Heavy metals (5.3.2.3). Use *Method I*. To mix 2 g of the sample with 20 mL of water, add 25 mL of 3 M hydrochloric acid, and heat to boiling point for total dissolution of the sample. Allow to cool and add ammonium hydroxide until pH 7,0. Filter, reduce the volume of the filtrate at 25 mL and filter again, If necessary, obtain Solution. At most 0,001% (10 ppm)

Loss on drying(5.2.10). Determine in minimum temperature of 250 °C, to constant weight. For the dihydrate form the loss is between 19,0% and 23,0 %. For the anhydrous form, at most 1,5%.

DOSAGE

Weigh exactly, about 0,15 g of the sample and to dissolve in 120 mL of water. To proceed as described in *Complexometric Titrations (5.3.3.4)* for *Calcium*,

using 0,1 M SV disodium edetate. Each mL of 0,1 M SV disodium edetate equals to 13,614 mg of CaSO₄.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

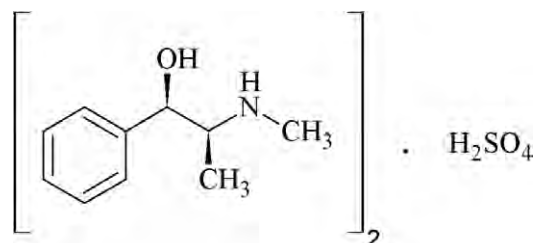
Observe legislation in force.

CATEGORY

Adjuvant.

EPHEDRINE SULPHATE

Ephedrini sulfas



(C₁₀H₁₅NO)₂·H₂SO₄; 428,54 ephedrine sulphate; 03311
 Sulphate of (aR)-a-[(1S)-1-(methylamino)ethyl]
 benzenomethanol (1:2)
 [134-72-5]

It contains, at least, 98,0% and, at most, 101,0% of (C₁₀H₁₅NO) H₂SO₄ in relation to the dried substance.

DESCRIPTION

Physico-chemical characteristics. Fine powder or white crystals, odorless and darkens when exposed to light. Melting temperature (5.2.2): about 245 °C, with decomposition.

Solubility. Very water soluble and slightly soluble in ethanol.

Physical and chemical constants.

Specific rotation (5.2.8): -32° to -30°, in relation to the desiccated substance. To determine in 5% solution (w/v) in water.

IDENTIFICATION

The identification test **A.** in relation to the dried substance **B.**, **C.** e **D.**

A. The infrared absorption spectrum (**5.2.14**) of the sample, dispersed in potassium bromide, shows highest absorption at the same tablets of wavelength and with the same relative intensities observed in the spectrum ephedrine sulphate SQR, prepared in a similar manner.

The ultraviolet absorption spectrum (**5.2.14**), in the range of 200 to 400 nm, of a Solution to 0,1% (w/v) in water, displays maximum and minimum identical to those observed in the spectrum of solution similar of ephedrine sulphate SQR.

To dissolve 10 mg in 1 mL of water, add 0,1 mL cupric sulphate SR and 1 mL 20% sodium hydroxide (w/v). Produces red-purple color. Add 1 mL of ethyl ether and stir well. The ethereal layer becomes purple and the water becomes blue.

Responds to the reactions of the sulphate ion (**5.3.1.1**).

PURITY TESTS

Acidity or Alkalinity. To dissolve 1 g em 20 mL of distilled water and add 1 drop of methyl red SI. Of the Solution is red or pink, should change to yellow by the addition of, at most, 0,2 mL of 0.02M sodium hydroxide. If it gets yellow, it should change to red by the addition of, at most, 0,1 mL of 0.04 M sulfuric acid.

Chlorides. At most 0,15%.

Loss on drying(5.2.9). Determine in 1 g of sample. Dry in an oven at 105 °C, por 3 hours. At most 2,0%.

Sulphated ashes(5.2.10). Determine in 1g of the substance. At most 0,1%.

DOSAGE

To proceed as described in *Titration in non-aqueous media* (**5.3.3.5**). To transfer about 0,3 g of the sample, accurately weighed, to a separatory funnel and dissolve in 10 mL of water, Add 3 g of sodium chloride and 5 mL of sodium hydroxide M. Extract with four portions of 25 mL of chloroform. Stir the chloroform extracts gathered with 10 mL saturated solution of sodium chloride solution and filter through cotton wool soaked with chloroform. Extract the aqueous layer with 10 mL of chloroform and collect to the chloroform extract. To add methyl red SI e Titrate with 0.1 M SV perchloric acid. To prepare um branco para a correção necessária. Each mL of 0.1 M SV perchloric acid equals to 21,426 mg of $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$.

PACKING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Adrenergic (bronchodilator).

INJECTABLE SOLUTION EPHEDRINE SULPHATE

It contains, at least, 95,0% and, at most, 105,0% of the stated amount of $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$.

IDENTIFICATION

A. To mix 1 mL of the sample with 5 mL of ethanol, and evaporate in a water bath. The infrared absorption spectrum (**5.2.14**) the residue, dispersed in potassium bromide, has highest absorption at the same wavelengths and with sane relative intensities observed in the spectrum of ephedrine sulphate SQR, prepared in a similar manner.

B. Responds to the reactions of the sulphate ion (**5.3.1.1**).
CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test. **pH (5.2.19).** 4,5 to 7,0.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

Bacterial endotoxins (5.5.2.2). At most 1,7 UE/ mg of ephedrine sulphate.

DOSAGE

Quantitatively transfer the equivalent to 250 mg of ephedrine sulphate to a separation funnel. To add 10 mL of water, 3 g of sodium chloride, 5 mL of sodium hydroxide M and extract with four portions of 25 mL of chloroform. Collect the chloroform extracts and stir with 10 mL saturated solution sodium chloride and filter through a cotton soaked with chloroform. Separate phases and add 10 mL of chloroform to the aqueous phase. Collect the chloroform extracts, add methyl red SI and titrate with 0.1 M SV perchloric acid in dioxane. Perform blank test and make the necessary corrections. Each mL of 0.1 M SV perchloric acid equals to 21,426 mg of $(CHNO)_2H_2SO$

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

STREPTOMYCIN SULPHATE IN POWDER TO INJECTABLE SOLUTION

Streptomycin sulphate powder for injectable solution is a sterile powder of streptomycin sulphate to be reconstituted in water for injection. Contains, at least 90,0% and, at most 115,0% of the stated amount of $C_{12}H_{39}N_7O_{12}$.

IDENTIFICATION

A. To dissolve 10 mg of the powder em 5 mL of water, add 1 mL of sodium hydroxide M and heat in a water bath for 5 minutes. Allow to cool, add 2 mL of a 2% ammonium ferric sulphate solution (w/v) in 0.5 M sulfuric acid. Violet color is produced.

B. To dissolve 0,1 g of the powder of the sample em 2 mL of water, To add 1 mL of a 10 % 1-naphtol solution (w/v) in ethanol and 2 mL of aqueous solution of 2% sodium hypochlorite(w/v). Reddish color is produced.

C. Responds to the reactions of the sulphate ion (5.3.1.1).
CHARACTERISTICS

pH (5.2.19). 5,0 a 8,0. Determine after reconstitution with diluent.

Determination of weight(5.1.1). It complies with the test.

Uniformity of single doses (5.1.6). It complies with the test.

PURITY TESTS

Loss on drying (5.2.9). Determine in 100 mg da sample. Dry in an oven at 60 °C, under reduced pressure (not exceeding 5 mmHg), for três hours. At most 5,0%.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test. Use method of membrane filtration.

Bacterial endotoxins (5.5.2.2). Contains, at most, 0,25 UE/mg of streptomycin.

DOSAGE

To employ one of the methods described below.

Note: to perform the following tests, use minimum sampling of 10 bottles.

A. To proceed as described in *The visible absorption spectrophotometry* (5.2.14). Prepare Standard solution and Stan-

dard solution to 0,2% (w/v) in water. Transfer 5 mL of each Solution for 25 mL volumetric flasks. Add to each flask 1 mL of sodium hydroxide M and heat por 4 minutes in a boiling water bath. Allow to cool in cold water until room temperature. Add, to each

flask, 2 mL of 2% ammonium ferric sulphate solution (w/v) in 0.5 M sulfuric acid. Complete the volume with water, homogenize and allow to stand for 10 minutes. Prepare the blank at the same time, by adding 5 mL of water in a 25 mL volumetric flaske and proceed as described previously from "Add at each 1 mL flask...". Measure the absorbance ins at 520 nm (5.2.14), using the blanck for zero adjustment. Calculate the power in Mg/mL of streptomycin $C_{12}H_{39}N_7O_{12}$ on the sample, from the readings obtained and of power of the standard.

B. To proceed as described in *Microbiological assay by agar diffusion* (5.5.3.3.1), after reconstituting the contents of the flasks as indicated by the producer.

Microorganism: Bacillus subtilis ATCC 6633

Standard solution: To dissolve amount, accurately weighed, of streptomycin SQR sulphate in *Potassium phosphate buffer 0,1 M, sterile, pH 8,0 (Solution 2)* in order to obtain solution a 1 mg/mL. Dilute successively with the *Potassium phosphate buffer 0,1 M, sterile, pH 8,0 (Solution 2)* to obtain solutions in a concentration range suitable for analytical curve.

Standard solution: weigh equivalent amount of sample and dilute, successively, with the *Potassium phosphate 0,1 M buffer, sterile, pH 8,0 (Solution 2)* to obtain Solution containing 1 mg/mL of base. Dilute with the *0,1 M Potassium phosphate buffer, sterile, pH 8,0 (Solution 2)* and obtain solutions in the concentration ranges 2,0 Mg/mL, 1,0 Mg/mL and 05 Mg/mL.

Procedure: To add 20 mL of base medium number 5 in each plate, wait to solidify, add 5 mL de inoculum number 5 and proceed as described in *Microbiological assay by agar diffusion* (5.5.3.3.1). Calculate the amount, in mg of streptomycin ($C_{12}H_{39}N_7O_{12}$) in the powder to injectable solution, from the standard power and of the responses obtained to the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In airtight containers, protected from moisture and to the room temperature.

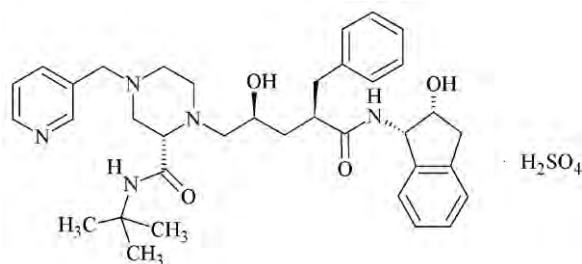
LABELLING

To comply with the legislation in force.

S

INDINAVIR SULPHATE

Indinaviri sulfas



$C_{36}H_{47}N_5O_4 \cdot H_2SO_4$; 711,87

Indinavir sulphate; 04883

Sulphate of 2,3,5-trideoxy-A?-[(1S,2R)-2,3-dihydro-2-hydroxy-1H-inden-1-yl]-5-[(2S)-2-[[[(1,1-dimethylethyl)amino]carbonyl]-4-(3-pyridinylmethyl)-1-piperazinyl]-2-(phenylmethyl)]-

D-eritro-pentanamide (1:1)

[157810-81-6]

It contains, at least, 98,5% and, at most, 101,5% of $C_{36}H_{47}N_5O_4 \cdot H_2SO_4$ and, at least, 13,2% and, at most, 14,4% of sulphate, relative to anhydrous substance e free of ethanol.

DESCRIPTION

Physical Characteristics. Amorphous powder, white or almost white.

Solubility. Easily soluble in water, soluble in methanol, very slightly soluble in acetonitrile and hexane.

Physical and chemical constants.

Melting range (5.2.2): 150 °C to 154 °C, with decomposition.

Specific rotation (5.2.8): between +122° and +129°, in Aqueous solution to 1% (w/v), relative to anhydrous substance and free of ethanol. Perform the reading at 25 °C in the wavelength of 365 nm.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, only has highest absorption at the same wavelengths and with same relative intensities of those observed in the spectrum of indinavir SQR sulphate.

B. The ultraviolet absorption spectrum (5.2.14), of 200 nm to 400 nm, of the solution of the sample to 0,004% (w/v) in 0.1 M hydrochloric acid, displays the highest in 210 nm and 260 nm, identical to those observed in the spectrum of Solution similar of indinavir SQR sulphate.

C. The retention time of the main peak of the chromatogram of the *Standard solution*, obtained in method **B.** of *DOSAGE*, corresponds the one of the main peak of the *Standard solution*.

D. A Solution of the sample to 0,5% (w/v) responds to the reactions of the sulphate ion (5.3.1.1).

PURITY TESTS

Content of ethanol. To proceed as described in *Gas chromatography* (5.2.17.5). Use gas chromatograph provided with flame ionization detector; chromatographic column 30 m long and 0.25 mm inner diameter, filled with polyethylene glycol, with a thickness of 0.25 µm film and; temperature of the column of 45 °C, injector temperature of 260 °C and detector temperature of 280 °C; Use nitrogen as carrier gas; carrier gas flow of 10 mL/minute. At the end of each run, the column temperature is increased to 230 °C and maintained for 18 minutes.

Standard solution: To transfer, exactly, about 0,2 g of the sample to a 10 mL volumetric flask, dissolve in dimethylsulfoxide and top up with the same solvent. Homogenize.

Standard solution: To transfer, exactly, about 6,5 mL of absolute ethanol to a 100 mL volumetric flask, complete the volume com dimethylsulfoxide and homogenize. To transfer 5 mL of the resulting solution to a 25 mL volumetric flask and complete the volume com dimethylsulfoxide. Homogenize.

Procedure: to separately inject, 1 µL of the *Standard solution* and of *Standard sample*, record the chromatograms e measure the areas under the peaks. To calculate the amount of ethanol na sample from the responses obtained with the *Standard solution* and the *Standard solution*. Between 5,0% and 8,0%.

Chromatographic purity. To proceed as described in *High performance liquid chromatography* (5.2.17.4), using chromatograph equipped with a UV detector with 220 nm; 250 mm length column and 4,6 mm of inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at room temperature; flow of Mobile phase of 1 mL/minute.

Eluent A: To dissolve 0,54 g of monobasic potassium phosphate and 2,79 g of dibasic potassium phosphate in 2000 mL of water.

Eluent B: acetonitrile.

Diluent: mixture of Eluent A and Eluent B (50:50).

Gradient and of Mobile phase: adopt the gradient system described in the following table:

Time (minutes)	Eluent A (%)	Eluent B (%)	Elution
0 - 40	80 ^ 30	20 ^ 70	linear gradient
40 - 45	30	70	isocratic
45 - 47	30 ^ 80	70 ^ 20	linear gradient
47 - 52	80	20	isocratic

Test Solution: To transfer, exactly, about 50 mg of the sample to a 100 mL volumetric flask and to dissolve in

70 mL of *Diluyente*. Top up with the same solvent. Homogenize, obtaining Solution to 500 pg/mL.

Standard solution: To prepare Solution of indinavir SQR sulphate to 500 pg/mL i *Diluent*.

Inject replicates of 20 pL of the *Standard solution*. The column efficiency is not less than 4000 theoretical plates/meter. The retention factor for the peak corresponding to indinavir is between 3 and 6. The tailing factor is not more than 2,0.

Procedure: inject 20 pL of the *Test Solution*, record the chromatograms and measure the areas under the peaks. Do not consider the peaks representing the solvents. The area of any single peak, except the main peak, is not greater than 0.1% in the total area of the peaks obtained. The sum of the areas of all peaks, other than the main peak is not higher than 0.5% of the total area of the peaks obtained.

Heavy metals (5.3.2.3). Use *Method I*. At most 0,001% (10 ppm).

Water (5.2.20.1). At most 1,5%.

Sulphated ashes (5.2.10). At most 0,1%.

DOSAGE

Sulphate

Weigh exactly, about 0,5 g of the sample, transfer para beaker and to dissolve em 60 mL of mixture of methanol and water (50:50). Use specific electrode for lead and adequate reference electrode. Tritate with 0.1 M SV lead nitrate determining the end point potentiometrically. Each mL of 0.1 M SV lead nitrate equals to 9,604 mg of sulphate.

Indinavir Sulphate

To proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with a UV detector with 260 nm; 250 mm length column and 4.6 mm of inner diameter, packed with silica chemically bonded the group octilsilano (5 pm), kept at 40 °C; flow of Mobile phase of 1 mL/minute.

Dibutyl ammonium phosphate buffer: To dissolve 3 g of phosphoric acid and 1,7 mL of dibutyl amine in 900 mL of water. Adjust the pH in (6,5 ± 0,05) with M sodium hydroxide.

Mobile phase: mixture of dibutyl ammonium phosphate buffer e acetonitrile (55:45).

Standard solution: To transfer, exactly, about 58 mg of the sample to a 100 mL volumetric flask, add 70 mL of *Mobile phase*. Stir mechanically 15 minutes and leave in ultrasound for 15 minutes. Complete the volume with *Mobile phase*. Homogenize.

Standard solution: To prepare Solution of indinavir SQR sulphate to 0,58 mg/mL in *Mobile phase*, equal to 0,5 mg of indinavir per mm.

Inject replicates of 10 pL da *Standard solution*. The column efficiency is not less than 4000 theoretical plates/meter. The tailing factor is not more than 2,0. The relative standard deviation of the replicates areas of the peaks recorded is not higher than 1,0%.

Procedure: separately inject, 10 pL of the *Standard solution* and of *Standard sample*, record the chromatograms and measure the areas under the peaks. Calculate the content of $C_{36}H_{47}N_5O_4 \cdot H_2SO_4$ in the sample from the responses obtained with the *Standard solution* and the *Standard solution*.

PACKAGING AND STORAGE

In airtight containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antiretroviral.

MAGNESIUM SULPHATE HEPTAHYDRATE

Magnesii sulfas heptahydricus

$MgSO_4 \cdot 7H_2O$; 246,47
magnesium sulphate heptahydrate; 08168
Magnesium salt of sulfuric acid hydrate (1:1:7)
[10034-99-8]

It contains, at least, 99,0% and, at most, 100,5% of $MgSO$ in relation to the desiccated substance.

DESCRIPTION

Physical Characteristics. White powder, crystalline, or Colourless shiny crystals, of bitter and saline taste.

Solubility. Easily soluble in water, Very hot water soluble, Practically insoluble in ethanol.

IDENTIFICATION

A. Responds to the reactions of the magnesium ion (5.3.1.1).

B. Responds to the reactions of the sulphate ion (5.3.1.1).

S

PURITY TESTS

Aspect of the Solution. To dissolve 5 g of the sample in water and dilute to 50 mL with the same solvent. The solution obtained is clear (5.2.25) and colorless (5.2.12).

Acidity or Alkalinity. To 10 mL of the solution obtained in *Aspect of the Solution*, add a drop of phenol red SI. Not more than 0,2 mL of 0.01 M hydrochloric acid or 0.01 M sodium hydroxide is necessary to change the color of the indicator.

Arsenic (5.3.2.5). Determine in 15 mL of the Solution obtained in *Aspect of the Solution*. To proceed as described in *Spectrophotometric Method, Method I*. At most 0,0002% (2 ppm).

Chlorides (5.3.2.1). Determine in 1,2 g of the sample. At most 0,03% (300 ppm).

Iron (5.3.2.4). Determine in 5 mL of the Solution obtained in the *Aspect of the Solution*. To proceed as described in *Method I* using 10 mL of *Standard solution of iron (1 ppm Fe)*. At most 0,002% (20 ppm).

Heavy metals (5.3.2.3). At most 0,001% (10 ppm).

Loss on drying (5.2.9). Weigh exactly, about 1 g of the sample and transfer to crucible previously calcined, cooled in a desiccator and tared. Dry in an oven at 105 °C, for 2 hours, and then, incinerate in muffle at 400 °C, to constant weight. Between 48,0% and 52,0%.

DOSAGE

Proceed in accordance described in *Complexometric Titrations (5.3.3.4)* for *Magnesium*. Weigh exactly, about 0,45 g of the sample. Each mL of 0.1M SV disodium edetate equals to 12,036 mg of MgSO_4 .

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

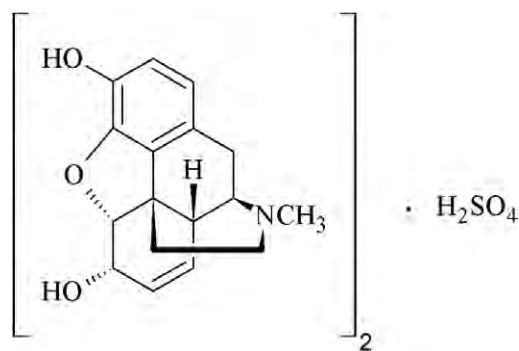
To comply with the legislation in force.

THERAPEUTIC CLASS

Osmotic laxative, electrolyte used in therapy

MORPHINE SULPHATE

Morphini sulfas



$(\text{C}_{17}\text{H}_{19}\text{NO}_3)_2 \cdot \text{H}_2\text{SO}_4$; 668,75

$(\text{C}_{17}\text{H}_{19}\text{NO}_3)_2 \cdot \text{H}_2\text{SO}_4 \cdot 5\text{H}_2\text{O}$; 758,83

morphine sulphate; 06114

morphine sulphate pentahydrate; 09532

Sulphate of (5 α ,6 α)-7,8- didehydro -4,5-epoxy-17-methyl-morphinan -3,6-diol (1:2)

[64-31-3]

Sulphate of (5 α ,6 α)-7,8- didehydro-4,5-epoxy-17-methyl-morphinano-3,6-diol hydrate (1:2:5)

[6211-15-0]

It contains, at least, 98,0% and, at most 102,0% of $(\text{C}_{17}\text{H}_{19}\text{NO}_3)_2 \cdot \text{H}_2\text{SO}_4$, relative to anhydrous substance.

DESCRIPTION

Physical Characteristics. White crystalline powder, odorless.

Solubility. Easily soluble in water, relslightly soluble in ethanol, very slightly soluble in toluene, insoluble in chloroform and ethyl ether.

Physical and chemical constants. *Specific rotation (5.2.8):* -107° to -110°, relative to anhydrous substance. Determine in Solution a 2% (w/v) in water.

IDENTIFICATION

The identification test A. can be omitted if the tests B., C., D., and E are carried out. The identification tests B., C. and D. can be omitted if the tests A. and E are carried out.

A. The infrared absorption spectrum (5.2.14) of the dried sample at 145°C for one hour, and dispersed in potassium bromide, only shows maximum absorption in the same wavelength and with same relative intensities of those observed in the spectrum of morphine sulphate SQR, prepared in a similar manner.

B. The ultraviolet absorption spectrum (5.2.14), in the range of 250 nm to 300 nm, of the standard solution to 0,01% (w/v)

prepared in water, ethyl ethershows maximum absorption in 285 nm identical to the spectrum of Solution similar of morphine SQR sulphate.

C. The retention time of the main peak of the chromatogram of the *Standard solution* obtained in method **B.** of *DOSAGE*, corresponds the one of the main peak of the *Standard solution*.

D. In a porcelain capsule, add a 1 mg of the sample, 0,5 mL of Solution of formaldehyde. Purple coloration which becomes violet was developed.

E. Responds to the reactions of the sulphate ion (**5.3.1.1**).

F. Responds to the alkaloid reactions (**5.3.1.1**).

PURITY TESTS

Aspect of the Solution. The 2% Solution (w/v) of the sample in water is clear.

Acidity. To 10 mL of the solution described in *Aspect of the Solution*, add 0,05 mL of methyl red SI. Not more than 0,2 mL of 0.02M sodium hydroxide is necessary to develop the yellow color.

Related substances. To proceed as described in *Thin layer chromatography*(**5.2.17.1**), using silica gel GF₂₅₄, as support, and ammonia mixture, acetona, ethanol, water and toluene (2,5:32,5:24,5:10,5:35) as Mobile phase. Apply, separately, to the plate 10µL of each one of the freshly prepared solutions described below.

Solution (1): Solution to 20 mg/mL of the sample in mixture of water and bethanol (1:1).

Solution (2): To dissolve 25 mg of codeine phosphate in 5 mL of the *Solution (1)*, dilute 0,2 mL to 10 mL in a mixture of water and ethanol (1:1).

Solution (3): Diluir 0,1 mL of the *Solution (1)* em 20 mL in mixture of water e ethanol (1:1).

Solution (4): Diluir 2 mL of the *Solution (3)* em 5 mL in mixture of water e ethanol (1:1).

Solution (5): Diluir 2 mL da *Solution (3)* em 10 mL in mixture of water e ethanol (1:1).

Develop the chromatogram. Remove the plate, allow to air dry. Nebulize with iodine bismuthate of potassium SR and allow to air dry for 15 minutes. Nebulize with 3% Hydrogen peroxide (w/v) SR. Any secondary spot obtained in the chromatogram with the *Solution (1)*, is not more intense than that obtained with the *Solution (2)* (0,5%). Any secondary spot obtida with the *Solution (1)* can not be more intense than that obtained with the *Solution (3)* (0,5%) and not more than two spots can be more intense than that obtained with the *Solution (4)* (0,2%). The test is not valid unless the spot obtained in chromatogram with the *Solu-*

tion (2) shows two clearly separated spots and that the spot obtained in chromatogram with the *Solution (5)* is clearly visible.

Water (5.2.20.1). Determine in 0,2 g of the sample. Between 10,4% e 13,4%.

Sulphated ashes(5.2.10). Determine in 1 g of sample. At most 0,1%.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Titration in nonaqueous medium*(**5.3.4.5**). Weigh exactly, about 0,5 g of the sample, To dissolve em 120 mL of acetic anhydride. Tritate with 0.1 M SV perchloric acid determining the end point potentiometrically. Each mL of 0.1 M SV perchloric acid equals to 66,880 mg of (C₁₇H₁₉NO₃)₂.H₂SO₄.

B. To proceed as described in *High performance liquid chromatography* (**5.2.17.4**), using chromatograph equipped with a UV detector with 284 nm; 300 mm column and 3,9 mm of inner diameter, packed with silica chemically bonded to octadecylsilane group; flow of *Mobile phase* 1,5 mL/minute.

Mobile phase: To dissolve, exactly, about 0,73 g of sodium heptanesulphonate and, 720 mL of water. To add 280 mL of methanol and 10 mL of glacial acetic acid.

Standard solution: To dissolve amount, accurately weighed, of the sample in the *Mobile phase*, in order to obtain Solution containing 0,24 mg/mL.

Standard solution: To dissolve amount, accurately weighed, of morphine SQR sulphate in *Mobile phase*, in order to obtain Solution containing 0,24 mg/mL.

The relative retention times are about 1,0 minute for the morphine sulphate. The relative standard deviation for the for replicate areas of the peaks recorded is not higher than 2,0%.

Procedure: to separately inject, 25 µL of the *Standard solution and of Standard sample*, record the chromatograms and measure the average área of the peaks. Calculate the content of (C₁₇H₁₉NO₃)₂.H₂SO₄ in the Standard solution from the responses obtained for the *Standard solution* and the *Standard solution*.

PACKAGING AND STORAGE

In containers protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Opioid analgesic.

MORPHINE SULPHATE TABLETS

It contains, at least, 92,5% and, at most 107,5% of the stated amount of $(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O$.

IDENTIFICATION

A. To weigh and pulverize the tablets. Weigh the equivalent to 20 mg of morphine sulphate, add 5 mL of water and stir. Filter, and add to the filtrate 0,05 mL of ferric chloride SR. The color blue is developed.

B. To weigh and pulverize tablets. Weigh the equivalent to 10 mg of morphine sulphate and add 10 mL of water. Filter, and add 5 mL of the filtrate, 0,15 mL 1% potassium ferricyanide (w/v) and 0,05 mL of ferric chloride SR. It is immediately developed the green color, which rapidly changes to blue.

C. The titrated of the tablets should respond the reactions of the sulphate ion (**5.3.1.1**).

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness test (5.1.3.1). It complies with the test.

Test of friability (5.1.3.2). It complies with the test.

Test of disintegration (5.1.4.1). It complies with the test.

Uniformity of single doses (5.1.6). It complies with the test. Proceed in accordance with the described in method **B.** of *DOSAGE*.

TEST OF DISSOLUTION (5.1.5)

Means of dissolution: phosphate buffer pH 6,5, 900 mL
Apparatus: blades, 50 rpm

Time: 45 minutes

Procedure: after the test, remove the aliquot from the means of dissolution and filter. Proceed as described in method **B.** of *DOSAGE*, except that the volume of injection should be 200 μ L. To calculate the amount of $(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O$ dissolved in the medium, comparing the chromatograms obtained with the Solution of morphine sulphate SQR, in the concentration of 0,0033% (w/v), prepared in the same solvent.

Tolerance: not less than 70% (Q) of the stated amount of $(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O$ dissolve within 45 minutes.

PURITY TESTS

Related substances. To proceed as described in *Thin layer chromatography* (**5.2.17.1**), using silica gel GF₂₅₄, as support, and mixture of ethanol to 70% (v/v), toluene, acetone, 13,5 M ammonia (35:35:32,5:2,5) as Mobile phase. Apply,

separately, to the plate 50 μ L of Each one of the freshly prepared solutions described below.

Solution (1): Weigh the equivalent to 25 mg of morphine sulphate in order to obtain a Solution a 1 mg/mL of the sample em ethanol.

Solution (2): To dissolve amount sufficient of codeine sulphate SQR in *Solution (1)* in order to obtain a Solution of codeine sulphate to 1 mg/mL. Remove an aliquot of a Solution and to dissolve in ethanol, in order to obtain a Solution of codeine sulphate to 0,005 mg/mL.

Solution (3): To transfer an aliquot of 2 mL from the *Solution (2)* and dilute in 5 mL of ethanol.

Develop the chromatogram. Remove plate, allow to air dry. Nebulize with potassium iodide and bismuth subnitrate SR and allow to air dry for 15 minutes. Nebulize with 3% Hydrogen peroxide (w/v). The spot corresponding to codeine and morphine has a uniform bluish gray and pink, respectively. Any secondary spot obtained in chromatogram with the *Solution (1)*, is not more intense than that of codeine obtained with the *Solution (2)* (0,5%). Any secondary spot obtained is not more intense than that obtained with the *Solution (2)* (0,5%) and no more than two spots are more intense than that obtained with the *Solution (3)* (0,2%). The test is not valid unless the spot obtained in chromatogram with the *Solution (2)* shows two clearly separated spots.

DOSAGE

To employ one of the methods described below.

A. To weigh and pulverize 20 tablets. To transfer amount of powder equal to 0,1 g of morphine sulphate to 25 mL of water and 5 mL of sodium hydroxide M. To add 1 g of ammonium sulphate and stir mechanically until complete dissolution. If necessary, leave it on ultrasound for 10 minutes. Add 20 mL of ethanol and extract with successive amounts of 40, 20, 20, and 20 mL of mix of chloroform and ethanol (3:1). Wash each extract with the same 5 mL of water, Filter and evaporate the solvent of the filtrate. Dissolve the residue obtained in 10 mL of 0.05M SV hydrochloric acid, boil and allow to cool and add 15 mL of water. Titrate the excess hydrochloric acid with 0.05 M SV sodium hydroxide, using methyl red SI, as indicator. Each mL of 0.05M SV hydrochloric acid equals to 18,970 mg of $(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O$.

B. Proceed in accordance with the described in method **B.** of *DOSAGE* of the *Morphine sulphate monograph*. Prepare the solutions as described below.

Standard solution: To weigh and tablets. To dissolve amount accurately weighed of morphine sulphate in *Mobile phase*, in order to obtain solution containing 0,3 mg/mL.

Standard solution: To dissolve amount accurately weighed of morphine SQR sulphate in the *Mobile phase*, in order to obtain solution containing 0,3 mg/mL.

The retention time is about 6 minutes for the morphine sulphate. The relative standard deviation for the for replicate areas of the peaks recorded is not higher than 2,0 %.

PACKAGING AND STORAGE

In containers protected from light.

LABELLING

To comply with the legislation in force.

MORPHINE SULPHATE INJECTABLE SOLUTION

It contains, at least, 90,0% and, at most 110,0% of the stated amount of $(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O$.

IDENTIFICATION

A. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and mixture of acetone, methanol and ammonium hydroxide (50:50:1) as Mobile phase. Apply, separately, to the plate 20 | L of each one of the freshly prepared solutions described below.

Solution (1): Dissolve amount accurately weighed of the sample in mixture of methanol and water (1:1), in order to obtain solution to 0,05% (w/v).

Solution (2): Dissolve amount accurately weighed of morphine SQR sulphate in mixture of methanol and water (1:1), in order to obtain solution to 0,05% (w/v).

Develop the chromatogram. Remove plate, allow to air dry. Examine under ultraviolet light of low wavelength (254 nm). The main spot obtained with the *Solution (1)* Examine under ultraviolet light of low wavelength with the *Solution (2)*.

B. The retention time of the main peak of the chromatogram of the *Standard solution* obtained in method de *DOSAGE*, corresponds the one of the main peak of the *Standard solution*.

C. Evaporate to dryness, in a water bath, a volume equal to 5 mg of morphine sulphate. Dissolve the residue so obtained in 5 mL of water and add 0,15 mL 1% potassium ferricyanide (w/v) and 0,05 mL of ferric chloride SR. The color bluish gray is developed, wich quickly changes to blue.

D. Responds to the reactions of the sulphate ion (5.3.1.1).
CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

pH (5.2.19). 2,5 a 6,5.

TEST OF PURITY

Related substances. Proceed in accordance described in the *Test of Related substances* of monograph of morphine sulphate *tablets*. Apply, separately, to the plate 10 µL of each one of the freshly prepared solutions described below.

Solution (1): Dilute the volume of the Injectable solution in mixture of ethanol and water (1:1) in order to obtain a Solution of morphine sulphate to 1% (w/v).

Solution (2): Dissolve amount suficient and of codeine sulphate SQR in *Solution (1)*, obtaining a Solution of codeine sulphate 10 mg/mL. Remove an aliquot of the Solution and dissolve in mixture of ethanol and water (1:1) obtaining a Solution of codeine sulphate to 0,05 mg/mL.

Solution (3): Remove an aliquot of 2 mL da *Solution (2)* and dilute em 5 mL of mixture of ethanol and water (1:1).

The test is only valid, IF the chromatogram obtained with the *Solution (2)* Disregard any spot that has retention factor (Rf) less than 0,1.

TEST OF BIOLOGICAL SAFETY

Sterility (5.5.3.2.1). It complies with the test. Use method of direct inoculation or membrane filtration.

Pyrogen (5.5.2.1). It complies with the test.

Bacterial endotoxins (5.5.2.2). It complies with the test. At most 14,29 EU/mg of morphine sulphate.

DOSAGE

Proceed in accordance with the described in method **B.** of *DOSAGE* of the monographa of morphine sulphate. Prepare the solutions as described below.

Standard solution: Transfer volume of the Injectable solution equal to 25 mg of morphine sulphate to a 100 mL volumetric flask, using the *Mobile phase* as solvent, to obtain a final concentration of 0,25 mg/ mL.

Standard solution: Dissolve amount accurately weighed of Morphine sulphate SQR in the *Mobile phase*, in order to obtain a Solution with final concetration of 0,25 mg/mL.

PACKAGING AND STORAGE

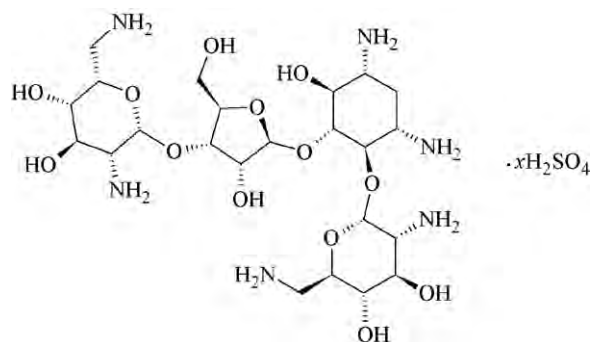
In glass containers type I, in a cool place and protected from light. Avoid freezing.

LABELLING

To comply with the legislation in force.

NEOMYCIN SULPHATE

Neomycini sulfas



$C_{23}H_{46}N_6O_{13} \cdot xH_2SO_4$; 614,64 (base)
Neomycin sulphate; 06284
[1405-10-3]

Neomycin sulphate is mixture of sulphates of substances produced by *Streptomyces fradiae* being its main component the sulphate of 2-deoxy-4-O- (2,6-diamine-2,6-dideoxy-α-D-glucopyranosyl)-5-O-[3-O-(2,6-diamine-2,6-dideoxy-γ-L-idopiranosil)-6-D-ribofuranosyl]-D-streptamine (neomycin B). Features power output of, at least, 0,6 mg/mg of neomycin, in relation to the desiccated substance.

DESCRIPTION

Physical Characteristics. Crystalline powder, yellowish white, hygroscopic.

Solubility. Very water soluble, very slightly soluble in ethanol, Practically insoluble in acetone, chloroform and ethyl ether.

Physical and chemical constants. *Specific rotation* (5.2.8): +53,5° to +59,0°, in relation to the desiccated substance. Determine in Aqueous solution to 10% (w/v).

IDENTIFICATION

A. Proceed as described in *Thin layer chromatography* (5.2.17.1), using silica gel H, as support, and mixture of methanol, ammonium hydroxide, chloroform and water (6:3:2:1), as *Mobile phase*. Apply, separately, to the plate, 5 mL of one of the solutions, freshly prepared, described below.

Solution (1): Prepare Solution to 20 mg/mL of the sample in water.

Solution (2): Prepare Solution to 20 mg/mL of neomycin sulphate SQR in water.

Develop the chromatogram. Remove plate, dry out to hot air. Nebulize the plate with ninhydrin to 1% (w/v) in

1-butanol and heat TO 105 °C for dois minutes. The main spot obtained with the *Solution (1)*, Examine under ultraviolet light of low wavelength with the *Solution (2)*.

B. The *Solution (1)* obtained in method **A.** of *Identification* to 5% (w/v) in water responds to the reactions of the sulphate ion (5.3.1.1).

PURITY TESTS

pH (5.2.19). 5,0 a 7,5. Determine in Aqueous solution to 1% (w/v).

Related substances 1. Proceed as described in *Thin layer chromatography* (5.2.17.1), using silica gel G, as support, and mixture of methylene chloride, ammonium hydroxide and methanol (10:20:20), as *Mobile phase*. Apply, separately, to the plate, 5 pL of each one of the solutions, freshly prepared, described below.

Solution (1): Prepare Solution to 25 mg/mL of the sample in water.

Solution (2): Prepare Solution to 0,5 mg/mL of neamine SQR in water.

Solution (3): Mix 0,5 mL of *Solution (1)* with 0,5 mL of *Solution (2)*.

Develop the chromatogram. Remove plate, allow it dry between 100 °C and 105 °C for 10 minutes. Nebulize with solution of stannous chloride and ninhydrin and heat to 100 °C for 15 minutes. Nebulizer again with the same solution and heat to 110 °C for 15 minutes. Any spot corresponding to neamine obtained in chromatography with the *Solution (1)* is not more intense than that obtained with the *Solution (2)* (2%). The test is only valid if the chromatogram obtained with the *Solution (3)* presents two major spots well defined.

Related substances 2. To proceed as described in *Thin layer chromatography* (5.2.17.1), using silica gel G, as support, and mixture of methanol and 20% Sodium chloride (w/v) (20:80), as *Mobile phase*. Apply, separately, to the plate, 5 pL of each one of the solutions, freshly prepared, described below.

Solution (1): To prepare Solution a 8 mg/mL of the sample in water.

Solution (2): To prepare Solution a 1,2 mg/mL of framycetin sulphate SQR in water.

Solution (3): To mix 5 mL of the *Solution (2)* with 25 mL of water.

Solution (4): To prepare Solution to 8 mg/mL of neomycin sulphate SQR in water.

Develop the chromatogram. Remove plate, allow it dry between 100 °C e 105 °C for 10 minutes and Nebulize with acetic ethanolic ninhydrin SR. To heat between 100 °C and 105 °C for 15 minutes. The main spot obtained with the *Solution (1)* should be examined under ultraviolet light of low wavelength with the *Solution (4)*. The spot with the Rf

smaller (neomycin C impurity) than the main spot is not more intense than that obtained with the *Solution (2)* (15%), but is more intense than the main spot obtained with the *Solution (3)* (3%). The test is only valid if the chromatogram obtained with *Solution (4)* presents spot with R_f less than the main spot.

Sulphate. To dissolve, exactly, about 0,25 g of sample in 100 mL of water and adjust to pH 11,0 with concentrated ammonia solution. Add 10 mL of 0.1 M SV barium chloride and about 0.5 mg of Phthalein purple. Titrate with 0.1M SV disodium edetate. Add 50 mL of ethanol when the color starts to change and continue the titration until the color continues to change until the coloration bluish violet fades. Perform blank test and make the necessary corrections. Each mL of 0.1 M SV barium chloride equals to 9,606 mg of sulphate (SO_4). It contains, at least 27% and, at most, 31% of sulphate (SO_4), in relation to the desiccated substance.

Loss on drying(5.2.9). Determine in 0,1 g of the sample. Dry in an oven in vacuo at 60 °C, to a pressure that does not exceed 5 mm of mercury, during three hours. At most 8,0%.

Sulphated ashes (5.2.10). Determine in 1,0 g of the sample. At most 1%.

BIOLOGICAL SAFETY TESTS

Sterile neomycin sulphate, the sample fulfills with the sterility and Bacterial endotoxins tests. Neomycin sulphate to be sterilized during the process of preparing parenteral dosage forms, the sample complies with the Bacterial endotoxins test.

Sterility (5.5.3.2.1). It complies with the test. Use method of membrane filtration.

Bacterial endotoxins (5.5.2.2). At most 1,30 UE/ mg of neomycin.

DOSAGE

To proceed as described in *Microbiological assay by agar diffusion (5.5.3.3.1)*, using cylinders.

Microorganism: *Staphylococcus epidermidis* ATCC 12228 or *Staphylococcus aureus* ATCC 6538P.

Culture media: Sterile physiological solution for standardization of the inoculum and the culture medium number 11 for the base layer and inoculum preparation.

Standard solution: Weigh exactly, the equivalent to 25 mg of neomycin and transfer to a 25 mL volumetric flask with the aid of a 0,1M Potassium phosphate buffer, sterile, pH 8,0 (*Solution 2*). Top up with the same and homogenize. Dilute, successively, until concentration of 0,5 pg/mL, 1 pg/mL and 2 pg/mL, using the *Solution 2* as diluent, when using the *Staphylococcus epidermidis* ATCC 12228 microorganism or until concentration of 5 pg/mL, 10 pg/mL and 20 pg/mL, using *Solution 2* as diluent, when using *Staphylococcus aureus* ATCC 6538P microorganism.

Standard solution: Weigh exactly, the equivalent to 25 mg of neomycin SQR and transfer to a 25 mL volumetric flask with the aid of *Solution 2*. Top up with the same solvent and homogenize. Dilute, successively, until concentrations of 0,5 pg/mL, 1 pg/mL and 2 pg/mL, using the *Solution 2* as diluent, when using *Staphylococcus epidermidis* ATCC 12228 microorganism or until concentration of 5 pg/mL, 10 pg/mL and 20 pg/mL, using *Solution 2* as diluent when using *Staphylococcus aureus* ATCC 6538P microorganism.

Procedure: To add 20 mL culture medium number 11 in each plate, wait to solidify, add 5 mL of 1% inoculum and proceed as described in *Microbiological assay by agar diffusion (5.5.3.3.1)*. Calculate the content in pg of neomycin, the sample from the standard power and of the responses obtained to the *Standard solution* and with the *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light. When the substance is intended for the production of parenterals, the label must indicate whether the product is sterile or should be sterilized during the process.

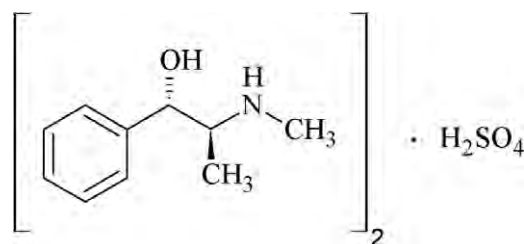
LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antibiotic.

PSEUDOEPHEDRINE SULPHATE Pseudoephedrini sulfas



$(\text{C}_{10}\text{H}_{15}\text{NO})_2 \cdot \text{H}_2\text{SO}_4$; 428,54

Pseudoephedrine sulphate; 07522

Sulphate of (aS)-a-[(1S)-1-(methylamino)ethyl]-benzenomethanol (1:2)
[7460-12-0]

It contains, at least, 98,0% and, at most, 100,5% of $(\text{C}_{10}\text{H}_{15}\text{NO})_2 \cdot \text{H}_2\text{SO}_4$, in relation to the desiccated substance.

DESCRIPTION

Physical Characteristics. White crystalline powder.

Solubility. Slightly soluble in water, easily soluble in ethanol and ethyl ether.

Physical and chemical constants.

Melting range (5.2.2). 174 °C to 179 °C.

Specific rotation (5.2.8). +56,0° to +59,0°, in relation to the desiccated substance. Determine in Solution to 5 % (w/v) in water.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the spectrum of sulphate of pseudoephedrine SQR, prepared in a similar manner.

B. The ultraviolet absorption spectrum (5.2.14), in the range of 200 to 400 nm, of Solution to 0,05% (w/v) in water, displays maximum in 257 nm, calculated as desiccated substance not differing more than 3%.

C. Responds to the reactions of the sulphate ion (5.3.1.1).

PURITY TESTS

pH (5.2.19). 5,0 a 6,5. Determine in Aqueous solution a 5% (w/v).

Heavy metals (5.3.2.3). Treat a Solution of 1 g of the sample in 20 mL of ethanol to 50% (v/v) with 5 mL of Solution of 5% sodium hydroxide (w/v) and five drops of sodium sulfide SR. Use *Standard Lead solution (10 ppm Pb)* in the preparation if the standard. At most 0,001% (10 ppm).

Loss on drying (5.2.9). Determine in 2 g of the sample, and oven at 05 °C, under reduced pressure, for 2 hours. At most 2,0%.

Sulphated ashes (5.2.10). At most 0,1%.

DOSAGE

To dissolve 0,15 g of the sample in 50 mL of glacial acetic acid and Titrate with 0.1 M SV perchloric acid. Determine the end point, potentiometrically. Perform blank test and make the necessary adjustments. Each mL of 0.1 M SV perchloric acid equals to 42,854 mg of sulphate of pseudoephedrine $(C_{10}H_{15}NO)_2H_2SO_4$

PACKAGING AND STORAGE

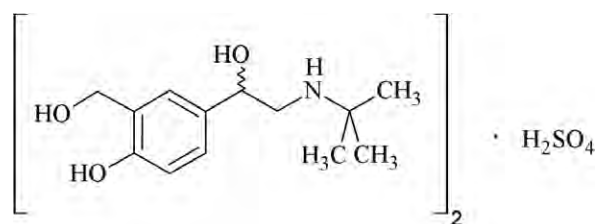
In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Decongestant.

SALBUTAMO SULPHATE**Salbutamoli sulfas**

$(C_{13}H_{21}NO_3)_2 \cdot H_2SO_4$; 576,70 salbutamol sulphate; 07867 Sulfato de a¹-[[[1,1-dimethyletil]amino]metil]-4-hidroxi-1,3-benzenodimetanol (1:2) [51022-70-9]

It contains, at least, 98,0% and, at most, 101,0% of $(C_{13}H_{21}NO_3)_2 \cdot H_2SO_4$, relative to anhydrous substance.

DESCRIPTION

Physical Characteristics. Crystalline powder, white or almost white.

Solubility. Easily soluble in water, slightly soluble in ethanol, ethyl ether and chloroform.

Physical and chemical constants. **Melting range (5.2.2):** 157 °C to 158 °C.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, only has highest absorption at the same wavelengths and with same relative intensities of those observed in spectrum of salbutamol sulphate SQR, prepared in a similar manner.

B. The ultraviolet absorption spectrum (5.2.14), in the range of 230 nm to 400 nm, of Solution of the sample to 0,008% (w/v) in 0.1 M hydrochloric acid, ethyl ether shows maximum absorption in about 276 nm. The absorbância in 276 nm is of 0,44 to 0,51.

C. To dissolve 10 mg of the sample in 50 mL 2 % sodium tetraborate (w/v). To add 1 mL of 4-aminoantipyrine to 3% (w/v), 10 mL of 2% potassium ferricyanide (w/v) and 10 mL of chloroform. Stir and allow separating the layers. The chloroform layer develops a reddish-orange color.

D. To dissolve amount of sample equal to 4 mg of salbutamol in 10 mL of water and filter. The filtrate obtained responds to the reactions of the sulphate ion (5.3.1.1).

PURITY TESTS

Aspect of the Solution. The Solution to 1% (w/v) in water free of carbon dioxide is clear (5.2.25).

Acidity or Alkalinity. To transfer 0,25 g of the sample to 25 mL volumetric flask and complete the volume with

water free of carbon dioxide. Add 10 mL of this Solution, 0,15 mL of methyl red SI and 0,2 mL of 0.01 M sodium hydroxide. The solution becomes yellow. Not more than 0,4 mL of 0.01 M hydrochloric acid is necessary to change the color to red.

Related substances. To proceed as described in *Thin layer chromatography* (5.2.17.1), using silica gel GF₂₅₄, as support, and mixture of methylisobutylketone, isopropyl alcohol, ethyl acetate, water and ammonium hydroxide (50:45:35:18:3), as Mobile phase. Apply, separately, to the plate, 50 mL of each one of the solutions, freshly prepared, described below.

Solution (1): To dissolve amount accurately weighed of the sample in methanol and dilute with the same solvent, in order to obtain Solution to 20 mg/mL.

Solution (2): To dissolve amount accurately weighed of salbutamol sulphate SQR in methanol and dilute with the same solvent, in order to obtain Solution to 0,1 mg/mL.

Develop the chromatogram. Remove plate, allow to air dry. Expose to iodine vapor. Any secondary spot obtained in chromatogram with the *Solution (1)*, different of the main spot, is not more intense than that obtained with the *Solution (2)* (0,5%) and the sum of the intensities of all secondary spots present is not greater than 2,0%.

Water (5.2.20.1). At most 0,5%.

Sulphated ashes(5.2.10). Determine in 1 g of sample. At most 0,1%.

DOSAGE

To proceed as described in *Titrations in non-aqueous media* (5.3.3.5). Weigh exactly, about 0,9 g of the sample, transfer to 250 mL erlenmeyer flask and dissolve in 50 mL of glacial acetic acid. Add two drops of Oracet Blue B SI and Titrate with 0.1 M SV perchloric acid. Perform blank test and make the necessary corrections. Each mL of 0.1 M SV perchloric acid equals to 57,670 mg of (C₁₃H₂₁NO₃)₂.H₂SO₄.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antiasthmatic.

SALBUTAMOL SULPHATE ORAL SOLUTION

Contains SALBUTAMOL SULPHATE equal to, at least, 90,0% and, at most, 110,0% of the stated amount of salbutamol (C₁₃H₂₁NO₃). Contains preservatives and sweeteners. The Oral Solution may contain sugar.

IDENTIFICATION

A. The absorption spectrum in the visible (5.2.14), in the range 400 nm to 800 nm, of the standard solution obtained in method of *DOSAGE*, displays maximum in 605 nm, similar to that observed in the spectrum of the standard solution.

A. Use volume of oral solution equal to 4 mg of salbutamol. Dissolve in 10 mL of water and filter. The filtrate responds to the reactions of the sulphate ion (5.3.1.1).

B. To a volume of oral solution equal to 10 mg of salbutamol, add 50 mL of 2 % sodium tetraborate (w/v) in water. Proceed as described in test **C.** of *Identification of the monograph of salbutamol sulphate*.

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

pH (5.2.19). 3,3 a 5,0.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll microorganisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic microorganisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Visible absorption spectrophotometry* (5.2.14). Protect the solutions from light. To transfer volume of oral solution equal to 8 mg of salbutamol to a 100 mL volumetric flask. To add 70 mL of water. Leave it on ultrasound for 5 minutes and top up with the same solvent. Homogenize. To transfer 2 mL of that solution to a 250 mL separatory funnel containing 80 mL of water. To add 4 mL of 5% sodium bicarbonate (w/v), 4 mL of sulphate of *N,N*-dimethyl-*p*-phenylenediamine to 0,1% (w/v) and 4 mL potassium ferricyanide at 8% (w/v). Stir and allow to stand for 20 minutes, in the absence of light. Extract with 2 portion of 10 mL of chloroform, collect the extracts in a 25 mL volumetric flask and top up with the same solvent. Homogenize. Prepare standard solution in the same concentration, using the same procedure. Measure the absorbance of the resulting solutions in

605 nm, using chloroform for zero adjustment. Calculate the amount of salbutamol ($C_{13}H_{21}NO_3$) in the oral solution from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Proceed as described in *DOSAGE* in monograph of *salbutamol sulphate tablets*. Prepare *Standard solution* as described below.

Standard solution: Transfer volume of oral solution equal to 6 mg of salbutamol to a 200 mL. Add 150 mL of *Diluent*. Stir. Top up with the same solvent, obtaining Solution to 30 µg of salbutamol per mL. Homogenize and filter.

Procedure: separately inject, 20 µL of the *Standard and sample solutions*, record the chromatograms and measure the areas under the peaks. Calculate the amount of salbutamol ($C_{13}H_{21}NO_3$) in the oral solution oral from the responses obtained for the *Standard solution e Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

SODIUM SULPHATE

Natrii sulfas

Na_2SO_4 ;
142,04
 $Na_2SO_4 \cdot 10H_2O$; 322,19
Sodium sulphate; 08173
Sodium salt of sulfuric acid(2:1)
[7757-82-6]
Sodium salt of sulfuric acid hydrate (2:1:10)
[7727-73-3]

It contains, at least 98,5% and, at most, 101% of Na_2SO_4 , calculated in the anhydrous base.

DESCRIPTION

Physical Characteristics. White crystalline powder or Transparent colorless crystals; saline taste, slightly bitter. Hygroscopic.

Solubility. Easily soluble in water

IDENTIFICATION

A. A Solution 1:20 responds to the test for sulfate ion (5.3.1.1).

B. A Solution 1:20 responds to the test for sodium ion (5.3.1.1).

PURITY TESTS

Acidity or Alkalinity. To 10 mL of a solution containing 1 g in 20 mL of water, add A drop of bromothymol blue SI. Are necessary, at most, 0,5 mL of 0.01 M hydrochloric acid or 0,5 mL of 0.01 M sodium hydroxide to change the color of the solution.

Chloride (5.3.2.1). At most 0,02% (200 ppm).

Heavy metals (5.3.2.3). Dissolve 2 g in 10 mL of water, add 2 mL of 0,1 M hydrochloric acid and complete the volume to 25 mL. At most 0,001% (10 ppm).

Loss on drying (5.2.9). Desiccate at 105 °C for 4 hours. For dehydrated form, the loss is in between 51% and 57 %. For the anhydrous form, at most 0,5%.

DOSAGE

Weigh the equal to 0,4 g of the anhydrous or desiccated sample and dissolve in 200 mL of water and add 1 mL of hydrochloric acid. Heat until boiling and, gradually, add small portions, with constant stirring, of a solution in excess of barium chloride (about 8 mL). Heat the mixture in a water bath for 1 hour. Allow to settle, filter the precipitate and wash with water until the washing waters are free of Chlorides. Dry, calcine and weigh. The mass of barium sulphate obtained multiplied by 0,6086 represents the equivalent of Na_2SO_4 .

PACKAGING AND STORAGE

In airtight containers, with a temperature not exceeding 30 °C.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Laxative.

FERROUS SULPHATE

Ferrosi sulfas

$FeSO_4$; 151,91
ferrous sulphate; 08176
Salt of iron (2+) of Sulfuric acid (1:1)
[7720-78-7]
It contains, at least, 86,0% and, at most, 90,0% of $FeSO_4$.

DESCRIPTION

Physical Characteristics. White powder, yellow-gray.

Solubility. Soluble in water, insoluble in ethanol.

IDENTIFICATION

A. Responds to the reactions of the ferrous ion (5.3.1.1).

B. Responds to the reactions of the sulphate ion (5.3.1.1).

PURITY TESTS

Insoluble Substances. Dissolve 2 g of the sample in 20 mL of 1% sulfuric acid (v/v), heat until boiling and leave it in a water bath, in a covered beaker, for 1 hour. Filter and wash with 1% sulfuric acid (v/v). Dry the filter at 105 °C. At most, 1 mg of residue (0,05%).

Ferric ion. Dissolve, in a erlenmeyer flask with lid, 5 g of the sample in mixture of hydrochloric acid and water free of carbon dioxide (1:10) and add 3 g of potassium iodide. Cover the flask and allow to stand, protected from light, for 5 minutes. Titrate the released iodine with 0,1 M SV tiosodium sulphate, using 0,5 mL of starch SI as indicator, added near the end of the titration. Prepare blank test omitting the addition of the sample. The difference between the titrations represents the amount released by the ferric ion. At most 4,5 mL of 0,1 M SV tiosodium sulphate are spent (0,5%).

Copper. To proceed as described in *Atomic absorption spectrophotometry* (5.2.13.1), Use *Method II*. Transfer 2 g of the sample to a 100 mL volumetric flask, dissolve 5 % nitric acid v/v) and top up with the same solvent. At the same time, transfer 0,393 g of pentahydrate cupric sulphate to a 100 mL volumetric flask, dissolve in water and top up with the same solvent, obtaining copper standard solution (1000 ppm Cu). Dilute this solution in 5 % nitric acid (v/v), in order to obtain the standard solutions. Measure the absorbances of the solutions in 324,7 nm. At most 0,005% (50 ppm).

Manganese. To dissolve 1 g of the sample in 40 mL of water, Add 10 mL nitric acid and boil until it ceases releasing red smoke. Add 0,5 g of ammonium peroxydisulfate and boil for 10 minutes. Eliminate any pinkish eventually formed, by adding, dropwise, 5% sodium sulfite (w/v). Heat to boiling point until disappearance of the odor of sulfur dioxide. Add 10 mL of water, 5 mL of phosphoric acid and 0,5 g of sodium periodate, heat to boiling point for 1 minute and allow to cool to room temperature. The solution obtained is not more intensely colored than the standard solution prepared in the same conditions, using 1 mL of 0.02 M SV potassium permanganate, and the same amounts of reagents.

Basic sulfate. Dissolve 2g of sample mixing 7.5mL of water free of carbon dioxide and 0.5mL of sulfuric acid 0.5M. Preparation is slightly turbid.

Zinc. 5mL of solution obtained in tests in *Heavy metals*, add 1mL of Potassium ferrocyanide SR, dilute to 13mL with water, and let it rest for 5 minutes. Any turbid produced is not most intense than the obtained by 10mL mix of zinc pattern solution (10 ppm Zn), 2mL of Hydrochloric

acid 7 M and 1mL of Potassium ferrocyanide SR. At most 0.05% (500 ppm).

Arsenic (5.3.2.5) Dissolve 1g of sample in 10mL of water and 15mL of Tin-hydrochloric acid SR Distill 20mL of this solution. When distillate, add 0.15mL of Bromine water SR, remove Bromine excess with 0.15mL of Tin chloride (II) SR and dilute it with water to 75mL. Use 25mL of obtained solution and proceed as described in *Visual method*. At most 0.0003% (3 ppm).

Heavy metals (5.3.2.3) Dissolve 1g of sample in 10mL Hydrochloric acid 7 M, add 2mL Concentrated hydrogen peroxide and boil it until its volume is reduced to 5mL. Let it cool, dilute with Hydrochloric acid 7 M to 20mL and extract with three portions of 20mL of Methyl Isobutyl Ketone recently distilled, and Hydrochloric acid 7 M (100:1). Let it rest, separate aqueous phase and evaporate it until half volume. Let it cool and dilute it with water to 25mL, obtaining the test solution. Neutralize 7.5mL of test solution with Ammonia SR and dilute it to 15mL with water. Use 12mL of this solution and proceed as described in *I Method*. At most 0,005% (50 ppm).

ASSAY

Dissolve, about 0.5g, exactly, of sample mixed in water and sulfuric acid M (30:20). Titular com Ammonium cerium sulfate 0,1 M SV, use Ferroin. SI as an indicator. Each mL of Ammonium cerium sulfate 0,1 M SV is equal to 15.190mg of FeSO₄.

PACKING AND STORAGE

In well closed container.

TAGGING

See legislation in force.

THERAPEUTIC CLASS

Anti-anemic.

TABLETS OF FERROUS SULFATE

It contains minimum of 95.0% and at most of 110.0%. of FeSO₄·7H₂O specified amount. Tablets should be coated.

IDENTIFICATION

A. Weight and spray the tablets. Agitate the amount of powder equal to 0.1g of Elemental iron with 20mL of water and filtrate. Responds to ferrous ion reactions (5.3.1.1).

B. Weight and spray the tablets. Agitate the amount of powder equal to 0.1g of Elemental iron with 20mL of Hydrochloric acid SR and filtrate. Responds to sulfate ion reactions. (5.3.1.1).

FEATURES

Determination of Weight (5.1.1) Meet the test.

Disintegration test (5.1.4) Meet the test.

Uniformity of unitary dosages (5.1.6) Meet the test.

ASSAY

Weight and spray 20 tablets. Transfer a power amount equal to 0.5g of Ferrous Sulfate to a beaker containing a 20mL mix of sulfur acid *M* and 80mL of water recently boiled and cooled. Immediately filtrate the solution. Rinse the filter and precipitate with small portions of the mix. Gather the filtrated and washing waters, add Orthophenanthroline SI and titrate immediately with Cerium sulfate 0,1 *M* SV. Each Cerium sulfate mL 0,1 *M* is equal to 27,80mg of Heptahydrate ferrous sulfate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$).

PACKING AND STORAGE

In well closed container.

TAGGING

See legislation in force. It should be specified on tag the Ferrous sulfate quantities (FeSO_4) and Elemental iron (Fe) per tablet.

HEPTAHYDRATED FERROUS SULFATE

Ferrosi sulfas heptahydricus

$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$; 278,01

Fe; 55⁴,85²

Heptahydrated ferrous sulfate; 08177

Iron salt (2+) of Heptahydrated sulfuric acid (1:1:7)
[7782-63-0]

It contains minimum of 98.0% and at most of 105.0%. of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$

DESCRIPTION

Physical characteristics. Crystal light green powder or bluish-green crystal, odorless, astringent taste, fluorescent in dry air. It oxidate fast in contact with humid air, forming basic brownish-yellow ferrous sulfate

Solubility. Easily soluble in water, practically insoluble in ethanol.

IDENTIFICATION

A. Solution obtained in *Solution aspect* responds reactions of ion ferrous (5.3.1.1).

B. Solution obtained in *Solution aspect* responds reactions of ion sulfate (5.3.1.1).

PURITY TESTS

Solution aspects Dissolve 2.5g of sample in water free of Carbon dioxide, add 0.5mL of Sulfuric acid *M* and dilute to 50mL with water. Obtained preparation is no longer opalescent than *Suspension of reference II* (5.2.25).

pH (5.2.19). 3.0 to 4.0. Determine sample in solution at 5% (p/v) in water free of Carbon dioxide.

Arsenic (5.3.2.5) Transfer 1g of sample to a 100mL round bottom flask provided by distillation system. Add 40mL of Sulfuric acid 4.5 *M*, 2mL of Potassium bromide at 30% (p/v) and immediately connect flask to distillation system. Add glass beads, heat the flask under low flame until dissolution of sample and distill until obtain 25mL of distillate. Transfer distillate to the Arsine generator flask and rinse condenser and other distillation system part with small portions of water, adding washing waters to the Arsine generator flask. Agitate flask in circular movements, add Bromide water SR until obtain a slightly yellow coloring and dilute with water at 35mL. Proceed as described in *Spectrophotometric Method, Method I*. At most 0,0003% (3 ppm).

Chlorides (5.3.2.1) Determine in 1.2g of sample, using 1mL of pattern Hydrochloride acid (HCl 0,01 *M* SV), to the pattern preparation. At most 0.03% (300 ppm).

Ferric Ion. Transfer 5g of sample to covered Erlenmeyer and dissolve with 10mL Hydrochloride acid mix and 100mL of water free of Carbon dioxide. Add 3g of Potassium iodide, cover it and let it rest away from light for 5 minutes. Titrate Iodine liberated with Sodium thiosulfate 0,1 *M* SV using, 0.5mL of Starch SI as indicator, adding close to the end point. Perform a white test and make corrections when necessary. At most 4.5mL of Sodium Thiosulfate 0,1 *M* SV are spent on titration (0,5%).

Manganese. Dissolve 1g of sample in 40mL of water, add 10mL of nitric acid and boil it until liberation of red smoke. Add 0.5g of Ammonium peroxydisulfate and heat until boil for 10 minutes. Eliminate any pink coloring

which eventually formates, adding a Sodium sulphite solution drop to drop. Heat it to boiling point until disappearance of Sulfur dioxide odor. Add 10mL of water, 5mL of Phosphoric acid and 0.5g of Sodium periodate, heat it to boiling point for 1 minute and cool it in room temperature. Solution obtained is not the most intensely colored than the pattern solution prepared in same conditions, using 1mL of Potassium permanganate 0,02 *M* SV and the same reagent amount.

Zinc. Dissolve 1g of sample in 10mL Hydrochloric acid SR, add 2mL Concentrated hydrogen peroxide and boil it until its volume is reduced to 5mL. Cool it, dilute to 20mL with Hydrochloric acid SR, transfer to separatory funnel and agitate for 3 minutes with three portions of 20mL saturated Methyl Isobutyl Ketone with Hydrochloric acid (prepared agitating 100mL of Methyl Isobutyl Ketone recently distilled with 1mL of Hydrochloric acid SR). Let it rest,

separate aqueous layer and reduce its volume to half in water bath. Cool it, transfer quantitatively to 25mL volumetric flask and complete volume with water. Add to 5mL of this solution 1mL of Potassium ferrocyanide SR and dilute it to 13mL with water. After 5 minutes, any turbid produced is not most intense than the produced by 10mL mix of zinc pattern solution (10 ppm Zn), 2mL of Hydrochloric acid SR and 1mL of Potassium ferrocyanide SR. At most 0.05% (500 ppm).

Heavy metals (5.3.2.3) Dissolve 2g of sample in 1mL Sulfuric acid SR mix and 40mL of water. Add 0.05 g of Hydroxylamine hydrochloride and heat until boil for 1 minute. Cool it in room temperature, transfer quantitatively to volumetric flask of

50 mL with water assistance to complete volume with the same solvent (*Solution A*). Transfer 30mL of *Solution A* to Nessler tube of 50mL and adjust pH between 3.0 and 4.0 with Ammonium hydroxide 6 M or Acetic acid M. Add 2mL of Acetate buffer pH 3.5, dilute it with water to 40mL and homogenize. For the pattern preparation, transfer 15mL of *Solution A* for 50mL Nessler tube, dilute to 25mL with water, adjust pH between 3.0 and 4.0 Ammonium hydroxide 6 M or Acetic acid M, add 2mL of Acetate buffer pH 3.5, 3mL of *Lead pattern solution* (10 ppm Pb), dilute to 40mL with water and homogenize. Add to pattern and sample 10mL of Hydrogen sulfide SR, complete volumes with water and homogenize. Let it rest for 2 minutes. See tubes from up to down, over white surface. Any brown coloring developed on sample preparation is not most intense than the developed on pattern preparation. At most 0.005% (50 ppm).

ASSAY

Dissolve, about 0.5g of sample exactly mixing 25mL of sulfuric acid M and 25mL of water free of Carbon dioxide. Add 2 drops of Ferroin 51 and titrate immediately with Ammonium cerium sulfate 0,1 M SV until it turns from reddish-orange to pale green. Each Ammonium cerium sulfate mL 0,1 M SV is equal to 27.801mg of Heptahydrate ferrous sulfate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) and 5.585mg of Elemental iron (Fe).

PACKING AND STORAGE

In well closed container.

TAGGING

See legislation in force.

THERAPEUTIC CLASS

Anti-anemic.

ORAL SOLUTION OF FERROUS SULFATE

It contains heptahydrate Ferrous sulfate equal to, 94,0% minimum and at most 106,0% of stated amount of Elemental iron (Fe).

IDENTIFICATION

A. Responds to ferrous ion reactions (5.3.1.1).

B. Responds to sulfate ion reactions (5.3.1.1).

FEATURES

Determination of Volume (5.1.2) Meet the test.

pH (5.2.19). 1.8 to 5.3.

BIOLOGICAL SAFETY TESTS

Total number counting of mesophilic microorganisms (5.5.3.1.2) Meet the test.

Research of pathogenic microorganisms (5.5.3.1.3). Meet the test.

ASSAY

Transfer volume, exactly measured, of equivalent oral solution about 0,125g of Elemental iron (Fe) to Erlenmeyer, add 80mL of water free of Carbon dioxide and 20mL of Sulfuric acid M. Add two Ferroin SI drops and immediately titrate with Ammonium cerium sulfate 0,1 M SV until it turns from reddish-orange to pale-green. Each mL of Ammonium cerium sulfate 0,1 M SV is equal to 5.585 mg of Elemental iron FeSO_4 .

PACKING AND STORAGE

In well closed container, away from light.

TAGGING

See legislation in force. It should be specified on tag the Heptahydrate ferrous sulfate quantities ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) and Elemental iron (Fe) per oral solution mililitre.

SELENIUM SULFIDE Selenii disulfidum

SeS_2 ; 143,09
Se; 78,96
Selenium sulfide; 08182
Selenium sulfide
[7488-56-4]

It contains 52.0% minimum and at most 55.5% of Selenium (Se)

DESCRIPTION

Physical characteristics. Orange or reddish-brown powder.

Solubility. Practically insoluble in water and practically insoluble in organic solvents.

IDENTIFICATION

Boil 50mg of sample with 5mL of Nitric acid for 30 minutes. Dilute to 50mL with water and filtrate. For each 5mL of filtrate, add 10mL of water and 5g Urea. Heat until boiling point, let it cool and add 2mL of Potassium iodide at 0.8% (p/v). A yellow coloring is produced and dims fast (Selenium presence). This solution is used for the test of *Identification*.

B. Let the solution obtained in test **A.** of *Identification* rest for 10 minutes e filtrate. filtrate obtained responds to Ion sulfate reactions (**5.3.1.1**).

PURITY TESTS

Soluble Selenium compounds. Proceed as described on *Spectrofotometric of Visible absorption* (**5.2.14**).

Sample solution: weight 10g of sample, add 100mL of water and stir. Let it under constant agitation for one hour and filtrate. For each 10mL of filtrate, add 2mL of Formic acid, complete to 50mL with water and adjust the pH with Formic acid in 2.5 ± 0.5 . Add 2mL of 3,3'-diaminobenzidine tetrahydrochloride SR. Let it rest for 45 minutes and adjust the pH with Ammonium 6M between 6.5 ± 0.5 . Agitate the solution for one minute with 10mL of Toluene and allow phases separation. Dispose the aqueous phase.

Pattern solution: use 10mL of a Selenious acid solution containing 0.5 µg/mL of Selenium. Proceed as preparation of sample solution before adding 2mL of Formic acid.

To determine absorbance of *Pattern solution* and *Sample solution* in 420 nm. Use white with same composition of the sample solution. Absorbance of *Sample solution* is not higher than *Pattern solution*. At most 0.0005% (5 ppm).

ASSAY

Weight about 100g of sample exactly, add 25mL of fuming nitric acid and heat it in water bath for one hour. Let it cool, transfer to a 250mL volumetric flask containing 100mL of water and complete to volume 250mL with water. Transfer 50mL of solution, add 25mL of water and 10g of Urea and heat until boiling point. Let it cool, add 3mL of Starch SI, 10mL of Potassium iodine solution at 10% (p/v) and titrate immediately with volumetric solution of Sodium thiosulfate 0,1 M SV. Perform the test in white. Each mL of Sodium thiosulfate 0,1 M is equal to 1.974mg of Se.

PACKING AND STORAGE

In well closed containers.

TAGGING

See legislation in force.

THERAPEUTIC CLASS

Antifungal.

SODIUM SULPHITE

Natrii sulfit

Na₂SO₃; 126,04
Sodium sulphite; 08187
Sodium salt of Sulfurous acid (2:1)
[7757-83-7]

It contains minimum of 95.0% and at most of 100.5%. of Na₂SO₃.

DESCRIPTION

Physical characteristics. White and odorless powder.

Solubility. Easily soluble in water, very little soluble in ethanol.

IDENTIFICATION

A. Solution at 5% (p/v) respond ion sodium reactions (**5.3.1.1**).

B. Solution at 0.9% (p/v) respond ion sulphite reactions (**5.3.1.1**).

C. Dissolve 5g of sample in water and complete volume to 100mL with the same solvent. To a 5mL aliquot, add 0.5 mL of Iodine 0,05 M. Resulting solution is colorless and respond to ion sulfate reactions (**5.3.1.1**).

PURITY TESTS

Solution aspects Dissolve 10g of sample in 25mL of water and carefully add 15mL of Hydrochloric acid. Heat until boiling point. Coll it and complete volume to 100mL with water. Obtained preparation is clear (**5.2.25**) and colorless (**5.2.12**).

Selenium. In 3g of sample add 10mL of Formaldehyde solution and, carefully 2mL of Hydrochloric acid. Heat in water bath for 20 minutes. If pink coloring is developed, this should not be most intense than the pattern solution prepared simultaneously at the same conditions, with 1g of sample added of 0.2mL of Selenium pattern solution (100 ppm Se). At most 0.001% (10 ppm).

Thiosulfates. Dissolve 2 g of the sample into 100mL water. Add 10mL of Formaldehyde solution and 10mL of Acetic acid. Wait for 5 minutes. Add 0.5mL os Starch SI and titrate with Iodine 0.05 M SV. Perform the test in

white. Difference between the volumes spent in titration is not higher than 0.15mL.

Zinc. Proceed as described in *Atomic absorption spectrophotometry (5.2.13.1)*, use the *II Method*. At most 0,0025% (25 ppm)

Sample solution: dilute 2mL of obtained solution *Solution aspect* at 10mL with water.

Reference Solution: prepare the reference solutions using a Zinc pattern solution (100 ppm Zn), diluting it with water when necessary.

Measure absorbance in 213,9nm using a hollow-cathode lamp as source of radiation and air-acetylene flame.

Iron (5.3.2.A). Use *Method I*. Determine in 10mL of obtained solution in *Solution aspect*. Proceed as described in *Iron limit test*, using 10mL of *Iron pattern solution (1 ppm of Fe)*. At most 0.001% (10 ppm).

Heavy metals (5.3.2.3) Use *Method I*. Transfer 20mL of obtained solution in *Solution aspect* for a 50mL Nessler

tube. Complete volume to 25mL with water and proceed as described in *Heavy metal limit test*. At most 0.001% (10 ppm).

ASSAY

Weight exactly about 0.25g of sample and transfer to an Erlenmeyer containing 50mL of Iodine 0,05 M SV. Agitate until complete dissolution. titrate Iodine excess with Sodium thiosulfate 0,1 M SV, using 1mL of Starch SI as an indicator. Perform the test in white. Each mL of Iodine 0,05 M SV is equal to 6.302mg of Na₂SO₃.

PACKING AND STORAGE

In well closed container.

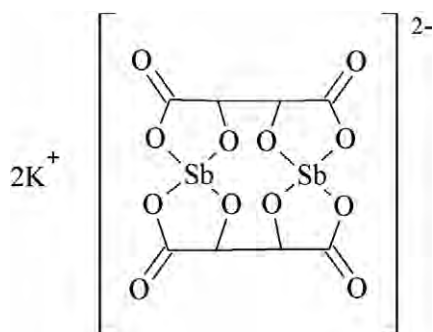
TAGGING

See legislation in force.

CATEGORY

Antioxidant.

POTASSIUM ANTIMONY TARTRATE



$C_8H_4K_2O_{12}Sb_2$; 613,83

$C_8H_4K_2O_{12}Sb_2 \cdot 3H_2O$; 667,87

Potassium Antimony Tartrate; 00352

bis[u-[(2.R,3.R)-2,3-Di(hidroxi-KO)butanedioate(4-)-
 $KO^1:xO^4$]]di-potassium(2-) antimonate (1:2)
 [11071-15-1]

bis[u-[(2.R,3.R)-2,3-Di(hidroxi-KO)butanedioate((4-)-
 $KO^1:xO^4$]]]hidrated di-potassium(2-) antimonate (1:2:3)
 [28300-74-5]

It contains 99.0% minimum and 103.0% at most

$C_8H_4K_2O_{12}Sb_2 \cdot 3H_2O$

DESCRIPTION

Physical characteristics. White or colorless, crystal powder. **Solubility.** Easily soluble in water.

IDENTIFICATION

A. Dissolve a small amount of a Tartrate salt in two drops of Sodium periodate at 5% (p/v). Add a drop of Sulfuric acid 0.5 M and after 5 minutes, add a few drops of Sulfurous acid, followed by a few drops of Fuchsin bleached SR. Pink coloring formation occurs in 15 minutes.

B. When heated to incandescence, it occurs the burn with burned sugar odor release, leading to a dark waste. When this waste is taken to the flame, it presents a violet coloring.

C. Dissolve 1 g of the sample in 100mL of water. To acidify solution with Hydrochloric acid and add Hydrogen sulfide SR A formation of an orange precipitated occurs, soluble Sodium hydroxide 0,05 M.

PURITY TESTS

Acidity or alkalinity. Dissolve 1g of sample in 50mL in water free of organic compounds and titrate with Hydrochloric acid 0,01 M or with Sodium hydroxide 0,01 M in pH 4,5. More than 2mL is not necessary.

Arsenic (5.3.2.5) Use *Metod II*. Dissolve 0.1g of the sample in 5mL of Hydrochloric acid. Add 10mL of a solution recently prepared of 20g of Stannous chloride in 30mL of Hydrochloric acid. Transfer to a color comparison tube and

let it rest for 30 minutes. White surface formed is not most intense than the produced when an equivalent solution containing 15 µg of Arsenic is used. At most 0.015% (150 ppm).

Lead (5.3.2.12) At most 0.002% (20 ppm).

Loss by Desiccation (5.2.9) To determine in 2g of sample. To desiccate in kiln at 105 °C until constant weight. At most 2.7%.

ASSAY

Weight about 0.5g exactly of sample and dissolve in 50mL of water. Add 5g of Potassium sodium tartrate, 2g of Sodium borate, 3mL of Iodized starch SI and titrate immediately with Iodine 0,1 M SV until a persistent coloring blue appear. Each mL of Iodine 0,1 M SV is equal to 16,70 mg of $C_8H_4K_2O_{12}Sb_2 \cdot 3H_2O$

PACKING AND STORAGE

In well closed container.

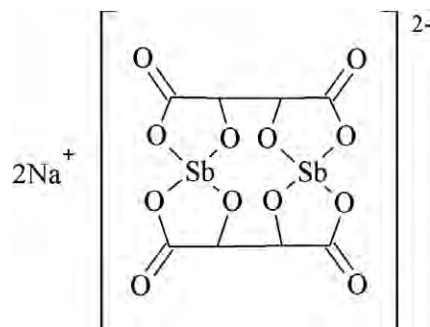
TAGGING

See legislation in force.

THERAPEUTIC CLASS

Antiparasitic

SODIUM ANTIMONY TARTRATE



$C_8H_4Na_2O_{12}Sb_2$; 581,61

Sodium Antimony Tartrate; 09845

bis[u-[(2.R,3.R)-2,3-Di(hidroxi-KO)butanedioate(4-)-
 $KO^1:xO^4$]]di-sodium(2-) antimonate (1:2)
 [34521-09-0]

It contains, minimum of 98,0% and at most 101,0% of $C_8H_4Na_2O_{12}Sb_2$ in relation to desiccated substance.

DESCRIPTION

Physical characteristics. White or colorless crystal powder.

Solubility. Easily soluble in water.

IDENTIFICATION

A. Dissolve a small amount of a Tartrate salt in two drops of Sodium periodate at 5% (p/v). Add a drop of Sulfuric acid 0.5 M and after 5 minutes, add a few drops of Sulfurous acid, followed by a few drops of Fuchsin bleached SR. Pink coloring formation occurs in 15 minutes.

B. Responds to ion antimony reactions (5.3.1.1).

C. Responds to ion sodium reactions (5.3.1.1).

PURITY TESTS

Acidity or alkalinity. Dissolve 1g of sample in 50mL in water free Carbon dioxide and titrate with Hydrochloric acid 0,01 M or with Sodium hydroxide 0,01 M in pH 4,5. More than 2mL is not necessary.

Arsenic (5.3.2.5) Weight 0,375g of sample and proceed as described in *Arsenic limit test, Metod II*. At most 0.0008% (8 ppm).

Lead (5.3.2.12) At most 0.002% (20 ppm).

Loss by Desiccation (5.2.9) To determine in 2g of sample. To desiccate in kiln at 105 °C until constant weight. At most 6%.

ASSAY

Weight about 0.5g exactly of sample and dissolve in 50mL of water. Add 5g of Potassium sodium tartrate, 2g of Sodium borate, 3mL of Iodized starch SI and titrate immediately with Iodine 0,1 M SV until a persistent coloring blue appear. Each mL of Iodine 0,1 M SV is equal to 14,54 mg of $C_8H_4Na_2O_{12}Sb_2$.

PACKING AND STORAGE

In well closed container.

TAGGING

See legislation in force.

THERAPEUTIC CLASS

TABLETS OF METOPROLOL TARTRATE

It contains minimum of 95,0% and at most 105,0% of the stated amount of $(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6$.

IDENTIFICATION

A. Weight and spray the tablets. Transfer the powder amount equal to 40mg of Metoprolol tartrate for separatory funnel. Add 25mL of water and 4mL of diluted Ammonium

hydroxide (1:3). Extract with 20mL of Chloroform filtrating the Chloroform extract obtained via Anhydrous sodium sulfate previously moisturized with Chloroform. Evaporate the Chloroform until it is dry, freeze the waste at -18°C for 30 minutes and let it reach room temperature. Infrared absorption spectrum (5.2.14) of waste, disperse in Potassium bromide, present absorption maximum only on same waves length and with same intensity related to the observed on Metoprolol tartrate spectrum SQR, prepared identically.

B. Ultraviolet absorption spectrum (5.2.14) of sample solution obtained on method A. of *Essay*, shows maximum and minimum identical of the observed on Metoprolol tartrate spectrum SQR.

FEATURES

Determination of Weight (5.1.1). Meet the test.

Hardness test (5.1.3.1). Meet the test.

Friability test (5.1.3.2). Meet the test.

Disintegration test (5.1.4.1). Meet the test.

Uniformity of unitary dosages (5.1.6) Meet the test.

DISSOLUTION TEST (5.1.5)

Mean of dissolution: simulated gastric fluid (without enzyme), 900mL

Appliances: basket 100rpm *Time:* 30 minutes

Procedure: after the test, remove aliquot, filtrate and dilute on *Mean of dissolution* until adequate concentration. Measure absorbance in 275nm (5.2.14), using *Mean of dissolution* for zero adjust. Calculate the amount of $(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6$ dissolved on mean, comparing to the obtained readings with Metoprolol tartrate SQR solution on 0,01% (p/v) concentration prepared on the mean of dissolution.

Tolerance: not less than 75% (Q) of the stated amount of $(C_8H_7NO)_2 \cdot C_4H_6O_6$ dissolving in 30 minutes.

BIOLOGICAL SAFETY TESTS

Total number counting of mesophilic microorganisms (5.5.3.1.2). Meet the test.

Research of pathogenic microorganisms (5.5.3.1.3). Meet the test.

DOSAGE

Use one of the following methods.

A. Proceed in accordance with the described in Absorption spectrophotometry in ultraviolet (5.2.14). Weigh and

spray 20 tablets. Transfer quantity of powder equivalent to 75 mg of metoprolol tartrate to a volumetric flask of 200 mL and add 150 mL of absolute ethanol. Homogenize and leave in ultrasonic bath for 15 minutes. Complete the volume with absolute ethanol, homogenize and filter. Transfer 20 mL of the filtered to a volumetric flask of 50 mL, complete the volume with absolute ethanol and homogenize. Prepare standard solution in the same conditions. Measure the absorbencies of the solutions in 274 nm, using absolute ethanol for adjustment of the zero. Calculate the amount of $(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6$ in the tablets, from the obtained readings.

B. Proceed in accordance to the described in Chromatography on high efficiency liquid (5.2.17.4). Use chromatograph provided with ultraviolet detector on 254 nm; column of 250 mm of length and 4,6 mm of internal diameter, packed with silica chemically bonded to the octadecilsilane group (3 μ m to 10 μ m), maintained on room temperature; Mobile phase flux of 1,0 mL/minute.

Mobile phase: dissolve 961 mg of 1-pentanossulfonato of monohydrated sodium and 82 mg of anhydrous sodium acetate in a mix of 550 mL of methanol and 470 mL of water, add 0,57 mL of glacial acetic acid and homogenize:

Diluent: prepare a mix of methanol and chloridric acid 0,1 M (1:1).

Sample solution: weigh and spray 20 tablets. Transfer quantity of powder equivalent to 50 mg of metoprolol tartrate to the volumetric flask of 50 mL, add 30 mL of Diluent and leave in ultrasound for 30 minutes. Complete the volume with the Diluent, homogenize and filter. Dilute until concentration of 0,5 mg/mL, using Mobile phase as solvent.

Standard solution: dissolve exact weighed amount of metoprolol tartrate SQR in Diluent, in such way to obtain solution on 1 mg/mL. Dilute until concentration of 0,5 mg/mL, using Mobile phase as solvent.

Procedure: inject, separately, 20 μ L of the standard and sample solutions, register the chromatograms and measure the areas under the peaks. Calculate the quantity of $(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6$ in the tablets from the answers obtained for the standard and sample Solutions.

PACKAGING AND STORING

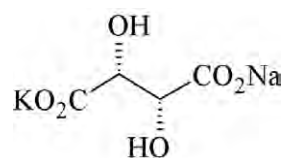
In well closed recipients, protected from light.

LABELLING

Observe the current legislation.

POTASSIUM AND SODIUM TARTRATE

Kalii natrii tartras



$C_4H_4KNaO_6$; 210,16

4 4 6

$C_4H_4KNaO_6 \cdot 4H_2O$; 282,22

potassium and sodium tartrate; 09846

Sodium salt and acid potassium

dihydroxybutanedioic (1:1:1)

[304-59-6]

Sodium and Potassium salt from the acid (2R,3R)-2,3-

(2R,3R)-2,3-

hydrated dihydroxybutanedioic (1:1:1:4)

[6381-59-5]

Contains at least, 99,0% and at the maximum, 102,0% of $C_4H_4KNaO_6$ calculated in the anhydrous base.

DESCRIPTION

Physical characteristics. Crystalline powder, white or colorless, transparent crystals.

Solubility. Highly soluble in water, practically insoluble in ethanol.

IDENTIFICATION

A. In 10 mL of a solution on 5% (p/v), add 10 mL of acetic acid 6 M. A crystalline white precipitate is formed within 15 minutes.

B. Answers to the reactions of tartrate ion (5.3.1.1).

C. Answers to the reactions of potassium ion (5.3.1.1).

D. Answers to the reactions of sodium ion (5.3.1.1).

PURITY TESTS

Acidity or alkalinity. Dissolve 5 g of the sample into 100 mL of water. On 5 mL of this solution, add 0,1 mL of Phenolphthalein SI. At the most, 0,5 mL of Hydrochloric acid 0,01 M or sodium hydroxide 0,01 M to change the color of the indicator.

Ammonia (5.3.2.6) In 5 mL of the solution obtained in the test *Acidity or alkalinity* to make *Limit test for Ammonia*. At most 0.004% (40 ppm).

Barium and oxalates. On 5 mL of the solution obtained in the test *Acidity or alkalinity* Add 3 mL of calcium sulfate SR. Leave it in rest for 5 minutes. Any opalescence on the preparation is not more intense than the one obtained with the mix of 3 mL calcium sulfate SR and 5 mL of distilled water.

Calcium (5.3.2.7). Determinate in 0,5 g of the sample. At most 0.02% (200 ppm).

Chlorides (5.3.2.1). At most 0.01% (100 ppm).

Sulfates (5.3.2.2). Determinate in 4,8 g of the sample. Use 0,5 mL of standard sulfuric acid. At most 0.005% (50 ppm).

Heavy metals (5.3.2.3). Use *Method III*. At most 0.001% (10 ppm).

Water (5.2.20.1). Between 21,0% and 27,0%

ASSAY

To weight exactly, around 2 g of the sample in a weighed porcelain crucible and slowly make ignition until the salt is charred, protecting the salt from the flame the whole time. Cool the crucible, put it in a glass beaker and break the charred dough with a glass baton. Without removing the glass baton or the crucible, add 50 mL of water and sulfuric acid 0,25 *M* SV, cover the beaker and boil the solution for 30 minutes. Filter and wash with hot water until the last washing is neutral to litmus paper. Cool the filtered and the washings. Title the excess of acid with sodium hydroxide 0,5 *M* SV using as an indicator, a mix of 10 mL of methyl red SI and 10 mL of Mehtylene blue SR1. Make blank test. Each mL of sulfuric acid 0,25 *M* SV is equivalent to 52,54 mg of C₁₆H₉N₄Na₃O₉S₂.

PACKING AND STORAGE

In closed recipients

LABELING

Observe the current legislation

CATHEGORY

Cathartic.

TARTRAZINE



C₁₆H₉N₄Na₃O₉S₂; 534,36 *CI19140*

Sodium salt of the acid 4,5-dihydro-5-oxo-1-(4-sulphophenyl)-4-[2-(4-sulphophenyl)diazemyl]-1H-pyrazole-3-carboxylic (3:1)

[1934-21-0]

Contains, at least, 85% of C₁₆H₉N₄Na₃O₉S₂.

DESCRIPTION

Physical characteristics. Thin powder, orange, bright and hygroscopic. Yellow aqueous solution.

Solubility. Soluble in water, methanol and glycerol. Somewhat soluble in ethanol. Insoluble in ethyl ether, acetone, mineral oil and fats.

IDENTIFICATION

The absorption spectrum is visible on the ultraviolet (5.2.14), in the range of 200 nm to 700 nm, of solution to 0,001% (p/v) in ammonium acetate 0,02 *M* (pH 5,6), exhibits maximum in 426 nm, 257 nm and 203 in one and leasts of 311 nm and 221 nm, identical to the ones observed in the spectrum of solution similar to tartrazine SQR.

PURITY TESTS

Subsidiary colouring. Proceed in accordance with the described in *Chromatography in slim layer* (5.2.17.1), using silica gel G, as support, and mix of 1-butanol, ethanol, water, ammonium hydroxide (50:25:25:10), as mobile phase. Apply, separately the plate, 2μL of each of the solutions recently prepared as described next:

Solution (1): 0,25 g of sample in 10 mL of sodium hydroxide 0,5 *M*.

Solution (2): 0,05 g of standard tartrazine in 10 mL of sodium hydroxide 0,5 *M*.

Solution (3): dilute *Solution (2)* in such way to obtain a solution on 0,05 mg/mL, with the same diluent.

Solution (4): dilute *Solution (1)* in such way to obtain a solution on 0,25 mg/mL, with the same diluent.

Develop the chromatogram. Remove the plate, let it dry in the air. Examine under ambience light the ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in position, color and intensity to that obtained with *Solution (2)*. The secondary stains obtained with *Solution (1)* must not be more intense than these obtained with *Solution (3)* and *Solution (4)*.(1%).

Alternatively, a mix of 1-butanol, water, glacial acetic acid (20:12:5) can be used as mobile phase. Instead of silica gel, chromatographic paper can be used, using the conditions previously described and observing the stains, also by transparency.

Lead, copper, tin and zinc. Proceed as described in *Spectrophotometry of atomic absorption* (5.2.13). Weight 2 g of the sample, use a silic crucible and burn, blandly, over the asbestos screen (± 350 °C); take it to the muffle for 12 hours, without exceeding the temperature of 450 °C. Remove the crucible and cool. Mix the residue with around 2 mL of water and add

two drops of magnesium nitrate to 50% (p/v). Dry over electrical plate and return to the muffle during 3 to 4 hours, or until the residue is white, or yellow. Next, cool, drip 1 to 2 mL of nitric acid and 1 mL of water and heat over electrical plate until is almost dry. Dissolve the metallic nitrates with 5 mL of water. If necessary, centrifuge. Take to the spectrophotometer of atomic absorption, previously calibrated and execute the reading of the concentration of each of the metals. At most 0.001% (10 ppm) of lead, 0.002% (20 ppm) of copper, 0.025% (250 ppm) of tin and 0.005% (50 ppm) of zinc.

Chlorides and sulfates. Weigh 0,5 g of the sample, dissolve in 200 mL of water, acidify with 8 mL of nitric acid on 25% (v/v) and titrate with silver nitrate 0,1 M SV in potentiometer with combined silver electrode. Each mL of silver nitrate 0,1 M SV is equivalent to 5,85 mg of NaCl.

Weigh 0,5 g of the sample and dissolve with 100 mL of water in water-bath. Add 35 g of sodium chloride, exempt of sulfates and shake well. Transfer for the volumetric flask of 200 mL and complete the volume with saturated solution of sodium chloride. Homogenize. After 1 hour, filter with filter paper and transfer aliquot of 100 mL to beaker of 600 mL, dilute up to 300 mL with water and acidify with hydrochloric acid SR, adding light excess. Heat to boiling and drip, with shaking, 25 mL of barium chloride on 12% (p/v), or until there is no more precipitation. Leave in rest during four hours. Separate the barium sulfate for filtration, wash with hot water, dry the paper with residue, transfer to a dry crucible, previously weigh and calcine in muffle on 500°C during 1 hour. Cool in desiccator and weigh. Calculate the tenor of sulfates by the expression:

$$\frac{N \times 0,6085 \times 100}{p} = \% \text{ sulfates}$$

in which

N = grams of barium sulfate; p = grams of the sample used in the precipitation.

At most, 6% of chlorides and sulfates.

Substances insoluble in water. Dissolve 5 g of the sample in 200 mL of hot water (80-90 °C) with shaking. Cool to room temperature. Filter by filtering plate, previously dried and weigh. Wash with cold water until the washing waters become colorless. Dry the filter with the residue in stove in 120°C during four hours and weigh. At most, 0,5%.

Arsenic (5.3.2.5). Use *Method I*. Determine in 1 g of the sample. At most 0.0001% (1 ppm).

Heavy metals (5.3.2.3). Use *Method III*. Determine in 0,5 g of the sample. At most 0.004% (40 ppm).

ASSAY

Effect the dilutions as described in *Identification*, and read the absorbance on maximum peak in around 426 nm (5.2.14). Calculate the tenor of the coloring by the expression:

$$\frac{A \times 100}{536,6 \times p} \quad \% \text{ of tartrazine in the sample in } 519 \text{ nm}$$

in which

p = weight of the sample in grams in the executed dilution.

Alternatively one can consider $A(1\% \text{ } 1 \text{ cm}) = 536.6$ in 426 nm.

PACKING AND STORAGE

In well closed recipient, protected from light.

LABELING

Observe the current legislation.

CATEGORY

Coloring.

PURIFIED HYDROPHILIC GAUZE TISSUE

100% cotton tissue, simple, low density of threads by centimeter, screen like, targeted (exempt of starch, dextrin, corrective coloring, optical blue, alkalis and acids), odorless and tasteless.

The purified hydrophilic gauze is a white tissue of many threads counting and weights, in many lengths and widths. In **Table 1** there is designation, for each commercial type, the number of threads and the respective grammage.

t

Table 1 - Commercial types of gauze with respective numbers of threads and grammage.

<i>Type of gauze</i>	<i>Minimum number of threads of warp by 10 cm</i>	<i>Minimum number of threads of chain by 10 cm</i>	<i>Minimum number of threads by 100 cm² of area</i>	<i>Grammage (g/m²)</i>	<i>Variation in percentage (%)</i>
I	158	138	296	73,0	± 6
II	138	138	276	66,5	± 6
III	118	79	197	38,5	± 6
IV	89	69	158	31,0	± 6
V	79	59	138	26,8	± 6
VI	74	54	128	25,2	± 6
VII	74	34	108	21,3	± 6
VIII	69	29	98	19,3	± 6
IX	59	29	88	16,6	± 6

CHARACTERISTICS

Condition the sample for, at least, 4 hours in standard atmosphere of relative $65\% \pm 2\%$, to $20 \pm 2^\circ\text{C}$, before executing the tests of *Thread counting*, *Grammage* and *Absorbing power*. Remove the sample from its packages before submitting it to the conditioning atmosphere. If the sample is in form of rolls, cut the necessary amount for the execution of tests, excluding the first and last two meters, when the total amount of available sample allows.

Thread counting. Collect amount with at least 50 cm of length and width equal to the tissue. Put the sample, with no wrinkles and no tension, over a plain surface. Start counting in the space between two threads. Do not execute the counting in the are of the selvages. Put the scale over the sample and count the number of threads comprehended in 5 cm. Count on the sense of warp, throughout the width of the sample. The counting must be executed in five different parts of the sample. Count on the sense of chain, throughout the length of the sample. The counting must be executed in five different parts of the sample. Divide the number of threads of each measure by 5 cm, in order to determine the number of threads by centimeter.

Calculate the arithmetic average of the five counts executed in each sense. The average, multiplied by 10 must be within the variation interval of **Table 1**.

Length. Unfold or unroll the sample, expand without stretching and measure the length throughout the core thread, using a graduated ruler. It must present at least 98% of the declared length.

Width. Remove sample with at least 50 cm of length, in total width of the tissue and 1 meter from the roll tips. Measure the width with help of the graduated ruler, in at least three points with equal intervals and not superior to 10 cm, distributed throughout the sample. The average of the three measures must not present difference superior to 1.6 mm of the width written in the label.

Grammage. Cut three evidence bodies from the sample with area equal to 100 cm². Weigh each evidence body in scale with precision of 0.001 g. Calculate the average of

mass obtained and multiply by 100 to express the result in grams for square meter. The grammage complies with the specification indicated in table in **Table 1**.

Absorbing power. Fill with water to temperature of approximately 20°C in a recipient of 11 to 12 cm of diameter. Fold, with a pinch, a square from the sample with around 1 g and analyze the surface. Carefully deposit the sample square over the water surface. Determine with a stopwatch the time necessary for total submersion of the sample. The time of immersion, expressed by the average of times registered in the course of three tests, must not exceed 10 seconds.

PURITY TESTS

Substances soluble in water. Transfer exactly, around 20 g of the sample to a beaker of 1000 mL containing 500 mL of purified water. Heat to boiling, during 15 minutes, adding boiling water to preserve the initial volume. Filter the hot water through a funnel, squeezing the sample retained with a pistil, in such way to remove all the water. Wash with two portions of 200 mL of boiling water, pressing the gauze after each washing. Collect the filtered with volumetric flask of 1000 mL and complete the volume with water. Transfer 400 mL of the extract to the porcelain capsule previously weigh and evaporate until residue in water-bath.

Residue after desiccation: Dry the residue obtained in *Substances soluble in water* in stove to 105°C until constant weight. Calculate the percentage of residue in relation to the mass of initial sample. Must be at least 0.25% of the initial weight.

Residue after incineration: Dry the residue obtained in *Residue after desiccation* in muffle to 600°C until constant weight. Calculate the percentage of residue in relation to the mass of initial sample. Must be at least 0.075% of the initial weight.

Acidity or alkalinity. Cut the sample of 10 g of tissue with tolerance of $\pm 0,1$ g. Boil, moderately 250 mL of purified water in a beaker. Immerse the sample, cover the beaker with Petri dish or clock glass and boil for more 5 minutes. Maintaining the beaker and the content covered, cool until

room temperature. Remove the sample with a pinch or tong and squeeze all the excess

of liquid from the beaker. Determine the pH of the aqueous extract potentiometrically (5.2.19). The value of pH must be between 5,0 and 8,0.

Dextrin or starch. Drip over the sample two or three drops of iodine SR. The coloration of the solution in the tissue, after 30 seconds, remains yellow. Alteration of green tones indicates dextrin residues, blue coloration or violet indicates the presence of starch.

Determination of sulfated ashes (2.2.10). Weigh exactly, around 5 g of the sample and transfer to the crucible previously weigh. Wet with 0,5 mL of sulfuric acid *M* and calcine, carefully, under direct flame, until darkening of the sample. Cool, add to the residue three to five drops of sulfuric acid *M*, and slowly heat until there is no more liberation of white smoke. Incinerate to 800°C until constant weight. The residue must be at least 0.2% of the initial weight.

Fat substances. Weigh exactly, around 10 g of the sample and adapt it to the Soxhlet extractor. Weigh a flask with flat bottom of 250 mL containing pearls of glass or pieces of porcelain and add 180 mL of ethyl ether to it. Adapt the flask to the Soxhlet extractor and to the heating blanket with temperature adjustment and heat the compound for 5 hours, maintaining, at least four refluxes fby the hour (the ethereal extract must not present signs of blue, green or brown coloration). Remove the heating blanket after the extraction period and let cool the compound, in such way so that some milliliters of ethyl ether stay in the flask. Disconnect the extractor from the flask and evaporate the ether using a light flux of nitrogen by the interior of the flask, carefully, always in the interior of the exhaustion chapel. Dry the flask in a stove to 105°C until constant weight. Must be at least 0,7%.

Corrective colorings. Transfer 10 g of the sample to the percolator. Proceed slowly to the extraction of ethanol until the obtaining of 50 mL of alcoholic extract. The percolate, observed over white background, in column of 20 cm high, can present light yellow coloration, but no green or blue coloration.

BIOLOGICAL SECURITY TESTS

Sterility (5.5.3.2.1). Gauze declared sterile complies with the test.

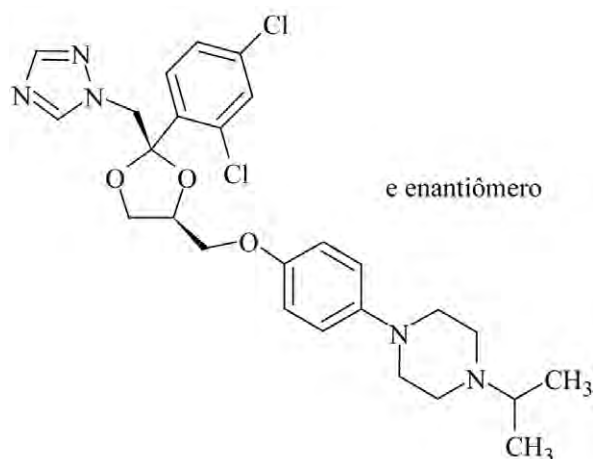
PACKING AND CONDITIONING

In well closed packages. Gauze declares sterile is packaged in such way to maintain the sterility until it is opened for used.

LABELING.

Observe the current legislation.

TERCONAZOL Terconazolium



$C_{26}H_{31}Cl_2N_5O_3$; 532,46 terconazol; 08417
rel-1-[4-[[[(2R,4S)-2-(2,4-Dicloro-phenyl)-2-(1H-1,2,4-triazol-1-ilmetil)-1,3-dioxolan-4-il]metoxi]phenyl]-4-(1-metiletil)-piperazine
[67915-31-5]

Contains, at least, 99,0% and, at most, 101,10% of $C_{26}H_{31}Cl_2N_5O_3$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or almost white powder. Presents polymorphism.

Solubility. Practically insoluble in water, easily soluble in methylene chloride, soluble in acetone, partially soluble in ethanol.

Physicochemical constants.

Specific rotatory power (5.2.8): $-0,10^\circ$ to $+0,10^\circ$, in relation to the desiccated substance. Determine the solution to 10% (p/v) in methylene chloride.

IDENTIFICATION

A. The absorption spectrum in infrared (5.2.14) of the sample, releases potassium bromide, present absorption maximums only in equal wave lengths and with the same relative intensities of these observed in the spectrum of terconazol SQR, prepared in identical way. If the spectrum obtained present differences, dissolve the sample and the standard, separately, in a minimum volume of acetone. Let evaporate until dryness and execute new spectrum with the residues.

B. Proceed in accordance with the described in *Chromatography in slim layer* (5.2.17.1), using silica gel G, as support, and mix ammonium acetate SR dioxane and methanol (20:40:40), as mobile phase. Apply; separately the plate, 5 mL of each of the solutions recently prepared as described next:

Solution (1): dissolve 30 mg of the sample in methanol and dilute to 5 mL with the same solvent.

Solution (2): dissolve 30 mg of terconazole SQR in methanol and dilute to 5 mL with the same solvent.

Solution (3): dissolve 30 mg of terconazole SQR and 30 mg of ketokonazole and dilute to 5 mL with the same solvent.

Develop the chromatogram. Remove the plate, let dry to the air and heat for 15 minutes. Expose to the vapor of iodine until the stains appear. The main stain obtained with *Solution (1)* corresponds in position, color and intensity to that obtained with *Solution (2)*. The test will only be valid if the chromatogram obtained with *Solution (3)* present two stains visibly separated.

C. On 30 mg of the sample, in porcelain crucible, add 0,3 g of anhydrous sodium carbonate. Heat to red for 10 minutes. Let cool. Extract the residue with 5 mL of nitric acid SR and filter. For 1 mL of the filtered add 1 mL of water. Answers to the reactions of chloride ion (5.3.1.1).

PURITY TESTS

Related substances. Proceed in accordance to the described in the method **B.** of *Assay*. Prepare *Solution (1)*, *Solution (2)* and *Solution (3)* as described in the following.

Solution (1): dissolve, exactly, around 0.1 g of the sample in methanol and dilute to 10 mL with the same solvent.

Solution (2): transfer 1 mL of *Solution (1)* for volumetric flask of 100 mL and complete the volume with methanol. Transfer 2.5 mL of *Solution (1)* for volumetric flask of 10 mL and complete the volume with methanol.

Solution (3): dissolve 2.5 mg of terconazole SQR and 2 mg of ketokonazole and dilute to 100 mL with the same solvent.

Inject 20 mL of *Solution (3)*. The retention time is of around 6 minutes for ketoconazole and 7.5 minutes for terconazole. The resolution between the peaks of ketoconazole and terconazole must not be smaller than 10. Make the necessary adjustments.

Procedure: inject, separately, 20 mL of methanol as white, 20 | L of *Solution (1)* and 20 | L of *Solution (2)*. The area of any peak, obtained in chromatogram with *Solution (1)* main peak, is not bigger than that the are under the main peak, obtained in chromatogram with *Solution (2)* (0,25%). The sum of the area of all peaks, except for main peak, obtained in chromatogram with *Solution (1)*, is not bigger than two times the area under the main peak, obtained in chromatogram with *Solution (2)* (0,5%). Ignore any peak obtained with white or with are smaller than 0,2 the are under the main peak, obtained in chromatogram with *Solution (2)*.

Loss by desiccation (5.2.9). Determinate in 1 g of the sample. Desiccate in stove between 100°C and 105°C until constant weight. At most, 0,5%.

Sulfated ashes (2.5.10) Determinate in 1 g of the sample. At most, 0,1%.

ASSAY

A. Proceed in accordance to the described in *Titration in non-aqueous environment (5.3.3.5)*. Dissolve, exactly, around 0,15 g of the sample in 70 mL of mix of glacial acetic acid and methyl-ethyl- (9:1). Titrate with perchloric acid 0,1 M SV, determining the final point potentiometrically, in the second point of inflection. Each mL of perchloric acid 0,1 MSV is equivalent to 17,749 mg of $C_{26}H_{31}Cl_2N_5O_3$.

B. Proceed in accordance to the described in *Chroato-graphy in liquid of high efficiency (5.2.17.4)*. Use chromatograph provided with ultraviolet detector in 220 nm; column of 125 mm of length and 4,6 mm of internal diameter, packaged with silica chemically linked to the deactivated octadecilsane group (5 | m), maintained in room temperature; flux of *Mobile phase* of 2 mL/minute.

Eluent A: solution of hydrogen-sulfate of tetra-butyl-ammonium to 3,4 mg/mL.

Eluent B: acetonitrile.

Gradient of Mobile Phase: adopt the system of gradient described in the following table:

Time (minutes)	Eluent A (%)	Eluent B (%)	Elution
0 – 10	95 → 50	5 → 50	Linear gradient
10 – 15	50	50	Isocratic
15 – 20	95	5	Stabilization

Sample solution: dissolve, exactly, sample amount of methanol in such way to obtain solution in around 0,5 mg/mL. Transfer 5 mL of this solution for volumetric flask of 50 mL and complete the volume with methanol, obtaining solution on 50 g/mL.

Sample solution: dissolve, exactly, amount of terconazole SQR in methanol in such way to obtain solution in around 0,5 mg/mL. Transfer 5 mL of this solution for volumetric flask of 50 mL and complete the volume with methanol, obtaining solution on 50 g/mL.

Resolution solution: dissolve 2.5 mg of terconazole SQR and 2 mg of ketokonazole and dilute to 100 mL with the same solvent.

Inject replicates of 20 pL of *Resolution solution*. The retention time is of around 6 minutes for ketoconazole and 7.5 minutes for terconazole. The relative standard deviation of the replicated areas of peaks registered is not bigger than 2,0%. The resolution between the peaks of ketoconazole

and Terconazole must not be smaller than 10. Make the necessary adjustments.

Procedure: inject, separately, 20 mL of *Standard solutions* and *sample*, register the chromatograms and measure the areas under the peaks. Calculate the tenor of $C_{26}H_{31}Cl_2N_5O_3$ in the sample from the answers obtained with *Standard solutions* and *sample*.

PACKING AND STORAGE

In well closed recipient, protected from light.

LABELING

Observe the current legislation.

THRAPEUTIC CLASS ANTIFUNGAL.

CREAM TERCONAZOLE

Contains, at least, 90,0% and, at most, 120,0% of the declared quantity of $C_{26}H_{31}Cl_2N_5O_3$.

IDENTIFICATION

A. The absorption spectrum in ultraviolet (5.2.14), in the range of 200 nm to 400 nm, of *Sample solution* obtained in method **A.** of *Assay*, exhibits maximum of absorption in 226,6 nm, identical to the observed in the spectrum of *Standard solution*.

B. The retention time of the main peak of the chromatogram of *Sample Solution*, obtained in method **B.** of *Array*, corresponding to that of the main peak of *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

BIOLOGICAL SECURITY TESTS

Counting of the total number of micro-organisms mesophile aerobical (5.5.3.1.2). Complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). Complies with the test.

ASSAY

A. *Proceed in accordance to the described in Spectrophotometry in absorption in ultraviolet(5.2.14).* Transfer, exactly, quantity of cream equivalent to around 1,4 mg of terconazole to volumetric flask of 100 mL and add 60 mL of chloridric acid 0,1 M. Shake for 30 minutes to disperse the cream and complete the volume with the same solvent. Filter. Dilute, successively, with the same

solvent, concentration up to 0.0014% (p/v). Prepare standard solution in the same concentration, using the same

solvent. Measure the absorbency of the resulting solutions in 226,6 nm, using chloridric acid 0,1 M for adjustment of the zero. Calculate the amount of $C_{26}H_{31}Cl_2N_5O_3$ in the cream, from the obtained readings.

B. *By Chromatography in liquid of high efficiency (5.2.17.4).* Proceed in accordance with the described in the method **B.** of *Array* of the monograph of *Terconazole*. Prepare the *Sample solution* as described in the following.

Sample solution: Transfer, exactly, quantity of cream equivalent to around 1,4 mg of terconazole to volumetric flask of 100 mL and add 60 mL of chloridric acid 0,1 M. Shake for 30 minutes to disperse the cream and complete the volume with methanol and homogenize. Filter, ignoring the first 5 mL. Transfer 25 mL of this solution for volumetric flask of 50 mL and complete the volume with methanol, obtaining solution on 50 g/mL. Transfer 15 mL of this solution for volumetric flask of 50 mL and complete the volume with methanol, obtaining solution on 50 g/mL.

Procedure: inject, separately, 20 mL of *Standard solutions* and *sample*, register the chromatograms and measure the areas under the peaks. Calculate the tenor of $C_{26}H_{31}Cl_2N_5O_3$ in the sample from the answers obtained with *Standard solutions* and *sample*.

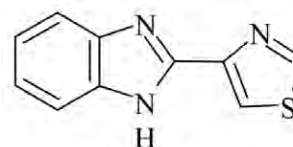
PACKING AND STORAGE

In well closed recipient, protected from light.

LABELING

Observe the current legislation.

TIABENDAZOLE Tiabendazolium



$C_{10}H_7N_3S$; 201,25
tiabendazole; 08493
2-(4-Tiazolil)-1H-benzimidazol
[148-79-8]

Contains, at least, 98,0% and, 101,0% of at most, CHNS, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White or almost white crystalline powder.

Solubility. Practically insoluble in water, little soluble in chloroform, ethanol and wthyl ether. Soluble in diluted mineral acids.

Physicochemical constants. *Fusion range (5.2.2):* 296 °C to 303 °C.

IDENTIFICATION

A. The absorption spectrum in infrared (5.2.14) of the sample, desiccated to 105°C until constant weight, releases potassium bromide, present absorption maximums only in equal wave lengths and with the same relative intensities of these observed in the spectrum of tiabendazole SQR, prepared in identical way.

B. Weigh 25 mg of the sample, dissolve in chloridric acid 0,1 M and dilute, successively, in the same solvent until the concentration of 0,0005% (p/v). The absorption spectrum in ultraviolet (5.2.14) of the obtained solution, in the range of 200 nm to 400 nm, exhibits absorption maximums in 302 nm, identical to the observed in the spectrum of similar solution of tiabendazole SQR.

C. The main stain of chromatogram of *Solution (2)* obtained in *Related substances* corresponds in position, color and intensity, that obtained with *Solution (3)*.

D. Dissolve 10 mg of the sample in 5 mL of chloridric acid M, add 5 mg of phenilenodyamine hydrochloride *p*-and shake until dissolution. Add around 0.1 g of ground zinc, mix and leave in rest for 2minutes. Add 5 mL of ammoniac ferric sulphate SR just prepared. Blue or blue-violet coloration develops.

PURITY TESTS

Related substances. Proceed in accordance with the described in *Chromatography in slim layer (5.2.17.1)*, using silica gel HF₂₅₄, as support, and mix of water, acetone, glacial acetic acid and toluene (2,5:10:25:62,5), as mobile phase. Apply, separately the plate, 20µL of each of the solutions recently prepared as described next:

Solution (1): transfer 0,1 g of the sample to the volumetric flask of 10 mL. Dissolve in methanol and complete the volume with the same solvent.

Solution (2): transfer 1 mL of *Solution (1)* for volumetric flask of 10 mL and complete the volume with methanol.

Solution (3): transfer 25 mg of tiabendazole SQR to the volumetric flask of 25 mL. Dissolve in methanol and complete the volume with the same solvent.

Solution (4): transfer 1 mL of *Solution (2)* for volumetric flask of 10 mL and complete the volume with methanol.

Solution (5): transfer 1 mL of *Solution (2)* for volumetric flask of 25 mL and complete the volume with methanol.

Develop the chromatogram. Remove the plate, let it dry in the air. Examine under ultraviolet light (254 nm).

Any secondary stain obtained in chromatogram with *Solution (1)*, different fro the main stain, is not more intense than that obtained with *Solution (4)* (1%), and only one stain is more intense than that obtained in the chromatogram with *Solution (5)* (0,4%).

Heavy metals (5.3.2.3). Use *Method III*. In At most 0.001% (10 ppm).

Water (5.2.20.1). At most, 0,5%.

Sulfated ashes (2.5.10). At most, 0,1%.

ASSAY

Proceed in accordance to the described in *Titration in non-aqueous environment (5.3.3.5)*. Dissolve, exactly, around 0,15 g of the sample in 30 mL of mix of glacial acetic acid. Titrate with perchloric acid 0,1 M SV, determining the final point potentiometrically or using methyl-rosanilinium chloride SI until change of color from blue to blue-green. Make test in blank and make the necessary corrections. Each mL of perchloric acid 0,1 MSV is equivalent to 20,130 mg of C₁₀H₇N₃S.

PACKING AND STORAGE In well closed recipient, protected from light.

LABELING

Observe the current legislation.

THRAPEUTIC CLASS

Anthelmintic.

TIABENDAZOLE - TABLETS

Contains, at least, 90,0% and, at most, 110,0% of the declared quantity of C₁₀H₇N₃S.

IDENTIFICATION

A. A. The absorption spectrum in ultraviolet (5.2.14), in the range of 200 nm to 400 nm, of Sample solution obtained in method **B.** of *Assay*, exhibits maximum of absorption in 302 nm, identical to the observed in the spectrum of Standard solution. The difference between the absorbencies must not be bigger than 3,0%.

B. The retention time of the main peak of the chromatogram of *Sample Solution*, obtained in method **B.** of *Array*, corresponding to that of the main peak of *Standard solution*.

C. Weigh and spray the tablets. Use amount of powder equal to 20 mg of tiabendazole. Add 5 mL of chloridric

acid *M*, 5 mg of dimethyl-*p*-phenylenodiamine and shake. Add 0.1 g of grounded zinc, mix, wait for 2 minutes and add 10 mL of ammoniac ferric sulphate SR just prepared. Intense blue or violet color is produced.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unitary doses (5.1.6). Complies with the test. Proceed in accordance to the described in the method **B.** of *Assay*.

ASSAY

Use one of the methods described in the following.

A. Proceed in accordance to the described in *Titration in non-aqueous environment* (5.3.3.5). Weigh and spray the 20 tablets. Use amount of powder equivalent to 0,15 g de tiabendazole and proceed in accordance to described in *Assay* of monograph of *Tiabendazole*.

B. Proceed as described in *Spectrophotometry of atomic absorption in ultraviolet* (5.2.14). Weigh and spray the 20 tablets. Transfer amount of powder equivalent to 0,1 g of tiabendazole to volumetric flask of 100 mL. Add 75 mL of chloridric acid 0,1 *M*, heat in water-bath, for 15 minutes, shaking occasionally, cool, complete the volume to 100 mL with chloridric acid 0,1 *M* and filter. Transfer 10 mL of the filter to a volumetric flask of 100 mL and complete the volume with chloridric acid 0,1 *M*. Of this solution, pipette 5 mL, transfer to the volumetric flask of 100 mL and complete the volume with the same solvent, obtaining the solution to 0.0005% (p/v). Prepare standard solution in the same concentration, using the same solvent. Measure the absorbency of the resulting solutions in 302 nm, using chloridric acid 0,1 *M* for adjustment of the zero. Calculate the amount of $C_{10}H_7N_3S$ in the tablets, from the obtained readings.

C. Proceed in accordance to the described in *Chroato-graphy in liquid of high efficiency* (5.2.17.4). Use chromatograph provided with ultraviolet detector in 254 nm; column of 300 mm of length and 4,0 mm of internal diameter, packaged with silica chemically linked to the deactivated octadecilisane group (5 | m), maintained in room temperature; flux of *Mobile phase* of 2 mL/minute.

Phosphate buffer pH 3,5: dissolve 13,8 g of mono-basic sodium phosphate in 2000 mL of water. Adjust the pH of the solution with phosphoric acid to $3,5 \pm 0,05$.

Mobile phase: mix of phosphate buffer pH 3,5 and methanol (54:46).

Sample solution: Weigh and spray the 20 tablets. Transfer quantity of powder equivalent to 0,2 g of tiabendazole, to a volumetric flask of 1000 mL, add 100 mL of chloridric acid 0.1 *M*, homogenize and heat in water-bath, for 30 minutes. Wait until cool to room temperature and complete the volume with water. Homogenize and filter, ignoring the first 20 mL of the filtered.

Sample solution: dissolve quantity of tiabendazole SQR, exactly weigh, in chloridric acid 0,1 *M* and make quantitative dilutions, if necessary, until obtaining solution in 2 mg/mL. Transfer 5 mL of this solution for volumetric flask of 50 mL and complete the volume with methanol, obtaining solution on 0,2 mg/mL. Homogenize.

The efficiency of the column must not be smaller than 960 theoretical plates. The tail factor to the peak of tiabendazole is not bigger than 2,0%. The relative standard deviation of the replicated areas of peaks registered is not bigger than 2,0%.

Procedure: inject, separately, 20 mL of *Sample solutions* and *standard*, register the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{10}H_7N_3S$ in the tablets, from the answers obtained for the standard solutions and sample,

PACKING AND STORAGE

In well closed recipient, protected from light.

LABELING

Observe the current legislation.

TIABENDAZOL OINTMENT

Contains, at least, 90,0% and, at most, 110,0% of the declared quantity of $C_{10}H_7N_3S$.

IDENTIFICATION

A. The absorption spectrum in ultraviolet (5.2.14) in the range of 200 nm to 400 nm, of the sample obtained in *Assay*, exhibits the absorption maximum in 302 nm, identical to the observed in standard solution spectrum.

B. To ignore the amount of ointment equivalent to 10 mg of tiabendazole in 5 mL of chloridric acid *M*, add 5 mg of dimethyl hydrochloride-*p*-phenylenodiamine and homogenize. Add around 0.1 g of ground zinc, shake and leave in rest for 2 minutes. Add 5 mL of ammoniac ferric sulphate SR. Intense blue or blue-violet coloration develops.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

BIOLOGICAL SECURITY TESTS

Count of total feasible micro-organisms (5.5.3.1.2). Complies with the test.

Survey and identification of pathogens (5.5.3.1.3). Complies with the test.

DOSAGE

To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To weigh an amount of ointment equivalent to 50 mg of thiabendazole and quantitatively transfer to a 250 mL separatory funnel by using 50 mL of ethyl ether. To stir to dissolve the ointment and extract with 4 portions (40 mL each) of 0.1 M hydrochloric acid. To collect the aqueous extract in 250 mL volumetric flask and gently heat to eliminate the residues of ethyl ether. To cool down and top up with 0.1 M hydrochloric acid. To transfer 5 mL of this solution to a 200 mL volumetric flask and top up with 0.1 M hydrochloric acid, to obtain 0.0005% concentration (w/v). To prepare a standard solution at the same concentration by using the same solvent. To measure the absorbances of the solutions at 302 nm, by using 0.1 M hydrochloric acid to adjust zero. To calculate the amount of $C_{10}H_7N_3S$ from the readings obtained.

PACKAGING AND STORAGE

In tightly closed container, protected from excessive heat.

LABELLING

To comply with the legislation in force.

THIABENDAZOLE, ORAL SUSPENSION

It contains at least 90.0 % and at most 110.0 % of the amount stated of $C_{10}H_7N_3S$.

IDENTIFICATION

A. The ultraviolet absorption spectrum (5.2.14), ranging from 200 nm to 400 nm, of sample solution obtained in *Dosage* method **A.** exhibits maximum at 302 nm, similar to that observed in the thiabendazole SQR spectrum.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method **B.** corresponds to that of main peak of *Standard solution*.

C. To transfer a volume of oral suspension equivalent to 50 mg of thiabendazole to test tube, add 10 mL of M hydrochloric acid, and vigorously stir it. To transfer 5 mL to test tube, add 5 mg of dimethyl *p*-phenylenediamine, and stir it. To add 0.1 g of zinc powder and stir. Let it rest for 2 minutes. To add 5 mL of ferric ammonium sulfate SR. An intense blue or blue-violet color is produced.

CHARACTERISTICS

Appearance. To completely empty the contents of vials as specified in table 1 *Determination of volume* (5.1.2), previously stirred in clean, dry, corresponding beakers with lid.

To immediately observe under suitable conditions of visibility. The content should flow fluidly, the suspension should be homogeneous, viscous, free of lumps, and foreign matter. After 24 hours, it may have slight sedimentation resuspended upon stirring.

Determination of volume (5.1.2). It complies with the test.

pH (5.2.19). 3.4 to 4.2. To determine in the reconstituted oral suspension as informed on the label.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To employ one of the methods described below.

A. To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To transfer the volume of oral suspension equivalent to 0.25 mg of thiabendazole to a 100 mL volumetric flask, add 75 mL of 0.1 M hydrochloric acid, and put in a water bath for 15 minutes, by occasionally stirring. To cool down at room temperature. To top up with 0.1 M hydrochloric acid and filter it. To successively dilute in 0.1 M hydrochloric acid to obtain 0.0005% concentration (w/v). To prepare a standard solution at the same concentration by using the same solvent. To measure the absorbances of the resulting solutions at 302 nm, by using 0.1 M hydrochloric acid to adjust zero. To calculate the amount of $C_{10}H_7N_3S$ in the oral suspension, from the readings obtained.

To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 254 nm; 300 mm column and 4 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μ m), kept at room temperature; flow of *Mobile phase* of 2.0 mL/minute.

Phosphate buffer pH 3.1: to dissolve 13.8 g of monobasic sodium phosphate monohydrate in 2000 mL of water. To adjust the pH to 3.10 ± 0.05 with phosphoric acid.

Mobile phase: mixture of *Phosphate buffer pH 3.1* and methanol (65:35).

Sample solution: to transfer volume of oral suspension equivalent to 500 mg of thiabendazole to a 250 mL vol-

umetric flask, top up to volume with 0.1 *M* hydrochloric acid, and make it homogeneous. To transfer 5 mL of this solution to 50 mL volumetric flask and top up with water, thus obtaining 0.2 mg/mL solution. To make it homogeneous.

Standard solution: to dissolve accurately weighed amount of thiabendazole SQR in 0.1 *M* hydrochloric acid to obtain a 2 mg/mL. To transfer 5 mL of this solution to a 50 mL volumetric flask and top up with water, thus obtaining 0.2 mg/mL solution. To make it homogeneous.

To inject replicates of 20 µL of the *Standard solution*. The column efficiency is greater than 960 theoretical plates. The tailing factor for thiabendazole peak should not be greater than 2.0. The relative standard deviation for replicate areas of the peaks recorded should not be greater than 2.0 %.

Procedure: to separately inject 20 µL of *Sample and standard solutions*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of C₁₀H₇N₃S in the oral solution, from the responses obtained from *Sample and standard solutions*.

PACKAGING AND STORAGE

In tightly closed container, protected from excessive heat.

LABELLING

To comply with the legislation in force.

STRONG IODINE TINCTURE

Iodine tincture consists of 6.5 g of iodine in the presence of sodium iodide and diluted ethanol. It contains at least 5.85 g and at most 7.15 g of iodine in 100 mL of solution. The sodium iodide may be replaced by potassium iodide, and the composition is at least 2.25 g of iodine in 100 mL of solution.

IDENTIFICATION

A. To add a drop of sample to 0.2% starch solution (w/v). Blue color is produced.

B. To evaporate to dryness, in a water bath, 3 mL of sample. The residue responds to the reaction 1 of sodium ion (5.3.1.1).

C. The residue obtained in test **B.** *Identification* responds to the reactions of iodide ion (5.3.1.1).

PURITY TESTS

Alcohol (5.3.3.8). To proceed as described in *Determination of alcohol*. From 82% to 88.5% (v/v).

DOSAGE

Iodine. To transfer 5 mL of the strong iodine tincture to an erlenmeyer flask containing 20 mL of water. To add 3 drops of starch SI and titrate with 0.1 *M* SV sodium thiosulfate. Each mL of 0.1 *M* SV sodium thiosulfate is equal to 12.69 mg of iodine (I).

Sodium iodide or potassium iodide. To transfer 5 mL of the strong iodine tincture to an erlenmeyer flask containing 30 mL of water and add 10 mL of hydrochloric acid. To titrate with 0.05 *M* SV potassium iodate to obtain light brown. To add 5 mL of chloroform and keep titrating, by vigorously stirring to the chloroform layer discolors. From the volume of 0.05 *M* SV potassium iodate spent, subtract half volume of 0.1 *M* SV sodium thiosulfate spent in the test for dosage of iodine. Each mL of 0.05 *M* SV potassium iodate remaining is equivalent to 16.6 mg of potassium iodide (KI) or 15.0 mg of sodium iodide (NaI).

PACKAGING AND STORAGE

In tightly closed containers, protected from heat.

LABELLING

To comply with the legislation in force.

WEAK IODINE TINCTURE

Weak iodine tincture consists of 2 g iodine in the presence of 2.4 g of sodium iodide in 100 mL of 50% ethanol (v/v). It contains at least 1.8 g and at most 2.2 g of iodine in 100 mL of solution. The sodium iodide may be replaced by potassium iodide, and the composition is at least 1.35 g in 100 mL of solution.

IDENTIFICATION

A. To add a drop of sample to 0.2% starch solution (w/v). Blue color is produced.

B. To evaporate to dryness, in a water bath, 3 mL of sample. The residue responds to the reaction 1 of sodium ion (5.3.1.1) and the reactions of iodine (5.3.1.1).

PURITY TESTS

Alcohol (5.3.3.8). To proceed as described in *Determination of alcohol*. From 44% to 50%.

DOSAGE

Iodine. To transfer 5 mL of the weak iodine tincture to an erlenmeyer flask containing 20 mL of water. To add 3 drops of starch SI and titrate with 0.1 *M* SV sodium thiosulfate. Each mL of 0.1 *M* SV sodium thiosulfate is equal to 12.69 mg of iodine (I).

t

Sodium iodide or potassium iodide. To transfer 5 mL of the weak iodine tincture to an erlenmeyer flask containing 30 mL of water and add 10 mL of hydrochloric acid. To titrate with 0.05 M SV potassium iodate to obtain light brown. To add 5 mL of chloroform and keep titrating, by vigorously stirring to the chloroform layer discolors. From the volume of 0.05 M SV potassium iodate spent, subtract volume of 0.1 M SV sodium thiosulfate spent in the test for dosage of iodine.

Each mL of 0.05 M SV potassium iodate remaining is equivalent to 15.0 mg of sodium iodide (NaI) or 16.6 mg of potassium iodide (KI).

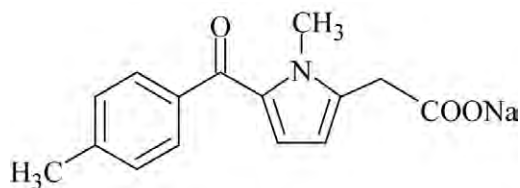
PACKAGING AND STORAGE

In tightly closed containers, protected from heat.

LABELLING

To comply with the legislation in force.

TOLMETIN SODIUM Tolmetinum natricum



$C_{15}H_{14}NNaO_3$; 279.27

$C_{15}H_{14}NNaO_3 \cdot 2H_2O$; 315.30

tolmetin sodium; 08743

Sodium salt of 1-methyl-5-(4-methylbenzoyl)-1H-pyrrole-2-acetic acid (1:1)

[35711-34-3]

Sodium salt of 1-methyl-5-(4-methylbenzoyl)-1H-pyrrole-2-acetic acid hydrate (1:1:2)

[64490-92-2]

It contains at least 98.0% and at most 102.0% of $C_{15}H_{14}NNaO_3$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, orange or slightly yellowish powder.

Solubility. Freely soluble in water and methanol. Sparingly soluble in ethanol, very slightly soluble in chloroform.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative

intensities to those observed in the tolmetin sodium SQR spectrum similarly prepared.

B. The ultraviolet absorption spectrum (5.2.14) ranging from 200 nm to 400 nm, of 10 µg/mL solution (w/v) in phosphate buffer M/15 pH 7.0, exhibits maxima only in the same wavelength of a similar tolmetin sodium SQR solution.

C. To weigh 1 g of the sample and dissolve it in 20 mL of water. It responds to the reactions of sodium ion (5.3.1.1).

PURITY TESTS

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using plate covered with about 0.25 mm of silica gel as support and a mixture of chloroform and glacial acetic acid (95:5) as mobile phase. To separately apply to the plate 20 µL of each of the solutions freshly prepared, as described below.

Solution (1): to dissolve 0.125 g of the sample in 10 mL of methanol (12.5 mg/mL).

Solution (2): to dissolve accurately weighed amount of tolmetin sodium SQR in methanol to obtain a 12.5 mg/mL solution. To quantitatively dilute a portion of this solution in methanol, to obtain 62.5 µg/mL solution.

To develop the chromatogram until the solvent reaches 3/4 of the length of the plate. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). The principal spot obtained with *Solution (1)* corresponds to that obtained in the chromatogram with *Solution (2)*. Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (2)* (2%).

Volatile organic impurities. To proceed as described in Gas chromatography (5.2.17.5). To use chromatograph equipped with flame ionization detector, by using mixture of nitrogen, synthetic air, and hydrogen (1:1:10) as the auxiliary gas to flame detector; 30 m long capillary column and 0.53 mm inner diameter, filled with stationary linked phase of 5% phenyl polysiloxane and 95% methyl polysiloxane, with a film thickness of 5 µm; column temperature from 35 °C to 260 °C (35 °C kept for 5 minutes, then rises to 175 °C at 8 °C per minute, and rises to 260 °C at 35 °C and kept at this temperature for at least 16 minutes); injector temperature at 70 °C and detector temperature at 260 °C; use helium as carrier gas; flow of carrier gas of 1 mL/minute.

Sample solution: to accurately dissolve about 1 g of sample in 50 mL of water, free of organic compounds.

Standard solution: to prepare a solution in water free of organic compounds containing, in each mL, 10 µg of methylene chloride, 1 µg of chloroform, 2 µg of benzene, 2 µg of dioxane, and 2 µg of trichlorethylene.

To separately inject 1 µL of *Sample solution* and *Standard solution* into the gas chromatograph. To obtain the chromatograms and measure the area under the peaks. Based on the retention time, identify any peak in the chromatogram of the *Sample solution*. To compare the chromatograms of the *Sample solution* and *Standard solution* to establish the presence and identification of peaks in the chromatogram. Limits: Benzene (2 ppm), chloroform (50 ppm), dioxane (100 ppm), methylene chloride (500 ppm), and trichlorethylene (80 ppm). It complies with the test.

Heavy metals (5.3.2.3). To use *Method III*. To use 1 g of sample. At most 0.002% (20 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in a vacuum oven at 60 °C for 4 hours. From 10.4% to 12.4%.

DOSAGE

To proceed as described in *Titration in non-aqueous medium (5.3.3.5)*. To accurately weigh about 0.3 g of the sample and dissolve, by heating, in 150 mL of glacial acetic acid. To cool down at room temperature and titrate with 0.1 M SV perchloric acid, to potentiometrically determine the endpoint. Each mL of 0.1 M SV perchloric acid is equal to 27.93 mg of C₁₅H₁₄NNaO₃. To perform sample blank correction.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Anti-inflammatory

TOXOID, ADSORBED TETANUS Toxoidum tetanicum adsorbatum

The tetanus toxoid is a tetanus anatoxin diluted in buffered saline solution and adsorbed by aluminum hydroxide or aluminum phosphate, and may contain a preservative. It is an opalescent, slightly brown suspension, which does not have lumps or foreign particles.

The preparation of tetanus toxin is based on the seed lot system, which is an amount of lyophilized ampoules containing lyophilized *Clostridium tetani*, of uniform composition, from a lyophilized strain of known origin. The culture media used for the batch preparation of seed and inoculum production should allow the growth of *C. tetani*. The culture medium for preparation of tetanus toxin must not contain animal proteins and be free of substances capable of inducing toxic and/or allergic reactions to human being. Tetanus toxin is a toxic filtrate from the culture media

for toxin preparation and aseptically collected into a single process. At the end of culture and lysis of the bacterial cells, there is culture purity by microscopic examination or inoculation of the sample in suitable culture media. The limit of flocculation (Lf/mL) is evaluated by employing the Ramon's technique.

The purified toxoid is prepared from an individual collection or a mixture of individual collections of anatoxin. After sterilizing filtration process, a preservative agent may be added.

It is not allowed the use of phenol, because it affects the antigenic properties of the product. The purified anatoxin is evaluated as the concentration of antigen (Lf/mL), sterility, and corresponding tests.

The tetanus anatoxin is obtained by detoxifying the concentrated tetanus toxin by adding chemical agents under suitable pH and temperature conditions. Formaldehyde at 35 °C is the most widely used chemical agent. Lf/mL, pH, and specific toxicity are the controls performed.

IDENTIFICATION

A. To dissolve the sample with sodium citrate with pH 9.0 to obtain a 10% solution (w/v). To keep at 37 °C for approximately 16 hours and centrifuge it. To use the supernatant for identification. Other suitable methods may be used to separate the adjuvant. To prepare 1% agar gel (w/v) in buffered saline solution and distribute over microscope slide, to result in a thin layer. To place it in an oven at 37 °C without drying it. To add a volume of 4 mL of agar on slide and put it at 2 °C - 8 °C in a humidified chamber for 1 hour. To make holes in the gel, by keeping the same distance between the central hole and peripheral ones. To fill the central hole with the reference tetanus antitoxin and peripherals ones with several sample dilutions. As a positive control, fill one of the holes with fluid tetanus toxoid. To incubate at 37 °C for 24 hours in a humid chamber and perform a reading in contrast lighting. To note the presence of line of precipitation and reaction of identity between the components analyzed.

B. To determine the limit of flocculation (Lf/mL) by employing the Ramon's technique.

C. To comply with one of the tests described in *Dosage*.

CHARACTERISTICS

pH (5.2.19). 6.0 to 7.0.

Determination of volume (5.1.2). It complies with the test.

Limit of flocculation - Ramon's Technique. To distribute several amounts of standardized tetanus antitoxin into test tubes. To add a constant volume of 1 mL of the sample to each tube. To make it homogeneous and put in a water bath at 45 °C - 50 °C. To constantly observe and write down the first tube showing flocculation and time required. To deter-

mine the Lf/mL in the sample, by multiplying the reference volume of antitoxin added to the tube by its concentration in Lf.

A dose for human use do not contain more than 25 Lf.

PHYSICAL AND CHEMICAL TESTS

Aluminum. To proceed as described in the monograph *Vaccines for human use*. At most 1.25 mg/human single-dose.

Producer is allowed to use the result from the product before bottling.

Residual formaldehyde. To proceed as described in the monograph *Vaccines for human use*. At most 200 ppm. Producer is allowed to use the result from the product before bottling.

Antigenic purity. To determine the content of protein nitrogen (5.3.3.2) and express the concentration in mg/ mL. The antigenic purity is determined by the relationship of concentration of antigen in Lf/mL and the concentration of protein nitrogen found. The product is an antigenic purity of at least 1000 Lf/mg of protein nitrogen.

Thimerosal. To proceed as described in the monograph *Vaccines for human use*. At most 200 ppm. Producer is allowed to use the result from the product before bottling.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). To proceed as described in the monograph *Vaccines for human use*.

Reversal of toxicity. To dilute the sample to 25 Lf/mL in saline solution and distributing into two vials. To keep one of them at 4 °C - 8 °C and the other one at 37 °C for 6 weeks. To subcutaneously inject the contents of each via into 5 guinea pigs (from 250 to 350 g), and the volume of the inoculum is 5 mL per animal. To weigh the animals on 1st, 2nd, 7th, 14th, and 21st day. The animals may not show signs of tetanus intoxication and should gain weight.

Specific toxicity. Do not dilute the anatoxin if it is not concentrated. To dilute the sample to 100 Lf/mL in saline solution. To subcutaneously inoculate 5 mL of the dilution into at least 5 guinea pigs (from 250 to 350 g). To observe the animals for 4 weeks. At least 80% of the inoculated animals have to survive during the observation period, and no signs of tetanus intoxication must be observed.

Specific toxicity. To proceed as previously described for tetanus anatoxin. Sample is diluted to 500 Lf/mL and each guinea pig is inoculated with a volume of 1 mL.

DOSAGE

A. Determination of the antitoxic titer in immunized animals sera

Immunization and bleeding of animals: to inoculate 0.75 mL (half of the total human dose) of the sample subcutaneously into each of the 6 guinea pigs (from 450 to 550 g). Six weeks after inoculation, collect 5 mL of blood from each animal by cardiac puncture and extract the serum. To mix equal volumes of sera from at least four guinea pigs.

Control L+/10/50 of the standardized tetanus toxin: to distribute the constant volumes of reference tetanus antitoxin among a series of test tubes, as measured by international standard, so that the volume to be inoculated contains 0.1 IU.

To add variable amounts of standardized tetanus toxin and to equal volumes of all tubes with buffered saline solution containing 1% (w/v) of peptone. To make it homogeneous and incubate at 37°C for 60 minutes. To subcutaneously inoculate a constant volume of each dilution into each of 10 Swiss albino mice (from 17 to 22 g). To observe the animals for 96 hours after inoculation.

Serum titration: To distribute variable amounts of serum into a series of test tubes. To add constant volume of standardized tetanus toxin, so that the volume to be inoculated per animal contains L+/10/50 (limit death). To equal the volumes of all tubes with buffered saline solution containing 1% (w/v) of peptone. To make it homogeneous and incubate at 37°C for 60 minutes. To subcutaneously inoculate each mixture into, at least, 10 Swiss albino mice (from 17 to 22 g). To observe the animals for 96 hours after inoculation and record the number of the living ones in each mixture. The values of mean effective doses (ED₅₀) of the sample and reference antitoxin are determined by proven statistical methods, to understand the transformation of data from linear regression (probits, logits, or angular transformations). To calculate the immunogenic activity by the equation:

$$AI = A/B \times C$$

where

AI = immunogenic activity in IU/mL;

A = ED₅₀ of the reference antitoxin;

B = ED₅₀ of the sample;

C = IU/mL of the antitoxin reference.

At least 2 IU/mL or 40 IU/human single-dose. Producer is allowed to use the result from the product before bottling.

B. Challenge method in mice. This determination confirms the immunogenic activity of the product by comparing with a reference tetanus toxoid measured by an international standard. To separate 9 groups of at least 20 mice (from 11 to 14 g) to test, and a group of 12 animals, with no inoculation, to control challenge toxin. To make 4 sample dilutions with saline solution, by using a dilution factor 2. To similarly proceed with reference tetanus toxoid. To subcutaneously immunize with a volume of 0.5 mL of each sample dilution per animal. To dilute the standardized tetanus toxin in buffered saline solution containing 1% (w/v)

of peptone, after 28 days of inoculation, so as to contain 100 DL_{50}/mL (lethal dose 50%), and subcutaneously inoculate each immunized mouse with a volume of 0.5 mL of standardized challenge toxin. To observe the animals until 96 hours after inoculation and record the number of the living ones for each dilution. Simultaneously, as the challenge dose control, dilute 1:50, 1:100, and 1:200 from the toxin solution containing 200 DL_{50}/mL by using the same diluent.

To subcutaneously inoculate 0.5 mL of each dilution in group of 12 separate animals, divided into groups of four animals. To observe the animals until 96 hours after inoculation and record the number of the dead ones for each dilution. All controlled animals inoculated with the challenge to 1:50 dilution must die, and none of the animals inoculated with a 1:200 dilution should die. To calculate the Effective Dose (ED_{50}) of the test sample and the reference toxoid, by using statistical analysis method to understand the transformation of data from linear regression (probits, logits, and angular transformations). The response range (survival rate) must be between the highest and lowest dilution used in the test sample and standard test forming the regression curve that should present linear relationship. Confidence limits should not be large; they must indicate better test accuracy as smaller their limits. To calculate the immunogenic activity by the equation:

$$AI = A / C \times B$$

where

AI = immunogenic activity in IU/mL;

A = ED_{50} of the reference antitoxin;

B = ED_{50} of the sample;

C = IU/mL of the antitoxin reference.

where

AI = immunogenic activity in IU/mL;

AI = immunogenic activity in IU/mL;

A = ED_{50} of the reference antitoxin;

B = ED_{50} of the sample;

C = IU/mL of the antitoxin reference.

To calculate the Effective Dose (ED_{50}) of the sample and the reference toxoid, by using statistical analysis method to understand the transformation of data from linear regression (probits, logits, and angular transformations). The response range (survival rate) must be between the 10% e 90%, by forming the regression curve that should present linear relationship. Confidence limits should not be large; they must indicate better test accuracy as smaller their limits. To calculate the immunogenic activity by the equation:

$$AI = A / B \times C$$

At least 2 IU/mL or 40 IU/human single-dose. Producer is allowed to use the result from the product before bottling.

B. Challenge in guinea pig. This determination confirms the immunogenic activity of the product by comparing with a reference tetanus toxoid measured by an interna-

tional standard. To separate 8 groups of at least 16 guinea pig (from 250 to 350 g) to test, and a group of 12 animals, with no inoculation, to control challenge toxin. To make 4 sample dilutions with saline solution, by using a dilution factor 2. To similarly proceed with reference tetanus toxoid. To subcutaneously immunize with a volume of 1 mL of each sample dilution per animal. To dilute the standardized tetanus toxin in buffered saline solution containing 1% (w/v) of peptone, after 28 days of inoculation, so as to contain 100 DL_{50}/mL and subcutaneously inoculate each immunized guinea pig with a volume of 1 mL of standardized challenge toxin. To observe the animals until 96 hours after inoculation and record the number of the living ones for each dilution. Simultaneously, as the challenge dose control, dilute 1:50, 1:100, and 1:200 from the toxin solution containing 100 DL_{50}/mL by using the same diluent. To subcutaneously inoculate 1 mL of each dilution in group of 12 separate animals, divided into groups of 4 animals. To observe the animals until 96 hours after inoculation and record the number of the dead ones for each dilution. All controlled animals inoculated with the challenge to 1:50 dilution must die, and none of the animals inoculated with a 1:200 dilution should die.

At least 40 IU/human single-dose. Producer is allowed to use the result from the product before bottling.

PACKAGING AND STORAGE

In accordance with the monograph *Vaccines for human use*.

LABELLING

To comply with the legislation in force.

TRETINOIN CREAM

It contains at least 90.0 % and at most 120.0 % of the amount stated of $C_{20}H_{28}O_2$.

IDENTIFICATION

The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

DOSAGE

To proceed as described in *High performance liquid chromatography (5.2.17.4)*. To protect the sample and corresponding solutions from direct light. To use chromatograph equipped with an ultraviolet detector at 365 nm; 150 mm chromatograph column and 3.9 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (4 μm), kept at room temperature; flow of *Mobile phase* of 1 mL/min.

Phosphate buffer: to dissolve 1.38 g of monobasic sodium phosphate in 1000 mL of water. To adjust the pH to 3.0 with diluted phosphoric acid.

Diluent: mixture of water and 10% phosphoric acid (v/v) (9:1).

Mobile phase: mixture of *Phosphate buffer* and tetrahydrofuran (55:45). To make the necessary adjustments so that the retention time is 15 minutes.

Sample solution: to transfer an accurately weighed amount of cream equivalent to 1 mg of tretinoin to a 50 mL amber volumetric flask and add of 20 mL of tetrahydrofuran. To stir to disperse the cream and top up with the same solvent. To make it homogenous and filter it. To transfer 5 mL of this solution to a 25 mL amber volumetric flask and top up with a mixture of tetrahydrofuran and *Diluent* (3:2). To make it homogeneous and filter.

Standard solution: to dissolve an accurately weighed amount of tretinoin SQR in tetrahydrofuran, to obtain a 0.4 mg/mL solution. To successively dilute this solution with a mixture of tetrahydrofuran and *Diluent* (3:2), to obtain a 4 µg/mL solution.

Procedure: to separately inject 25 µL of *Sample and standard solutions*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of $C_{20}H_{28}O_2$ in the cream, from the responses obtained for the *Sample and standard solutions*. The relative standard deviation for injections in duplicate should be at most 2.0%.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

TRETINOIN GEL

It contains at least 90.0 % and at most 120.0 % of the amount stated of $C_{20}H_{28}O_2$.

IDENTIFICATION

A. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using F_{254} silica gel as support and a mixture of cyclohexane and isopropyl alcohol (10:90) as mobile phase. To separately apply to the plate 10 µL of each of the solutions freshly prepared, as described below.

Solution (1): to dissolve an amount of gel equal to about 1.25 mg of tretinoin in hot methanol. To cool down at room temperature. To extract with 3 portions (50 mL each) of hexane. To wash the extract with 20 mL of water and filter by using sodium sulfate anhydrous.

To evaporate the filtrate to dryness in a rotary evaporator. Temperature should not exceed 60 °C. To dissolve the residue in 5 mL of methanol.

Solution (2): 0.25 mg/mL tretinoin SQR solution in methanol.

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*.

B. The ultraviolet absorption spectrum (5.2.14) of the sample solution obtained in *Dosage*, ranging from 300 nm to 450 nm, exhibits maxima and minima only in the same wavelength observed in the standard solution spectrum.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

PURITY TESTS

Related substances. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 353 nm; 150 mm column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (10 µm), kept at room temperature; flow of *Mobile phase* of 1.4 mL/minute.

Mobile phase: 0.5% glacial acetic acid (v/v) solution in a mixture of methanol and water (77:23).

Solution (1): to use *Solution (1)* as described in method A. *Identification*.

Solution (2): to dilute 3 mL of *Solution (1)* to 100 mL with methanol.

Procedure: to separately inject 20 mL of *Solutions (1) and (2)*, record the chromatograms, and measure the areas under the peaks. The sum of the areas under the secondary peaks obtained with *Solution (1)* is not greater than the area under the main peak obtained with *Solution (2)*.

DOSAGE

To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To protect the sample and corresponding solutions from direct light. To dissolve an amount of gel equivalent to about 0.5 mg of tretinoin in chloroform and top up to 100 mL with the same solvent. To prepare a standard solution at the same concentration by using the same solvent. To measure the absorbances of the resulting solutions at 365 nm, by using chloroform to adjust zero. To calculate the amount of $C_{20}H_{28}O_2$ in the gel from the readings obtained. Alternatively, make the calculations by taking into account that $A(1\%, 1\text{ cm}) = 1430$ at 365 nm, in chloroform.

PACKAGING AND STORAGE

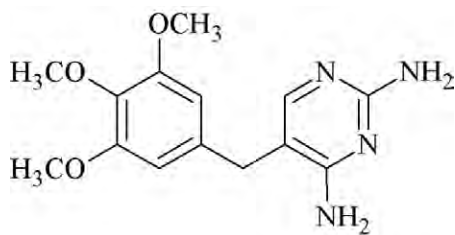
In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

TRIMETHOPRIM

Trimethoprimum



$C_{14}H_{18}N_4O_3$; 290.32
 trimethoprim; 08921
 5 - [(3,4,5-trimethoxyphenyl) methyl]
 -2,4-pyrimidinediamine
 [738-70-5]

It contains at least 98.5% and at most 101.0% of $C_{14}H_{18}N_4O_3$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline, white or yellowish-white powder. It is virtually odorless. It shows polymorphism.

Solubility. Very slightly soluble in water, slightly soluble in chloroform and methanol, sparingly soluble in ethanol and acetone, practically insoluble in ethyl ether and carbon tetrachloride.

Physical and chemical constants.

Melting range (5.2.2): 199 °C to 203 °C.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the trimethoprim SQR spectrum similarly prepared.

B. To transfer about 0.1 g of the sample to a 100 mL volumetric flask and add 25 mL of ethanol. To put it in ultrasound for 10 minutes and top up with 0.1 M sodium hydroxide. To transfer 2 mL of this solution to a 100 mL volumetric flask and top up with 0.1 M sodium hydroxide, to obtain a 0.002% solution (w/v). The ultraviolet absorption spectrum (5.2.14), from 230 nm to 350 nm, of 0.002% solution (w/v), has maximum at 287 nm, similar to those

observed in the spectrum of a similar solution of trimethoprim SQR.

The absorption maximum should not differ by more than 3%.

C. The retention time of the main peak in the chromatogram of *Test solution* obtained in method B., *Related substances*, corresponds to that of trimethoprim peak of *Resolution solution*.

PURITY TESTS

Related substances.

A. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel as support and a mixture of chloroform, methanol, and 6 M ammonium hydroxide (95:7.5:1) as mobile phase. To separately apply to the plate 10 µL of each of the solutions freshly prepared, as described below.

Solution (1): to transfer 0.2 g of sample to 10 mL volumetric flask, dissolve it in a mixture of chloroform and methanol (9:1), and top up with the same solvent.

Solution (2): to transfer 1 mL of *Solution (1)* to 10 mL volumetric flask and top up with a mixture of chloroform and methanol (9:1).

Solution (3): to transfer 20 mg of trimethoprim to 10 mL volumetric flask, dissolve it in a mixture of chloroform and methanol (9:1), and top up with the same solvent.

Solution (4): to transfer 1 mL of *Solution (3)* to 10 mL volumetric flask, and top up with a mixture of chloroform and methanol (9:1). To transfer 1 mL of this solution to 10 mL volumetric flask and top up with the same solvent, to obtain 20 µg/mL.

To develop the chromatogram. To remove the plate, and let it drying. To nebulize with a mixture of 1.9 g of ferric chloride in 20 mL of water and 0.5 g of potassium ferricyanide in 10 mL of water. Any spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, should not be more intense than the spot in the chromatogram obtained with *Solution (4)* (0.1%). The sum of the intensities of secondary spots obtained in the chromatogram of *Solution (1)* is at most 0.5%.

B. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped with an ultraviolet detector at 280 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm), kept at room temperature; flow of *Mobile phase* of 1.3 mL/minute.

Perchlorate buffer pH 3.6: to dissolve 1.405 g of sodium perchlorate in 950 mL of water, adjust the pH to 3.6 with phosphoric acid, and dilute to 1000 mL with water.

Mobile phase: mixture of *Perchlorate buffer pH 3.6* and methanol (7:3). To make the adjustments as required.

Test solution: to accurately transfer about 25 mg of the sample to a 25 mL volumetric flask by using 15 mL of *Mobile phase*. To put it in ultrasound for 10 minutes and top up with the same solvent.

Resolution solution: to dissolve accurately weighed amounts of trimethoprim SQR and diaveridine in *Mobile phase* and dilute with the same solvent, to obtain respectively 10 µg/mL and 5 µg/mL solutions for each substance.

To inject replicates of 20 µL of the *Resolution solution*. The resolution between the diaveridine and trimethoprim SQR peaks is not less than 2.5. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2.0 %.

Procedure: to inject 20 µL of *Test solution*, record the chromatograms at least 11 times the retention time of the main peak, and measure the areas under the peaks. To calculate the percentage of each impurity in the sample according to the equation:

$$100 \{Fr_i / [\Sigma(Fr_i) + Fr_i]\}$$

where

F = relative response factor, which is 0.5 to every peak with relative retention times of 0.9, 2.3, 2.7, or 10.3 in relation to trimethoprim, and is equal to 1.0 to any other peak;

r_i = area under the peak for any individual impurity obtained in *Test solution*;

r_i = area under the trimethoprim peak obtained in *Test solution*.

At most 0.1% of any individual impurity. The sum of the percentages of all impurities is no greater than 0.2%. To disregard the peaks related to solvent.

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 105 °C for 4 hours or to obtain constant weight. At most 0.5%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. To dissolve 0.3 g of the sample in 60 mL of glacial acetic acid. To titrate with 0.1 M SV perchloric acid. To potentiometrically determine the endpoint. Each mL of 0.1 M SV perchloric acid is equal to 29.032 mg of $C_{14}H_{18}N_4O_3$.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

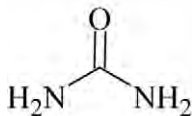
To comply with the legislation in force.

THERAPEUTIC CLASS

Antibacterial.

UREA

Ureum



CH₄N₂O; 60.06
urea; 01711
Urea
[57-13-6]

It contains at least 99.0% and at most 100.5% of CH₄N₂O, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White, crystalline powder or transparent crystals, slightly hygroscopic. Virtually odorless, but it can gradually develop ammonia odor after long-term storage.

Solubility. Freely soluble in water, soluble in ethanol, practically insoluble in chloroform and ethyl ether.

Physical and chemical constants.

Melting range (5.2.2): 132 °C to 135 °C.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in potassium bromide, has absorption maximum only at the same wavelength, with the same relative intensities to those observed in the urea SQR spectrum similarly prepared.

B. To heat 0.5 g of the sample in a test tube. Liquefaction occurs upon releasing ammonia. To keep heating to liquid becomes turbid and cool down. To dissolve the melt mass in 10 mL of water, add 1 mL of sodium hydroxide SR and a drop of cupric sulfate SR. Reddish-violet color is developed.

C. To dissolve 0.1 g of the sample in 1 mL of water and add 1 mL of nitric acid SR. White crystalline precipitate of urea nitrate is produced.

PURITY TESTS

Residue insoluble in ethanol. To dissolve 5 g of the sample in 50 mL of gently warmed ethanol. Should any insoluble residue be observed, filter the solution through tared filter paper. To wash the residue and filter paper with 20 mL of gently warmed ethanol and desiccate at 105 °C for 1 hour. At most 2 mg (0.04%).

Ammonia (5.3.2.6). To dissolve 10 g of the sample in 50 mL of water. To use 0.1 mL of this solution. At most 0.05% (500 ppm).

Chlorides (5.3.2.1). To determine in 5 g of the sample. At most 0.007% (70 ppm).

Sulfates (5.3.2.2). To determine in 2 g of the sample. To use 0.4 mL of 0.005 M sulfuric acid standard solution. At most 0.012% (120 ppm).

Heavy metals (5.3.2.3). To use *Method I*. To determine in 2 g of the sample (w/v). At most 0.001% (10 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample in oven at 105 °C for 2 hours. At most 1.0%.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0.1%.

DOSAGE

To accurately weigh about 0.5 g of the sample, dissolve in water, and dilute to 200 mL with the same solvent. To proceed as described in *Determination of nitrogen by the Kjeldahl method - semi-micro determination (5.3.3.2.2)* by using 2 mL of the resulting solution. Each mL of 0.005 M SV sulfuric acid is equivalent to 0.303 mg of CH₄N₂O.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Keratolytic.

VACCINES, HUMAN USE

Vaccina ad usum humanum

Vaccines for human use are, as a rule, prophylactic medicines able to induce immunity against a specific infectious agent. Its safety and efficacy must be proven through studies approved by the national quality control authority.

Vaccines may consist of inactivated micro-organisms, attenuated micro-organisms, substances produced by them, and antigenic fractions. The methods employed for preparation of vaccines depends on each type of product, and comply with standards of good manufacturing practices for pharmaceutical products.

Throughout the process of vaccine production, certain substances, such as stabilizers, adjuvants, and preservatives, can be added. In the final product, very low concentrations of antibiotics are allowed, except for streptomycin and penicillin and its byproducts. Should serum of animal origin be used in the production process, the final product can not have more than 50 ng/dose of proteins derived from serum. Should albumin be used, demonstrate absence of hepatitis B, hepatitis C, and HIV 1 and 2 antibodies.

BACTERIAL VACCINES

Bacterial vaccines are produced in liquid or solid form, by using appropriate strains and inactivated bacteria, attenuated bacteria (live) or their antigenic components. They are a colorless liquid or with different degrees of opacity or lyophilized.

For preparation of such vaccines, both all the micro-organisms cultivated in suitable culture media and fractions of microbial agents can be used. Inactivated vaccines must be prepared by chemical or physical methods, to not destroy its antigen ability. The live bacterial vaccines are produced by using attenuated strains capable of inducing immunity before the micro-organism of the same species or related virus species.

BACTERIAL TOXOIDS

The bacterial toxoids are toxins detoxified by physical and chemical treatments, which despite losing its ability toxic they retain immunogenic activity. The production is based on a seed lot system of strains in specific microorganisms cultivated in culture media free of substances which may cause toxic effects, allergic reactions, and other undesirable reaction to humans.

The toxoids may be liquid or lyophilized, and in both cases they may be purified or adsorbed. The adsorbed are white or slightly brownish opalescent suspension and may form sediment at the bottom of the bottle.

VIRAL VACCINES

Viral vaccines consist of attenuated or inactivated virus suspension, or their fractions, which can be lyophilized or

as suspension. Very low concentrations of antibiotics may be present, except streptomycin, penicillin, and its byproducts. Product can not have more than 50 ng/dose of proteins derived from animal serum. Should albumin be used, demonstrate absence of hepatitis B, hepatitis C, and HIV 1 and 2 antibodies.

The production of vaccine is based on the seed lot system, and virus strain used should demonstrate adequate immunogenicity as well as being safer to human beings. The replication of the vaccine virus strain is obtained from suitable host system (animal, bird embryos or cell culture), and production methodologies are given in the monographs of each product.

In the case of using mammalian cell culture for replication of the vaccine virus, separate 5% or 500 mL for control purposes, whichever is greater in volume. At the end of production of the vaccine, these cell cultures may not exhibit cytopathogenic effect (CPE). Moreover, aliquots from the growth medium are inoculated in suitable culture media, in order to confirm the absence of contaminating microorganisms (fungi, bacteria, and mycoplasmas). Cells should also demonstrate the absence of other contaminants, especially viruses from animal species, from which the cell culture was derived by hemadsorption test with guinea pig red blood cells and inoculation into cell cultures, laboratory animals, and embryonated eggs.

If the cell culture used is from primary embryonic lineage of birds, in addition to the controls mentioned in the previous paragraph, the farms supplying eggs must demonstrate adequate conditions of production in environments free of specific pathogens. Often, the birds are monitored for infections caused by retroviruses, Newcastle virus, parainfluenza virus, variola virus, encephalitis virus, Infectious laryngotracheitis virus, reticuloendotheliosis virus, Marek's virus, adenovirus, influenza virus, mycobacteria, *Haemophilus paragallinarum*, *Salmonella gallinarum*, *Salmonellapullorum*, *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, among other poultry pathogens.

In the case of cell culture used is from primary lineage of rabbit's kidney (*Oryctolagus cuniculus*), in addition to the controls mentioned in the third paragraph, rabbits must be raised under appropriate microbiological control and regularly monitored for infections caused by fungi, bacteria, and viruses, such as coccidiosis, myxomatosis, smallpox, fibromatosis, herpes, tuberculosis, *Nosema cuniculi*, toxoplasmosis, among other infections caused by micro-organisms which occur naturally in rabbits.

For monkey's kidney, animals have to be healthy and have never been used for other purposes. The animals before having their kidneys removed, must be kept in quarantine for a period of not less than six weeks and demonstrated to be free of hepatitis B antibodies (*herpes viruses*) and immunodeficiency virus.

If human diploid cells or continuous lineage cells are used, they must coming from a cell bank certified by the national

control authority and demonstrate the absence of contaminating microorganisms, as described in the third paragraph. There may be tumorigenic and identified according to the species of origin. The number of passages of human diploid cells may not exceed two-thirds of its maximum number of passage and its karyotype has to be normal. When the vaccine is produced in continuous lineage cell, the pool of virus must be purified by a method certifying that the residual DNA is less than 100 pg per dose in the final product.

The trypsin and serum used in cell culture preparation should be free of contaminating microorganisms (bacteria, fungi, mycoplasma, and viruses). Furthermore, the serum should from herds with certificates of absence of bovine spongiform encephalopathy.

COMBINED VACCINES

Combined vaccines consist of a mixture of two or more different antigens and may be lyophilized or as suspension. These immunobiologicals may have attenuated microorganisms, inactivated microorganisms, substances produced by them, and antigenic fractions in their formula. The production process and quality control shall comply with the specific monograph of each product in the vaccine.

IDENTIFICATION

To proceed as described in the specific monograph.

CHARACTERISTICS

To proceed as described in the specific monograph.

PHYSICAL AND CHEMICAL TESTS

Aluminum.

A. To proceed as described in *Visible absorption spectrophotometry* (5.2.14).

Acetate buffer: to dissolve 27.5 g of ammonium acetate in 50 mL of bidistilled water and add 0.5 mL of 25% hydrochloric acid (w/v). To top up the volume to 100 mL with bidistilled water.

Carbonate buffer: to dissolve 20 g of ammonium carbonate in 20 mL of diluted ammonia solution (dilute 17.5 mL of 10% ammonium hydroxide (w/v) in 32.5 mL of bidistilled water) and top up to 100 mL with distilled water.

To transfer 1 mL of sample to a Kjeldahl flask and add 2 mL of nitric acid. To digest the mixture to the solution becomes clear. To transfer to 25 mL volumetric flask and top up with *Acetate buffer*. To transfer 2 mL of this solution to a 50 mL volumetric flask and add 2 mL of 1% thioglycolic acid solution (v/v) freshly prepared. To wait for 2 minutes, add 15 mL of aluminon reagent, and heat it in a water bath (100 °C) for 15 minutes. To cool down, add 10 mL of *Carbonate buffer*, and top up with bidistilled water. To prepare the blank

with bidistilled water instead of sample. The readings of the sample and standards are performed in a spectrophotometer at 530 nm wavelength, by using blank to adjust zero. To calculate the concentration of aluminum (III) in the sample by graphic interpolation or linear regression. The result is expressed in mg of aluminum (III) per dose.

B. To proceed as described in *Atomic absorption spectrophotometry* (5.2.13). To transfer 2 mL of sample to a Kjeldahl flask and add 4 mL of nitric acid. To digest the mixture to the solution becomes clear. To transfer to 25 mL volumetric flask and top up with bidistilled water. To simultaneously prepare the blank containing bidistilled water instead of sample and calibration curve of aluminum at 20, 40, 60, and 80 ppm concentrations. To add certain amount of ionization suspension to the sample, calibration curve solutions, and blank a certain amount of ionization suppressor, so as to contain 2000 ppm of potassium at the end. To determine the concentration of aluminum (III) in the sample by using atomic absorption spectrophotometer at 309.3 nm wavelength, of 0.2 nm slit opening, 10 mA lamp for aluminum, and nitrous oxide/ acetylene flame.

Phenol. To dilute the sample to obtain concentration of phenol between 5 and 30 ppm. To add 5 mL of borate buffer, pH 9.0, 5 mL of 0.1% 4-aminoantipyrine (w/v), and 5 mL of aqueous solution of 5% potassium ferricyanide (w/v). To simultaneously prepare the blank and calibration curve of phenol at concentrations ranging from 5 to 30 ppm. To read the absorbances of the sample and standards at a 546 nm wavelength 10 minutes after the end of the reaction, by using the blank to adjust zero. To use reading of standards to construct a calibration curve. To calculate the concentration of phenol in the sample by graphic interpolation or linear regression. Producer is allowed to use the result from the product before bottling.

Residual formaldehyde. To slowly, by stirring, 3 mL of 2.5% trichloroacetic acid (v/v) to 1 mL of the sample. To let it rest for 5 minutes, centrifuge it at 2000 g for 10 minutes, and transfer the supernatant to a test tube.

To simultaneously prepare the calibration curve of formaldehyde at 2.5, 5, 7.5, 10 mL/mL concentrations with the volume of 4 mL/tube. To prepare the blank with bidistilled water instead of sample. To add 4 mL of Hantzsch reagent to each of 6 test tubes prepared above, put them in water bath at 58 °C for 5 minutes, and cool down. To immediately read the absorbances of the standard and sample, at a 412 nm wavelength, by using the blank to zero the instrument. To use reading of standards to construct a calibration curve. The concentration of residual formaldehyde in the sample is determined by graphic interpolation or linear regression.

Protein nitrogen (5.3.3.2). It complies with the test.

Thimerosal.

A. To proceed as described in *Visible absorption spectrophotometry* (5.2.14). After thorough homogenization of the

sample, transfer 1 mL in duplicate to beakers and add 3 mL of purified water (1:4 dilution), then take 1 mL of this solution and transfer to a digestion tube. To add 1 mL of purified water and 2 mL of a mixture of equal volumes of analytical standard sulfuric acid and analytical standard nitric acid. To boil the mixture for 10 minutes. To cool down. To add 10 mL of purified water and 2 mL of 50% hydroxylamine hydrochloride (w/v). To boil again for 1 minute, cool down, and transfer the liquid to a separatory funnel by filtering through cotton. To wash the tube with 70 mL of purified water and similarly transfer to the separatory funnel. To add 10 mL of dithizone solution (1:7) and vigorously stir for 1 minute. To let it rest for 1 minute, filter through cotton, and collect the organic phase (chloroform) to erlenmeyer flask. To immediately proceed the reading of filtrate by using a spectrophotometer at 490 nm. Preparation of standards and calibration curve: To prepare a stock thimerosal solution (1200 µg/mL in thimerosal or 600 µg/mL of Mercury). To prepare standard solutions by using this stock solution in 100 mL volumetric flasks. To construct a calibration curve with 6 µg Hg/mL to 24 µg Hg/mL concentrations. After the preparation of standard solutions, proceed as described for the sample. The blank is prepared by using 2 mL of purified water instead of sample. To use the reading of standards to construct a calibration curve and determine the concentration of thimerosal in the sample by graphic interpolation or linear regression.

B. To proceed as described in *Atomic absorption spectrophotometry* (5.2.13.1). To quantitatively, transfer 1 mL of sample to 50 mL volumetric flask, add 0.5 mL of nitric acid, and top up with bidistilled water. To prepare blank with bidistilled water. From a stock solution of 1000 ppm Hg, prepare an intermediate standard of 1 ppm Hg and remove different aliquots from it, according to the working range, by transferring them to reaction cells containing potassium permanganate solution. To determine the absorbance at 253.6 nm in atomic absorption spectrophotometer with an energy source with hollow cathode mercury lamp (6 mA), opening H07, and nitrogen as carrier gas.

Residual moisture. To transfer 80 mg of sample to weighting filter previously dried and tared. To keep the sample for 3 hours in an atmosphere of phosphorous pentoxide anhydrous, under pressure not exceeding 5 mm of mercury, at 60 °C. The weighting filter is cooled down for 20 minutes in a desiccator containing silica gel and immediately weighed. The heating and cooling stages are repeated to obtain constant weight. The amount of residual moisture is the average percentage weight loss of at least three evaluations of the sample. The volumetric method for *Determination of water* (5.2.20.1) may also be used.

BIOLOGICAL SAFETY TEST

Bacterial endotoxins. To proceed as described in the specific monograph.

Sterility (5.5.3.2.1). It complies with the test.

Pyrogens. To proceed as described in the specific monograph.

Nonspecific toxicity. To proceed as described in the specific monograph.

DOSAGE

To proceed as described in the specific monograph.

THERMOSTABILITY

To proceed as described in the specific monograph.

PACKAGING AND STORAGE

The temperature and expiry date are stated by the manufacturer of the vaccine, based on experimental evidence approved by the national control authority.

LABELLING

To comply with the legislation in force.

VACCINE, ADSORBED, DIPHTHERIA AND TETANUS, ADULT

Vaccinum diphtheriae et tetani adsorbatum

Vaccine is a mixture of diphtheria and tetanus anatoxins diluted in buffered saline solution and adsorbed by aluminum hydroxide or aluminum phosphate, and they may contain a preservative. It is an opalescent, slightly brown suspension, which does not have lumps or foreign particles.

Diphtheria component: The purified diphtheria anatoxin meets the specifications of production and controls described in the monograph *Adsorbed vaccine, diphtheria and infantile tetanus*.

Tetanic component: the purified tetanus anatoxin meets the specifications of production and controls described in the monograph *Adsorbed tetanus toxoid*.

The vaccine is prepared by dilution and adsorption on aluminum compounds, with certain quantities of purified diphtheria toxoid and tetanus toxoid. A dose for human use contains 2 Lf for diphtheria component and at most 25 Lf for tetanus component.

The product is bottled in suitable containers, labeled, and subjected to the controls required.

IDENTIFICATION

It complies with Identification described in the monograph *Vaccine, adsorbed, diphtheria and tetanus, infantile*.

CHARACTERISTICS

pH (5.2.19). 6.0 to 7.0.

Determination of volume (5.1.2). It complies with the test.

PHYSICAL AND CHEMICAL TESTS

Aluminum. To proceed as described in the monograph *Vaccines for human use*. At most 1.25 mg/human single-dose. Producer is allowed to use the result from the product before bottling.

Residual formaldehyde. To proceed as described in the monograph *Vaccines for human use*. At most 200 ppm. Producer is allowed to use the result from the product before bottling.

Thimerosal. To proceed as described in the monograph *Vaccines for human use*. At most 200 ppm. Producer is allowed to use the result from the product before bottling.

BIOLOGICAL SAFETY TEST

To proceed as described in *Biological safety tests*, in the monograph *Adsorbed vaccine, diphtheria and infantile tetanus*.

DOSAGE

The vaccine's immunogenic activity is determined for each component. There are no international reference standards for combined vaccines. The activity of each component is expressed in international units by comparing the reference standards calibrated and the reference standards of the components.

Diphtheria component: To proceed as described in *Dosage*, by using one of the methods described in the monograph *Adsorbed vaccine, diphtheria and infantile tetanus*.

A. At least 0.5 IU/mL.

B. At least 2 IU/human single-dose.

Producer is allowed to use the result from the product before bottling.

Tetanic component. To proceed as described in *Dosage*, by using one of the methods in the monograph *Adsorbed tetanus toxoid*.

A. At least 2 IU/mL.

B. At least 40 IU/human single-dose.

C. At least 40 IU/human single-dose.

Producer is allowed to use the result from the product before bottling.

PACKAGING AND STORAGE

In accordance with the monograph *Vaccines for human use*.

LABELLING

To comply with the legislation in force.

VACCINE, ADSORBED, DIPHTHERIA AND TETANUS, INFANTILE

Vaccinum diphtheriae et tetani adsorbatum

Vaccine is a mixture of diphtheria and tetanus anatoxins diluted in buffered saline solution and adsorbed by aluminum hydroxide or aluminum phosphate, and they may contain a preservative. It is an opalescent, slightly brown suspension, which does not have lumps or foreign particles.

Diphtheria component: The preparation of diphtheria toxin is based on the seed lot system, which is an amount of ampoules containing lyophilized *Corynebacterium diphtheriae*, of uniform composition, from a lyophilized strain of known origin. The culture media used for the batch preparation of seed and inoculum production should allow the growth of *C. diphtheriae*. The culture medium for preparation of diphtheria toxin must not contain animal proteins and be free of substances capable of inducing toxic and/or allergic reactions to human being. Diphtheria toxin is a toxic filtrate from the culture media for toxin preparation and aseptically collected into a single process. At the end of culture and lysis of the bacterial cells, there is culture purity by microscopic examination or inoculation of the sample in suitable culture media. The limit of flocculation (Lf) is measured by using the Ramon's technique, as described in monograph *Adsorbed tetanus toxoid*. The purification of the toxin is achieved by physical or chemical methods, and the sample is subjected to controls of Lf/mL and pH.

The diphtheria anatoxin is obtained by detoxifying the concentrated diphtheria toxin by adding chemical agents under suitable pH and temperature conditions.

Formaldehyde at 35 °C is the most widely used chemical agent. Lf/mL, pH, and specific toxicity are the controls performed.

The purified anatoxin is prepared from an individual collection or a mixture of individual collections of anatoxin. After sterilizing filtration process, a preservative agent may be added. It is not allowed the use of phenol, because it affects the antigenic properties of the product. Product samples are evaluated for antigen concentration (Lf/mL), sterility, and controls.

The diphtheria vaccine for infantile use is prepared by dilution and adsorption on aluminum compounds, with certain amounts of diphtheria and tetanus anatoxins. The human

single-dose must contain up to 30 and 25 Lf, respectively, to the diphtheria and tetanus components.

IDENTIFICATION

Diphtheria component

A. To dissolve the sample in sodium citrate with pH 9.0 to obtain a 10% solution (w/v). To keep at 37 °C for approximately 16 hours and centrifuge it. To use the supernatant for identification. Other suitable methods may be used to separate the adjuvant. To prepare 1% agar gel (w/v) in buffered saline solution and distribute over microscope slide, to result in a thin layer. To place it in an oven at 37 °C without drying it. To add a volume of 4 mL of agar on slide and put it at 2 °C - 8 °C in a humidified chamber for 1 hour. To make holes in the gel, by keeping the same distance between the central hole and peripheral ones. To fill the central hole with the reference diphtheria antitoxin and peripherals ones with several sample dilutions. As a positive control, fill one of the holes with fluid diphtheria toxoid. To incubate at 37 °C for 24 hours in a humid chamber and perform a reading in contrast lighting. To note the presence of line of precipitation and reaction of identity between the components analyzed.

B. To determine the limit of flocculation (Lf/mL) by employing the Ramon's technique.

C. To proceed as described in *Dosage*.

Tetanic component. To proceed as described in *Identification*, in the monograph *Adsorbed tetanus toxoid*.

CHARACTERISTICS

pH (5.2.19). 6.0 to 7.0.

Determination of volume (5.1.2). It complies with the test.

PHYSICAL AND CHEMICAL TESTS

Aluminum. To proceed as described in the monograph *Vaccines for human use*. At most 1.25 mg/human single-dose. Producer is allowed to use the result from the product before bottling.

Residual formaldehyde. To proceed as described in the monograph *Vaccines for human use*. At most 200 ppm. Producer is allowed to use the result from the product before bottling.

Antigenic purity. To determine the content of protein nitrogen (5.3.3.2) and express the concentration in mg/ mL. The antigenic purity is determined by the relationship of concentration of antigen in Lf/mL and the concentration of protein nitrogen found. The product is an antigenic purity of at least 1500 Lf/mg of protein nitrogen.

Thimerosal. To proceed as described in the monograph *Vaccines for human use*. At most 200 ppm. Producer is allowed to use the result from the product before bottling.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

Diphtheria component

Reversal of toxicity. To proceed as described in the monograph *Adsorbed tetanus toxoid*. The animals may not show signs of diphtheria intoxication and should gain weight.

Specific toxicity.

Subcutaneous test: To dilute the sample to 100 Lf/mL in saline solution. To subcutaneously inoculate 5 mL of the dilution into at least 5 guinea pigs (from 250 to 350 g). To observe the animals for 4 weeks. At least 80% of the inoculated animals have to survive during the observation period, and no signs of diphtheria intoxication must be observed.

Intradermal test: To dilute the sample to 100 Lf/mL in saline solution. To intradermally inoculate 0.2 mL of the dilution into a guinea pig previously shaved. As a control, inoculate the same volume of saline solution into the same animal. After 48 hours of observation, no erythema must be formed in specific locations of inoculation.

Specific toxicity. To perform *Subcutaneous Test* as previously described for diphtheria anatoxin. Sample is diluted to 500 Lf/mL and each guinea pig is inoculated with a volume of 1 mL.

Tetanic component

To proceed as described in *Biological safety tests*, in the monograph *Adsorbed tetanus vaccine*.

DOSAGE

The vaccine's immunogenic activity is determined for each component. There are no international reference standards for combined vaccines. The activity of each component is expressed in international units by comparing the reference standards calibrated and the reference standards of the components.

Diphtheria component

A. Determination of the antitoxic titer in immunized animals sera

Immunization and bleeding of animals: To inoculate 0.75 ml (half of the total human dose) of the sample subcutaneously into each of the six guinea pigs ranging from 450 to 550 g. Four weeks after inoculation, collect 5 mL blood from each animal by cardiac puncture and extract the se-

rum. To mix equal volumes of sera from at least four guinea pigs.

Control L+/50 of the standardized diphtheria toxin: To distribute the constant volumes of reference diphtheria antitoxin among a series of test tubes, as measured by international standard, so that the volume to be inoculated contains 1 IU. To add variable amounts of standardized diphtheria toxin and to equal volumes of all tubes with buffered saline solution containing 1% (w/v) of peptone. To make it homogeneous and incubate at 37°C for 60 minutes. To inoculate the constant volume of each dilution subcutaneously into each of four guinea pigs ranging from 250 to 350 g. To observe the animals for 96 hours after inoculation.

Serum titration: To distribute variable amounts of serum into a series of test tubes. To add constant volume of standardized diphtheria toxin, so that the volume to be inoculated per animal contains 1 L+/50 (limit death). To equal the volumes of all tubes with buffered saline solution containing 1% (w/v) of peptone. To make it homogeneous and incubate at 37°C for 60 minutes. To inoculate each mixture subcutaneously into, at least, four guinea pigs ranging from 250 to 350 g. To observe the animals for 96 hours after inoculation and record the number of the living ones in each mixture. The values of mean effective doses (ED₅₀) of the sample and reference antitoxin are determined by proven statistical methods, to understand the transformation of data from linear regression (probits, logits, or angular transformations). To calculate the immunogenic activity by the equation:

$$AI = \frac{A}{B} \times C$$

where

AI = immunogenic activity in IU/mL;
A = ED₅₀ of the sample;
B = ED₅₀ of the reference antitoxin;
C = IU/ml of the antitoxin reference.

At least 2 IU/mL. Producer is allowed to use the result from the product before bottling.

B. Challenge in guinea pig To prove the immunogenic activity of the product to be tested by comparing with reference diphtheria toxoid. To separate 8 groups of at least 16 guinea pigs ranging from 250 to 350 g. To perform 4 dilutions of the test sample with sodium chloride solution 0.85% (w/v) by using a dilution factor 2. To proceed similarly with reference diphtheria toxoid. To inoculate subcutaneously a volume of 1 mL per animal for each dilution. To separate a group of 12 animals with no inoculation, to control challenge toxin. To dilute the standardized diphtheria toxin in buffered saline solution containing 1% (w/v) of peptone, after 28 days of inoculation, so as to contain 100 LD₅₀/mL (lethal dose 50%), and inoculate each immunized guinea pig subcutaneously with a volume of 1 mL of dose of challenge toxin. To observe the animals until 96 hours after inoculation and record the number of the living ones

for each dilution. Simultaneously, as the challenge dose control, dilute 1:100 from the toxin solution containing 100 LD₅₀/mL by using the same diluent. To inoculate 1 mL of the dilution subcutaneously into each of five guinea pigs. To observe the animals until 96 hours after inoculation and record the number of the dead ones for each dilution. To calculate the Effective Dose 50% (ED₅₀) of the test sample and the reference toxoid, by using statistical analysis method to understand the transformation of data from linear regression (probits, logits, and angular transformations). The response range (survival rate) must be between the highest and lowest dilution used in the sample and standard test forming the regression curve that should present linear relationship. Confidence limits should not be large; they must indicate better test accuracy as smaller their limits. To calculate the immunogenic activity by the equation:

$$AI = \frac{A}{B} \times C$$

where

AI = immunogenic activity in IU/human single-dose;
A = ED₅₀ of the sample;
B = ED₅₀ of the reference toxoid;
C = IU/human single-dose of the reference toxoid.

At least 30 IU/human single-dose. Producer is allowed to use the result from the product before bottling.

Tetanic component

To proceed to *Dosage*, by using one of the methods described in the monograph *Adsorbed tetanus toxoid*. At least 2 IU/mL (method A). At least 40 IU/human single-dose (method B). At least 40 IU/human single-dose (method C). Producer is allowed to use the result from the product before bottling.

PACKAGING AND STORAGE

In accordance with the monograph *Vaccines for human use*.

LABELLING

To comply with the legislation in force.

VACCINE, ADSORBED, DIPHTHERIA, TETANUS, AND PERTUSSIS

Vaccinum diphtheriae et tetani et pertussis adsorbatum

Vaccine is a mixture of diphtheria and tetanus anatoxins and whole dead cell suspension (*Bordetella pertussis*), diluted in buffered saline solution and adsorbed by aluminum hydroxide or aluminum phosphate, and it may contain a preservative. It is an opalescent, slightly brown suspension, which does not have lumps or foreign particles.

Diphtheria component: The purified diphtheria anatoxin meets the specifications of production and controls described in the monograph *Adsorbed vaccine, diphtheria and infantile tetanus*.

Tetanic component: The purified tetanus anatoxin meets the specifications of production and controls described in the monograph *Adsorbed tetanus toxoid*.

Pertussis component: pertussis vaccine is a homogeneous suspension of whole dead cell suspension of one or more strains of *B. pertussis* in saline solution. The strains used to prepare the vaccines are identified by complete records, including its origin, insulation characteristics, and all evidence conducted periodically to check the characteristics of the strains. The strains must be lyophilized in phase I, and contain at least agglutinogens 1, 2, and 3. In addition, they have to be maintained at a maximum temperature of 4 °C.

The production of vaccine is based on a seed lot system, which must have the same characteristics of the original batch. The culture medium used for the *B. pertussis* culture must allow the maintenance of the agglutinogens and immunogenic activity. Such medium can not increase the particular toxicity of the strain; it should be free from animal protein, as well as substances capable of inducing toxic/allergic reactions to humans. At the end of culture, the bacteria are collected and washed to remove substances from the culture medium, and resuspended in isotonic saline solution. Samples of the individual collections are evaluated for opacity and bacterial purity. The suspension may be inactivated by heating at 56 °C for a specified time or detoxified by adding chemicals, under appropriate conditions of pH, temperature, and treatment time. Samples of suspension are evaluated as for bacterial inactivation, by sowing in appropriate culture medium, purity, identification, sterility, and related controls.

The adsorbed vaccine, diphtheria, tetanus, and pertussis is prepared by dilution and adsorption in aluminum compounds, with certain quantities of diphtheria and tetanus anotoxins and whole dead cells (*Bordetella pertussis*). The human single-dose must contain up to 30 and 25 Lf, respectively, to the diphtheria and tetanus components.

IDENTIFICATION

To dissolve the sample with sodium citrate with pH 9.0 to obtain a 10% solution (w/v). To keep at 37 °C for approximately 16 hours and centrifuge it. To use the supernatant to identify each component, either diphtheria or tetanus. To resuspend the precipitate to identify the pertussis component. Other suitable methods may be used to separate the adjuvant.

Diphtheria component: To proceed as described in *Identification, in the monograph Adsorbed vaccine, diphtheria and infantile tetanus*.

Tetanic component. To proceed as described in *Identification, in the monograph Adsorbed tetanus toxoid*.

Pertussis component:

A. To transfer 50 µL of the sample on a microscope slide and add the same volume of polyvalent antiserum of

B. *B. pertussis*. To make the mixture homogeneous in a circular movement for a minute, and keep the material at rest for three minutes. To observe the agglutination of the sample for five minutes at most.

B. To proceed as per Dosage for Pertussis component.

CHARACTERISTICS PH (5.2.19). 6.0 TO 7.0.

Determination of volume (5.1.2). It complies with the test.

Lymphocytosis promoting factor (LPF)

Suitable methods are used, such as induction of lymphocytosis in mice, to observe the level of active factor in the vaccine and evidences of histamine sensitizing activity in mice.

Presence of agglutinin

To transfer 50 µL of the sample on three microscope slides and add 50 µl of mono-specific agglutinin serum 1, 2, and 3 over the samples in each slide. To make it homogeneous for a minute and let at rest for three minutes. To observe the agglutination of the sample in the three slides for five minutes at most. The strain of *B. pertussis* must present agglutination with the three specific monovalent sera.

PHYSICAL AND CHEMICAL TESTS

Aluminum. To proceed as described in the monograph *Vaccines for human use*. At most 1.25 mg/human single-dose. Producer is allowed to use the result from the product before bottling.

Residual formaldehyde. To proceed as described in the monograph *Vaccines for human use*. At most 200 ppm. Producer is allowed to use the result from the product before bottling.

Opacity. To perform within 15 days after preparation of suspension. To measure with standard turbidimetric distributed by the National Institute of Health (NIH) or equivalent approved by the national control agency. The value of 10⁶ opacimetric units is assigned to this pattern when examined by photometry, by using green filter, at a wavelength of 530 nm. Such a degree of opacity is approximately 10⁹ bacteria/mL. To put 1 mL of sample in a test tube and add saline solution according to opacity similar to the pattern. To visually compare the opacity with the reference opacity. The opacity unit (UOP) is determined by the equation:

$$\text{UOP/mL} = \frac{\text{final volume of the diluted sample} \times 10}{\text{initial volume}}$$

For the pertussis component, the concentration of bacteria must be at most 20 UOP/dose.

Thimerosal. To proceed as described in the monograph *Vaccines for human use*. At most 200 ppm. Producer is allowed to use the result from the product before bottling.

BIOLOGICAL SAFETY TEST

Sterility. To proceed as described in the monograph *Vaccines for human use*.

Toxicity (5.5.2.3). To individually weight the animals. The weight of each animal has to be higher than the initial one. No animal can die or have any change in health status during the observation period. In case of product fails to meet the requirements, conduct a new test with the same procedure and criteria.

Diphtheria component

To proceed as described in *Biological safety tests, in the monograph Adsorbed vaccine, diphtheria and infantile tetanus*.

Tetanic component

To proceed as described in *Biological safety tests, in the monograph Adsorbed tetanus vaccine*.

Pertussis component:

Detection of heat-labile toxin (dermonecrotic toxin).

The subcutaneous inoculation of the sample in the suckling mouse's nuchal area is the most sensitive method for detecting the heat-labile toxin. The pertussis vaccine should not contain biologically-active heat-labile toxin.

Specific toxicity. To dilute the sample in saline solution to obtain corresponding maximum concentration at

20 UOp/dose. To use two groups of at least 10 susceptible Swiss albino mice ranging from 14 to 16 g. Immediately before inoculation, determine the total weight of the animals. To inoculate 0.5 mL of the diluted sample intraperitoneally into each mouse from the first group. To proceed as described for the second group, by inoculating saline solution containing the same amount of preservative as the inoculum injected into animals of the test group. To determine the total weight of each group of mice on the 3rd and 7th days after inoculation. The product is considered non-toxic if (a) on the 3rd day the total weight of the group is smaller than its initial weight; (b) on the 7th day the average weight gain of the group inoculated with the sample is not less than 60% of the average weight gain of the negative control group; and (c) no more than 5% of the animals inoculated with the sample have died.

DOSAGE

The vaccine's immunogenic activity is determined for each component. There are no international reference standards for combined vaccines. The activity of each component is expressed in international units by comparing the reference standards calibrated and the reference standards of the components.

Diphtheria component: To proceed as described in *Dosage*, by using one of the methods described in the monograph *Adsorbed vaccine, diphtheria and infantile tetanus*.

A. At least 0.5 IU/mL.

B. At least 2 IU/human single-dose.

Producer is allowed to use the result from the product before bottling.

Tetanic component. To proceed as described in *Dosage*, by using one of the methods in the monograph *Adsorbed tetanus toxoid*.

A. At least 2 IU/mL.

B. At least 40 IU/human single-dose.

C. At least 40 IU/human single-dose.

Producer is allowed to use the result from the product before bottling.

Pertussis component. The immunogenic activity is determined by setting the comparative evaluation in relation to the standardized reference vaccine against the international standard for the antipertussis vaccine. To use susceptible Swiss albino mice ranging from 12 to 16 g from homogeneous group of standardized lineage. Animals should preferably be of the same gender. Should they be from different gender, distribute them equally. To use at least 20 animals for each dilution of the sample and reference vaccine. To control the challenge dose, separate groups with at least 10 mice.

Immunization of animals: To make three serial dilutions of the sample and reference pertussis vaccine in buffered saline solution with dilution factor 5, so that the dilutions can ensure, respectively, protection of 70% to 80%, 40% to 50%, and 10% to 20%. To intraperitoneally inoculate 0.5 mL of the dilutions into each mouse of the immunization group. To keep animals of the control group with no inoculation. The interval between immunization and challenge is 14 to 17 days.

Challenge: to reconstitute one or more ampoules from a batch *B. pertussis* with an aqueous solution containing casein peptone 1% (w/v) and sodium chloride 0.6% (w/v), with pH 7.0 to 7.2.

To sow in test tubes and plates containing appropriate medium. To incubate at 35 °C for 48 hours. To make a passag-

ing of culture on plates and tubes with Bordet-Gengou agar or similar and incubate at 35 °C for 24 hours. To make a second passaging under the same conditions described and incubate it for 18 hours. The cultures obtained on the plates are used to observe the colonies and identify them by comparing serum agglutination with antiserum specific for the strain. Alternatively, aliquots of suspension for the challenge may be frozen and kept in liquid nitrogen and after freezing and dilution, they can be used directly as a challenge culture. To prepare suspension by using suitable diluent allowing micro-organisms to remain viable, so as to contain 10 UOp/mL, by comparing with the 5th International Reference Preparation for Opacity. Suspension is then adjusted so that each challenge dose contains 100 to 1000 LD₅₀ (median lethal dose) in 30 mL. To intracerebrally inoculate the challenge dose into each immunized mouse. To intracerebrally inoculate the serial dilutions of the challenge dose, in each of the control groups, in order to obtain estimated LD₅₀. To grow dilution of the challenge dose in Bordet-Gengou, to determine the number of colony forming units (CFU) contained therein. The value of mean effective dose (ED₅₀) of the test sample is determined by proven statistical methods, to understand the transformation of data from linear regression (probits, logits, or angular transformations). The test is valid if the ED₅₀ of the vaccine is between the highest and lowest immunizing dose, the standard deviation of the ED₅₀ is between 65% and 156%, the dilution with lowest concentration of the challenge suspension control contains between 10 and 50 CFU/30 mL, the challenge dose is between 100 and 1000 LD₅₀, the LD₅₀ contains up to 300 colony forming units, and the dose response curves for the product and reference pertussis vaccine does not differ significantly from the parallelism and linearity ($p < 0.05$). The immunogenic activity is calculated by the equation below:

$$AI = \frac{A}{B} \times C$$

where

AI = immunogenic activity in IU/human single-dose;

A = ED₅₀ of the sample;

B = ED₅₀ of the reference pertussis vaccine;

C = IU/human single-dose of the reference vaccine.

At least 4 IU/human single-dose and a maximum of 18 IU/human single-dose. If the determined immunogenic activity is to not meet the requirements, the test can be repeated. Product meets the requirements if the geometric average of the results of two, three, or four valid tests is at least 4 IU/human single-dose. Producer is allowed to use the result from the product before bottling.

PACKAGING AND STORAGE

In accordance with the monograph *Vaccines for human use*.

LABELLING

To comply with the legislation in force.

VACCINE, BCG Vaccinum BCG

The lyophilized BCG vaccine is a live vaccine derived from the culture of *Bacillus Calmette and Guérin*, attenuated strain of *Mycobacterium bovis*, with safety and efficacy recognized for providing protection to humans against tuberculosis. The lyophilized is a desiccated bacillus mass with powder consistency, whitish or pale yellow color. When it is reconstituted, it appears slightly blurred with homogeneous aspect.

The production of vaccine is based on the seed lot system. No more than eight subcultures can be made from the original strain. The selected strain must retain its stability and maintain its non-pathogenic nature for both human and animal in tests. This vaccine must be produced by staff in good health conditions, free from infectious agents and particularly virulent strains of *Mycobacterium tuberculosis*. The bacterium is inoculated into a suitable culture medium, free from substances that might cause toxic/allergic reactions to humans. The culture and culture medium of each container are visually examined for appearance, thus presenting bacterial veil on the surface and clear culture medium. The cultures are transferred to a fresh medium and after growth they are tested for sterility and visually evaluated as for the transparency and appearance of the bacterial veil. After filtration of the bacterial veil, this is resuspended in appropriate medium and subjected to the bacterial respiration, opacity, and sterility tests. The bacterial suspension is diluted to the appropriate number of doses and, prior to bottling, the product is evaluated as for the number of colony forming units and sterility. Product is bottled in amber glass ampoules or vials, with pharmaceutical grade, lyophilized, labeled, and subjected to the controls required.

IDENTIFICATION

By using a microscopy, observe smear obtained after reconstitution of the vaccine and stained by Ziehl-Nielsen technique. Only acid-fast bacilli are detected.

As a complement, observe the colony morphology sown in Lowenstein-Jensen medium, used in *Dosage* (colony forming units). The colonies are rough, predominantly scattered and non-pigmented.

CHARACTERISTICS

pH (5.2.19). 5.5 to 7.0. To determine with appropriate diluent after reconstitution of the vaccine.

Homogeneity. To use the slide prepared in *Identification*, to verify the bacilli dispersion in the suspension of the vaccine as per scale values, according to **Table 1**. Grade 5, maximum.

Table 1 - State of aggregation.

Grade	State of Aggregatio
0	Only scattered bacilli
1	Predominance of scattered bacilli; some small lumps
2	Predominance of scattered bacilli; some small and medium lumps
3	Scattered bacilli and small lumps
4	Scattered bacilli and small and medium lumps
5	Scattered bacilli and small, medium, and large lumps
6	Medium and large lumps

PHYSICAL AND CHEMICAL TESTS

Residual moisture. To proceed as described in the monograph *Vaccines for human use*. At most 3%.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

Virulent mycobacteria. To reconstitute the contents of the ampoules with the diluent recommended, in order to obtain 50 human doses. To subcutaneously inoculate 1 mL volume in each of six guinea pigs weighing from 250 g to 400 g, in the abdominal region, on the right side. To keep animals under observation for 42 days. To weigh, sacrifice, and necropsy the animals at the end of the period. To examine the site of inoculation, the regional, inguinal, axillary, mediastinal, lumbar, and portal nodes, as well as other organs, particularly the lungs, liver, spleen, and kidneys.

No guinea pig can show evidence of progressive tuberculosis and at least 2/3 of the animals must survive with weight gain at the end of the observation period. To repeat the test if more than 1/3 of the animals died or lost weight.

Skin reactivity. To reconstitute a sample and prepare dilutions 1:10 and 1:100, by using the diluent recommended. To intradermally inoculate 0.1 mL of each dilution into the left flank of four albino guinea pigs of the same gender, with at least 350 g each. The animals have to present a negative tuberculin reaction, and

they can not be previously subject to treatment that can present false negative. To proceed as described for the reference vaccine, by inoculating the same animal on the right flank. To observe the animals over four weeks and conduct weekly readings of the diameter of the lesions found in the inoculation areas. To calculate for each corresponding dilution, at the end of the observation period, an average of four readings of the vaccine and the reference vaccine. Vaccine meets the requirement if reaction from the sample and reference vaccine is similar.

DOSAGE

Number of colony forming units (CFU)

To reconstitute five ampoules of vaccine diluent recommended, by taking care to add it gently to avoid foaming. To transfer the contents from the ampoules to a single test tube, make it homogenous, and make 3 dilutions to obtain optimal number of colonies, around 40, by dismissing the counts above 100. To inoculate in Löwenstein-Jensen medium, by using five tubes for the 2 more concentrated dilutions and 10 tubes for those more diluted. To seal the tube and incubated it upright at 37 °C for four weeks. To simultaneously analyze a sample of reference vaccine. The limits are 2×10^6 to 10×10^6 UFC/mL.

THERMOSTABILITY

To incubate five ampoules of vaccine at 37 °C for four weeks and proceed according to *Dosage*. To compare the results obtained with the samples stored at 2 °C to 8 °C. The number of CFU/ mL can not be less than 20% in relation to the CFU/mL of the vaccine stored at 2 °C and 8 °C.

PACKAGING AND STORAGE

In accordance with the monograph *Vaccines for human use*.

LABELLING

To comply with the legislation in force.

VACCINE, ATTENUATED MUMPS *Vaccinum parotiditis vivum*

The attenuated mumps vaccine consists of live attenuated virus and is lyophilized. After reconstitution with an appropriate diluent, it presents an appearance of clear homogeneous suspension, and can demonstrate color due to the presence of a pH indicator.

The production of the vaccine is based on a seed lot system and the strain of virus used or five consecutive batches of the vaccine, and it may not induce neuro-pathogenicity in monkeys susceptible to mumps virus. In addition, the viral strain must demonstrate adequate immunogenicity and safety to humans. Replication of the virus is performed on susceptible cell culture. The viral suspension is identified and monitored for sterility. After clarification of the viral suspension by suitable method to remove cell debris, some stabilizing substances are added. They definitely do not change the efficacy and safety of the product. Before bottling and lyophilization, the product is analyzed for sterility, concentration of virus and proteins derived from the animal serum used.

The vaccine is bottled in suitable containers, lyophilized, labeled, and subjected to the controls required.

Further information on the criteria of production and its controls are indicated in the monograph *Vaccines for human use*.

IDENTIFICATION

To reconstitute the vaccine with suitable diluent and add an equal volume of serum containing neutralizing antibodies to mumps virus. To incubate it at 36 °C for an hour. After incubation, inoculate the mixture in susceptible cells culture and keep it at 36 °C for 10 days. To use as control the inoculated cell culture with vaccine virus and other non-inoculated, which presents, at the end of the test, the presence and absence of cytopathogenic effect, respectively. The absence of CPE in cell culture identifies the vaccine virus.

PHYSICAL AND CHEMICAL TESTS

Residual moisture. To proceed as described in the monograph *Vaccines for human use*. At most 2%.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

DOSAGE

To proceed the determination under direct light. To dilute two samples of the vaccine and a sample of the reference vaccine at intervals of at least 1.0 log in a suitable culture medium. To inoculate each dilution into at least 10 wells of microplate containing Vero cells in suspension and incubate at 36 °C for 10 days. To observe the cell cultures for the presence or absence of CPE and calculate titer of the vaccine by proven statistical method. The potency of the vaccine is the geometric mean value of the vials analyzed, expressed in CCID₅₀ (50% infectious dose in cell culture) per dose. For valid determination, it is necessary that (a) at the end of the test the control of cell culture presents unchanged monolayer; (b) the variation of the potency between the two samples of the vaccine is at most 0.5 log₁₀ CCID₅₀; (c) the variation in the titer of the reference vaccine is at most 0.5 log₁₀ CCID₅₀ in relation to its mean titer; and (d) the CPE is decreasing in relation to the increasing dilutions; (e) the dilutions used in the test are between 10% and 90% of inoculated cell cultures.

The potency of the vaccine is at least 10^{3.7} CCID₅₀/dose.

If it fails to meet the requirement, repeat the determination. The potency of the vaccine is the geometric mean of the two tests performed.

The method of plaque-forming units (PFU) can be used as well.

The value of potency for the approval of the product has to be correlated with the CCID₅₀.

THERMOSTABILITY

The test and *Dosage* are simultaneously performed. To incubate the sample of the vaccine at 37 °C for seven days, for analyzing according to the methodology described for the potency of the product. The vaccine can not lose more than 1.0 log₁₀ CCID₅₀/dose, in relation to the titer of the vaccine stored in appropriate temperature conditions. It can not have titer below requirement for the potency of the product.

PACKAGING AND STORAGE

In accordance with the monograph *Vaccines for human use*.

LABELLING

To comply with the legislation in force.

VACCINE, ATTENUATED YELLOW FEVER

Vaccinum febrile flavae vivum

The yellow fever vaccine consists of live attenuated virus and is lyophilized. After reconstitution with an appropriate diluent, it presents an appearance of homogeneous suspension, and can demonstrate color due to the presence of a pH indicator.

The production of the vaccine is based on a primary seed lot of lineage 17D, from which the secondary seed lot originates by means of passages in SPF embryonated chicken eggs (free from contaminating microorganisms). This lot must be assessed for neurovirulence in susceptible monkeys and it can not present strange microorganisms. Besides, the viral strain must demonstrate adequate immunogenicity and safety to humans.

Replication of the virus is performed on embryonated chicken eggs, free from specific pathogens, or susceptible cell culture. The viral suspension is identified and controlled for sterility. After clarification of the viral suspension by suitable method to remove cell debris, some stabilizing substances are added. They definitely do not change the efficacy and safety of the product. Before bottling and lyophilization, the product is analyzed for sterility, concentration of virus and protein nitrogen. If the production of vaccine occurs on embryonated eggs, 2% and not less than 20 eggs are separated to control. At the end of production of the vaccine, these eggs not infected with vaccine strain must demonstrate the absence of specific pathogens for fowls.

The vaccine is bottled in suitable containers, lyophilized, labeled, and subjected to the controls required.

Further information on the criteria of production and its controls are indicated in the monograph *Vaccines for human use*.

IDENTIFICATION

When neutralized with serum containing antibody specific for yellow fever virus, the vaccine inhibits the plaque-forming units (PFU) in susceptible cells, as described in *Dosage*.

PHYSICAL AND CHEMICAL TESTS

Residual moisture. To proceed as described in the monograph *Vaccines for human use*. At most 3%.

BIOLOGICAL SAFETY TEST

Bacterial endotoxin (5.5.2.2). It complies with the test. At most 10 UE/mL.

Sterility (5.5.3.2.1). It complies with the test.

Residual ovalbumin. Three vials of the same batch of lyophilized vaccine and standard ovalbumin are subjected to immunoassay ELISA method. The standard ovalbumin curve is on concentrations of 100 µL to 0.5 µL, with factor 2 dilution and the vaccine is diluted with phosphate buffered saline PBS/T20 0.05%/NFDM (phosphate buffered saline with Tween 20 and skimmed milk powder). To inoculate each dilution starting at 1:10 and use the factor 2 in two wells of the 96-well plate previously sensitized with anti-ovalbumin serum (rabbit) in sodium carbonate-bicarbonate buffer pH 9.6 and blocked with bovine albumin serum 3% (w/v). Incubation is carried out at 37 °C for 30 minutes. Plates are washed with phosphate buffered saline PBS /T20 0.05% (phosphate buffered saline with Tween 20), and anti-ovalbumin serum (rabbit) plus peroxidase in phosphate buffered saline PBS /T20 0.05%/ NFDM is added. Further incubation is carried out at 37 °C for 30 minutes and washed again. The reaction is shown with the substrate for the peroxidase in citrate-phosphate buffer pH 5.0 and is stopped with 2M sulfuric acid. Reading is taken in a microplate reader at a wavelength of 450/630 nm.

The content of residual ovalbumin is calculated by plotting the average absorbance against the standard concentration log, by using linear values corresponding to 50% of the endpoint.

The vaccine is considered satisfactory if the residual ovalbumin content is less than or equal to 5 µL/dose.

DOSAGE

At least two vials of lyophilized vaccine and one vial of reference vaccine are subjected to the plaque-forming units (PFU) method. To dilute the vaccine by applying a factor

4 and inoculate each dilution with at least three wells in 6-well microplate with monolayer of Vero cells previously sowed. The concentration of the cell lineage can range from 150.000 to 300.000 cells per mL, depending upon the day. After adsorption for 90 minutes at 36 °C in a CO₂ atmosphere (5%). Inoculums are removed, and a culture medium containing agarose or carboxymethylcellulose in appropriate concentration is added. Cells are incubated for 5-7 days at 36 °C in a CO₂ atmosphere (5%). After the incubation period, remove the growth medium, fix the cells with formaldehyde and stain with a vital dye.

The potency of the vaccine is calculated by averaging the number of plaques of at least two dilutions and the result is expressed in log PFU/dose. For valid determination, it is necessary that (a) the control of cell culture presents unchanged monolayer; (b) the variation of the potency between the two samples of the vaccine is at most 0.5 log₁₀ PFU; (c) the potency of the reference vaccine is at most 0.5 log₁₀ PFU in relation to its mean titer; and (d) the PFU is decreasing in relation to the increasing dilutions.

The potency in PFU/dose has to be equivalent to 1000 LD₅₀ in mice. If sample fails to meet the requirement, repeat the determination. The potency of the vaccine is the geometric mean of the two determinations carried out.

THERMOSTABILITY

The test and *Dosage* are simultaneously performed. To incubate at least two vaccine vials for 14 days at 36 °C and analyze as described in *Dosage*. The vaccine can not lose more than 1.0 log₁₀ PFU, in relation to the titer determined in the sample stored in appropriate temperature conditions. Moreover, it must not present titer below to that specified for the potency of the product.

PACKAGING AND STORAGE

In accordance with the monograph *Vaccines for human use*.

LABELLING

To comply with the legislation in force.

VACCINE, ATTENUATED 1, 2 AND 3 POLIO

Vaccinum poliomyelitis perorale typus I, II, III

The oral poliomyelitis vaccine is a mixture of attenuated poliovirus types 1, 2, and 3. It is a clear homogeneous aqueous suspension, and it may show color due to the presence of a pH indicator.

The production of the vaccine is based on the seed lot system, and each strain of the virus serotype can not have more than three subcultures from the original batch. They also may not induce the neuro-pathogenicity in monkeys susceptible to the three types of poliovirus. The viral strain

must demonstrate adequate immunogenicity and safety to humans.

Replication of each poliovirus is performed on susceptible cell culture. The viral suspension is identified and monitored for sterility. After clarification of the viral suspension by suitable method to remove cell debris, some stabilizing substances are added. They definitely do not change the efficacy of the product. Before the formulation, each purified viral suspension is evaluated for identification, viral concentration, sterility, consistency of viral characteristic, and neurovirulence in susceptible monkeys. After mixing it, the trivalent bulk vaccine is subjected to the control of viral concentration, sterility, content of stabilizer used, and pH.

The product is bottled in suitable containers, labeled, and subjected to the controls required.

Further information on the criteria of production and its controls are indicated in the monograph *Vaccines for human use*.

IDENTIFICATION

To dilute the sample, add an equal volume of mixed anti-poliovirus sera 1, 2, 3 and incubate at 37 °C for 1 hour. After incubation, inoculate the mixture in susceptible cells culture and incubate it at 35 °C for 7 days. As a control, use the cell culture inoculated with dilution of vaccine and other non-inoculated, which presents the presence and absence of cytopathogenic effect (CPE), respectively. The absence of CPE in the cell cultures inoculated with the mixture of vaccine and anti-poliovirus sera identifies the vaccine virus.

CHARACTERISTICS

Appearance. Mobile liquid and pinkish to reddish color.
Determination of volume (5.1.2). It complies with the test.

pH (5.2.19). 6.0 to 7.0.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

DOSAGE

To dilute in a suitable culture medium two samples of the vaccine to be analyzed and a sample of the reference vaccine. The interval between dilutions is at least 0.5 log₁₀, and the samples are diluted separately. To add equal volumes for the determination of each type of poliovirus

of each dilution of the vaccine to the suitable mixture of anti-poliovirus serum. Thus, for the determination of poliovirus type 1, add dilutions of the mixed sample to the anti-polio sera types 2 and 3; for poliovirus type 2, add dilutions to the mixed anti-polio sera types 1 and 3; and for poliovirus 3, add dilutions of the vaccine to the anti-polio sera types 1 and 2. To determine the total virus, add equal volumes of each dilution of the vaccine to the culture medium used for dilution. To incubate for 1 to 3 hours at 35 °C - 36 °C. After incubation, inoculate each dilution of the vaccine in a microplate, in eight wells, at least, with cell suspension containing Hep2_C. To incubate the microplates at 35 °C for 7 days. To observe the presence or absence of CPE in the cell cultures. To calculate the titer of each serotype by a proven statistical method.

The potency of the vaccine is the geometric mean value of the vials analyzed, expressed in CCID₅₀ (50% infectious dose in cell culture) per dose. For valid determination, it is necessary that (a) the control of cell culture presents unchanged monolayer; (b) the variation of the potency between the two samples of the vaccine is at most 0.5 log₁₀ CCID₅₀ for each serotype; (c) the potency of the reference vaccine is at most 0.5 log₁₀ CCID₅₀ in relation to the mean titer of each serotype; (d) the CPE is decreasing in relation to the increasing dilutions; and (e) the dilutions used in the test are between 10% and 90% of inoculated cell cultures.

The potency is at least 10⁶ to poliovirus type 1, 10⁵ to poliovirus type 2, and 10^{5.78} to poliovirus type 3. The confidence interval of 95% of the test may not differ by a factor of greater than 10^{0.5} of CCID₅₀ estimated for each type of virus in the vaccine. If the sample fails to meet the requirements, repeat the corresponding test of virus where the potency is below the minimum value specified. The potency is the geometric mean of the two determinations carried out.

THERMOSTABILITY

The test and *Dosage* are simultaneously performed. To incubate two samples of the vaccine at 37 °C for 48 hours, to determine the total virus content (type 1 + type 2 + type 3) by using the method described in *Dosage*. The vaccine can not lose more than 0.5 log₁₀ CCID₅₀ in relation to the titer of the total virus determined in the sample stored in appropriate temperature conditions. If it fails to meet the requirement, repeat the test. The final titer is the average of both tests conducted.

PACKAGING AND STORAGE

In accordance with the monograph *Vaccines for human use*.

LABELLING

To comply with the legislation in force.

VACCINE, INACTIVATED 1, 2, AND 3 POLIO

Vaccinum poliomyelitidis inactivatum

The inactivated poliomyelitis vaccine is a mixture of inactivated poliovirus types 1, 2, and 3. It is a clear liquid, and it may show color due to the presence of a pH indicator

The production of the vaccine is based on the seed lot system and each of the serotypes can not have more than 10 subcultures from the original batch. Replication of the virus is performed on susceptible cell culture. The viral suspension is identified and monitored for sterility. After clarification of the viral suspension by suitable method to remove cell debris, each viral suspension is concentrated and purified. The suspension of each type of virus is identified, evaluated for sterility, mycoplasma, and concentration of virus. Inactivation of each viral suspension is carried out separately by an appropriate method, such as the addition of chemical agents under appropriate conditions. Formaldehyde is the most widely used chemical agent. Before mixing the three types of inactivated poliovirus and adding preservative and other substances, each virus suspension is evaluated for effectiveness of inactivation. After mixing the three poliovirus and before bottling, controls are performed to detect the absence of infective particles in susceptible cells, sterility, and concentration of preservative.

The vaccine is bottled in suitable containers, lyophilized, labeled, and subjected to the controls required.

Further information on the criteria of production and its controls are indicated in the monograph *Vaccines for human use*.

IDENTIFICATION

It meets the requirements described in *Dosage*.

PHYSICAL AND CHEMICAL TESTS

Protein nitrogen. To proceed as described in *Determination of nitrogen by the Kjeldahl method (5.3.3.2)*. At most 10 µg/dose.

Residual formaldehyde. To proceed as described in the monograph *Vaccines for human use*. At most 200 ppm. Producer is allowed to use the result from the product before bottling.

Albumin, bovine. Up to 50 ng per human dose, determined by the *Immunochemical method (5.6.)*.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

DOSAGE

To use proven sensitivity enzyme immunoassay method for assessing the concentration of D antigen in each of the three poliovirus serotypes in the vaccine. To simultaneously evaluate the reference vaccine.

The potency is at least 40, 8, and 32 units of D antigen per dose for the poliovirus types 1, 2, and 3, respectively.

PACKAGING AND STORAGE

In accordance with the monograph *Vaccines for human use*.

LABELLING

To comply with the legislation in force.

VACCINE, RABIES (INACTIVATED) *Vaccinum rabiei ad usum humanum*

The vaccine is an inactivated suspension prepared from rabies virus replicated in cell culture. It can be presented in the lyophilized form or suspension. After reconstitution with an appropriate diluent, the lyophilized vaccine shows an appearance of clear homogeneous suspension, and can demonstrate color due to the presence of a pH indicator.

The production of the vaccine is based on the seed lot system of virus to be properly characterized. The batches are subject to the controls of viral identification, sterility, and infective potency. Besides, the viral strain must demonstrate adequate immunogenicity to humans. Replication of the virus is performed on susceptible cell culture. The viral suspension is monitored for sterility, viral identification, and infective potency. In the production process, an intermediate virus suspension is prepared at known concentrations and subjected to centrifugation, purification, and viral inactivation by validated method in which, usually, beta-propiolactone (1:4000) or UV irradiation is used. After inactivation, the product is concentrated, and sterility testing, viral inactivation, and immunogenic activity are performed. The final preparation should be isotonized, and it may contain preservatives and pH indicator. Before bottling, the product is subjected to the control of sterility, immunogenic activity, and preservatives.

The vaccine is bottled in suitable containers, and it can be lyophilized, labeled, and subjected to the controls required.

IDENTIFICATION

Determination of immunogenic activity can be used.

Bacterial endotoxins (5.5.2.2). At most 5 UE per dose.

PHYSICAL AND CHEMICAL TESTS

Phenol. Test applied when the preservative is present. To proceed as described in the monograph *Vaccines for human*



use. At most 0.15% (1500 ppm). Producer is allowed to use the result from the product before bottling.

Thimerosal. Test applied when the preservative is present. To proceed as described in the monograph *Vaccines for human use*. At most 0.015% (150 ppm). Producer is allowed to use the result from the product before bottling.

Residual moisture. Test applied to the lyophilized product. To proceed as described in the monograph *Vaccines for human use*. At most 3%.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

Pyrogens (5.5.2.1). It complies with the test. To inject into each rabbit a human dose of the vaccine diluted (1:10), in sterile and pyrogen-free saline solution.

Verification of viral inactivation.

To employ one of the methods described below.

A. To intracerebrally inoculate 10 µL of the sample into at least 20 suckling mice (5-10 days) and 30 µL into at least 20 Swiss albino mice ranging from 10 g to 15 g. To observe the inoculated animals for 21 days. During the observation period, the animals may not present neurological symptoms or death. If this occurs, perform the *Direct immunofluorescence* test in the brain of animals considered. The test meets the requirements if no inoculated animals present classic symptoms of rabies. Should evolving neurological symptoms or death of inoculated animals be verified, confirmation of rabies infection will depend on the positivity detected in the *Direct immunofluorescence* test.

Direct immunofluorescence: cut the mouse's brain under suspicion of rabies, so that the center sections will turn up. To prepare paired traces on microscope slide for microscopy properly identified. Meanwhile, prepare slides properly identified, traces for control, by using mice known as positive and negative for rabies, which respectively will serve as negative and positive controls. To allow the slides to dry for 30 minutes at 20 °C - 25 °C and fixed traces in acetone previously cooled at -20 °C, by keeping them incubated for 2-4 hours at -20 °C. To allow the slides to dry at 20 °C - 25 °C. To proceed color, by surrounding the traces with substance adequate to retain the conjugate during the incubation period. Enamel can be used. At the time of use thaw two aliquots of working conjugate dilution for identification of rabies virus (rabies virus antibodies generally labeled with fluorescein isothiocyanate) and diluted one

of the aliquots (1:5), with 20% suspension of brain tissue from uninfected mice (SCN). To similarly dilute another aliquot, but at 20% suspension of brain tissue from mice infected with rabies virus (SCI) and make it homogeneous. To position the slide so that its identification is toward the left side of the operator and deposit mixtures over the traces, by using distinct Pasteur pipettes. To cover the left trace

with mixture "conjugate + SCN." The right one with "conjugate + SCI." Then, incubated it in humid chamber for 30 minutes at 37 °C. After incubation, wash with phosphate buffered saline PBS (pH 7.6 - 8.0) and let soak for 10 minutes in the same diluent. To drain the diluent and wash with distilled water to prevent crystals. To allow to dry and set with glycerine buffered pH 8.5 - 9.0 to increase the intensity of fluorescence, by placing coverslip. To examine with a fluorescence microscope at 100 times magnification. Firstly, examine negative control trace treated with the mixture "conjugate + SCN." SCN is free from rabies virus, then the conjugate remains free to react with the antigen present in the trace, so there is fluorescence emission. Thus, the antigen will be shown as inclusions or fine green dust. Then, observe the positive control trace treated with the mixture "conjugate + SCI." The conjugate is adsorbed by the antigen present in the SCI, so there is no remaining available conjugate to react with the rabies antigen present in the trace. Consequently, there is no fluorescence emission. The antigen will not be shown in this trace. To observe traces from negative control slide in that order. No fluorescence should be observed after checking that the controls are satisfactory. To observe the traces of the material under test; the presence of rabies virus in the material analyzed, that is, the positivity is verified when test slide presents fluorescence only in trace that received the mixture "conjugate + SCN," which is not found where the mixture "conjugate + SCI" was placed; the absence of the rabies virus in the material examined, that is, the negative is detected when no fluorescence is observed.

B. Method for checking amplified viral inactivation

To inoculate equivalent amount of 25 doses of (*inactivated*) rabies vaccine, at least, into 5 cell cultures of the same type, used in the production of the vaccine or other cell culture with sensitivity similar to rabies virus. The ratio used is 3 cm² of culture per milliliter of vaccine. After adsorption of the virus, culture medium is added in a ratio not exceeding 1:3 in the volume of the vaccine used. The cultures are observed for 21 days, and the presence of rabies virus can be done by inoculating into mouse or by immunofluorescence.

Inoculation of mice on 14th and 21st days of culture: 0.03 ml from a mixture of samples of the culture supernatant is intracerebrally inoculated into 20 Swiss albino mice ranging from 12 to 15 g. The animals are observed for 14 days and any indication of rabies should be confirmed by immunofluorescence.

Immunofluorescence: cultures are examined on day 21, after inoculation, and the presence of rabies virus is detected by immunofluorescence test.

The test is considered satisfactory if at the end of the observation period neither rabies virus nor cytopathic effects in cultures is detected.

DETERMINATION OF IMMUNOGENIC ACTIVITY

Challenge method in mice: to prepare at least three dilutions of the sample and reference vaccine in phosphate buffered saline pH 7.6 and intraperitoneally inoculate 0.5 ml of each dilution into least 16 Swiss albino mice ranging from 10 g to 15 g. To keep 30 non-inoculated animals to control the titer of challenge virus. To make booster immunization by inoculating the same dilutions into each group of mice 7 days after the first immunization. Seven days after the second immunization, execute the challenge, by intracerebrally inoculating into each mouse 30 µL of volume containing approximately 50 LD₅₀ of fixed rabies virus CVS (*challenge virus standard*). To prepare two decimal dilutions from the challenge dilution and intracerebrally inoculate 30 µL of these dilutions and challenge dilution into three groups of 10 mice among non-immunized animals. To observe the animals for 14 days, and record the number of living ones of each mixture. The animals who have died before the fifth day after inoculation should not be considered upon calculating the immunogenic activity. To calculate the effective doses 50% (ED₅₀) of the sample and reference vaccine, as well as LD₅₀ of the challenge virus by statistically proven method. The response range (survival rate) must be between the highest and lowest dilution used in the sample and standard test forming the regression curve that should present linear relationship. To calculate the immunogenic activity by the equation:

$$AI \text{ (IU/mL)} = \frac{ED_{50} \text{ of the sample} \times \text{IU/mL of the reference vaccine}}{ED_{50} \text{ of the reference vaccine}}$$

AI = immunogenic activity

At least 2,5 IU/human single-dose. When referring to the value of immunogenic activity (IU/mL), inform the number of real LD₅₀ obtained in titration of the challenge virus, which is equal to the antilogarithm of the difference between the LD₅₀ calculated and the dilution of the challenge dose used. Confidence limits should not be below 25% or above 400% in relation to the activity determined. The titration of the challenge suspension must present at least 10 LD₅₀. The statistical analysis should show no deviations from linearity and parallelism of the dose-response curves.

THERMOSTABILITY

To incubate the sample at 36 °C ± 1 °C for four weeks and proceed to the *Determination of immunogenic activity*. At least 2,5 IU/human single-dose.

PACKAGING AND STORAGE

In accordance with the monograph *Vaccines for human use*.

LABELLING

To comply with the legislation in force.

VACCINE, ATTENUATED RUBELLA *Vaccinum rubellae vivum*

The vaccine consists of live attenuated virus and is lyophilized. After reconstitution with an appropriate diluent, it presents an appearance of clear homogeneous suspension, and can demonstrate color due to the presence of a pH indicator.

The production of the vaccine is based on a seed lot system and the strain of virus used or five consecutive batches of the vaccine, and it may not induce neuro-pathogenicity in monkeys susceptible to rubella virus. Besides, the viral strain must demonstrate adequate immunogenicity and safety for humans. Replication of the virus is performed on susceptible cell culture. The viral suspension is identified and monitored for sterility. After clarification of the viral suspension by suitable method to remove cell debris, some stabilizing substances are added. They definitely do not change the efficacy and safety of the product. Before bottling and lyophilization, the product is analyzed for sterility, concentration of virus, and proteins derived from the animal serum used in the culture.

The vaccine is bottled in suitable containers, lyophilized, labeled, and subjected to the controls required.

Further information on the criteria of production and its controls are indicated in the monograph *Vaccines for human use*.

IDENTIFICATION

To reconstitute the vaccine with suitable diluent and add an equal volume of serum containing neutralizing antibodies to rubella virus. To incubate for 90 minutes at 4 °C - 8 °C. After incubation, inoculate the mixture of the vaccine with serum in susceptible cells culture and keep it at 32 °C - 33 °C for 12 days. As a control, a cell culture inoculated with vaccine virus and other non-inoculated, respectively, have to present the presence and absence of cytopathogenic effect (CPE). The absence of CPE in cell culture identifies the vaccine virus.

PHYSICAL AND CHEMICAL TESTS

Residual moisture. To proceed as described in the monograph *Vaccines for human use*. At most 2%.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

DOSAGE

To proceed it under direct light. To dilute two samples of the vaccine to be analyzed and a sample of the reference vaccine at intervals of at least 1.0 log₁₀ in a suitable culture medium. To inoculate each dilution into at least 10 wells of a microplate containing RK-13 cells in suspension and incubate at 32 °C - 33 °C for 12 days. To observe the pres-

ence or absence of CPE in the cell cultures. To calculate the titer of the vaccine by proven statistical method. The potency of the vaccine is the geometric mean value of the vials analyzed, expressed in $CCID_{50}$ (50% infectious dose in cell culture) per dose. For valid determination, it is necessary that (a) the control of cell culture presents unchanged monolayer; (b) the variation of the potency between the two samples of the vaccine is at most $0.5 \log_{10} CCID_{50}$; (c) the potency of the reference vaccine is within a range of $0.5 \log_{10} CCID_{50}$ in relation to its mean titer; (d) the CPE is decreasing in relation to the increasing dilutions; and (e) the dilutions used in the test are between 10% and 90% of inoculated cell cultures.

The potency is at least $10^{3.7} CCID_{50}/dose$. If sample fails to meet the requirement, repeat the test. The potency is the geometric mean of the two determinations carried out.

The method of plaque-forming units (PFU) can also be used. The PFU's and $CCID_{50}$'s potency value for approval of the product has to be correlated.

THERMOSTABILITY

The test and *Dosage* are simultaneously performed. To incubate the sample at $37^{\circ}C$ for 7 days and proceed as set forth in *Dosage*. The vaccine can not lose more than $1.0 \log_{10} CCID_{50}$ in relation to the titer determined in the sample stored in appropriate conditions. Moreover, it must not present titer below to that specified for the approval of the *Dosage*. If it fails to meet the requirement, repeat the test. The final titer is the average of both tests conducted.

PACKAGING AND STORAGE

In accordance with the monograph *Vaccines for human use*.

LABELLING

To comply with the legislation in force.

VACCINE, ATTENUATED MEASLES *Vaccinum morbillorum vivum*

The vaccine consists of live attenuated virus and is lyophilized. After reconstitution with an appropriate diluent, it presents an appearance of clear homogeneous suspension, and can demonstrate color due to the presence of a pH indicator.

The production of the vaccine is based on a seed lot system and the strain of virus used or five consecutive batches of the vaccine, and it may not induce neuro-pathogenicity in monkeys susceptible to measles virus. Besides, the viral strain must demonstrate adequate immunogenicity and safety for humans. Replication of the virus is performed on susceptible cell culture. The viral suspension is identified and monitored for sterility. After clarification of the viral suspension by suitable method to remove cell debris, some stabilizing substances are added. They definitely do

not change the efficacy and safety of the product. Before bottling and lyophilization, the product is analyzed for sterility, concentration of virus, and proteins derived from the animal serum.

The vaccine is bottled in suitable containers, lyophilized, labeled, and subjected to the controls required.

Further information on the criteria of production and its controls are indicated in the monograph *Vaccines for human use*.

IDENTIFICATION

To reconstitute the vaccine with suitable diluent and add an equal volume of serum containing neutralizing antibodies to measles virus. To incubate it at $36^{\circ}C$ for an hour. After incubation, inoculate the mixture in susceptible cells culture and keep it at $36^{\circ}C$ for 7 days. To use as control a cell culture inoculated with vaccine virus and other non-inoculated, which presents, at the end of the test, the presence and absence of cytopathogenic effect (CPE), respectively. The absence of CPE in cell culture identifies the vaccine virus.

PHYSICAL AND CHEMICAL TESTS

Residual moisture. To proceed as described in the monograph *Vaccines for human use*. At most 3%.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

DOSAGE

To proceed it under direct light. To dilute two samples of the vaccine to be analyzed and a sample of the reference vaccine at intervals of at least $1.0 \log_{10}$ in a suitable culture medium. To inoculate each dilution in at least

10 wells of microplate containing Vero cells in suspension. To incubate it at $36^{\circ}C \pm 1^{\circ}C$ for 7 - 9 days. The cell cultures are observed for the presence or absence of CPE. The titer of the vaccine is calculated by using the estimation method of Spearman & Karber. The potency of the vaccine is the geometric mean value of the vials analyzed, expressed in $CCID_{50}$ (50% infectious dose in cell culture) per dose.

For valid determination, it is necessary that (a) at the end of the test, the control of cell culture presents unchanged monolayer; (b) the variation of the potency between the two samples of the vaccine is at most $0.5 \log_{10} CCID_{50}$; (c) the potency of the reference vaccine is within a range of $0.5 \log_{10} CCID_{50}$ in relation to its mean titer; (d) the CPE is decreasing in relation to the increasing dilutions; and (e) the dilutions used in the test are between 10% and 90% of inoculated cell cultures.

The potency of the vaccine must be at least $10^{3.7}$ CCID₅₀/dose for the Biken Cam 70 strain and $10^{3.0}$ CCID₅₀/dose for the other strains. If it fails to meet the requirement, repeat the determination of the potency. The result is the geometric mean of both tests conducted.

The method of plaque-forming units (PFU) can also be used. The PFU's and CCID₅₀'s potency value for approval of the product has to be correlated.

THERMOSTABILITY

The test and *Dosage* are simultaneously performed. To incubate the sample of the vaccine at $36\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ for 7 days, for analyzing according to the methodology described in *Dosage*. The vaccine can not lose more than 1.0 log_{10} CCID₅₀, in relation to the titer determined in the sample stored in appropriate temperature conditions. Moreover, it must not present titer below to that specified for the potency of the product.

PACKAGING AND STORAGE

In accordance with the monograph *Vaccines for human use*.

LABELLING

To comply with the legislation in force.

VACCINE, MEASLES, MUMPS, RUBELLA

Vaccinum parotiditis et rubellae et morbillorum vivum

The vaccine consists of a mixture of attenuated live measles, mumps, rubella viruses and is lyophilized. After reconstitution with appropriate diluent, it presents appearance of clear homogeneous suspension, and it may show color due to the presence of a pH indicator.

Each viral component in the vaccine is produced separately, as described in the specific monographs.

The vaccine is bottled in suitable containers, lyophilized, labeled, and subjected to the controls required.

IDENTIFICATION

To reconstitute the vaccine with suitable diluent and add an equal volume of serum containing neutralizing antibodies to mumps, rubella, and measles viruses. To keep it at $4\text{ }^{\circ}\text{C}$ a $8\text{ }^{\circ}\text{C}$ for 90 minutes. To inoculate into susceptible cells culture and keep it for 10 days. As a control test, the cell cultures inoculated with the vaccine non-neutralized with the mixture of sera containing antibodies to all three types of viruses and other non-inoculated must show the presence and absence of CPE, respectively. The absence of CPE in the cell cultures inoculated with the mixture of vaccine plus antibodies identifies the product.

PHYSICAL AND CHEMICAL TESTS

Residual moisture. To proceed as described in the monograph *Vaccines for human use*. At most 2%.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

DOSAGE

To proceed it under direct light. To dilute two samples of the vaccine to be analyzed and a sample of the reference vaccine at intervals of at most 1.0 log_{10} in a suitable culture medium. For the titration of each type of virus, each dilution of the vaccine, add equal volume of specific heterologous antiserum, according to the following table:

<i>Virus to be titred</i>	<i>Serum</i>
Mumps	anti-measles
Rubella	anti-mumps
Measles	anti-mumps

To keep the mixture at $4\text{ }^{\circ}\text{C}$ - $8\text{ }^{\circ}\text{C}$ for 90 minutes to obtain due neutralization. To inoculate each dilution in a microplate, in 10 wells, with susceptible cell suspension. For titration of the mumps virus and measles virus, the inoculation is performed in Vero cells, whereas rubella virus occurs in RK-13 cells. To incubate the microplate containing Vero cells at $36\text{ }^{\circ}\text{C}$ for 10 days and the microplates containing RK-13 cell at $32\text{ }^{\circ}\text{C}$ - $33\text{ }^{\circ}\text{C}$ for 12 days. To observe the cell cultures for the presence or absence of CPE and calculate titer of each virus in the vaccine according to proven statistical method. The potency of each virus is the value of the geometric mean in the vials analyzed, expressed in CCID₅₀ (50% infectious dose in cell culture) per dose. For valid determination, it is necessary that (a) at the end of the test, the control of cell culture presents unchanged monolayer; (b) the variation of the potency between the two samples of the vaccine is at most 0.5 log_{10} CCID₅₀ for each type of virus; (c) the variation in the titer of the reference vaccine is at most 0.5 log_{10} CCID₅₀ in relation to its mean titer for each type of virus; (d) the CPE is decreasing in relation to the increasing dilutions; and (e) the dilutions used in the test are between 10% and 90% of inoculated cell cultures.

The potency of the vaccine is at least $10^{3.7}$ CCID₅₀/dose for mumps virus and 10^3 CCID₅₀/dose for measles and rubella viruses. If the product fails to meet the potency requirements, the test is repeated for each type of virus not approved (no titer limit). The potency of the vaccine is the geometric mean of the two tests performed.

The method of plaque-forming units (PFU) can also be used. The PFU's and CCID₅₀'s potency value for approval of the product has to be correlated.

THERMOSTABILITY

The test and *Dosage* are simultaneously performed. To incubate the sample of the vaccine at $37\text{ }^{\circ}\text{C}$ for seven days,

for analyzing according to the methodology described for the potency of the product. The vaccine can not lose more than $1.0 \log_{10}$ CCID₅₀/dose, in relation to the titer determined in the sample stored in appropriate temperature conditions for each type of virus. Moreover, it must not present titer below to that specified for the approval of the *Dosage*. If it fails to meet the requirement, repeat the test. The final titer is the average of both tests conducted.

PACKAGING AND STORAGE

In accordance with the monograph *Vaccines for human use*.

LABELLING

To comply with the legislation in force.

VACCINE, MEASLES, RUBELLA *Vaccinum rubellae et morbillorum vivum*

The vaccine consists of a mixture of attenuated live measles and rubella viruses and is lyophilized. After reconstitution with an appropriate diluent, it presents an appearance of clear homogeneous suspension, and can demonstrate color due to the presence of a pH indicator.

Each viral component in the vaccine is produced separately, as described in the specific monographs.

The vaccine is bottled in suitable containers, lyophilized, labeled, and subjected to the controls required.

IDENTIFICATION

To reconstitute the vaccine with suitable diluent and add an equal volume of mixture of serum containing neutralizing antibodies to rubella and measles viruses. To keep at 4 °C - 8 °C for 90 minutes. To inoculate into susceptible cells culture and keep it for 10 days. As a control test, the cell cultures inoculated with the vaccine non-neutralized with the mixture of sera containing antibodies to both types of viruses and other non-inoculated must show the presence and absence of cytopathogenic effect (CPE), respectively. The absence of CPE in the cell cultures inoculated with the mixture of vaccine plus antibodies identifies the product.

PHYSICAL AND CHEMICAL TESTS

Residual moisture. To proceed as described in the monograph *Vaccines for human use*. At most 3%.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

DOSAGE

To proceed it under direct light. To dilute two samples of the vaccine to be analyzed and a sample of the reference

vaccine at intervals of at most $1.0 \log_{10}$ in a suitable culture medium.

To inoculate each dilution in a microplate, in 10 wells, with susceptible cell suspension. For titration of the measles virus, the inoculation is performed in Vero cells, whereas rubella virus occurs in RK-13 cells. To incubate the microplate containing Vero cells at 36 °C for 10 days and the microplates containing RK-13 cell at 32 °C - 33 °C for 12 days. To observe the cell cultures for the presence or absence of CPE and calculate titer of each virus in the vaccine according to proven statistical method.

The potency of the vaccine is the geometric mean value of the vials analyzed, expressed in CCID₅₀ (50% infectious dose in cell culture) per dose. For valid determination, it is necessary that (a) at the end of the test, the control of cell culture presents unchanged monolayer; (b) the variation of the potency between the two samples of the vaccine is at most $0.5 \log_{10}$ CCID₅₀ for each type of virus; (c) the variation in the titer of the reference vaccine is at most $0.5 \log_{10}$ CCID₅₀ in relation to its mean titer for each type of virus; (d) the CPE is decreasing in relation to the increasing dilutions; and (e) the dilutions used in the test are between 10% and 90% of inoculated cell cultures.

The potency of the vaccine must be at least $10^{3.7}$ CCID₅₀/dose for the Biken Cam 70 strain and 10^3 CCID₅₀/dose for the other strains of rubella and measles viruses. If the product fails to meet the potency requirements, the test is repeated for each type of virus not approved (no titer limit). The potency of the vaccine is the geometric mean of the two tests performed.

The method of plaque-forming units (PFU) can also be used. The PFU's and CCID50's potency value for approval of the product has to be correlated.

THERMOSTABILITY

The test and *Dosage* are simultaneously performed. To incubate the sample of the vaccine at 37 °C for seven days, for analyzing according to the methodology described for the potency of the product. The vaccine can not lose more than $1.0 \log_{10}$ CCID₅₀/dose, in relation to the titer determined in the sample stored in appropriate temperature conditions for each type of virus. Moreover, it must not present titer below to that specified for the approval of the *Dosage*. If it fails to meet the requirement, repeat the test. The final titer is the average of both tests conducted.

PACKAGING AND STORAGE

In accordance with the monograph *Vaccines for human use*.

LABELLING

To comply with the legislation in force.

VACCINE, ATTENUATED VARICELLA

Vaccinum varicellae vivum

The vaccine consists of live attenuated virus of the OKA strain and is lyophilized. After reconstitution with an appropriate diluent, it presents an appearance of clear homogeneous suspension, and can demonstrate color due to the presence of a pH indicator.

The production of the vaccine is based on a seed lot system and the strain of virus used may not induce neuro-pathogenicity in monkeys susceptible to varicella virus. Besides, the viral strain used in the production must demonstrate adequate immunogenicity and safety for humans. Replication of the virus is performed on susceptible human diploid cell culture. The viral suspension is identified and monitored for sterility. After clarification of the viral suspension by suitable method to remove cell debris, some stabilizing substances are added. They definitely do not change the efficacy and safety of the product. Before bottling and lyophilization, the product is analyzed for sterility, concentration of virus, and proteins derived from the animal serum.

The vaccine is bottled in suitable containers, lyophilized, labeled, and subjected to the controls required.

Further information on the criteria of production and its controls are indicated in the monograph *Vaccines for human use*.

IDENTIFICATION

When neutralized with serum containing antibody specific for varicella virus, the vaccine inhibits the plaque-forming units (PFU) in susceptible cells, as described in *Dosage*.

PHYSICAL AND CHEMICAL TESTS

Residual moisture. To proceed as described in the monograph *Vaccines for human use*. At most 3%.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

DOSAGE

At least two vials of lyophilized vaccine and one vial of reference vaccine are subjected to the plaque-forming units (PFU) method. To dilute the vaccine by applying factor no greater than 4 and inoculate each dilution into at least 3 wells in a plate containing monolayer of susceptible human diploid cell culture. After performing adsorption for about 90 minutes at 37 °C in a CO₂ atmosphere (5%), the inoculums are removed. Then, a culture medium containing agarose or carboxymethylcellulose, at appropriate concentration, is added. Cells are incubated for 5-7 days at 37 °C in a CO₂ atmosphere (5%). After the incubation period, remove the growth medium, fix the cells with formaldehyde and stain with a vital dye.

The potency of the vaccine is calculated by averaging the number of plaques of at least two dilutions and the result is expressed in log PFU/dose. For valid determination, it is necessary that (a) the control of cell culture presents unchanged monolayer; (b) the variation of the potency between the two samples of the vaccine is at most 0.5 log₁₀ PFU; (c) the potency of the reference vaccine is within a range of 0.5 log₁₀ PFU in relation to its mean titer; and (d) the number of PFU is decreasing in relation to the increasing dilutions.

The potency is at least 2 x 10³ UFP/dose. If sample fails to meet the requirement, repeat the determination. The potency of the vaccine is the geometric mean of the two determinations carried out.

PACKAGING AND STORAGE

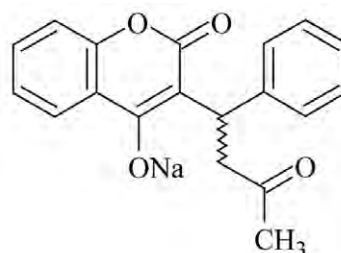
In accordance with the monograph *Vaccines for human use*.

LABELLING

To comply with the legislation in force.

WARFARIN, SODIUM

Warfarinum natricum



C₁₉H₁₅NaO₄; 330.31
warfarin, sodium; 09101
Sodium salt of 4-hydroxy -3-(3-oxo-1-phenylbutyl)-
-2H-1-benzopyran-2-one (1:1)
[129-06-6]

It contains at least 97.0% and no more than 102.0% of C₁₉H₁₅NaO₄, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White hygroscopic crystalline powder. It presents polymorphism.

Solubility. It is easily soluble in water and ethanol, soluble in acetone, slightly soluble in ethyl ether and chloroform.

Physical and chemical constants

Melting range (5.2.2): The precipitate obtained in the *Identification* test A., dried in an oven at 105 °C, melts at 159 °C - 163 °C.

IDENTIFICATION

A. To dissolve about 1 g of sample in 25 mL of water. To add 2 mL of hydrochloric acid and filter it. To use the filtrate to conduct the test. The absorption spectrum of the infrared (5.2.14) of the warfarin residue from the filtered, dispersed in mineral oil only has absorption maximum at the same wavelength, with the same relative intensities to those observed in the warfarin spectrum SQR similarly prepared.

B. To dissolve about 1 g of sample in 10 mL of water. To add 5 mL of nitric acid and filter it. To add 2 mL of potassium dichromate SR to the filtrate and stir for 5 minutes. Let it rest for 20 minutes. The solution obtained shows no blue-green color in comparison with white.

C. The filtrate used in the *Identification* test A. corresponds to identification reactions of the sodium ion (5.3.1.1).

D. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* method B. corresponds to that of main peak of *Standard solution*.

PURITY TESTS

Appearance of solution. To dissolve 1 g in 20 mL of water. The solution is clear (5.2.25) and colorless (5.2.12).

pH (5.2.19). 7.2 to 8.6. To determine in 1% aqueous solution (w/v).

Related substances. To proceed as described in *Thin-layer chromatography* (5.2.17.1) by using GF₂₅₄ silica gel as support and a mixture of glacial acetic acid, methylene chloride, and cyclohexane (20:50:50) as mobile phase. To apply separately to the plate 20 µL of each of the solutions freshly prepared, as described below.

Solution (1): to dissolve 0.20 g of the sample in acetone and dilute to 10 mL with the same solvent.

Solution (2): to dilute 2 mL of *Solution (1)* in 10 mL of acetone.

Solution (3): to dilute 1 mL of *Solution (2)* in 200 mL of acetone.

Solution (4): to dissolve 40 mg of the warfarin SQR in acetone and dilute to 10 mL with the same solvent.

Solution (5): to transfer 10 mg of acenocoumarol SQR and 1 mL of *Solution (1)* into 10 mL volumetric flask, dilute with acetone and top up with the same solvent.

To develop the chromatogram. To remove the plate, and let it drying. To examine chromatograms obtained under ultraviolet light (254 nm). Any spot in the chromatogram with *Solution (1)*, except for the principal spot, can not be more intense than that in the chromatogram with *Solution (3)* (0.1%). The test is not valid unless the chromatogram ob-

tained with *Solution (5)* shows two clearly separated spots, and the spot in the chromatogram obtained with *Solution (3)* is clearly visible.

Water (5.2.20.1). To determine in 1 g of the sample. At most 4.0%.

Heavy metals (5.3.2.3). To use *Method III*. To dissolve 4 g of the sample in 45 mL of water. To add 5 mL of glacial acetic acid and stir vigorously until the precipitate agglomerates. To filter and determine in 25 mL of the solution obtained, by using glacial acetic acid for pH adjustment. At most 0.001% (10 ppm).

Phenolic ketones. To weigh accurately about 1.25 g of the sample, transfer it to a 10 mL volumetric flask and dissolve it with 0.5 M sodium hydroxide. To top up it with the same solvent and make it homogeneous.

Let it rest for 15 minutes. To measure the absorbance of the resulting solution at 385 nm, by using 0.5 M sodium hydroxide to adjust zero. The absorbance at 385 nm is at most 0.20.

DOSAGE

A. To proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). To transfer, exactly, about 0.1 g of the sample to a 100 mL volumetric flask, dissolve and top up with 0.01 M sodium hydroxide. To dilute successively until reaching 0.001% concentration (w/v). To prepare a standard solution of warfarin sodium at the same concentration by using the same solvent. To measure the absorbance of the resulting solutions at 308 nm, by using 0.01 M sodium hydroxide to adjust zero. To calculate the C₁₉H₁₅NaO₄ concentration of the sample from the readings obtained.

B. To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using liquid chromatograph equipped with an ultraviolet detector at 280 nm; 250 mm column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group; flow of *Mobile phase* 1.0 mL/min.

Buffer pH 7.4: to dissolve 6.8 g of monobasic potassium phosphate in 250 mL of water. To add 160 mL of 0.2 M sodium hydroxide and top up with water to 1000 mL. To adjust pH to 7.4 with sodium hydroxide or phosphoric acid.

Mobile phase: mixture of methanol, water, and glacial acetic acid (68:32:1).

Diluent: mixture of *Buffer pH 7.4* and acetonitrile (85:15).

Sample solution: Dissolve accurately weighed amount of the sample with diluent to obtain a 1 mg/mL solution. To dilute successively in *Mobile phase*, to obtain 100 µg/mL solution.

Standard solution: to transfer 25 mg of warfarin SQR to 25 mL volumetric flask, add 15 mL of *Buffer pH 7.4* and leave it in ultrasound for 5 minutes to obtain a 1 mg/mL solution. To top up the volume with *Buffer pH 7.4*. To dilute successively in *Mobile phase*, to obtain 100 µg/mL solution.

Resolution Solution: to transfer 0.1 g of propylparaben SQR to 100 mL volumetric flask, add 50 mL of acetonitrile and leave it in ultrasound for 5 minutes. To top up with acetonitrile. To make it homogeneous. To transfer 2.5 mL of this solution to 25 mL volumetric flask, add 2.5 mL of 1 mg/mL *Standard solution* and top up with *Mobile phase*, thus obtaining a concentration of 100 µg/mL propylparaben and warfarin, respectively.

To inject replicates of 20 µL of *Resolution solution* and record the chromatograms. The resolution between the peaks of propylparaben and warfarin is not greater than 2.

The relative standard deviation for the replicate areas for the peaks recorded is not greater than 2.0%.

Procedure: To separately inject 20 µL of *Sample and standard solutions*, record the chromatograms, and measure the average area of the peaks. To calculate the content of $C_{19}H_{15}NaO_4$ from the sample, from the responses obtained for the *Sample and standard solutions*.

PACKAGING AND STORAGE

In containers protected from light.

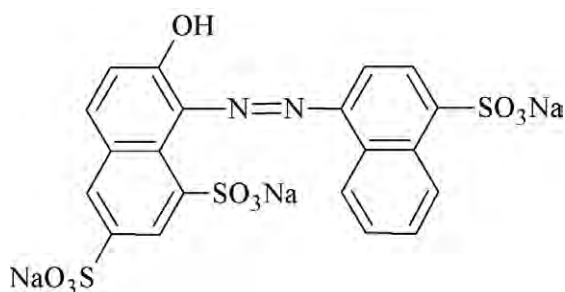
LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Anticoagulant.

PONCEAU 4R



$C_{20}H_{11}N_2Na_3O_{10}S_3$; 604,47
CI 16255

Sodium salt of 7-hydroxy-8-[2 - (4-sulfo-1-naphthalenyl) diazenil] -1,3-naphthalenedisulfonic (3:1)
[2611-82-7]

It contains at least 82% of $C_{20}H_{11}N_2Na_3O_{10}S_3$.

DESCRIPTION

Physical characteristics. Fine powder, red, hygroscopic. Red aqueous solution.

Solubility. It is soluble in water and methanol, and insoluble in ethanol, acetone, ether, and glycerol.

IDENTIFICATION

The ultraviolet absorption spectrum and visible (**5.2.14**), from 200 nm to 700 nm, 0.001% solution (w/v) in 0.02 M ammonium acetate (pH 5.6), exhibits maximum at 507 nm, 332 nm, 245 nm, and 215 nm, and minima at 375 nm, 300 nm, and 238 nm, similar to those observed in the spectrum of a similar solution of Ponceau 4R SQR.

PURITY TESTS

Subsidiary dyes. To proceed as described in *Thin-layer chromatography* (**5.2.17.1**) by using G silica gel as support and a mixture of 1-butanol, ethanol, water, and ammonium hydroxide (50:25:25:10) as mobile phase. To apply separately to the plate 2 µL of each of the solutions freshly prepared, as described below.

Solution (1): 10 mg/mL solution of the sample in water.

Solution (2) 10 mg/mL solution of Ponceau 4R in water.

Solution (3): to dilute 1 mL of *Solution (2)* to 50 mL with water.

To develop the chromatogram. To remove the plate, and let it drying. To examine at ambient light, under ultraviolet light (254 nm). The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*. Any secondary spot in the chromatogram obtained with *Solution (1)*, other than the principal spot, is not more intense than that obtained with *Solution (3)* (2%).

Lead, copper, tin, zinc. To proceed as described in *Atomic absorption spectrophotometry* (**5.2.13**). To weigh 2 g of the sample in a silica crucible and burn softly on asbestos canvas ($\pm 350^\circ\text{C}$); take it to the oven for 12 hours, at maximum temperature of 450°C . To remove the crucible and let it cool down. To mix the residue with about 2 mL of water and add two drops of 50% magnesium nitrate (w/v). To dry it over the electric plate and return to oven for 3 - 4 hours, or until the residue is white or yellowish. Then, cool down, drip 1 to 2 mL of nitric acid and 1 mL of water, and heat on a electric plate until almost dry it. To dissolve the metal nitrate with 5 mL of water. If necessary, centrifuge it. To take it to an atomic absorption spectrophotometer, previously calibrated, to read the concentration of both metals. Maximum of 0.001% (10 ppm) lead, 0.002% (20 ppm) copper, 0.025% (250 ppm) tin, and 0.005% (50 ppm) zinc.

Chlorides and sulfates. To weigh 0.5 g of sample, dissolve in 200 mL of water, acidify with 8 mL of 25% nitric acid (v/v), and titrate with 0.1 M SV silver nitrate in a potentiometer pot with combined silver electrode. Each mL of 0.1 M SV silver nitrate is equivalent to 5.85 mg of NaCl.

To weigh 0.5 g of the sample and dissolve it in 100 mL of water, in a water bath. To add 35 g of sulfate-free sodium chloride, and stir it well. To transfer to 200 mL volumetric flask and top up with saturated sodium chloride solution. To make it homogeneous. After 1 hour, filter it by using a filter paper and transfer 100 mL aliquot of the filtrate to 600 mL beaker, dilute to 300 mL with water, and acidify with hydrochloric acid SR, by adding slight excess. To heat to boiling and by stirring drip 25 mL of 12% barium chloride (w/v) or until no further precipitation occurs. Let it rest for 4 hours. To separate barium sulfate by filtration, wash with hot water, dry the paper with the residue, transfer to dry crucible previously weighed, and calcined in an oven,

at 500 °C for 1 hour. To cool down in desiccator and weigh it. To calculate the sulfate content, use the following equation:

$$\frac{N \times 0,6085 \times 100}{p} = \% \text{ sulfates}$$

where

N = barium sulfate in grams;

p = sample used in the precipitation in grams. Maximum of 8% of chlorides and sulfates.

Substances insoluble in water. To dissolve by stirring 5 g of the sample in 200 mL of hot water (80-90 °C). To cool down at room temperature. To filter it by filter plate, previously dried and weighed. To wash with cold water until the washings become colorless. To dry the filter with the residue in an oven at 120 °C for 4 hours and weigh it. At most 0,2%.

Heavy metals (5.3.2.3). To use *Method III*. To determine 0.5 g of the sample. At most 0.004% (40 ppm).

Arsenic (5.3.2.5). To use *Method I*. To determine 1 g of the sample. At most 0.0001% (1 ppm).

Loss on drying (5.2.9). To determine 0.5 g of the sample.

To desiccate in an oven at 120 °C for 4 hours or at 135 °C for 3 hours. At most 10%.

DOSAGE

To proceed as described in *Visible absorption spectrophotometry (5.2.14)*. To prepare sample solution as described in *Identification*. To prepare a standard solution at the same concentration by using the same solvent. To measure the absorbance of the resulting solutions at 507 nm, by using 0.02 M ammonium acetate (pH 5.6) to adjust zero. To calculate the $C_{20}H_{11}N_2Na_3O_{10}S_3$ concentration of the sample

from the readings obtained. Alternatively, make the calculations taking into account that $A(1\%, 1\text{ cm}) = 442.5$ at 507 nm, in 0.02 M ammonium acetate (pH 5.6).

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

CATEGORY

Dye.

PONCEAU 4R, ALUMINUM LAKE

Dye consists mainly of sodium salt of 7-hydroxy-8-[2 - (4-sulfo-1-naphthalenyl) diazenil] -1,3 - naphthalene (3:1) - Ponceau red 4R - over

alumina substrate. It contains at least 95% and at most 105% of dye content stated on the label.

DESCRIPTION

Physical characteristics. Fine powder, somewhat red. Hygroscopic.

Solubility. It is practically insoluble in water and ethanol. It is soluble in M sodium hydroxide, but the dye decomposes slowly at this alkaline pH.

IDENTIFICATION

A. The ultraviolet absorption spectrum and visible (**5.2.14**), from 200 nm to 700 nm, 0.001% solution (w/v) in 0.02 M ammonium acetate (pH 5.6), exhibits maximum at 507 nm, 332 nm, 245 nm, and 215 nm, and minima at 375 nm, 300 nm, and 238 nm, similar to those observed in the spectrum of a similar solution of Ponceau 4R SQR.

B. To transfer 0.15g of the sample to 60 mL beaker and dissolve it in about 20 mL of 30% acetic acid (w/v), hot, until it becomes opalescent. To cool down and divide the solution into two tubes. To add 2 mL of morin solution in 3 mg/mL ethanol, freshly prepared, in one of them. To observe the green fluorescence developing under ultraviolet light (254 nm), by comparing with the non-reactive tube.

PURITY TESTS

Subsidiary dyes. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using G silica gel as support and a mixture of 1-butanol, ethanol, water, and ammonium hydroxide (50:25:25:10) as mobile phase. To apply separately to the plate 2 µL of each of the solutions freshly prepared, as described below.

Solution (1): 0.25 g of the sample in 10 ml of 0.5 M sodium hydroxide.

Solution (2): 0.05 g of standard Ponceau 4R in 10 ml of 0.5 M sodium hydroxide.

Solution (3): to dilute *Solution (2)* so as to obtain a 1.0 mg/ml solution with the same diluent.

Solution (4): to dilute *Solution (1)* so as to obtain a 0.5 mg/ml solution with the same diluent.

To develop the chromatogram. To remove the plate, and let it drying. To examine at ambient light, under ultraviolet light (254 nm). The principal spot obtained with *Solution (1)* corresponds in position, color, and intensity to that obtained with *Solution (2)*. Any secondary spot in the chromatogram obtained with *Solution (1)* must not be more intense than that obtained with *Solution (3)* and *Solution (4)* (2%).

Chlorides and sulfates. To weigh 10 g of the sample, stir it with 250 mL of water, and leave it in contact for 30 minutes. To filter it. To measure 50 mL of the filtrate, equivalent to 2 g of the sample, dilute to 200 ml with water, and acidify with 8 ml of

25% nitric acid (v/v), and titrate with 0.1 M SV silver nitrate in a potentiometer pot with combined silver electrode. Each mL of 0.1 M SV silver nitrate is equivalent to 5.85 mg of NaCl.

To measure remaining 50 mL of the filtrate, dilute to 300 mL with water, acidify with hydrochloric acid SR plus 1 mL of excess. To heat to boiling and by stirring drip 25 mL of 12% barium chloride (w/v). Let it rest for 4 hours. To separate barium sulfate by filtration, wash with hot water, dry the paper with the residue, transfer to dry crucible previously weighed, and calcined in an oven, at 500 °C for 1 hour. To cool down in desiccator and weigh it. The calculate the sulfate content, use the following equation:

$$\frac{N \times 0,6086 \times 100}{p} = \% \text{ sulfates}$$

where

N = barium sulfate in grams;

p = sample used in the precipitation in grams.

Maximum of 2% of chlorides and sulfates.

Loss on drying (5.2.9). To weigh about 0.5 g. To desiccate the sample at 120 °C for 4 hours or at 135 °C for 3 hours. At most 20%.

Sulfated ashes (5.2.10). To determine in 0.1 of the sample.

It should contain between 40 and 55%.

DOSAGE

To dilute as described in Method A. in *Identification*, and read the absorbance at the peak maximum at around 507 nm (5.2.14). To calculate the dye content by the following equation:

$$\frac{A \times 100}{442,5 \times p} = \% \text{ de vermelho ponceau 4R na amostra em } 507 \text{ nm}$$

where

p = sample weight in grams in the dilution made.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

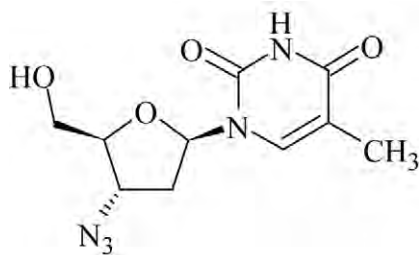
To comply with the legislation in force.

CATEGORY

Dye.

ZIDOVUDINE

Zidovudinum



$C_{10}H_{13}N_5O_4$; 267.24
 zidovudine; 09256
 3'-Azido-3'-deoxythymidine
 [30516-87-1]

It contains at least 97.0% and no more than 102.0% of $C_{10}H_{13}N_5O_4$, in relation to the desiccated substance.

DESCRIPTION

Physical and chemical characteristics. Crystalline and brown powder. It presents polymorphism. *Melting temperature (5.2.2):* around 124 °C.

Solubility. It is slightly soluble in water, and soluble in ethanol.

Physical and chemical constants.

Specific rotation (5.2.8): +60,5° to +63,0°, at 25 °C in relation to the desiccated substance. To determine at 1% solution (w/v) in ethanol.

IDENTIFICATION

A. The infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium chloride only has absorption maximum at the same wavelength, with the same relative intensities to those observed in the zidovudine SQR similarly prepared.

PURITY TESTS

Appearance of solution. To dissolve 0.5 g in 50 mL of water, by heating, if necessary. The solution is not darker than that mixture of 1 mL of *Standard solution color SC G (5.2.12)* with 7 mL of water.

Related substances 1. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF₂₅₄ silica gel as support and a mixture of methanol and methylene chloride (10:90) as mobile phase. To apply separately to the plate 10 µL of each of the solutions freshly prepared, as described below.

Solution (1): to dissolve 0.2 g of the sample in methanol and dilute to 10 mL with the same solvent.

Solution (2): to dissolve in methanol 20 mg of thymine SQR, 20 mg of impurity A (1 - [(2R, 5S)-5-hydroxymethyl-2, 5 - dihydro-2-furyl]-5-methyl pyrimidine-2, 4 (1H, 3 #-)-dione), 20 mg of triphenylmethanol, add 1 mL of *Solution (1)* and dilute to 100 mL with methanol.

Solution (3): to dilute 5 mL of *Solution (2)* to 10 mL with methanol.

To develop the chromatogram. To wait for the rise of the solvent up to 12 cm above the line of application. To remove the plate, allow to air dry for 5 minutes, and examine under ultraviolet light (254 nm). In the chromatogram obtained with *Solution (1)*, the spot corresponding to impurity A is not more intense than the corresponding spot in the chromatogram obtained with *Solution (3)* (0.5%). Any spot, except for the principal one and spots corresponding to zidovudine and thymine impurities are not more intense than the spot corresponding to zidovudine in the chromatogram obtained with *Solution (3)* (0.5%). To aerosolize the plate with a 1% vanillin solution (w/v) in sulfuric acid. In the chromatogram obtained with *Solution (1)* any spot corresponding to the triphenylmethanol is no more intense than the corresponding spot in the chromatogram obtained with *Solution (3)* (0.5%). The test is valid if the chromatogram obtained with *Solution (2)* presents 4 clearly separated spots corresponding to thymine SQR, to impurity A, to zidovudine, and to triphenylmethanol, in order of increasing retention factor (R_f).

Related substances 2. To proceed as described in *Dosage*. To prepare the solutions as described below:

Test solution: to dissolve accurately weighed amount of the sample in *Mobile phase* to obtain a 1 mg/mL solution.

Diluted test solution: to transfer to 0.25 mL of *Test solution* to 50 mL volumetric flask and top up with *Mobile phase*.

Thymine solution: to dissolve accurately weighed amount of thymine SQR in methanol to obtain a 0.2 mg/mL solution. To transfer 5 mL of this solution to 50 mL volumetric flask and top up with *Mobile phase*, thus obtaining 20 µg/mL.

Impurity B solution: to dissolve accurately weighed amount of impurity B (1 - (3-chlorine-2,3-dideoxy-β-D-ribofuranosyl)-5-methyl pyrimidine-2, 4 (1H, 3H)-dione) to obtain a 1 mg/mL solution. to transfer to 5 mL of this solution to 50 mL volumetric flask and top up with *Mobile phase*.

To inject replicates of 10 µL of *Resolution solution* described in *Dosage*. The tailing factor should not be greater than 1.5 to zidovudine peak and impurity B peak. The resolution between zidovudine and impurity B should not be less than 1.4. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2.0%.

Procedure: To separately inject 10 µL of each of the solutions described above. To record the chromatograms 1.5 times the retention time of the main peak obtained with the

Test solution. The substances are eluted in the following order: thiamine, zidovudine, and impurity B. In the chromatogram obtained with the *Test solution*, the area of any peak corresponding to thymine is not greater than the area under the peak in the chromatogram obtained with *Thymine solution* (2.0%). The area of any peak corresponding to impurity B obtained with *Test solution* is not greater than the area under the corresponding peak in the chromatogram obtained with *Impurity B solution* (1.0%). The area of any other peak obtained with the *Test solution*, except the main peak, is not greater than the area under the peak of the chromatogram obtained with the *Diluted test solution* (0.5%). The sum of the areas of all the peaks, except the main peak, obtained with *Test solution*, is not greater than 6 times the area under the peak obtained with the *Diluted test solution* (3.0 %). To discard any peak with an area less than 10% of the area under the peak obtained in the chromatogram of the *Diluted test solution*.

Heavy metals (5.3.2.3). To determine in 1 g of the sample. To transfer to a silica crucible and mix it with 0.5 g of magnesium oxide. To incinerate to dark red on flame. To proceed to obtain homogeneous white or gray mass. If after 30 minutes of ignition color remains as before, cool down, use a glass rod to mix it in the crucible, and then incinerate again. To incinerate in an oven at 500-600 °C for about 1 hour. The residue obtained is dissolved with twice 5 mL of 0.2 M hydrochloric acid, by adding 0.1 mL of phenolphthalein SI. To add 6 M ammonium hydroxide until the pink color appears. To cool it down. To bleach the solution with glacial acetic acid and add another 0.5 mL of the acid. If necessary, filter and dilute the solution with water to a volume of 20 mL. To transfer 12 mL of the solution obtained to a Nessler tube, add 2 mL of acetate buffer pH 3.5 and immediately make it homogeneous.

Standard preparation: to mix 2 mL of *Standard lead solution* (10 ppm Pb) with 0.5 g of magnesium oxide in a silica crucible. Then, dry the mixture in an oven at 105 °C To incinerate it afterwards. To proceed the technique to prepare the sample from "The residue thus obtained is dissolved..." To add 2 ml of the sample solution to 10 mL of the solution obtained.

Procedure: To add 1.2 mL of thioacetamide SR to each tube related to the standard and sample. The brown color that develops in the sample should not be more intense than that obtained with the standard one. At most 0.002% (20 ppm).

Loss on drying (5.2.9). To determine in 1 g of the sample. To desiccate in an oven at 100 °C - 105 °C until obtaining constant weight. At most 1 %.

Sulfated ashes (5.2.10). To determine in 1 g of the sample. At most 0,25%.

DOSAGE

To proceed as described in *High performance liquid chromatography* (5.2.17.4), by using chromatograph equipped

with an ultraviolet detector at 265 nm; 250 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm); flow of *Mobile phase* of 1.2 mL/min.

Mobile phase: mixture of methanol and water (20:80).

Sample solution: to dissolve accurately weighed amount of the sample in *Mobile phase* to obtain a 1 mg/mL solution. To transfer 10 mL of this solution to 50 mL volumetric flask and top up with *Mobile phase*, thus obtaining 0,2 mg/mL solution.

Standard solution: to dissolve accurately weighed amount of zidovudine SQR in *Mobile phase* to obtain a 0,2 mg/mL solution.

Resolution Solution: to transfer 5 mg of impurity B (1 - (3-chlorine -2,3-dideoxy-β-D-ribofuranosyl)-5- methyl pyrimidine 2.4-(1H, 3H)-dione) to a 50 mL volumetric flask, add 25 mL of the *Standard solution* and top up with *Mobile phase*.

To inject replicates of 10 µL of the *Resolution solution*. The tailing factor should not be greater than 1.5 to zidovudine peak and impurity B peak. The resolution between zidovudine and impurity B should not be less than 1.4. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2.0%.

Procedure: to separately inject 10 µL of *Sample and standard solutions*, record the chromatograms, and measure the average area under the peaks. To calculate the content of C₁₀H₁₃N₅O₄ from the sample, from the responses obtained for the *Sample and standard solutions*.

PACKAGING AND STORAGE

To store in tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

THERAPEUTIC CLASS

Antiretroviral.

ZIDOVUDINE, CAPSULES

It contains at least 90.0 % and at most 110 % of the amount stated of C₁₀H₁₃N₅O₄.

IDENTIFICATION

A. To weigh capsules, remove the content, and weigh them again. To make the contents of the capsules homogeneous.

To transfer amount of powder equivalent to 0.3 g of zidovudine to 200 mL volumetric flask. To add 50 mL of

methanol-water (75:25), leave it in ultrasound for 5 minutes, and top up with methanol. To decant and successively dilute the supernatant with methanol-water (75:25) to the 0.0015% concentration (w/v). The ultraviolet absorption spectrum (5.2.14), for the solution obtained in the range from 200 nm to 400 nm, exhibits maxima and minima only in the same wavelength of a similar zidovudine SQR solution.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Procedure: to separately inject 10 µL of *Solutions* (1) and (2), record the chromatograms, and measure the areas under the peaks. To calculate the amount, in mg, of thymine in the sample from the equation.

$$1000 \times C \times \left[\frac{r_1/r_2}{T} \right]$$

where

C = concentration in mg/mL of thymine in *Solution* (2);
 r_1 and r_2 = response of peaks relating to the thymine from *Solutions* (1) and (2), respectively;
 T = amount of zidovudine, in milligrams, in the mass of powder used to prepare the *Solution* (1), as set out in *Dosage*. At most 3,0%.

Uniformity of dosage units (5.1.6). It complies with the test.

Procedure for content uniformity. To proceed as described in *Dosage*. To prepare the *Sample solution* as described below.

Sample solution: to individually weigh each capsule, transfer the contents to a 100 mL volumetric flask, and weigh it again. To add 30 mL of methanol-water (75:25), leave it in ultrasound for 20 minutes, and top up with the same solvent. To filter it. To dilute with the same solvent to a 1 mg/mL concentration.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL

Equipment: blades, 50 rpm

Time: 45 minutes

Procedure: After testing, remove aliquot from the dissolution medium, filter and dilute, if necessary, in metha-

nol-water (75:25) to reach suitable concentration. Then, proceed as described in *Dosage*. To calculate the amount of $C_{10}H_{13}N_5O_4$ dissolved in the medium from the responses obtained from *Sample and standard solutions*.

Tolerance: not less than 75% (Q) of the stated amount of $C_{10}H_{13}N_5O_4$ dissolves within 45 minutes.

PURITY TESTS

Limit of thymine. To proceed as described in *Dosage*. To prepare *Solutions* (1) and (2) as described below.

Solution (1): to use the *Sample solution* obtained in *Dosage*.

Solution (2): to use the *Standard solution* obtained in *Dosage*.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3). It complies with the test.

DOSAGE

To proceed as described in *High performance liquid chromatography* (5.2.17.4). To proceed as described in *Dosage*, in the *Zidovudine* monograph. To prepare the *Sample and standard solutions* as described below.

Sample solution: to weigh 20 capsules, remove the content, and weigh them again. To make the content of the capsules homogeneous. To transfer amount of powder equivalent to 100 mg of zidovudine to 100 mL volumetric flask, and add 30 mL of mixture of methanol and water (75:25). To leave it in ultrasound for 20 minutes and top up with the same solvent. To filter it. To transfer 10 mL of the filtrate to a 100 mL volumetric flask and top up with the same solvent.

Standard solution: To dissolve 10 mg of thymine in methanol and quantitatively transfer it to 50 mL volumetric flask. To top up with the same solvent. To transfer 1 mL of this solution and 10 mg of zidovudine SQR to 100 mL volumetric flask, dissolve 25 mL of mixture of methanol and water (75:25), and top up with the same solvent. To make it homogeneous.

To inject replicates of 10 µL of the *Standard solution*. The relative retention times are about 0.2 for thymine and 1.0 for zidovudine. The resolution between zidovudine and thymine peaks should not be less than 5.0. The tailing factor to zidovudine peak should not be greater than 2.0. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2 %.

Procedure: to separately inject 10 µL of *Sample and standard solutions*, record the chromatograms, and measure the

the areas under the peaks. To calculate the amount of $C_{10}H_{13}N_5O_4$ in the capsules from the responses obtained from *Sample and standard solutions*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

ZIDOVUDINE, INJECTABLE SOLUTION

It contains at least 90.0 % and at most 110 % of the amount stated of $C_{10}H_{13}N_5O_4$.

IDENTIFICATION

A. To transfer the volume of injectable solution equivalent to 20 mg of zidovudine SQR to 200 mL volumetric flask, and top up with a mixture of methanol and water (75:25). To transfer 15 mL of this solution to 100 mL volumetric flask and top up with the same solvent. To make it homogeneous. The ultraviolet absorption spectrum (5.2.14) of the resulting solution, ranging from 200 nm to 400 nm, exhibits maxima and minima only in the same wavelength of a similar zidovudine SQR solution.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peak of *Standard solution*.

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

pH (5.2.19). 3.0 to 4.0. To determine in mixture the volume of the injectable solution containing 0.15 g of zidovudine and 5 mL of 0.12 M potassium chloride.

BIOLOGICAL SAFETY TEST

Sterility (5.5.3.2.1). It complies with the test.

Bacterial endotoxins (5.5.2.2). At most 1.0 UE/ mg of zidovudine.

PURITY TESTS

Limit of thymine. To proceed as described in *Dosage*. To prepare *Solutions (1)* and *(2)* as described below.

Solution (1): to use the *Sample solution* obtained in *Dosage*.

Solution (2): to use the *Sample solution* obtained in *Dosage*.

Procedure: to separately inject 10 µL of *Solutions (1)* and *(2)*, record the chromatograms, and measure the areas under the peaks. To calculate the amount, in mg, of thymine in the sample from the equation.

$$1000 \times C \times \left[\frac{r_1/r_2}{Q} \right]$$

where

C = concentration in mg/mL of thymine in *Solution (2)*;

r_1 and r_2 = response of peaks relating to the thymine from *Solutions (1)* and *(2)*, respectively;

Q = amount of zidovudine, in milligrams, in the volume used in injectable solution used to prepare the *Solution (1)*.

At most 1.0%.

DOSAGE

To proceed as described in the *Zidovudine* monograph, in *Dosage*. To prepare the *Sample and standard solutions* as described below.

Standard solution: to dissolve 10 mg of thymine SQR in methanol and dilute to 50 mL with the same solvent. To transfer 1 mL of this solution and 10 mg of zidovudine SQR to 100 mL volumetric flask, dissolve 25 mL of mixture of methanol and water (75:25), and top up with the same solvent. To make it homogeneous.

Sample solution: to transfer the exact volume of injectable solution equivalent to about 25 mg of zidovudine to 250 mL volumetric flask and top up with *Mobile phase*. To make it homogeneous.

Procedure: to separately inject 10 µL of *Sample and standard solutions*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of $C_{10}H_{13}N_5O_4$ in the injectable solutions from the responses obtained from *Sample and standard solutions*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELLING

To comply with the legislation in force.

ZIDOVUDINE, ORAL SOLUTION

It contains at least 90.0 % and at most 110 % of the amount stated of $C_{10}H_{13}N_5O_4$.

IDENTIFICATION

A. To proceed as described in *Thin-layer chromatography (5.2.17.1)* by using GF_{254} silica gel as support and a mixture of acid butyl, n-heptane, acetone and ammonium hydroxide

(40:30:30:10) as *Mobile phase*. To apply separately, as band, 5 μ L of each of the solutions freshly prepared, as described below.

Solution (1): 5 mg/mL solution of the sample in mixture of methanol and water (75:25).

Solution (2): 5 mg/mL solution of zidovudine SQR in mixture of methanol and water (75:25).

To develop the chromatogram. To remove the plate, and let it drying. To examine under ultraviolet light (254 nm). The principal spot obtained with *Solution (1)* corresponds in position and intensity to that obtained with *Solution (2)*.

B. The retention time of the main peak in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of peak of *Standard solution*.

CHARACTERISTICS

Determination of volume (5.1.2). It complies with the test.

pH (5.2.19). 3.0 to 4.0. To determine in volume of oral solution containing 0.15 g of zidovudine plus 5 mL of 0.12 M potassium chloride.

BIOLOGICAL SAFETY TEST

Counting of the total number of mesophyll aerobic micro-organisms (5.5.3.1.2). It complies with the test.

Survey of pathogenic micro-organisms (5.5.3.1.3).

It complies with the test.

PURITY TESTS

Limit of thymine. To proceed as described in *Dosage*. To prepare *Solutions (1)* and *(2)* as described below.

Solution (1): to use the *Sample solution* obtained in *Dosage*.

Solution (2): to use the *Sample solution* obtained in *Dosage*.

Procedure: to separately inject 10 μ L of *Solutions (1)* and *(2)*, record the chromatograms, and measure the areas under the peaks. The area related to the thymidine peak from *Solution (1)* is not greater than 3% of the area related to the thymidine peak from *Solution (2)*.

DOSAGE

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 240 nm; 125 mm chromatograph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μ m), at room temperature; flow of *Mobile phase* of 1 mL/min.

Mobile phase: mixture of 0.04 M sodium acetate, methanol, acetonitrile, and glacial acetic acid (900:90:10:2).

Sample solution: to transfer the volume of zidovudine (oral solution) equivalent to 0,1 mg of zidovudine to 100 mL volumetric flask, and top up with *Mobile phase*. To make it homogeneous. to transfer 5 mL of this solution to 50 mL volumetric flask and top up with *Mobile phase*. To make it homogeneous.

Stock standard solution: 1 mg/mL zidovudine SQR solution in *Mobile phase*.

Thymine standard solution: to transfer the exact amount of about 20 mg of thymine to 200 mL volumetric flask. To add 150 mL of *Mobile phase* and leave it in ultrasound for 10 minutes. To top up with *Mobile phase*. To make it homogeneous.

Standard solution: to transfer 10 mL of this *Stock standard solution* and 2 mL of *Thymine standard solution* to 100 mL volumetric flask and top up with *Mobile phase*. To make it homogeneous.

To inject replicates of 10 μ L of the *Standard solution*. The relative retention times are about 0.12 for thymine and 1.0 for zidovudine. The resolution between thymine and zidovudine peaks should not be less than 4.0. The tailing factor to zidovudine peak should not be greater than 2.0. The relative standard deviation for replicate areas of the peaks recorded is not greater than 2.0 %.

Procedure: to separately inject 10 μ L of *Sample solution* and *Standard solution*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of zidovudine ($C_{10}H_{13}N_5O_4$) in oral solution from the responses from the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

ZIDOVUDINE AND LAMIVUDINE, TABLETS

It contains at least 90.0 % and at most 110 % of the amount stated of zidovudine ($C_{10}H_{13}N_5O_4$) and lamivudine ($C_8H_{11}N_3O_3S$).

IDENTIFICATION

The retention times of the main peaks in the chromatogram of the *Sample solution* obtained in the *Dosage* corresponds to that of main peaks of *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). It complies with the test.

Hardness Test (5.1.3.1). It complies with the test.

Friability test (5.1.3.2). It complies with the test.

Disintegration test (5.1.4.1). It complies with the test.

Uniformity of dosage units (5.1.6). It complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL

Equipment: blades, 50 rpm

Time: 60 minutes

Procedure: After testing, remove aliquot from the dissolution medium and dilute, if necessary, in *Mobile phase* to reach suitable concentration. To proceed as described in *Dosage*.

Tolerance: not less than 80% (Q) of the stated amount of zidovudine ($C_{10}H_{13}N_5O_4$) and lamivudine ($C_8H_{11}N_3O_3S$) dissolve within 60 minutes.

DOSAGE

To proceed as described in *High performance liquid chromatography (5.2.17.4)*, by using chromatograph equipped with an ultraviolet detector at 270 nm; 125 mm chromato-

graph column and 4.6 mm inner diameter, packed with silica chemically bonded to the octadecylsilane group (5 μ m), at room temperature; flow of *Mobile phase* of 1 mL/min.

Mobile phase: mixture of 0.1 M sodium acetate buffer, methanol, and glacial acetic acid (65:35:0.1).

Sample solution: to weigh and spray 20 tablets. To transfer amount of powder equivalent to 75mg

of zidovudine and 37.5 mg of lamivudine to 100 mL volumetric flask. To add 70 mL of water and leave it in ultrasound for 30 minutes. To top up with the same solvent, make it homogeneous, and filter it. To transfer 1 mL of the filtrate to 25 mL volumetric flask and top up with *Mobile phase*, thus obtaining 30 μ L/mL of zidovudine and 15 μ L/mL of lamivudine.

Stock standard solution: To accurately weigh about 75 mg of zidovudine SQR and 37.5 mg of lamivudine SQR and transfer to a 100 mL volumetric flask by using 70 mL of water. To leave it in ultrasound for 15 minutes and top up with the same solvent, thus obtaining 0.75 mg/mL zidovudine and 0.375 mg/mL of lamivudine.

Standard solution: To transfer 1 mL of *Stock standard solution* to 25 mL volumetric flask and top up with *Mobile phase*, thus obtaining a standard solution (30 μ L/mL of zidovudine and 15 μ L/mL of lamivudine).

To inject replicates of 20 μ L of the *Standard solution*. The relative retention times are about 1.0 for lamivudine and 1.8 for zidovudine. The relative standard deviation for the replicate areas for the peaks recorded is not greater than 2.0%.

Procedure: to separately inject 20 μ L of *Sample and standard solutions*, record the chromatograms, and measure the areas under the peaks. To calculate the amount of zidovudine and lamivudine in the tablets from the responses obtained from *Sample and standard solutions*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELLING

To comply with the legislation in force.

INDEX

1 - (2,6-DICHLOROPHENYL) -1,3-DIHYDRO-2H-INDOL-2-ONE (REAGENTS AND REAGENT SOLUTIONS)	451
1,1,1- TRICHLOROETHANE (REAGENTS AND REAGENT SOLUTIONS)	498
1,1,3,3- TETRAMETHYLBUTYLAMINE (REAGENTS AND REAGENT SOLUTIONS)	496
1,10- PHENANTHROLINE (REAGENTS AND REAGENT SOLUTIONS)	457
1,1- DIMETHYLAMYLIMINE (REAGENTS AND REAGENT SOLUTIONS)	453
1,3- DINITROBENZENE (REAGENTS AND REAGENT SOLUTIONS)	454
1,3- DINITROBENZENE SR (REAGENTS AND REAGENT SOLUTIONS)	454
1,3- NAPHTHALENEDIOL (REAGENTS AND REAGENT SOLUTIONS)	474
1,8- CINEOLE (REAGENTS AND REAGENT SOLUTIONS)	443
1,8- DIAMINONAPHTHALENE (REAGENTS AND REAGENT SOLUTIONS)	450
1-BUTANOL (REAGENTS AND REAGENT SOLUTIONS)	440
1-CHLORINE-2,4-DINITROBENZENE (REAGENTS AND REAGENT SOLUTIONS)	449
SODIUM 1-HEXANE SULFONATE (REAGENTS AND REAGENT SOLUTIONS)	464
1- NAPHTHYLAMINE (REAGENTS AND REAGENT SOLUTIONS)	474
1- NAPHTHOL (REAGENTS AND REAGENT SOLUTIONS)	474
1- NAPHTHOL SR (REAGENTS AND REAGENT SOLUTIONS)	474
1- NAPHTHOLBENZEIN (INDICATORS AND INDICATOR SOLUTIONS)	417
1- NAPHTHOLBENZEIN SI (INDICATORS AND INDICATOR SOLUTIONS)	417
1- NAPHTHOLPHTHALEIN (INDICATORS AND INDICATOR SOLUTIONS)	418
1- NAPHTHOLPHTHALEIN SI (INDICATORS AND INDICATOR SOLUTIONS)	418
SODIUM 1-OCTANE SULFONATE (REAGENTS AND REAGENT SOLUTIONS)	478
SODIUM 1-PENTANE SULFONATE (REAGENTS AND REAGENT SOLUTIONS)	480
2,5- DIMETHYLPHENOL (REAGENTS AND REAGENT SOLUTIONS)	453
2,5- DIBROMOQUINONE-4-CHLORIMIDE (REAGENTS AND REAGENT SOLUTIONS)	450
2,6- DICHLOROINDOPHENOL, SODIUM (REAGENTS AND REAGENT SOLUTIONS)	451
2,6-DICHLOROQUINONE-4-CHLOROIMIDE (REAGENTS AND REAGENT SOLUTIONS)	451
2,6- DIMETHYLANILINE (REAGENTS AND REAGENT SOLUTIONS)	453
2,7- NAPHTHALENEDIOL (REAGENTS AND REAGENT SOLUTIONS)	474
AMINO HEPTANE (REAGENTS AND REAGENT SOLUTIONS)	434
2- AMINOPYRIDINE (REAGENTS AND REAGENT SOLUTIONS)	434
2- PHENOXYETHANOL (REAGENTS AND REAGENT SOLUTIONS)	458
2- MERCAPTOETHANOL (REAGENTS AND REAGENT SOLUTIONS)	471
2- NAPHTHOL (REAGENTS AND REAGENT SOLUTIONS)	474
2- NAPHTHOL SR (REAGENTS AND REAGENT SOLUTIONS)	474
2- NAPHTHOL SR1 (REAGENTS AND REAGENT SOLUTIONS)	474
2- NITROBENZALDEHYDE (REAGENTS AND REAGENT SOLUTIONS)	478
3,3 ' - DIAMINO BENZIDINE TETRAHYDROCHLORIDE	495
3,3 ' - DIAMINO BENZIDINE TETRAHYDROCHLORIDE SR	495
3-METHYL-2-PENTANONE (REAGENTS AND REAGENT SOLUTIONS)	472
4,4-METHYLENEBIS-N,N-DIMETHYLANILINE (REAGENTS AND REAGENT SOLUTIONS)	471
4-AMINOANTIPYRINE (REAGENTS AND REAGENT SOLUTIONS)	434
4- AMINOPHENOL (REAGENTS AND REAGENT SOLUTIONS)	434
4- DIMETHYLAMINOCINNAMALDEHYDE (REAGENTS AND REAGENT SOLUTIONS)	453
4- METHYLPENTAN-2-OL (REAGENTS AND REAGENT SOLUTIONS)	472

A

AVOCADO	557
ACETAL (REAGENTS AND REAGENT SOLUTIONS)	421
ACETALDEHYDE (REAGENTS AND REAGENT SOLUTIONS)	421
ACETANILIDE (REAGENTS AND REAGENT SOLUTIONS)	421
ACETATE 0.05 M PH 4.5 (BUFFERS)	507
ACETATE, AMMONIUM (REAGENTS AND REAGENT SOLUTIONS)	421
ACETATE, AMMONIUM PH 8.5 (BUFFERS)	509
ACETATE, AMMONIUM SR (REAGENTS AND REAGENT SOLUTIONS)	422
ACETATE, BORNYL (REAGENTS AND REAGENT SOLUTIONS)	422
ACETATE, BUTYL (REAGENTS AND REAGENT SOLUTIONS)	422
ACETATE, CELLULOSE (REAGENTS AND REAGENT SOLUTIONS)	422
ACETATE, LEAD SR (APPROXIMATELY 0.25 M) (REAGENTS AND REAGENT SOLUTIONS)	422
ACETATE, LEAD, PAPER (REAGENTS AND REAGENT SOLUTIONS)	422
ACETATE, LEAD, SATURATED SOLUTION (REAGENTS AND REAGENT SOLUTIONS)	422
ACETATE, LEAD, TRIHYDRATE (REAGENTS AND REAGENT SOLUTIONS)	422
ACETATE, CHLORHEXIDINE (REAGENTS AND REAGENT SOLUTIONS)	422
ACETATE, CHLORHEXIDINE AT 0.1% (W/V) (REAGENTS AND REAGENT SOLUTIONS)	422
ACETATE, COPPER (REAGENTS AND REAGENT SOLUTIONS)	422
ACETATE, CORTISONE (REAGENTS AND REAGENT SOLUTIONS)	422
ACETATE, CORTISONE, INJECTABLE (REAGENTS AND REAGENT SOLUTIONS)	423
ACETATE, DES-OXYCORTONE (REAGENTS AND REAGENT SOLUTIONS)	423
ACETATE, DEXAMETHASONE - CREAM	561
ACETATE, ETHYL (REAGENTS AND REAGENT SOLUTIONS)	423
ACETATE, PHENYLMERCURIC (REAGENTS AND REAGENT SOLUTIONS)	423
ACETATE, INDOPHENOL SR (REAGENTS AND REAGENT SOLUTIONS)	423
ACETATE, MAGNESIUM (REAGENTS AND REAGENT SOLUTIONS)	423
ACETATE, MENTHYL (REAGENTS AND REAGENT SOLUTIONS)	423
ACETATE, MERCURY (REAGENTS AND REAGENT SOLUTIONS)	423
ACETATE, MERCURY SR (REAGENTS AND REAGENT SOLUTIONS)	423
ACETATE, METHYL (REAGENTS AND REAGENT SOLUTIONS)	423
ACETATE, POTASSIUM (REAGENTS AND REAGENT SOLUTIONS)	424
ACETATE, POTASSIUM SR (REAGENTS AND REAGENT SOLUTIONS)	424
ACETATE, PREDNISOLONE (REAGENTS AND REAGENT SOLUTIONS)	424
ACETATE, SODIUM	561
ACETATE, SODIUM (REAGENTS AND REAGENT SOLUTIONS)	424
ACETATE, SODIUM 0.1 M PH 5.0 (BUFFERS)	507
ACETATE, SODIUM PH 4.5 (BUFFERS)	507
ACETATE, SODIUM SR (APPROXIMATELY 0.02 M) (REAGENTS AND REAGENT SOLUTIONS)	424
ACETATE, URANYL (REAGENTS AND REAGENT SOLUTIONS)	424
ACETATE, URANYL AND ZINC SR (REAGENTS AND REAGENT SOLUTIONS)	424
ACETATE, ZINC (REAGENTS AND REAGENT SOLUTIONS)	424
ACETATE PH 3.0 (BUFFERS)	506
ACETATE PH 3.5 (BUFFERS)	506
ACETATE PH 4.0 (BUFFERS)	507
ACETATE PH 4.4 (BUFFERS)	507
ACETATE PH 6.0 (BUFFERS)	507
ACETATE PH 7.0 (BUFFERS)	508
ACETAZOLAMIDE	562
ACETYLACETONE (REAGENTS AND REAGENT SOLUTIONS)	424
ACETYLCYSTEINE	563

ACETYL METHIONINE	564
ACETONE (REAGENTS AND REAGENT SOLUTIONS)	424
ACETONE, DEHYDRATED (REAGENTS AND REAGENT SOLUTIONS)	424
ACETONE, BUFFERED SR (REAGENTS AND REAGENT SOLUTIONS)	424
ACETONITRILE (REAGENTS AND REAGENT SOLUTIONS)	425
ACYCLOVIR	565
ACYCLOVIR, TABLETS	566
ACIYCLOVIR, CREAM	568
ACID 1,2 - CYCLOHEXYLENE DINITRILE TETRACETIC (REAGENTS AND REAGENT SOLUTIONS)	426
ACID 3,5 - DINITROBENZOIC (REAGENTS AND REAGENT SOLUTIONS)	427
ACID 7 - AMINODESACETOXYCEPHALOSPORANIC (REAGENTS AND REAGENT SOLUTIONS)	425
ACETIC ACID 6M (REAGENTS AND REAGENT SOLUTIONS)	425
ACETIC ACID, DILUTED (REAGENTS AND REAGENT SOLUTIONS)	425
ACETIC ACID, GLACIAL (REAGENTS AND REAGENT SOLUTIONS)	425
ACETIC ACID M (REAGENTS AND REAGENT SOLUTIONS)	425
ACETIC ACID SR (REAGENTS AND REAGENT SOLUTIONS)	425
ACETIC ACID, AMMONIUM ACETATE (BUFFERS)	509
ACETYLSALICYLIC ACID	568
ACETYLSALICYLIC ACID, TABLETS	569
ASCORBIC ACID	570
ASCORBIC ACID (REAGENTS AND REAGENT SOLUTIONS)	425
ASCORBIC ACID, TABLETS	571
ASCORBIC ACID, INJECTABLE SOLUTION	572
BENZOIC ACID	572
BENZOIC ACID (REAGENTS AND REAGENT SOLUTIONS)	425
BORIC ACID	573
BORIC ACID (REAGENTS AND REAGENT SOLUTIONS)	425
ACID, BORIC, SATURATED SOLUTION (REAGENTS AND REAGENT SOLUTIONS)	425
ACID, HYDROBROMIC (REAGENTS AND REAGENT SOLUTIONS)	425
ACID, CAFFEIC (REAGENTS AND REAGENT SOLUTIONS)	426
ACID, CALCON CARBOXYLIC (REAGENTS AND REAGENT SOLUTIONS)	426
ACID, CYCLOBUTANE-1,1-DICARBOXYLIC (REAGENTS AND REAGENT SOLUTIONS)	426
ACID, CINNAMIC (REAGENTS AND REAGENT SOLUTIONS)	426
ACID, CITRIC	574
ACID, CITRIC, MONOHYDRATE (REAGENTS AND REAGENT SOLUTIONS)	426
ACID, HYDROCHLORIC (REAGENTS AND REAGENT SOLUTIONS)	426
ACID, HYDROCHLORIC, BROMINATED (REAGENTS AND REAGENT SOLUTIONS)	426
ACID, HYDROCHLORIC, DILUTED (REAGENTS AND REAGENT SOLUTIONS)	426
ACID, HYDROCHLORIC M (REAGENTS AND REAGENT SOLUTIONS)	426
ACID, HYDROCHLORIC M SV (VOLUMETRIC SOLUTIONS)	501
ACID, HYDROCHLORIC, METHANOLIC 0.01 M (REAGENTS AND REAGENT SOLUTIONS)	426
ACID, HYDROCHLORIC PH 2.0 (BUFFERS)	506
ACID, HYDROCHLORIC SR (REAGENTS AND REAGENT SOLUTIONS)	426
ACID, HYDROCHLORIC, TIN SR (REAGENTS AND REAGENT SOLUTIONS)	426
ACID, CHLOROGENIC (REAGENTS AND REAGENT SOLUTIONS)	427
ACID, CHLOROPLATINIC (REAGENTS AND REAGENT SOLUTIONS)	427
ACID, CHROMIC (REAGENTS AND REAGENT SOLUTIONS)	427
ACID, DEHYDROCHOLIC	575
ACID, EDETIC (REAGENTS AND REAGENT SOLUTIONS)	427
ACID, STEARIC	576
ACID, PHENOL DISULFONIC SR (REAGENTS AND REAGENT SOLUTIONS)	427
PHENOXYACETIC ACID (REAGENTS AND REAGENT SOLUTIONS)	427

ACID, HYDROFLUORIC (REAGENTS AND REAGENT SOLUTIONS)	427
ACID, FOLIC	577
ACID, FOLIC, TABLETS	578
ACID, FORMIC (REAGENTS AND REAGENT SOLUTIONS)	427
ACID, PHOSPHOMOLYBDIC (REAGENTS AND REAGENT SOLUTIONS)	427
ACID, PHOSPHOMOLYBDIC SR (REAGENTS AND REAGENT SOLUTIONS)	427
ACID, PHOSPHORIC (REAGENTS AND REAGENT SOLUTIONS)	428
ACID, PHOSPHORIC SR (REAGENTS AND REAGENT SOLUTIONS)	428
ACID, PHOSPHOTUNGSTIC SR (REAGENTS AND REAGENT SOLUTIONS)	428
ACID, PHTHALIC (REAGENTS AND REAGENT SOLUTIONS)	428
ACID, GALLIC (REAGENTS AND REAGENT SOLUTIONS)	428
ACID, HYPOPHOSPHOROUS (REAGENTS AND REAGENT SOLUTIONS)	428
ACID, HYDRIODIC (REAGENTS AND REAGENT SOLUTIONS)	428
ACID, LACTIC	579
ACID, LACTIC (REAGENTS AND REAGENT SOLUTIONS)	428
ACID, METAPHOSPHORIC (REAGENTS AND REAGENT SOLUTIONS)	428
ACID, METAPHOSPHORIC - ACETIC SR (REAGENTS AND REAGENT SOLUTIONS)	428
ACID, METHANESULFONIC (REAGENTS AND REAGENT SOLUTIONS)	428
ACID, NALIDIXIC	580
ACID, NALIDIXIC, TABLETS	581
ACID, NALIDIXIC, ORAL SUSPENSION	582
ACID, NITRIC (REAGENTS AND REAGENT SOLUTIONS)	429
ACID, NITRIC, FUMING (REAGENTS AND REAGENT SOLUTIONS)	429
ACID, NITRIC SR (REAGENTS AND REAGENT SOLUTIONS)	429
ACID, OXALIC (REAGENTS AND REAGENT SOLUTIONS)	429
ACID, OXALIC 0.05 M SV (VOLUMETRIC SOLUTIONS)	501
ACID, OXALIC SR (REAGENTS AND REAGENT SOLUTIONS)	429
ACID, PARA-AMINO BENZOIC	582
ACID, PERCHLORIC (REAGENTS AND REAGENT SOLUTIONS)	429
ACID, PERCHLORIC 0.1 M SV (VOLUMETRIC SOLUTIONS)	502
ACID, PERCHLORIC (REAGENTS AND REAGENT SOLUTIONS)	429
ACID, PERCHLORIC SR (REAGENTS AND REAGENT SOLUTIONS)	429
ACID, PERFORMIC (REAGENTS AND REAGENT SOLUTIONS)	429
ACID, PERIODIC (REAGENTS AND REAGENT SOLUTIONS)	429
ACID, P-HYDROXYBENZOIC (REAGENTS AND REAGENT SOLUTIONS)	428
ACID, PICRIC (REAGENTS AND REAGENT SOLUTIONS)	429
ACID, PICRIC SR (REAGENTS AND REAGENT SOLUTIONS)	429
ACID, PICRIC SR1 (REAGENTS AND REAGENT SOLUTIONS)	429
ACID, P-TOLUENESULFONIC (REAGENTS REAGENTS AND SOLUTIONS)	431
ACID, ROSMARINIC (REAGENTS AND REAGENT SOLUTIONS)	430
ACID, SALICYLIC	583
ACID, SALICYLIC (REAGENTS AND REAGENT SOLUTIONS)	430
ACID, SELENIOUS (REAGENTS AND REAGENT SOLUTIONS)	430
ASCORBIC ACID	584
ACID, SULPHAMIC (REAGENTS AND REAGENT SOLUTIONS)	430
ACID, SULFANILIC (REAGENTS AND REAGENT SOLUTIONS)	430
ACID, SULFANILIC, DIAZOTIZED (REAGENTS AND REAGENT SOLUTIONS)	430
ACID, SULFANILIC SR (REAGENTS AND REAGENT SOLUTIONS)	430
ACID, SULFURIC (REAGENTS AND REAGENT SOLUTIONS)	430
ACID, SULFURIC, DILUTED (REAGENTS AND REAGENT SOLUTIONS)	430
ACID, SULFURIC, NITROGEN-FREE (REAGENTS AND REAGENT SOLUTIONS)	430
ACID, SULFURIC M SV (VOLUMETRIC SOLUTIONS)	502

ACID, SULFURIC, METHANOLIC 0.1 M (REAGENTS AND REAGENT SOLUTIONS)	430
ACID, SULFURIC, METHANOLIC SR (REAGENTS AND REAGENT SOLUTIONS)	430
ACID, SULFURIC SR (REAGENTS AND REAGENT SOLUTIONS)	431
ACID, SULFURIC, ETHANOLIC SOLUTION (REAGENTS AND REAGENT SOLUTIONS)	431
ACID, SULFURIC / METHANOL SR (REAGENTS AND REAGENT SOLUTIONS)	430
ACID, SULFUROUS (REAGENTS AND REAGENT SOLUTIONS)	431
ACID, TARTARIC (REAGENTS AND REAGENT SOLUTIONS)	431
ACID, THIOGLYCOLIC (REAGENTS AND REAGENT SOLUTIONS)	431
ACID, TRICHLOROACETIC	585
ACID, TRICHLOROACETIC SR (REAGENTS AND REAGENT SOLUTIONS)	431
ACID, TRICHLOROACETIC - CHLORAMINE - T SR (REAGENTS AND REAGENT SOLUTIONS)	431
ACID, TRIFLUOROACETIC (REAGENTS AND REAGENT SOLUTIONS)	431
ACID, UNDECYLENIC	585
ACRYLAMIDE (REAGENTS AND REAGENT SOLUTIONS)	432
ACRYLAMIDE / BISACRYLAMIDE (29:1) AT 30% (W/V) SR (REAGENTS AND REAGENT SOLUTIONS)	432
SUITABILITY OF PHARMACOPOEIA METHODS	245
AGAR (REAGENTS AND REAGENT SOLUTIONS)	432
AGAROSE, GEL (REAGENTS AND REAGENT SOLUTIONS)	432
AGAROSE - DEAE FOR ION EXCHANGE CHROMATOGRAPHY (REAGENTS AND REAGENT SOLUTIONS)	432
BROMINE WATER (REAGENTS AND REAGENT SOLUTIONS)	432
CHLORINE WATER SR (REAGENTS AND REAGENT SOLUTIONS)	432
AMMONIA-FREE WATER (REAGENTS AND REAGENT SOLUTIONS)	432
CARBON DIOXIDE-FREE WATER (REAGENTS AND REAGENT SOLUTIONS)	432
NITRATE-FREE WATER (REAGENTS AND REAGENT SOLUTIONS)	432
PARTICLE-FREE WATER (REAGENTS REAGENTS AND SOLUTIONS)	432
WATER FOR INJECTION	586
WATER FOR PHARMACEUTICAL PURPOSE	391
PURIFIED WATER	587
ULTRAPURE WATER	588
ALANINE	588
METHYL ORANGE (CI 13025) (INDICATORS AND INDICATOR SOLUTIONS)	413
METHYL ORANGE SI (INDICATORS AND INDICATOR SOLUTIONS)	413
METHYL ORANGE, SOLUTION (INDICATORS AND INDICATOR SOLUTIONS)	413
XYLENOL ORANGE (INDICATORS AND INDICATOR SOLUTIONS)	413
XYLENOL ORANGE SI (INDICATORS AND INDICATOR SOLUTIONS)	413
ALBENDAZOLE	589
ALBENDAZOLE, TABLETS	590
ALBENDAZOLE, ORAL SUSPENSION	592
ALBUMIN, BOVINE (REAGENTS AND REAGENT SOLUTIONS)	432
ALBUMIN, HUMAN (REAGENTS AND REAGENT SOLUTIONS)	432
ALBUMIN, HUMAN, REAGENT SOLUTION (REAGENTS AND REAGENT SOLUTIONS)	432
ALBUMIN - PHOSPHATE PH 7.2 (BUFFERS)	508
ALCOHOL, BENZYL	592
ALCOHOL, ETHYL	593
ALCOHOL, ISOAMYL (REAGENTS AND REAGENT SOLUTIONS)	432
ALCOHOL, ISOBUTYL (REAGENTS AND REAGENT SOLUTIONS)	433
ALCOHOL, ISOPROPYL (REAGENTS AND REAGENT SOLUTIONS)	433
ALCOHOL, N-AMYL (REAGENTS AND REAGENT SOLUTIONS)	433
ALCOHOL, N-PROPYL (REAGENTS AND REAGENT SOLUTIONS)	433
ALCOHOL, POLYVINYL (REAGENTS AND REAGENT SOLUTIONS)	433
ALCOHOL, TERT-AMYL (REAGENTS AND REAGENT SOLUTIONS)	433
ALCOHOL, TERT-BUTYL (REAGENTS AND REAGENT SOLUTIONS)	433

ALCOHOLOMETRY	146
ALCOHOLOMETRY (ANNEX D)	525
ROSEMARY VOLATILE OIL	595
PURIFIED AND STERILE COTTON	598
ALIZARIN (INDICATORS AND INDICATOR SOLUTIONS)	413
ALIZARIN SI (INDICATORS AND INDICATOR SOLUTIONS)	414
ALOE	599
ALOE, DRY EXTRACT	602
ALTHEA	603
ALUMINUM, METALLIC (REAGENTS AND REAGENT SOLUTIONS)	433
ALUMINON (REAGENTS AND REAGENT SOLUTIONS)	433
AMARANTH	609
AMARANTH (CI 16185) (REAGENTS AND REAGENT SOLUTIONS)	434
AMARANTH, ALUMINUM LAKE	610
YELLOW TWILIGHT	611
YELLOW TWILIGHT, ALUMINUM LAKE	612
ALIZARIN YELLOW GG (CI 14025) (INDICATORS AND INDICATOR SOLUTIONS)	414
ALIZARIN YELLOW GG SI (INDICATORS AND INDICATOR SOLUTIONS)	414
ALIZARIN, DIMETHYL (CI 11020) (INDICATORS AND INDICATOR SOLUTIONS)	414
ALIZARIN, DIMETHYL SI (INDICATORS AND INDICATOR SOLUTIONS)	414
ALIZARIN, METANILE (CI 13065) (INDICATORS AND INDICATOR SOLUTIONS)	414
ALIZARIN, METANILE SI (INDICATORS AND INDICATOR SOLUTIONS)	414
YELLOW NAPHTHOL (CI 10315) (INDICATORS AND INDICATOR SOLUTIONS)	414
YELLOW TITAN (CI 19540) (INDICATORS AND INDICATOR SOLUTIONS)	414
YELLOW TITAN SI (INDICATORS AND INDICATOR SOLUTIONS)	414
YELLOW TITAN, PAPER (INDICATORS AND INDICATOR SOLUTIONS)	414
STARCH	614
STARCH (STARCH, SOLUBLE) (INDICATORS AND INDICATOR SOLUTIONS)	414
STARCH, IODINE SI (INDICATORS AND INDICATOR SOLUTIONS)	415
STARCH, IODINE SR (REAGENTS AND REAGENT SOLUTIONS)	434
STARCH, IODINE SR1 (REAGENTS AND REAGENT SOLUTIONS)	434
STARCH, IODINE, PAPER (INDICATORS AND INDICATOR SOLUTIONS)	415
STARCH, IODIDE-FREE SI (INDICATORS AND INDICATOR SOLUTIONS)	415
STARCH, IODIDE-FREE SR (REAGENTS AND REAGENT SOLUTIONS)	434
STARCH SI (INDICATORS AND INDICATOR SOLUTIONS)	414
STARCH, SOLUBLE (REAGENTS AND REAGENT SOLUTIONS)	434
STARCH SR (REAGENTS AND REAGENT SOLUTIONS)	434
STARCHES (REAGENTS AND REAGENT SOLUTIONS)	434
AMINO BUTANOL (REAGENTS AND REAGENT SOLUTIONS)	434
AMINOPHYLLINE	615
AMINOPHYLLINE, TABLETS	617
AMINOSALICYLATE, CALCIUM	618
AMMONIA 10 M (REAGENTS AND REAGENT SOLUTIONS)	434
AMMONIA 6 M (REAGENTS AND REAGENT SOLUTIONS)	434
AMMONIA SR (REAGENTS AND REAGENT SOLUTIONS)	434
AMMONIA, CONCENTRATED SOLUTION (REAGENTS AND REAGENT SOLUTIONS)	435
SAMPLING	196
AMOXICILIN AND CLAVULANATE POTASSIUM, TABLETS	619
AMOXICILIN AND CLAVULANATE POTASSIUM, POWDER FOR INJECTABLE SOLUTION	620
AMOXICILIN AND CLAVULANATE POTASSIUM, POWDER FOR ORAL SUSPENSION	621
AMOXICILIN, TRIHYDRATE	621
AMOXYCILLIN, TRIHYDRATE, CAPSULES	623

AMOXICILIN, TRIHYDRATE, POWDER FOR ORAL SUSPENSION	624
AMPICILLIN	625
AMPICILLIN, CAPSULES	627
AMPICILLIN, TABLETS	628
AMPICILLIN, POWDER FOR ORAL SUSPENSION	629
AMPICILLIN, SODIUM	630
AMPICILLIN, SODIUM, POWDER FOR INJECTABLE SOLUTION	632
AMPICILLIN, TRIHYDRATE	632
AMPICILLIN, TRIHYDRATE, CAPSULES	634
AMPICILLIN, TRIHYDRATE, TABLETS	635
AAMPICILLIN, TRIHYDRATE, POWDER FOR ORAL SUSPENSION	636
ANALYSIS OF AMINO ACIDS	190
ANALYSIS OF PHASE SOLUBILITY	127
ANALYSIS OF VARIANCE	343
ANALYSIS, ENANTIOMERIC	142
ANALYSIS, THERMAL	146
ANETWELL (REAGENTS AND REAGENT SOLUTIONS)	435
ANHYDRIDE, ACETIC (REAGENTS AND REAGENT SOLUTIONS)	435
ANHYDRIDE, ACETIC - PYRIDINE SR (REAGENTS AND REAGENT SOLUTIONS)	435
ANHYDRIDE, PHTHALIC (REAGENTS AND REAGENT SOLUTIONS)	435
ANHYDRIDE, PROPIONIC (REAGENTS AND REAGENT SOLUTIONS)	435
ANISE	637
STAR ANISE	642
ANISALDEHYDE (REAGENTS AND REAGENT SOLUTIONS)	435
ANISALDEHYDE SR (REAGENTS AND REAGENT SOLUTIONS)	435
ANISALDEHYDE SR1 (REAGENTS AND REAGENT SOLUTIONS)	435
ANISALDEHYDE, SOLUTION (REAGENTS AND REAGENT SOLUTIONS)	435
ANTIMONIATE, MEGLUMINE	647
ANTIMONIATE, MEGLUMINE, INJECTABLE SOLUTION	648
ANTITHROMBIN III (REAGENTS AND REAGENT SOLUTIONS)	435
ANTITHROMBIN III SR (REAGENTS AND REAGENT SOLUTIONS)	435
APROTININ (REAGENTS AND REAGENT SOLUTIONS)	435
ARNICA	648
ARSENIC	287
ARTEMETHER	657
ARTEMETHER, INJECTABLE SOLUTION	656
ARTESUNATE	657
ARTESUNATE, TABLETS	658
ASCORBATE, SODIUM	659
ASIATICOSIDE (REAGENTS AND REAGENT SOLUTIONS)	436
ASPARAGINE (REAGENTS AND REAGENT SOLUTIONS)	436
BANDAGE	660
ATENOLOL	661
ATENOLOL, TABLETS	662
ATENOLOL AND CHLORTHALIDONE, TABLETS	663
PERFORMANCE EVALUATION	328
OPERATIONAL EVALUATION OF THE MICROBIAL STATE OF PRODUCTS BOTTLED ASEPTICALLY	336
AZATHIOPRINE	665
AZIDE, SODIUM (REAGENTS AND REAGENT SOLUTIONS)	436
AZITHROMYCIN	666
AZITHROMYCIN, CAPSULES	667
AZITHROMYCIN, POWDER FOR ORAL SUSPENSION	667

ACID, BLUE 83 (REAGENTS AND REAGENT SOLUTIONS)	436
ACID, BLUE 90 (REAGENTS AND REAGENT SOLUTIONS)	436
ASTRA, BLUE (REAGENTS AND REAGENT SOLUTIONS)	436
BROMOPHENOL, BLUE (INDICATORS AND INDICATOR SOLUTIONS)	415
BROMOPHENOL, BLUE SI (INDICATORS AND INDICATOR SOLUTIONS)	415
BROMOTHYMOL, BLUE (INDICATORS AND INDICATOR SOLUTIONS)	415
BROMOTHYMOL, BLUE SI (INDICATORS AND INDICATOR SOLUTIONS)	415
COOMASSIE BLUE (REAGENTS AND REAGENT SOLUTIONS)	436
HYDROXY NAPHTHOL, BLUE (INDICATORS AND INDICATOR SOLUTIONS)	415
HYDROXY NAPHTHOL, BLUE SI (INDICATORS AND INDICATOR SOLUTIONS)	415
ORACET, BLUE B (INDICATORS AND INDICATOR SOLUTIONS)	415
ORACET, BLUE B SI (INDICATORS AND INDICATOR SOLUTIONS)	415
SULFANE, BLUE (REAGENTS AND REAGENT SOLUTIONS)	436
TETRAZOLIUM, BLUE (REAGENTS AND REAGENT SOLUTIONS)	436
THYMOL, BLUE (INDICATORS AND INDICATOR SOLUTIONS)	415
THYMOL, BLUE SI (INDICATORS AND INDICATOR SOLUTIONS)	415
NILE, BLUE A (CI 51180) (INDICATORS AND INDICATOR SOLUTIONS)	416
NILE, BLUE A SI (INDICATORS AND INDICATOR SOLUTIONS)	416

B

BALSAM, TOLU	670
BALSAM, CANADA (REAGENTS AND REAGENT SOLUTIONS)	436
BALSAM, PERU	669
BARBALOINE (REAGENTS AND REAGENT SOLUTIONS)	436
BARBATIMÃO	671
BARBITAL (REAGENTS AND REAGENT SOLUTIONS)	436
BARBITAL PH 7.4 (BUFFERS)	508
BARBITAL PH 8.4 (BUFFERS)	508
BARBITAL PH 8.6 (BUFFERS)	509
BARBITAL, SODIUM (REAGENTS AND REAGENT SOLUTIONS)	437
BARIUM SRA - 1 MG/ML (REAGENTS AND REAGENT SOLUTIONS)	437
VANILLA	676
BELLADONNA	679
BENJOIM	682
BENZENE (REAGENTS AND REAGENT SOLUTIONS)	437
BENZENSULFONAMIDE (REAGENTS AND REAGENT SOLUTIONS)	437
BENZYL (REAGENTS AND REAGENT SOLUTIONS)	437
BENZNIDAZOLE	683
BENZOATE, BENZYL (REAGENTS AND REAGENT SOLUTIONS)	437
BENZOATE, CWELLSTERYL (REAGENTS AND REAGENT SOLUTIONS)	437
BENZOATE, ESTRADIOL	684
BENZOATE, METHYL (REAGENTS AND REAGENT SOLUTIONS)	437
BENZOPHENONE (REAGENTS AND REAGENT SOLUTIONS)	437
BENZOYL METRONIDAZOLE	685
BENZOYL METRONIDAZOLE, ORAL SUSPENSION	686
BENZOIN (REAGENTS AND REAGENT SOLUTIONS)	437
BICARBONATE, POTASSIUM	687
BICARBONATE, SODIUM	689
BICARBONATE, SODIUM (REAGENTS AND REAGENT SOLUTIONS)	437
BICINCHONINATE, DISODIUM (REAGENTS AND REAGENT SOLUTIONS)	438
BIPHTHALATE, POTASSIUM (REAGENTS AND REAGENT SOLUTIONS)	438

BIPHTHALATE, POTASSIUM 0.05 M (REAGENTS AND REAGENT SOLUTIONS)	438
BIPHTHALATE, PH 4.4 (BUFFERS)	507
BIOCOMPATIBILITY OF RELATED MATERIALS	305
BIOCOMPATIBILITY	303
BISACODYL	689
BISACODYL, TABLETS	690
BISACODYL, SUPPOSITORIES	691
BISULFATE, POTASSIUM (REAGENTS AND REAGENT SOLUTIONS)	438
BISULFATE, SODIUM (REAGENTS AND REAGENT SOLUTIONS)	438
BISULFITE, SODIUM (REAGENTS AND REAGENT SOLUTIONS)	438
BITARTRATE, SODIUM (REAGENTS AND REAGENT SOLUTIONS)	438
BITARTRATE, SODIUM SR (REAGENTS AND REAGENT SOLUTIONS)	438
BIURET (REAGENTS AND REAGENT SOLUTIONS)	438
BIURET, REAGENT (REAGENTS AND REAGENT SOLUTIONS)	438
BOLDINE (REAGENTS AND REAGENT SOLUTIONS)	438
BOLDO	692
BOLDO, TINCTURE	698
BORATE, SODIUM	699
BORATE PH 8.0 (BUFFERS)	508
BORATE PH 9.0 (BUFFERS)	509
BORATE PH 9.6 (BUFFERS)	509
BORNEOL (REAGENTS AND REAGENT SOLUTIONS)	438
BROMATE, POTASSIUM (REAGENTS AND REAGENT SOLUTIONS)	439
BROMATE, POTASSIUM 0.1 M SV (VOLUMETRIC SOLUTIONS)	502
BROMAZEPAM	700
BROMAZEPAM, TABLETS	701
BROMELIN (REAGENTS AND REAGENT SOLUTIONS)	439
BROMELIN SR (REAGENTS AND REAGENT SOLUTIONS)	439
BROMIDE, DIMIDIUM (REAGENTS AND REAGENT SOLUTIONS)	439
BROMIDE, DIMIDIUM - SULFANE BLUE SR (REAGENTS REAGENTS AND SOLUTIONS)	439
BROMIDE, HEXADIMETHRINE (REAGENTS AND REAGENT SOLUTIONS)	439
BROMIDE, IODINE (REAGENTS AND REAGENT SOLUTIONS)	439
BROMIDE, IODINE SR (REAGENTS AND REAGENT SOLUTIONS)	439
BROMIDE, NEOSTIGMINE	702
BROMIDE, POTASSIUM (REAGENTS AND REAGENT SOLUTIONS)	439
BROMIDE, SODIUM	703
BROMIDE, TETRABUTYLAMMONIUM (REAGENTS AND REAGENT SOLUTIONS)	439
BROMIDE, TETRA HEPTYL AMMONIUM (REAGENTS AND REAGENT SOLUTIONS)	439
BROMIDE, MERCURY (REAGENTS AND REAGENT SOLUTIONS)	439
HYDROBROMIDE, CITALOPRAM	704
HYDROBROMIDE, HYOSCYAMINE	705
BROMINE (REAGENTS AND REAGENT SOLUTIONS)	440
BROMINE 0.05 M SV (VOLUMETRIC SOLUTIONS)	502
BROMINE 0.2 M IN GLACIAL ACETIC ACID (REAGENTS AND REAGENT SOLUTIONS)	440
BROMINE SR (REAGENTS AND REAGENT SOLUTIONS)	440
BROMOPRIDE	706
BROMOPRIDE, TABLETS	706
BROMOPRIDE, ORAL SOLUTION	707
BUTANE SULPHONATE, SODIUM (REAGENTS AND REAGENT SOLUTIONS)	440
BUTYL, HYDROXYANISOLE (REAGENTS AND REAGENT SOLUTIONS)	440
BUTYLAMINE (REAGENTS AND REAGENT SOLUTIONS)	440
BUTYLBROMIDE, SCOPOLAMINE	708

BUTYLBROMIDE, SCOPOLAMINE, TABLETS	709
BUTYLBROMIDE, SCOPOLAMINE, INJECTABLE SOLUTION	710
BUTYLPARABEN (REAGENTS AND REAGENT SOLUTIONS)	440

C

CAFFEINE	713
CALAMINE	714
CALCIFEROL (REAGENTS AND REAGENT SOLUTIONS)	440
CALCIUM SRA - 400 MG/ML (REAGENTS AND REAGENT SOLUTIONS)	440
CHALCONE (INDICATORS AND INDICATOR SOLUTIONS)	416
CHALCONE SI (INDICATORS AND INDICATOR SOLUTIONS)	416
CHALCONE, COMPOUND (INDICATORS AND INDICATOR SOLUTIONS)	416
CALENDULA	714
CINNAMON, CHINESE	718
CINNAMON, CEYLON	721
CAMPHENE (REAGENTS AND REAGENT SOLUTIONS)	440
CAMPHOR	724
CAMPHOR (REAGENTS AND REAGENT SOLUTIONS)	441
KAOLIN, LIGHT (REAGENTS AND REAGENT SOLUTIONS)	441
CAPACITY, VOLUMETRIC, TOTAL	288
LEMON GRASS	724
CAPSULES	
AMOXYCILLIN, TRIHYDRATE	623
AMPICILLIN	627
AMPICILLIN, TRIHYDRATE	634
AZITHROMYCIN	667
CEFACLOX	753
CEFADROXIL	757
HYDROCHLORIDE, AMITRIPTYLINE	810
HYDROCHLORIDE, CLINDAMYCIN	819
HYDROCHLORIDE, TETRACYCLINE	865
FLUCONAZOLE	967
INDOMETHACIN	1067
ISOTRETINOIN	1075
NIFEDIPINE	1156
PIROXICAM	1204
RIFAMPICIN	1258
RITONAVIR	1261
ZIDOVUDINE	1378
CAPTOPRIL	729
CAPTOPRIL, TABLETS	730
CARBAMAZEPINE	731
CARBAMAZEPINE, TABLETS	733
CARBONATE, BISMUTH, BASIC	734
CARBONATE, AMMONIUM (REAGENTS AND REAGENT SOLUTIONS)	441
CARBONATE, AMMONIUM SR (REAGENTS AND REAGENT SOLUTIONS)	441
CARBONATE, CALCIUM	735
CARBONATE, CALCIUM (REAGENTS AND REAGENT SOLUTIONS)	441
CARBONATE, STRONTIUM (REAGENTS AND REAGENT SOLUTIONS)	441
CARBONATE, LITHIUM (REAGENTS AND REAGENT SOLUTIONS)	441
CARBONATE, MAGNESIUM	736

CARBONATE, POTASSIUM	737
CARBONATE, POTASSIUM, ANHYDROUS (REAGENTS AND REAGENT SOLUTIONS)	441
CARBONATE, POTASSIUM, SESQUIHYDRATE (REAGENTS AND REAGENT SOLUTIONS)	441
CARBONATE, SODIUM	738
CARBONATE, SODIUM SR (REAGENTS AND REAGENT SOLUTIONS)	442
CARBONATE, SODIUM ANHYDROUS (REAGENTS AND REAGENT SOLUTIONS)	442
CARBONATE, SODIUM, DECAHYDRATE (REAGENTS AND REAGENT SOLUTIONS)	442
CARBONATE, SODIUM, MONOHYDRATE (REAGENTS AND REAGENT SOLUTIONS)	442
CARBONATE, SODIUM BICARBONATE PH 9.6 (BUFFERS)	509
CARBON, ORGANIC, TOTAL	160
CARBOPLATIN, INJECTABLE SOLUTION	739
CARDAMOM	740
CARQUEJA	744
CARRAGEENAN	747
CARVONE (REAGENTS AND REAGENT SOLUTIONS)	442
HORSE CHESTNUT	749
CATECHIN (REAGENTS AND REAGENT SOLUTIONS)	442
CEFACLOX	752
CEFACLOX, CAPSULES	753
CEFACLOX, ORAL SUSPENSION	754
CEFADROXIL	755
CEFADROXIL, CAPSULES	757
CEFADROXIL, TABLETS	758
CEFADROXIL, POWDER FOR ORAL SUSPENSION	759
CEPHALEXIN	760
CEPHALEXIN, TABLETS	762
CEPHALEXIN, POWDER FOR ORAL SUSPENSION	764
CEPHALIN (REAGENTS AND REAGENT SOLUTIONS)	442
CEPHALIN SR (REAGENTS AND REAGENT SOLUTIONS)	442
CEFALOTIN, SODIUM, POWDER FOR INJECTABLE SOLUTION	765
CEFOXITIN, SODIUM	766
CEFOXITIN, SODIUM, POWDER FOR INJECTABLE SOLUTION	767
CELLULOSE, CHROMATOGRAPHIC (REAGENTS AND REAGENT SOLUTIONS)	442
CENTELLA	768
KETOCONAZOLE, TABLETS	772
KETOCONAZOLE, SHAMPOO	772
KETOPROFEN	773
CHAPÉU-DE-COURO	774
LEAD SRA - 100 MG/ML (REAGENTS AND REAGENT SOLUTIONS)	442
CYANIDE, POTASSIUM (REAGENTS AND REAGENT SOLUTIONS)	442
CYANIDE, POTASSIUM SR (REAGENTS AND REAGENT SOLUTIONS)	443
CYANIDE, AMMONIA SR (REAGENTS AND REAGENT SOLUTIONS)	443
CYANOACETATE, ETHYL (REAGENTS AND REAGENT SOLUTIONS)	443
CYANOCOBALAMIN, INJECTABLE SOLUTION	780
CYCLOHEXANE (REAGENTS AND REAGENT SOLUTIONS)	443
CICLOPIROX OLAMINE	780
CICLOPIROX OLAMINE, TOPICAL SOLUTION	781
CIMETIDINE	782
CIMETIDINE, TABLETS	784
CIMETIDINE, INJECTABLE SOLUTION	784
CINNAMATE, BENZYL (REAGENTS AND REAGENT SOLUTIONS)	443
CINNAMATE, METHYL (REAGENTS AND REAGENT SOLUTIONS)	443

CINCHONINE (REAGENTS AND REAGENT SOLUTIONS)	443
CIPROFLOXACIN, INJECTABLE SOLUTION	785
CISPLATIN, INJECTABLE SOLUTION	787
CITRAL (REAGENTS AND REAGENT SOLUTIONS)	443
CITRATE, CUPRIC, ALKALINE SR (REAGENTS AND REAGENTS SOLUTIONS)	443
CITRATE, AMMONIUM SR (REAGENTS AND REAGENT SOLUTIONS)	443
CITRATE, LITHIUM	788
CITRATE, POTASSIUM	789
CITRATE, SODIUM	790
CITRATE, SODIUM (REAGENTS AND REAGENT SOLUTIONS)	444
CITRATE, PHOSPHATE, PH 5.0 (BUFFERS)	507
CITROPHOSPHATE PH 6.0 (BUFFERS)	507
CITROPHOSPHATE PH 7.0 (BUFFERS)	508
CITRONELLAL (REAGENTS AND REAGENT SOLUTIONS)	444
CITRONELLOL (REAGENTS AND REAGENT SOLUTIONS)	444
CLARITHROMYCIN	791
CLARITHROMYCIN, TABLETS	792
CLARITHROMYCIN, POWDER FOR ORAL SUSPENSION	793
CLASSIFICATION OF CLEAN ROOMS AND CONTROLLED RELATED ENVIRONMENTS	330
CLAVULANATE, POTASSIUM	794
CLOFAZIMINE	795
CLONAZEPAM, TABLETS	796
CLONAZEPAM, ORAL SOLUTION	798
CLOPIDOGREL, TABLETS	798
CHLORAMINE-T (REAGENTS AND REAGENT SOLUTIONS)	444
CHLORATE, POTASSIUM (REAGENTS AND REAGENT SOLUTIONS)	444
CHLORIDE, COBALTOUS (REAGENTS AND REAGENT SOLUTIONS)	444
CHLORIDE, COBALTOUS SR (REAGENTS AND REAGENT SOLUTIONS)	444
CHLORIDE, ACETYL (REAGENTS AND REAGENT SOLUTIONS)	444
CHLORIDE, ALUMINUM, HEXAHYDRATE (REAGENTS AND REAGENT SOLUTIONS)	444
CHLORIDE, ALUMINUM SR (REAGENTS AND REAGENT SOLUTIONS)	444
CHLORIDE, AMMONIUM	799
CHLORIDE, AMMONIUM (REAGENTS AND REAGENT SOLUTIONS)	445
CHLORIDE, AMMONIUM PH 10.0 (BUFFERS)	509
CHLORIDE, AMMONIUM PH 10.7 (BUFFERS)	509
CHLORIDE, AMMONIUM SR (REAGENTS AND REAGENT SOLUTIONS)	445
CHLORIDE, AMMONIUM - AMMONIUM HYDROXIDE (REAGENTS AND REAGENT SOLUTIONS)	445
CHLORIDE, BARIUM (REAGENTS AND REAGENT SOLUTIONS)	445
CHLORIDE, BARIUM 0.1 M SV (VOLUMETRIC SOLUTIONS)	502
CHLORIDE, BARIUM SR (REAGENTS AND REAGENT SOLUTIONS)	445
CHLORIDE, BENZALKONIUM (REAGENTS AND REAGENT SOLUTIONS)	445
CHLORIDE, BENZETHONIUM (REAGENTS AND REAGENT SOLUTIONS)	445
CHLORIDE, BENZETHONIUM 0.004 M SV (VOLUMETRIC SOLUTIONS)	502
CHLORIDE, BENZYL (REAGENTS AND REAGENT SOLUTIONS)	445
CHLORIDE, CALCIUM	800
CHLORIDE, CALCIUM (REAGENTS AND REAGENT SOLUTIONS)	445
CHLORIDE, CALCIUM, HEXAHYDRATE	801
CHLORIDE, CALCIUM SR (REAGENTS AND REAGENT SOLUTIONS)	446
CHLORIDE, CALCIUM, ANHYDROUS (REAGENTS AND REAGENT SOLUTIONS)	445
CHLORIDE, CAESIUM (REAGENTS AND REAGENT SOLUTIONS)	446
CHLORIDE, TIN (II) SR (REAGENTS AND REAGENT SOLUTIONS)	446
CHLORIDE, MAGNESIUM (REAGENTS AND REAGENT SOLUTIONS)	446

CHLORIDE, MERCURY (II) (REAGENTS AND REAGENT SOLUTIONS)	446
CHLORIDE, METHACHOLINE	802
CHLORIDE, METHYLENE (REAGENTS AND REAGENT SOLUTIONS)	446
CHLORIDE, METHYLENE, SATURATED WITH AMMONIA (REAGENTS AND REAGENT SOLUTIONS)	446
CHLORIDE, METHYLROSANILINIUM (CI 42555) (INDICATORS AND INDICATOR SOLUTIONS)	416
CHLORIDE, METHYLROSANILINIUM SI (INDICATORS AND INDICATOR SOLUTIONS)	416
CHLORIDE, METHYLTHIONIUM (REAGENTS AND REAGENT SOLUTIONS)	446
CHLORIDE, METHYLTHIONIUM SR (REAGENTS AND REAGENT SOLUTIONS)	446
CHLORIDE, METHYLTHIONIUM SR1 (REAGENTS AND REAGENT SOLUTIONS)	446
CHLORIDE, NICKEL (II) (REAGENTS AND REAGENT SOLUTIONS)	446
CHLORIDE, NITROBENZOYL (REAGENTS AND REAGENT SOLUTIONS)	446
CHLORIDE, GOLD (REAGENTS AND REAGENT SOLUTIONS)	446
CHLORIDE, GOLD SR (REAGENTS AND REAGENT SOLUTIONS)	447
CHLORIDE, PALLADIUM (REAGENTS AND REAGENT SOLUTIONS)	447
CHLORIDE, POTASSIUM (REAGENTS AND REAGENT SOLUTIONS)	447
CHLORIDE, POTASSIUM, SATURATED SOLUTION (REAGENTS AND REAGENT SOLUTIONS)	447
CHLORIDE, SODIUM	803
CHLORIDE, SODIUM (REAGENTS AND REAGENT SOLUTIONS)	447
CHLORIDE, SODIUM AT 0.9% (W/V) (REAGENTS AND REAGENT SOLUTIONS)	447
CHLORIDE, SODIUM, INJECTABLE SOLUTION	804
CHLORIDE, ZINC	804
CHLORIDE, STANNOUS (REAGENTS AND REAGENT SOLUTIONS)	447
CHLORIDE, STANNOUS SR (REAGENTS AND REAGENT SOLUTIONS)	447
CHLORIDE, STANNOUS SR1 (REAGENTS AND REAGENT SOLUTIONS)	447
CHLORIDE, FERRIC (INDICATORS AND INDICATOR SOLUTIONS)	416
CHLORIDE, FERRIC (REAGENTS AND REAGENT SOLUTIONS)	447
CHLORIDE, FERRIC, ACID SR (REAGENTS AND REAGENT SOLUTIONS)	447
CHLORIDE, FERRIC, METHANOLIC (REAGENTS AND REAGENT SOLUTIONS)	447
CHLORIDE, FERRIC SI (APPROXIMATELY 0.4 M) (INDICATORS AND INDICATOR SOLUTIONS)	416
CHLORIDE, FERRIC SR (APPROXIMATELY 0.4 M) (REAGENTS AND REAGENT SOLUTIONS)	447
CHLORIDE, MERCURY SR (APPROXIMATELY 0.2 M) (REAGENTS AND REAGENT SOLUTIONS)	447
CHLORIDE, PLATINUM SR (REAGENTS AND REAGENT SOLUTIONS)	448
HYDROCHLORIDE, (2-CHLOROETHYL), DIETHYLAMINE (REAGENTS AND REAGENT SOLUTIONS)	448
HYDROCHLORIDE, AMILORIDE AND HYDROCHLOROTHIAZIDE, TABLETS	805
HYDROCHLORIDE, AMIODARONE	806
HYDROCHLORIDE, AMITRIPTYLINE	808
HYDROCHLORIDE, AMITRIPTYLINE, CAPSULES	810
HYDROCHLORIDE, AMITRIPTYLINE, TABLETS	811
HYDROCHLORIDE, BENZOYL (REAGENTS AND REAGENT SOLUTIONS)	448
HYDROCHLORIDE, BIPERIDEN, TABLETS	812
HYDROCHLORIDE, BUPIVACAINE AND GLUCOSE, INJECTABLE SOLUTION	813
HYDROCHLORIDE, CYCLOBENZAPRINE	815
HYDROCHLORIDE, CYCLOBENZAPRINE, TABLETS	815
HYDROCHLORIDE, CIPROFLOXACIN, TABLETS	816
HYDROCHLORIDE, CIPROFLOXACIN, OPHTHALMIC SOLUTION	818
HYDROCHLORIDE, CLINDAMYCIN, CAPSULES	819
HYDROCHLORIDE, DIPHENHYDRAMINE	820
HYDROCHLORIDE, DIPHENHYDRAMINE, TABLETS	821
HYDROCHLORIDE, DIPHENHYDRAMINE, ORAL SOLUTION	822
HYDROCHLORIDE, DIMETHYL-P-PHENYLENEDIAMINE (REAGENTS AND REAGENT SOLUTIONS)	448
HYDROCHLORIDE, EPINASTINE	823
HYDROCHLORIDE, EPINASTINE, TABLETS	824

HYDROCHLORIDE, ETHAMBUTOL	825
HYDROCHLORIDE, ETHAMBUTOL, TABLETS	826
HYDROCHLORIDE, PHENYLHYDRAZINE (REAGENTSAND REAGENT SOLUTIONS)	448
HYDROCHLORIDE, PHENYLHYDRAZINE SR (REAGENTSAND REAGENT SOLUTIONS)	448
HYDROCHLORIDE, FEXOFENADINE	827
HYDROCHLORIDE, FEXOFENADINE, TABLETS	828
HYDROCHLORIDE, FLUOXETINE	829
HYDROCHLORIDE, FLUOXETINE, TABLETS	831
HYDROCHLORIDE, FLURAZEPAM, TABLETS	832
HYDROCHLORIDE ,HYDRALAZINE	833
HYDROCHLORIDE, HYDRALAZINE ,TABLETS	834
HYDROCHLORIDE, HYDRALAZINE , INJECTABLE SOLUTION	835
HYDROCHLORIDE, HYDRASTINE (REAGENTSAND REAGENT SOLUTIONS)	448
HYDROCHLORIDE, HYDROXYLAMINE (REAGENTSAND REAGENT SOLUTIONS)	448
HYDROCHLORIDE, HYDROXYLAMINE SR (REAGENTSAND REAGENT SOLUTIONS)	448
HYDROCHLORIDE, HYDROXYLAMINE SR1 (REAGENTSAND REAGENT SOLUTIONS)	448
HYDROCHLORIDE, IMIPRAMINE	836
HYDROCHLORIDE, IMIPRAMINE, TABLETS	838
HYDROCHLORIDE ,LIDOCAINE	839
HYDROCHLORIDE, LIDOCAINE, GEL	840
HYDROCHLORIDE, LIDOCAINE, OINTMENT	841
HYDROCHLORIDE, LIDOCAINE, INJECTABLE SOLUTION	842
HYDROCHLORIDE, MEFLOQUINE	843
HYDROCHLORIDE, MEFLOQUINE, TABLETS	844
HYDROCHLORIDE, METFORMIN	845
HYDROCHLORIDE, METFORMIN, TABLETS	846
HYDROCHLORIDE, METOCLOPRAMIDE, TABLETS	847
HYDROCHLORIDE, METOCLOPRAMIDE, INJECTABLE SOLUTION	848
HYDROCHLORIDE, METOCLOPRAMIDE, ORAL SOLUTION	849
HYDROCHLORIDE, O-PHENYLENEDIAMINE (REAGENTSAND REAGENT SOLUTIONS)	448
HYDROCHLORIDE, P-PHENYLENEDIAMINE (REAGENTS AND REAGENT SOLUTIONS)	448
HYDROCHLORIDE, PILOCARPINE	850
HYDROCHLORIDE, PYRIDOXINE	851
HYDROCHLORIDE, PYRIDOXINE, TABLETS	853
HYDROCHLORIDE, PROMETHAZINE	854
HYDROCHLORIDE, PROMETHAZINE, TABLETS	855
HYDROCHLORIDE, PROMETHAZINE, INJECTABLE SOLUTION	856
HYDROCHLORIDE, PROPRANOLOL	857
HYDROCHLORIDE, PROPRANOLOL, TABLETS	858
HYDROCHLORIDE, RANITIDINE	859
HYDROCHLORIDE, RANITIDINE ,TABLETS	860
HYDROCHLORIDE, SERTRALINE	861
HYDROCHLORIDE, SERTRALINE, TABLETS	862
HYDROCHLORIDE, TETRACYCLINE	863
HYDROCHLORIDE, TETRACYCLINE, CAPSULES	865
HYDROCHLORIDE, TETRYZOLINE	866
HYDROCHLORIDE, THIAMIN	867
HYDROCHLORIDE, THIAMIN, TABLETS	869
HYDROCHLORIDE, THIAMIN, INJECTABLE SOLUTION	870
HYDROCHLORIDE, TRAMADOL	870
HYDROCHLORIDE, TRAMADOL, INJECTABLE SOLUTION	871
HYDROCHLORIDE, VERAPAMIL	872

HYDROCHLORIDE, VERAPAMIL, TABLETS	873
HYDROCHLORIDE, VERAPAMIL, INJECTABLE SOLUTION	875
CHLORINE SR (REAGENTS AND REAGENT SOLUTIONS)	449
CHLOROBENZENE (REAGENTS AND REAGENT SOLUTIONS)	449
CHLOROFORM (REAGENTS AND REAGENT SOLUTIONS)	449
CHLOROFORM, ALCOHOL-FREE (REAGENTS AND REAGENT SOLUTIONS)	449
CHLOROTHIAZIDE (REAGENTS AND REAGENT SOLUTIONS)	449
CHLORPROPAMIDE	876
CHLORPROPAMIDE, TABLETS	877
CHLORTHALIDONE	878
CHLORTHALIDONE, TABLETS	880
CLOZAPINE	881
CLOZAPINE, TABLETS	882
COBALTINITRIDE, SODIUM (REAGENTS AND REAGENT SOLUTIONS)	449
COBALTINITRIDE, SODIUM SR (REAGENTS AND REAGENT SOLUTIONS)	449
COPPER (REAGENTS AND REAGENT SOLUTIONS)	449
COPPER SRA - 1 MG/ML (REAGENTS AND REAGENT SOLUTIONS)	449
COLCHICINE	884
COLCHICINE, TABLETS	885
COMBINED POTENCY ESTIMATES	347
TABLETS	
ACYCLOVIR	566
ACETYLSALICYLIC ACID	569
ASCORBIC ACID	571
ACID, FOLIC	578
ACID, NALIDIXIC	581
ALBENDAZOLE	590
AMINOPHYLLINE	617
AMOXICILLIN AND CLAVULANATE POTASSIUM	619
AMPICILLIN	628
AMPICILLIN, TRIHYDRATE	635
ARTESUNATE	658
ATENOLOL	662
ATENOLOL AND CHLORTHALIDONE	663
BISACODYL	690
BROMAZEPAM	701
BROMOPRIDE	706
BUTYLBROMIDE, SCOPOLAMINE	709
CAPTOPRIL	730
CARBAMAZEPINE	733
CEFADROXIL	758
CEPHALEXIN	762
KETOCONAZOLE	772
CIMETIDINE	784
CLARITHROMYCIN	792
CLONAZEPAM	796
CLOPIDOGREL	798
AMILORIDE HYDROCHLORIDE AND HYDROCHLOROTHIAZIDE	805
HYDROCHLORIDE, AMITRIPTYLINE	811
HYDROCHLORIDE, BIPERIDEN	812
HYDROCHLORIDE, CYCLOBENZAPRINE	815
HYDROCHLORIDE, CIPROFLOXACIN	816

HYDROCHLORIDE, DIPHENHYDRAMINE	821
HYDROCHLORIDE, EPINASTINE	824
HYDROCHLORIDE, ETHAMBUTOL	828
HYDROCHLORIDE, FEXOFENADINE	828
HYDROCHLORIDE, FLUOXETINE	831
HYDROCHLORIDE, FLURAZEPAM	832
HYDROCHLORIDE, HYDRALAZINE	834
HYDROCHLORIDE, IMIPRAMINE	838
HYDROCHLORIDE, MEFLOQUINE	844
HYDROCHLORIDE, METFORMIN	846
HYDROCHLORIDE, METOCLOPRAMIDE	847
HYDROCHLORIDE, PYRIDOXINE	853
HYDROCHLORIDE, PROMETHAZINE	855
HYDROCHLORIDE, PROPRANOLOL	858
HYDROCHLORIDE, RANITIDINE	860
HYDROCHLORIDE, SERTRALINE	862
HYDROCHLORIDE, THIAMIN	869
HYDROCHLORIDE, VERAPAMIL	873
CHLORPROPAMIDE	877
CLOZAPINE	882
COLCHICINE	885
DIAZEPAM	902
DICLOFENAC POTASSIUM	906
DIPHOSPHATE, CHLOROQUINE	907
DIPHOSPHATE, PRIMAQUINE	909
DIGOXIN	910
DIPYRONE	912
EFAVIRENZ	916
ERYTHROMYCIN ESTOLATE	936
ETHIONAMIDE	946
PHENYTOIN	955
PHENOBARBITAL	959
FLUNITRAZEPAM	968
FLUTAMIDE	975
FUROSEMIDE	990
GLIBENCLAMIDE	999
HALOPERIDOL	1014
IBUPROFEN	1053
ISONIAZID	1073
LAMIVUDINE	1080
LAMOTRIGINE	1082
LEFLUNOMIDE	1090
LEVONORGESTREL AND ETHINYLESTRADIOL	1093
LORATADINE	1098
MALEATE, DEXCHLORPHENIRAMINE	1106
MALEATE, ENALAPRIL	1109
MEBENDAZOLE	1120
METHYLDOPA	1144
METRONIDAZOLE	1146
MITOTANE	1153
NIMESULIDE	1159
NITROFURANTOIN	1167

OFLOXACIN	1177
PARACETAMOL	1190
PYRAZINAMIDE	1200
PYRIMETHAMINE	1203
PRAZIQUANTEL	1221
PREDNISONE	1224
SULFADIAZINE	1302
SULFAMETHOXAZOLE AND TRIMETHOPRIM	1305
SULFATE, MORPHINE	1318
SULFATE, FERROUS	1326
TARTRATE, METOPROLOL	1332
THIABENDAZOLE	1340
ZIDOVUDINE AND LAMIVUDINE	1381
CHEWABLE TABLETS	
ALUMINUM HYDROXIDE	1041
VAGINAL TABLETS	
NYSTATIN	1161
CONCENTRATE FOR DSS-EGPA SAMPLES (BUFFERS)	509
CONCENTRATE FOR DSS-EGPA SAMPLES AT REDUCING CONDITIONS (BUFFERS)	509
GENERAL CONDITIONS	237
CONDUCTIVITY OF WATER	143
TOTAL COUNTING OF MESOPHILIC MICRO-ORGANISMS	240
PARTICLE CONTAMINATION	77
COLOR OF LIQUID	92
BVF DYE (INDICATORS AND INDICATOR SOLUTIONS)	416
RELATED MATERIAL	304
CRATEGO	886
CREAM	
ACETATE, DEXAMETHASONE	561
ACYCLOVIR	568
TERCONAZOLE	1339
TRETINOIN	1347
VAGINAL CREAM	
NYSTATIN	1162
CHROMATE, POTASSIUM (REAGENTS AND REAGENT SOLUTIONS)	450
CHROMATE, POTASSIUM SR (REAGENTS AND REAGENT SOLUTIONS)	450
CHROMATOGRAPHY	105
GAS CHROMATOGRAPHY	114
GAS CHROMATOGRAPHY AT CONFINED SPACE (HEADSPACE)	117
HIGH PERFORMANCE LIQUID CHROMATOGRAPHY	109
ION CHROMATOGRAPHY	113
MICELLAR ELECTROKINETIC CHROMATOGRAPHY (MEKC)	139
THIN LAYER CHROMATOGRAPHY	105
COLUMN CHROMATOGRAPHY	107
PAPER CHROMATOGRAPHY	106
CHROMOTROPATE, DISODIUM (REAGENTS AND REAGENT SOLUTIONS)	450
TURMERIC	893

D

DAPSONE	899
DETRIS-HYDROCHLORIDE M PH 6.8 (BUFFERS)	507
DEOXYCHOLATE, SODIUM (REAGENTS AND REAGENT SOLUTIONS)	450
DETERMINATION OF WATER BY SEMI-MICRO METHOD	127
DETERMINATION OF HUMAN ANTITHROMBIN III	219
DETERMINATION OF IMMUNOGLOBULIN ANTICOMPLEMENTARITY	219
DETERMINATION OF HEMOLYTIC ACTIVITY	203
DETERMINATION OF MASS DENSITY AND SPECIFIC GRAVITY	86
DETERMINATION OF SPECIFIC GRAVITY	149
DETERMINATION OF FC FUNCTION OF IMMUNOGLOBULIN	227
DETERMINATION OF PARTICLE SIZE OF THE POWDER	92
DETERMINATION OF HEPARIN IN THE CLOTTING FACTORS	207
DETERMINATION OF HUMAN IMMUNOGLOBULIN ANTI-D	227
DETERMINATION OF MASS	81
DETERMINATION OF OSMOLALITY	148
DETERMINATION OF LOSS ON DRYING	91
DETERMINATION OF FREEZING TEMPERATURE	85
DETERMINATION OF BOILING TEMPERATURE AND DISTILLATION RANGE	84
DETERMINATION OF MELTING TEMPERATURE	149
DETERMINATION OF SOLIDIFICATION TEMPERATURE	149
DETERMINATION OF VISCOSITY	87
DETERMINATION OF 1,8-CINEOLE IN ESSENTIAL OILS	200
DETERMINATION OF ABSORPTION	283
DETERMINATION OF WATER	123
DETERMINATION OF WATER	150
DETERMINATION OF WATER IN VEGETABLE DRUGS	197
DETERMINATION OF ACID-INSOLUBLE ASHES	198
DETERMINATION OF SULFATED ASHES	198
DETERMINATION OF SULFATED ASH (RESIDUE INCINERATION)	91
DETERMINATION OF TOTAL ASHES	198
DETERMINATION OF SULPHUR DIOXIDE	188
DETERMINATION OF STEROLS IN FIXED OILS	159
DETERMINATION OF ACTIVATED CLOTTING FACTORS	213
DETERMINATION OF FOREIGN MATTER	197
DETERMINATION OF METHANOL AND 2-PROPANOL IN FLUID EXTRACTS	206
DETERMINATION OF METHOXYL	187
DETERMINATION OF NITROGEN BY KJELDAHL METHOD	181
DETERMINATION OF FIXED OILS	199
DETERMINATION OF VOLATILE OILS IN VEGETABLE DRUGS	198
DETERMINATION OF WEIGHT	59
DETERMINATION OF DRY MATTER IN FLUID AND SOFT EXTRACTS	207
DETERMINATION OF DRY MATTER IN DRY EXTRACTS	207
DETERMINATION OF MECHANICAL STRENGTH IN TABLETS	62
DETERMINATION OF ALCOHOL-EXTRACTABLE SUBSTANCES (ALCOHOLIC EXTRACT)	202
DETERMINATION OF UNSAPONIFIABLE MATTER	154
DETERMINATION OF TITER OF ANTI-A AND ANTI-B HEMAGGLUTININ (INDIRECT METHOD)	213
DETERMINATION OF VOLUME	61
DETERMINATION OF ALCOHOL	189
DETERMINATION OF FIBER LENGTH	283
DETERMINATION OF HUMAN VON WILLEBRAND FACTOR	207

DETERMINATION OF FACTOR II OF THE HUMAN BLOOD CLOTTING	209
DETERMINATION OF FACTOR IX OF THE HUMAN BLOOD CLOTTING	209
DETERMINATION OF FACTOR VII OF THE HUMAN BLOOD CLOTTING	210
DETERMINATION OF FACTOR VIII OF THE HUMAN BLOOD CLOTTING, LYOPHILISATE	212
DETERMINATION OF FACTOR X OF THE HUMAN BLOOD CLOTTING	211
DETERMINATION OF ACETYL INDEX	154
DETERMINATION OF BITTERNESS INDEX	202
DETERMINATION OF FOAM INDEX	201
DETERMINATION OF ESTERS INDEX	151
DETERMINATION OF HYDROXYL INDEX	153
DETERMINATION OF TUMESCENCE INDEX	204
DETERMINATION OF FOAM INDEX	151
DETERMINATION OF PEROXIDE INDEX	152
DETERMINATION OF REFRACTION INDEX	86
DETERMINATION OF REFRACTION INDEX	150
DETERMINATION OF SAPONIFICATION INDEX	150
DETERMINATION OF PH	121
DETERMINATION OF ROTATION	150
DETERMINATION OF ROTATION AND SPECIFIC ROTATION	90
DETERMINATION OF MELTING POINT OR RANGE	82
DETERMINATION OF TITER OF THE PREKALLIKREIN ACTIVATOR	218
DEXAMETHASONE	900
DEXAMETHASONE ELIXIR	901
DEXTROSE (REAGENTS AND REAGENT SOLUTIONS)	450
DEXTROSE 0.1% (W/V) (REAGENTS AND REAGENT SOLUTIONS)	450
DIACETATE, CHLORHEXIDINE (REAGENTS AND REAGENT SOLUTIONS)	450
DIAMETER OF SUTURES	280
DIAVERIDINE (REAGENTS AND REAGENT SOLUTIONS)	450
DIAZEPAM, TABLETS	902
DIAZEPAM, INJECTABLE SOLUTION	903
DIBUTYL-AMINE (REAGENTS AND REAGENT SOLUTIONS)	450
DICLOFENAC POTASSIUM	904
DICLOFENAC POTASSIUM, TABLETS	906
DICHLORIDE, ETHYLENE (REAGENTS AND REAGENT SOLUTIONS)	450
DIHYDROCHLORIDE, N-(1-NAPHTHYL) ETHYLENEDIAMINE (REAGENTS AND REAGENT SOLUTIONS)	451
DIHYDROCHLORIDE, N-(1-NAPHTHYL) ETHYLENEDIAMINE SR (REAGENTS AND REAGENT SOLUTIONS)	451
DICHLOROPHENOL-INDOPHENOL, STANDARD SOLUTION (VOLUMETRIC SOLUTIONS)	502
DICHROMATE, POTASSIUM (REAGENTS AND REAGENT SOLUTIONS)	451
DICHROMATE, POTASSIUM SR (REAGENTS AND REAGENT SOLUTIONS)	451
DIETHYLAMINE (REAGENTS AND REAGENT SOLUTIONS)	451
DIETHYLAMINOETHYL DEXTRAN (REAGENTS AND REAGENT SOLUTIONS)	451
DIETHYLDITHIOCARBAMATE, SILVER (REAGENTS AND REAGENT SOLUTIONS)	451
DIETHYLDITHIOCARBAMATE, SODIUM (REAGENTS AND REAGENT SOLUTIONS)	451
DIETHYLPHTALATE (REAGENTS AND REAGENT SOLUTIONS)	452
DIPHENYLAMINE SR (REAGENTS AND REAGENT SOLUTIONS)	452
DIPHENYL BENZIDINE (REAGENTS AND REAGENT SOLUTIONS)	452
DIPHENYL BORATE, AMINOETHANOL SR (REAGENTS AND REAGENT SOLUTIONS)	452
DIPHENYLCARBAZIDE (INDICATORS AND INDICATOR SOLUTIONS)	416
DIPHENYLCARBAZIDE (REAGENTS AND REAGENT SOLUTIONS)	452
DIPHENYLCARBAZIDE SI (INDICATORS AND INDICATOR SOLUTIONS)	416
DIPHENYLCARBAZIDE SR (REAGENTS AND REAGENT SOLUTIONS)	452
DIPHENYL CARBAZONE (INDICATORS AND INDICATOR SOLUTIONS)	416

DIPHENYL CARBAZONE (REAGENTS AND REAGENT SOLUTIONS)	452
DIPHENYL CARBAZONE, MERCURY SR (REAGENTS AND REAGENT SOLUTIONS)	452
DIPHENYLCARBAZONE SI (INDICATORS AND INDICATOR SOLUTIONS)	417
DIPHENYLCARBAZONE - BROMOPHENOL BLUE SR (REAGENTS AND REAGENT SOLUTIONS)	452
DIPHOSPHATE, CHLOROQUINE, TABLETS	907
DIPHOSPHATE, PRIMAQUINE	908
DIPHOSPHATE, PRIMAQUINE, TABLETS	909
DIGOXIN, TABLETS	910
DIMETHYLACETAMIDE (REAGENTS AND REAGENT SOLUTIONS)	453
DIMETHYLFORMAMIDE (REAGENTS AND REAGENT SOLUTIONS)	454
DIMETHYLSULFOXIDE (REAGENTS AND REAGENT SOLUTIONS)	454
DIOXANE (REAGENTS AND REAGENT SOLUTIONS)	454
DIOXIDE, SULPHUR (REAGENTS AND REAGENT SOLUTIONS)	454
DIOXIDE, MANGANESE (REAGENTS AND REAGENT SOLUTIONS)	454
DIOXIDE, SILICON	912
DIPYRONE, TABLETS	912
DIPYRONE, SODIUM, MONOHYDRATE	913
DIPYRONE, ORAL SOLUTION	914
DIPROPYLENEGLYCOL (REAGENTS AND REAGENT SOLUTIONS)	454
DISULFIDE, CARBON (REAGENTS AND REAGENT SOLUTIONS)	454
DITHIOL (REAGENTS AND REAGENT SOLUTIONS)	454
DITHIOL SR (REAGENTS AND REAGENT SOLUTIONS)	455
DITHIOTHREITOL (REAGENTS AND REAGENT SOLUTIONS)	455
DITHIZONE (REAGENTS AND REAGENT SOLUTIONS)	455
DITHIZONE SR (REAGENTS AND REAGENT SOLUTIONS)	455
DITHIZONE, CONCENTRATED SOLUTION (REAGENTS AND REAGENT SOLUTIONS)	455
DITHIZONE, DILUTED SOLUTION (REAGENTS AND REAGENT SOLUTIONS)	455
DITHIZONE, EXTRACTING SOLUTION (REAGENTS AND REAGENT SOLUTIONS)	455
DL-PHENYLALANINE (REAGENTS AND REAGENT SOLUTIONS)	457
DOSAGE FORM OF TOTAL PROTEIN	222
D-A-4 -HYDROXYPHENYL GLYCINE (REAGENTS AND REAGENT SOLUTIONS)	465

E

EDETATE, DISODIUM (REAGENTS AND REAGENT SOLUTIONS)	455
EDETATE, DISODIUM 0.05M M VS (VOLUMETRIC SOLUTIONS)	502
EDETATE, DISODIUM 0.1 M VS (VOLUMETRIC SOLUTIONS)	503
EDETATE, DISODIUM, SOLUTION 0.05 M (REAGENTS AND REAGENT SOLUTIONS)	455
EFAVIRENZ	915
EFAVIRENZ, TABLETS	916
ELECTROPHORESIS	129
ELECTROPHORESIS, CAPILLARY	136
ELECTROPHORESIS, CAPILLARY, IN FREE SOLUTION	138
ELECTROPHORESIS, DSS-EGPA (BUFFERS)	509
EMBONATE, PYRVINIUM	917
EMODIN (REAGENTS AND REAGENT SOLUTIONS)	455
ENDOTOXINS ,BACTERIAL	230
DILL	918
IODOMETRIC TEST, ANTIBIOTICS	191
LIMIT TEST, ALUMINUM	178
LIMIT TEST, AMMONIA	177
LIMIT TEST, ARSENIC	176

LIMIT TEST, CALCIUM	178
LIMIT TEST, LEAD	179
LIMIT TEST, CHLORIDE	169
LIMIT TEST, IRON	174
LIMIT TEST, PHOSPHATE	179
LIMIT TEST, MAGNESIUM	178
LIMIT TEST, MAGNESIUM AND ALKALINE EARTH METALS	178
LIMIT TEST, HEAVY METALS	171
LIMIT TEST, SULFATES	170
MICROBIOLOGICAL TEST, ANTIBIOTICS	261
MICROBIOLOGICAL TEST, AGAR DIFFUSION	264
MICROBIOLOGICAL TEST, TURBIDIMETRY	270
BIOLOGICAL TESTS	229
DIRECT TESTS	341
PHYSICAL AND CHEMICAL / PHYSICAL TESTS, FATS AND OILS	149
INDIRECT TESTS, "ALL OR NOTHING"	347
INDIRECT TESTS, QUANTITATIVE	341
LIMIT TEST, INORGANIC IMPURITIES	169
MICROBIOLOGICAL TEST	236
MICROBIOLOGICAL TEST, STERILE PRODUCTS	253
MICROBIOLOGICAL TEST, NON-STERILE PRODUCTS	236
CHEMICAL TESTS	180
SULPHUR (REAGENTS AND REAGENT SOLUTIONS)	455
EOSIN Y (CI 45380) (INDICATORS AND INDICATOR SOLUTIONS)	417
EOSIN Y SI (INDICATORS AND INDICATOR SOLUTIONS)	417
PHARMACEUTICAL EQUIVALENCE AND DRUG BIOEQUIVALENCE	387
ESCIN (REAGENTS AND REAGENT SOLUTIONS)	455
MEDICAL TAPE	922
SPECTROPHOTOMETRY, FLUORESCENCE	102
SPECTROPHOTOMETRY, ULTRAVIOLET, VISIBLE AND INFRARED	99
SPECTROMETRY, ATOMIC ABSORPTION	94
SPECTROMETRY, ATOMIC ABSORPTION WITH FLAME	94
SPECTROMETRY, ATOMIC ABSORPTION WITH GRAPHITE FURNACE	95
SPECTROMETRY, ATOMIC ABSORPTION WITH HYDRIDE GENERATION	95
SPECTROMETRY, ATOMIC ABSORPTION WITH COLD VAPOR GENERATION	95
SPECTROMETRY, ATOMIC EMISSION	96
SPECTROMETRY, OPTICAL EMISSION WITH INDUCTIVELY COUPLED PLASMA	97
SPECTROMETRY, MASS, WITH INDUCTIVELY COUPLED PLASMA	97
ESPINHEIRA SANTA	922
SPIRONOLACTONE	928
SQUALANE	929
TIN, METAL (REAGENTS AND REAGENT SOLUTIONS)	456
STEARATE, MACROGOL 40	930
STEARATE, METHYL (REAGENTS AND REAGENT SOLUTIONS)	456
ETHYL ESTER, TETRABROMO PHENOLPHTHALEIN (INDICATORS AND INDICATOR SOLUTIONS)	417
ETHYL ESTER, TETRABROMO PHENOLPHTHALEIN (REAGENTS AND REAGENT SOLUTIONS)	456
ETHYL ESTER, TETRABROMO PHENOLPHTHALEIN SI (INDICATORS AND INDICATOR SOLUTIONS)	417
STERILIZATION AND STERILITY ASSURANCE	321
STERILIZATION, HEAT	322
STERILIZATION, FILTRATION	323
STERILIZATION, IONIZING RADIATION	322
STEVIA	930

POTENCY ESTIMATE AND CONFIDENCE LIMITS	344
ESTOLATE, ERYTHROMYCIN	935
ESTOLATE, ERYTHROMYCIN (REAGENTS AND REAGENT SOLUTIONS)	456
ESTOLATE, ERYTHROMYCIN, TABLETS	936
ESTOLATE, ERYTHROMYCIN, ORAL SUSPENSION	936
ESTRADIOL	937
DATURA	938
ESTRONE	942
STRONTIUM SRA - 1 MG/ML (REAGENTS AND REAGENT SOLUTIONS)	456
ETHANOL (REAGENTS AND REAGENT SOLUTIONS)	456
ETHANOL, ABSOLUTE (REAGENTS AND REAGENT SOLUTIONS)	456
ETHANOL, GLYCERIN (REAGENTS AND REAGENT SOLUTIONS)	456
ETHER, PETROLEUM (REAGENTS AND REAGENT SOLUTIONS)	456
ETHER, ETHYL	943
ETHER, ETHYL (REAGENTS AND REAGENT SOLUTIONS)	456
ETHER, ISOPROPYL (REAGENTS AND REAGENT SOLUTIONS)	457
ETHYLENE GLYCOL (REAGENTS AND REAGENT SOLUTIONS)	457
ETHYLPARABEN (REAGENTS AND REAGENT SOLUTIONS)	457
ETHINYLESTRADIOL	944
ETHIONAMIDE	945
ETHIONAMIDE, TABLETS	946
EUGENOL (REAGENTS AND REAGENT SOLUTIONS)	457
INSPECTION, VISUAL / MICROSCOPIC	192
INSPECTION, VISUAL / ODOR / FLAVOR	192
EXAMPLE, COMBINED POTENCY ESTIMATES	372
EXAMPLE, DIRECT TEST	359
EXAMPLE, INDIRECT TESTS, "ALL OR NOTHING"	371
EXAMPLES, STATISTICAL CALCULATIONS APPLIED IN BIOLOGICAL TESTS	359
EXAMPLES, INDIRECT TESTS, QUANTITATIVE	360
EXTRACTS, LIQUID (EXTRACTA FLUIDA)	204
EXTRACTS, SOFT (EXTRACTA SPISSA)	205
EXTRACTS, DRY (EXTRACTA SICCA)	205
EXTRACTS, DRY (EXTRACTA SICCA)	206

F

BRAZILIAN PHARMACOPOEIA	21
FAST GREEN (CI 42053) (REAGENTS AND REAGENT SOLUTIONS)	457
FACTOR IX OF THE HUMAN BLOOD CLOTTING, LYOPHILISATE	949
FACTOR VII OF THE HUMAN BLOOD CLOTTING, LYOPHILISATE	950
FACTOR VIII OF THE HUMAN BLOOD CLOTTING, LYOPHILISATE	951
FACTOR XA BOVINE, SOLUTION (REAGENTS AND REAGENT SOLUTIONS)	457
FACTOR XA OF BOVINE BLOOD CLOTTING (REAGENTS AND REAGENT SOLUTIONS)	457
PHENINDIONE	952
PHENYTOIN	954
PHENYTOIN, TABLETS	955
PHENYTOIN, SODIUM, INJECTABLE SOLUTION	956
PHENOBARBITAL	957
PHENOBARBITAL, TABLETS	959
PHENOBARBITAL, ORAL SOLUTION	960
PHENOL	960
PHENOL (REAGENTS AND REAGENT SOLUTIONS)	457

PHENOLPHTHALEIN (INDICATORS AND INDICATOR SOLUTIONS)	417
PHENOLPHTHALEIN (REAGENTS AND REAGENT SOLUTIONS)	458
PHENOLPHTHALEIN AT 0.1% (W/V) (REAGENTS AND REAGENT SOLUTIONS)	458
PHENOLPHTHALEIN SI (INDICATORS AND INDICATOR SOLUTIONS)	417
PHENOLPHTHALEIN, PAPER (INDICATORS AND INDICATOR SOLUTIONS)	417
PHENOXYMETHYLPENICILLIN, POTASSIUM	961
FERRICYANIDE, POTASSIUM (REAGENTS AND REAGENT SOLUTIONS)	458
FERRICYANIDE, AMMONIACAL POTASSIUM (REAGENTS AND REAGENT SOLUTIONS)	458
FERROCYANIDE, POTASSIUM (REAGENTS AND REAGENT SOLUTIONS)	458
FERROCYANIDE, POTASSIUM SR (REAGENTS AND REAGENT SOLUTIONS)	458
FERROIN (INDICATORS AND INDICATOR SOLUTIONS)	417
FERROIN SI (INDICATORS AND INDICATOR SOLUTIONS)	417
FIBRINOGEN (REAGENTS AND REAGENT SOLUTIONS)	458
FIBRINOGEN, HUMAN, LYOPHILISATE	963
ADHESIVE TAPE	964
PHYTOMENADIONE	965
PHOROGLUCINE SR (REAGENTS AND REAGENT SOLUTIONS)	458
PHLOROGLUCINOL (REAGENTS AND REAGENT SOLUTIONS)	458
FLUCONAZOLE	966
FLUCONAZOLE, CAPSULES	967
SIMULATED GASTRIC FLUID (REAGENTS AND REAGENT SOLUTIONS)	458
SIMULATED GASTRIC FLUID (NO ENZYME) (REAGENTS AND REAGENT SOLUTIONS)	459
SIMULATED INTESTINAL FLUID (NO PANCREATIN) PH 7.5 (REAGENTS AND REAGENT SOLUTIONS)	459
FLUNITRAZEPAM, TABLETS	968
FLUNITRAZEPAM, INJECTABLE SOLUTION	969
FLUOCINOLONE, ACETONIDE	969
FLUORESCEIN, SODIUM	970
FLUORIDE, AMMONIUM (REAGENTS AND REAGENT SOLUTIONS)	459
FLUORIDE, CALCIUM (REAGENTS AND REAGENT SOLUTIONS)	459
FLUORIDE, SODIUM	971
FLUORIDE, SODIUM (REAGENTS AND REAGENT SOLUTIONS)	459
FLUORIDE, SODIUM, ORAL SOLUTION	972
FLUORIDE, SODIUM SR (REAGENTS AND REAGENT SOLUTIONS)	459
FLUORIDE, STANNOUS	972
FLUTAMIDE	974
FLUTAMIDE, TABLETS	975
FOLINATE, CALCIUM	976
FORMALDEHYDE, SOLUTION (REAGENTS AND REAGENT SOLUTIONS)	459
FORMAMIDE (REAGENTS AND REAGENT SOLUTIONS)	459
FORMATE, AMMONIUM (REAGENTS AND REAGENT SOLUTIONS)	459
PHOSPHATASE, ALKALINE, SOLUTION (REAGENTS AND REAGENT SOLUTIONS)	459
PHOSPHATE 0.025M PH 6.86 (BUFFERS)	507
PHOSPHATE, ALUMINUM	977
PHOSPHATE, DIBASIC AMMONIUM	978
PHOSPHATE, DIBASIC AMMONIUM (REAGENTS AND REAGENT SOLUTIONS)	459
PHOSPHATE, MONOBASIC AMMONIUM (REAGENTS AND REAGENT SOLUTIONS)	460
PHOSPHATE, DIBASIC CALCIUM, DIHYDRATE	978
PHOSPHATE, TRIBASIC CALCIUM	979
PHOSPHATE, CLINDAMYCIN	980
PHOSPHATE, CLINDAMYCIN, INJECTABLE SOLUTION	981
PHOSPHATE, CODEIN (REAGENTS AND REAGENT SOLUTIONS)	460
PHOSPHATE, POTASSIUM (REAGENTS AND REAGENT SOLUTIONS)	460

PHOSPHATE, DIBASIC POTASSIUM (REAGENTS AND REAGENT SOLUTIONS)	460
PHOSPHATE, MONOBASIC POTASSIUM (REAGENTS AND REAGENT SOLUTIONS)	460
PHOSPHATE, POTASSIUM PH 7.4 WITH POLYSORBATE 80 AT 2% (V/V) (BUFFERS)	508
PHOSPHATE, DIBASIC SODIUM	982
PHOSPHATE, DIBASIC SODIUM, DODECAHYDRATE SR (REAGENTS AND REAGENT SOLUTIONS)	460
PHOSPHATE, DIBASIC SODIUM, HEPTAHYDRATE SR (REAGENTS AND REAGENT SOLUTIONS)	460
PHOSPHATE, DIBASIC SODIUM, DIHYDRATE (REAGENTS AND REAGENT SOLUTIONS)	460
PHOSPHATE, DIBASIC SODIUM, DODECAHYDRATE (REAGENTS AND REAGENT SOLUTIONS)	460
PHOSPHATE, DIBASIC SODIUM, HEPTAHYDRATE (REAGENTS AND REAGENT SOLUTIONS)	460
PHOSPHATE, MONOBASIC SODIUM	983
PHOSPHATE, MONOBASIC SODIUM (REAGENTS AND REAGENT SOLUTIONS)	460
PHOSPHATE, MONOBASIC SODIUM, DIHYDRATE (REAGENTS AND REAGENT SOLUTIONS)	461
PHOSPHATE, MONOBASIC SODIUM, MONOHYDRATE (REAGENTS AND REAGENT SOLUTIONS)	461
PHOSPHATE, SODIUM, ORAL SOLUTION	984
PHOSPHATE, TRIBASIC SODIUM, DODECAHYDRATE (REAGENTS AND REAGENT SOLUTIONS)	461
PHOSPHATE, TETRABUTYLAMMONIUM (REAGENTS AND REAGENT SOLUTIONS)	461
PHOSPHATE, TRIBUTYL (REAGENTS AND REAGENT SOLUTIONS)	461
PHOSPHATE, DEXAMETHASONE DISODIUM	985
PHOSPHATE, EQUIMOLAR 0.05 M (REAGENTS AND REAGENT SOLUTIONS)	461
PHOSPHATE M/L5 PH 7.0 (BUFFERS)	508
PHOSPHATE PH 2.2 (BUFFERS)	506
PHOSPHATE PH 5.5 (BUFFERS)	507
PHOSPHATE PH 5.8 (BUFFERS)	507
PHOSPHATE PH 6.0 (BUFFERS)	507
PHOSPHATE PH 6.5 (BUFFERS)	507
PHOSPHATE PH 6.8 (BUFFERS)	507
PHOSPHATE PH 7.0 (BUFFERS)	508
PHOSPHATE PH 7.1 (BUFFERS)	508
PHOSPHATE PH 7.2 (BUFFERS)	508
PHOSPHATE PH 7.3 (BUFFERS)	508
PHOSPHATE PH 8.5 (BUFFERS)	509
PHOSPHATE PH 8.6 (BUFFERS)	509
PHOSPHATE, RIBOFLAVIN SODIUM	986
PHOSPHATE, SODIUM LAURYL SULFATE PH 11.0 (BUFFERS)	509
PHOSPHATE, SODIUM LAURYL SULFATE PH 6.8 (BUFFERS)	507
PHOSPHATE, BROMOCRESOL PURPLE SR (REAGENTS AND REAGENT SOLUTIONS)	461
PHOSPHATE, SALINE (PBS) (BUFFERS)	510
PHOSPHORUS, RED (REAGENTS AND REAGENT SOLUTIONS)	461
PHOTOMETRY, FLAME	96
FRUCTOSE (REAGENTS AND REAGENT SOLUTIONS)	461
FRUCTOSE AT 0.1 % (W/V) (REAGENTS AND REAGENT SOLUTIONS)	461
PHTHALALDEHYDE (REAGENTS AND REAGENT SOLUTIONS)	461
PHTHALATE, DIBUTYL (REAGENTS AND REAGENT SOLUTIONS)	462
PHTHALATE, ETHYL	988
PHTHALAZINE (REAGENTS AND REAGENT SOLUTIONS)	462
FUCHSIN, BASIC (CI 42510) (REAGENTS AND REAGENT SOLUTIONS)	462
FUCHSIN, BLEACHED SR (REAGENTS AND REAGENT SOLUTIONS)	462
BASIS	339
FUROSEMIDE	989
FUROSEMIDE, TABLETS	990
FUROSEMIDE, INJECTABLE SOLUTION	991

G

GALACTOSE (REAGENTS AND REAGENT SOLUTIONS)	462
GALACTOSE AT 0.1% (W/V) IN PYRIDINE (REAGENTS AND REAGENT SOLUTIONS)	462
GAS, ETHYLENE OXIDE	324
GAUZE, PETROLATUM	993
GEL	
HYDROCHLORIDE, LIDOCAINE	840
HYDROXIDE, ALUMINUM	1041
PIROXICAM	1205
TRETINOIN	1348
GELATIN (REAGENTS AND REAGENT SOLUTIONS)	462
GELATIN, GLYCERINATED (REAGENTS AND REAGENT SOLUTIONS)	462
GELATIN SR (REAGENTS AND REAGENT SOLUTIONS)	462
GENTIAN	993
GENERAL	39
GEMFIBROZIL	997
GLIBENCLAMIDE	998
GLIBENCLAMIDE, TABLETS	999
GLYCEROL	1001
GLYCEROL (REAGENTS AND REAGENT SOLUTIONS)	462
GLYCEROL, SUPPOSITORIES	1002
GLYCINE	1003
GLYCINE (REAGENTS AND REAGENT SOLUTIONS)	462
GLICLAZIDE	1003
GLUCONATE, COPPER	1005
GLUCONATE, MAGNESIUM	1006
GLUCOSE	1007
GLUCOSE (REAGENTS AND REAGENT SOLUTIONS)	463
GLUCOSE AT 0.1% (W/V) IN PYRIDINE (REAGENTS AND REAGENT SOLUTIONS)	463
GLUCOSE, INJECTABLE SOLUTION	1008
GLOSSARY OF SYMBOLS	338
GLUTARALDEHYDE (REAGENTS AND REAGENT SOLUTIONS)	463
GUAIACOL (REAGENTS AND REAGENT SOLUTIONS)	463
GUANINE (REAGENTS AND REAGENT SOLUTIONS)	463
GUARANA	1009
GUIDE FOR SELECTION OF PLASTIC AND OTHER POLYMERS; CLASS DESIGNATION FOR RELATED MATERIAL	307

H

HALOPERIDOL	1013
HALOPERIDOL, TABLETS	1014
HALOPERIDOL, INJECTABLE SOLUTION	1015
HALOPERIDOL, ORAL SOLUTION	1016
HALOTHANE	1017
HAMAMELIS, TINCTURE	1018
HEPARIN, CALCIUM	1019
HEPARIN, SODIUM	1024
HEPARIN, SODIUM (REAGENTS AND REAGENT SOLUTIONS)	463
HEPTANE (REAGENTS AND REAGENT SOLUTIONS)	463
HEPTANE SULPHONATE, SODIUM (REAGENTS AND REAGENT SOLUTIONS)	463
HEXANE (REAGENTS AND REAGENT SOLUTIONS)	464

HEXYLAMINE (REAGENTS AND REAGENT SOLUTIONS)	464
HEXYLRESORCINOL	1029
HYCLATE, DOXYCYCLINE	1030
GOLDEN SEAL	1032
HYDRATE, CHLORAL (REAGENTS AND REAGENT SOLUTIONS)	464
HYDRATE, CHLORAL (REAGENTS AND REAGENT SOLUTIONS)	464
HYDROCHLOROTHIAZIDE	1035
HYDROCHLOROTHIAZIDE, TABLETS	1036
HYDROCORTISONE	1037
HYDROQUINONE	1038
HYDROXYCOBALAMIN, INJECTABLE SOLUTION	1039
HYDROXIDE, ALUMINUM	1039
HYDROXIDE, ALUMINUM, CHEWABLE TABLETS	1039
HYDROXIDE, ALUMINUM, GEL	1041
HYDROXIDE, AMMONIUM (REAGENTS AND REAGENT SOLUTIONS)	464
HYDROXIDE, BARIUM (REAGENTS AND REAGENT SOLUTIONS)	464
HYDROXIDE, CALCIUM	1042
HYDROXIDE, CALCIUM (REAGENTS AND REAGENT SOLUTIONS)	464
HYDROXIDE, CALCIUM SR (REAGENTS AND REAGENT SOLUTIONS)	464
HYDROXIDE, CALCIUM, SATURATED SOLUTION (REAGENTS AND REAGENT SOLUTIONS)	464
HYDROXIDE, LITHIUM (REAGENTS AND REAGENT SOLUTIONS)	464
HYDROXIDE, MAGNESIUM	1043
HYDROXIDE, POTASSIUM	1044
HYDROXIDE, POTASSIUM (REAGENTS AND REAGENT SOLUTIONS)	465
HYDROXIDE, ETHANOLIC POTASSIUM 0.5 M SV (VOLUMETRIC SOLUTIONS)	503
HYDROXIDE, ETHANOLIC POTASSIUM 2 M (REAGENTS AND REAGENT SOLUTIONS)	465
HYDROXIDE, ETHANOLIC POTASSIUM SR (APPROXIMATELY 0.5 M) (REAGENTS AND REAGENT SOLUTIONS)	465
HYDROXIDE, POTASSIUM M SV (VOLUMETRIC SOLUTIONS)	503
HYDROXIDE, SODIUM (REAGENTS AND REAGENT SOLUTIONS)	465
HYDROXIDE, ETHANOLIC SODIUM 0.1 M SV (VOLUMETRIC SOLUTIONS)	503
HYDROXIDE, SODIUM M (REAGENTS AND REAGENT SOLUTIONS)	465
HYDROXIDE, SODIUM M SV (VOLUMETRIC SOLUTIONS)	503
HYDROXIDE, SODIUM SR (REAGENTS AND REAGENT SOLUTIONS)	465
HYDROXIDE, SODIUM, CONCENTRATED SOLUTION SR (APPROXIMATELY 10 M) (REAGENTS AND REAGENT SOLUTIONS)	465
HYDROXIDE, TETRABUTYLAMMONIUM (REAGENTS AND REAGENT SOLUTIONS)	465
HYDROXIDE, TETRABUTYLAMMONIUM 0.1 M SV (VOLUMETRIC SOLUTIONS)	503
HYDROXIDE, TETRAMETHYLAMMONIUM (REAGENTS AND REAGENT SOLUTIONS)	465
HYDROXYQUINOLINE (REAGENTS AND REAGENT SOLUTIONS)	466
HYDROXYTOLUENE, BUTYLATED (REAGENTS AND REAGENT SOLUTIONS)	466
HYPEROSIDE (REAGENTS AND REAGENT SOLUTIONS)	466
HYPOCHLORITE, SODIUM (REAGENTS AND REAGENT SOLUTIONS)	466
HYPOCHLORITE, SODIUM, DILUTED SOLUTION	1045
HYPOCHLORITE, SODIUM SR (REAGENTS AND REAGENT SOLUTIONS)	466
HYPOPHOSPHITE, SODIUM (REAGENTS AND REAGENT SOLUTIONS)	466
HYPOPHOSPHITE, SODIUM SR (REAGENTS AND REAGENT SOLUTIONS)	466
HISTAMINE	235
HISTORY	13
PEPPER MINT	1046
PEPPER MINT, VOLATILE OIL	1050

I

IBUPROFEN	1053
IBUPROFEN, TABLETS	1053
IDENTIFICATION OF STEROIDS BY THIN-LAYER CHROMATOGRAPHY	167
IDENTIFICATION OF PHENOTHIAZINES BY THIN-LAYER CHROMATOGRAPHY	168
IDENTIFICATION OF MICROBIAL ISOLATES	335
IDENTIFICATION OF FIXED OILS	155
IDENTIFICATION OF VEGETABLE OILS BY THIN-LAYER CHROMATOGRAPHY	155
IMIDAZOLE (REAGENTS AND REAGENT SOLUTIONS)	466
IMIDAZOLE PH 7.4 (BUFFERS)	508
IMINO DIBENZYL (REAGENTS AND REAGENT SOLUTIONS)	466
IMPURITIES, ALKALINE	155
IMMUNOGLOBULIN, HUMAN, ANTI-HEPATITIS A	1055
IMMUNOGLOBULIN, HUMAN, ANTI-HEPATITIS B	1056
IMMUNOGLOBULIN, HUMAN, ANTI-HEPATITIS B, INTRAVENOUS ADMINISTRATION	1056
IMMUNOGLOBULIN, HUMAN, ANTI-RABIES	1056
IMMUNOGLOBULIN, HUMAN, ANTI-RUBELLA	1058
IMMUNOGLOBULIN, HUMAN, ANTI-VARICELLA	1060
IMMUNOGLOBULIN, HUMAN, ANTI-VARICELLA, INTRAVENOUS ADMINISTRATION	1061
IMMUNOGLOBULIN, HUMAN, ANTI-ANTIGEN D	1055
IMMUNOGLOBULIN, HUMAN, ANTI-MEASLES	1058
IMMUNOGLOBULIN, HUMAN, ANTI-TETANUS	1059
IMMUNOGLOBULIN, HUMAN, NORMAL	1061
IMMUNOGLOBULIN, HUMAN, NORMAL, INTRAVENOUS ADMINISTRATION	1064
INDICATORS, BIOLOGICAL	326
INDICATORS AND INDICATOR SOLUTIONS	413
METHYL ORANGE (CI 13025)	413
METHYL ORANGE SI	413
METHYL ORANGE, SOLUTION	413
XYLENOL ORANGE	413
XYLENOL ORANGE SI	413
ALIZARIN	413
ALIZARIN SI	414
ALIZARIN YELLOW GG (CI 14025)	414
ALIZARIN YELLOW GG SI	414
DIMETHYL YELLOW (CI 11020)	414
DIMETHYL YELLOW SI	414
METANIL YELLOW (CI 13065)	414
METANIL YELLOW SI	414
NAPHTHOL YELLOW (CI 10315)	414
TITAN YELLOW (CI 19540)	414
TITAN YELLOW SI	414
TITAN YELLOW, PAPER	414
STARCH (STARCH SOLUBLE)	414
STARCH SI	414
STARCH, IODINE SI	415
STARCH, IODIDE-FREE SI	415
STARCH, IODIDE, PAPER	415
BROMOPHENOL BLUE	415
BROMOPHENOL BLUE SI	415
BROMOTHYMOL BLUE	415

BROMOTHYMOL BLUE SI	415
HYDROXY NAPHTHOL BLUE	415
HYDROXY NAPHTHOL BLUE SI	415
ORACET BLUE B	415
ORACET BLUE B SI	415
THYMOL BLUE	415
THYMOL BLUE SI	415
NILE BLUE A (CI 51180)	416
NILE BLUE A SI	416
CHALCONE	416
CHALCONE SI	416
CHALCONE, COMPOUND	416
CHLORIDE, METHYLROSANILINIUM (CI 42555)	416
CHLORIDE, METHYLROSANILINIUM SI	416
CHLORIDE, FERRIC	416
CHLORIDE, FERRIC SI (APPROXIMATELY 0.4 M)	416
DYE BVF	416
DIPHENYL CARBAZIDE	416
DIPHENYL CARBAZIDE SI	416
DIPHENYL CARBAZONE	416
DIPHENYL CARBAZONE SI	417
EOSIN Y (CI 45380)	417
EOSIN Y SI	417
ETHYL ESTER, TETRABROMO PHENOLPHTHALEIN	417
ETHYL ESTER, TETRABROMO PHENOLPHTHALEIN SI	417
PHENOLPHTHALEIN	417
PHENOLPHTHALEIN SI	417
PHENOLPHTHALEIN, PAPER	417
FERROIN	417
FERROIN SI	417
MAGNESON	417
MAGNESON SI	417
MAGNESON, REAGENT	417
1-NAPHTHOLBENZEIN	417
1-NAPHTHOLBENZEIN SI	417
1-NAPHTHOLPHTHALEIN	418
1-NAPHTHOLPHTHALEIN SI	418
ERIOCHROME BLACK T (CI 14645)	418
ERIOCHROME BLACK T SI	418
OXALATE, AMMONIUM	418
OXALATE, AMMONIUM SI	418
PURPLE, BROMOCRESOL	418
PURPLE, BROMOCRESOL SI	418
PURPLE, BROMOCRESOL, REAGENT	418
PURPLE, METACRESOL	418
PURPLE, METACRESOL SI	418
RESAZURIN	418
RESAZURIN SI	419
RESORCINOL	419
RESORCINOL SI	419
THYMOLPHTHALEIN	419
THYMOLPHTHALEIN SI	419

THIOCYANATE, AMMONIUM	419
THIOCYANATE, AMMONIUM SI	419
LITMUS	419
LITMUS SI	419
LITMUS, BLUE, PAPER	419
LITMUS, RED, PAPER	419
TROPEOLIN O (CI 14270)	419
TROPEOLIN O SI	419
TROPEOLIN OO (CI 13080)	420
BROMOCRESOL GREEN	420
BROMOCRESOL GREEN SI	420
MALACHITE GREEN, OXALATE	420
MALACHITE GREEN SI	420
METHYL GREEN (CI 42590)	420
METHYL GREEN SI	420
CRESOL RED	420
CRESOL RED SI	420
CONGO RED (CI 22120)	420
CONGO RED SI	420
CONGO RED, PAPER	420
PHENOL RED	421
PHENOL RED SI	421
METHYL RED (CI 13020)	421
METHYL RED SI	421
QUINALDINE RED	421
QUINALDINE RED SI	421
INDEX OF ACIDITY	150
INDIGO CARMINE (REAGENTS AND REAGENT SOLUTIONS)	467
INDIGO CARMINE SR (REAGENTS AND REAGENT SOLUTIONS)	467
INDIGO CARMINE SV (VOLUMETRIC SOLUTIONS)	504
INDOMETHACIN	1066
INDOMETHACIN, CAPSULES	1067
INDOMETHACIN, SUPPOSITORIES	1068
IODATE, POTASSIUM (REAGENTS AND REAGENT SOLUTIONS)	466
IODATE, POTASSIUM 0.02 M SV (VOLUMETRIC SOLUTIONS)	504
IODATE, POTASSIUM 0.1 M SV (VOLUMETRIC SOLUTIONS)	504
MERCURY, IODIDE (II) (REAGENTS AND REAGENT SOLUTIONS)	466
IODIDE, POTASSIUM	1069
IODIDE, POTASSIUM (REAGENTS AND REAGENT SOLUTIONS)	467
IODIDE, POTASSIUM, APPROXIMATELY M (REAGENTS AND REAGENT SOLUTIONS)	467
IODIDE, POTASSIUM AND SUBNITRATE, BISMUTH SR (REAGENTS AND REAGENT SOLUTIONS)	468
IODIDE, POTASSIUM, ALKALINE MERCURY SR1 (REAGENTS AND REAGENT SOLUTIONS)	467
IODIDE, POTASSIUM, MERCURY SR (REAGENTS AND REAGENT SOLUTIONS)	467
IODIDE, POTASSIUM SR (REAGENTS AND REAGENT SOLUTIONS)	467
IODIDE, SODIUM	1070
IODIDE, SODIUM (REAGENTS AND REAGENT SOLUTIONS)	467
IODIDE, SODIUM, IN ACETIC ACID (REAGENTS AND REAGENT SOLUTIONS)	467
IODIDE, TETRABUTYLAMMONIUM (REAGENTS AND REAGENT SOLUTIONS)	467
IODINE	1071
IODINE (REAGENTS AND REAGENT SOLUTIONS)	468
IODINE 0.05 M (REAGENTS AND REAGENT SOLUTIONS)	468
IODINE 0.05 M SV (VOLUMETRIC SOLUTIONS)	504

IODINE 0.1 M SV (VOLUMETRIC SOLUTIONS)	504
IODINE 0.5 % (W/V) IN CHLOROFORM (REAGENTS AND REAGENT SOLUTIONS)	468
IODINE 1 % (W/V) IN ETHANOL (REAGENTS AND REAGENT SOLUTIONS)	468
IODINE SR (REAGENTS AND REAGENT SOLUTIONS)	468
IODOBISMUTHATE, POTASSIUM (REAGENTS AND REAGENT SOLUTIONS)	468
IODOBISMUTHATE, AQUEOUS ACETIC POTASSIUM (REAGENTS AND REAGENT SOLUTIONS)	468
IODOBISMUTHATE, DILUTED POTASSIUM SR (REAGENTS AND REAGENT SOLUTIONS)	468
IODOBISMUTHATE, POTASSIUM SR1 (REAGENTS AND REAGENT SOLUTIONS)	468
ION, GROUPS AND FUNCTIONS	162
IRGANOX 1010 (REAGENTS AND REAGENT SOLUTIONS)	469
IRGANOX 1076 (REAGENTS AND REAGENT SOLUTIONS)	469
IRGANOX PS 800 (REAGENTS AND REAGENT SOLUTIONS)	469
ISONIAZID	1072
ISONIAZID, TABLETS	1073
ISOOCTANE (REAGENTS AND REAGENT SOLUTIONS)	469
ISOTHIOCYANATE, ALLYL	1074
ISOTHIOCYANATE, FLUORESCEIN (REAGENTS AND REAGENT SOLUTIONS)	469
ISOTRETINOIN, CAPSULES	1075

J

JABORANDI, TINCTURE	1077

L

LACTATE, CALCIUM	1079
LACTOSE (REAGENTS AND REAGENT SOLUTIONS)	469
LACTOSE AT 0.1% (W/V) IN PYRIDINE (REAGENTS AND REAGENT SOLUTIONS)	469
LAMIVUDINE	1079
LAMIVUDINE, TABLETS	1080
LAMOTRIGINE	1081
LAMOTRIGINE, TABLETS	1082
LANATOSÍDEO C	1083
BITTER ORANGE	1084
LAURATE, METHYL (REAGENTS AND REAGENT SOLUTIONS)	469
LAURYL SULFATE, SODIUM	1088
LAURYL SULFATE, SODIUM (REAGENTS AND REAGENT SOLUTIONS)	470
LAURYL SULFATE, SODIUM SR (REAGENTS AND REAGENT SOLUTIONS)	470
LECITHIN (REAGENTS AND REAGENT SOLUTIONS)	470
LEFLUNOMIDE	1089
LEFLUNOMIDE, TABLETS	1090
L-DOPA	1091
LEVONORGESTREL	1092
LEVONORGESTREL AND ETHINYLESTRADIOL, TABLETS	1093
LIDOCAINE	1095
ALLOY, NICKEL-ALUMINUM (REAGENTS AND REAGENT SOLUTIONS)	470
LIMITS, MICROBIAL	250
LIMITS, MICROBIOLOGICAL, ALERT AND ACTION IN CLEAN ROOMS AND AREAS	334
LIQUID, CLARITY	145
LINALOOL (REAGENTS AND REAGENT SOLUTIONS)	470
LITHIUM (REAGENTS AND REAGENT SOLUTIONS)	470
LITHIUM SRA - 2 MG/ML (REAGENTS AND REAGENT SOLUTIONS)	470

LODOBISMUTHATE, POTASSIUM SR (REAGENTS AND REAGENT SOLUTIONS)	468
LODOBISMUTHATE, POTASSIUM SR2 (REAGENTS AND REAGENT SOLUTIONS)	468
LORATADINE	1096
LORATADINE, TABLETS	1098
LORATADINE AND PSEUDOEPHEDRINE SULFATE, ORAL SOLUTION	1100
LORATADINE, ORAL SOLUTION	1099
LOSARTAN, POTASSIUM	1101

M

MACRO-DETERMINATION (METHOD I)	181
MACROGOL	1103
MACROGOL 1000 (REAGENTS AND REAGENT SOLUTIONS)	470
MACROGOL 300 (REAGENTS AND REAGENT SOLUTIONS)	470
MAGNESIUM SRA - 1 MG/ML (REAGENTS AND REAGENT SOLUTIONS)	470
MAGNESON (INDICATORS AND INDICATOR SOLUTIONS)	417
MAGNESON (REAGENTS AND REAGENT SOLUTIONS)	470
MAGNESON (INDICATORS AND INDICATOR SOLUTIONS)	417
MAGNESON, REAGENT (INDICATORS AND INDICATOR SOLUTIONS)	417
MALEATE, CHLORPHENIRAMINE	1104
MALEATE, DEXCHLORPHENIRAMINE	1105
MALEATE, DEXCHLORPHENIRAMINE, TABLETS	1106
MALEATE, DEXCHLORPHENIRAMINE, ORAL SOLUTION	1107
MALEATE, ENALAPRIL	1108
MALEATE, ENALAPRIL, TABLETS	1109
MALEATE, LEVOMEPRIMAZINE	1110
PASSION FRUIT, SOUR	1111
PASSION FRUIT, SWEET	1116
MEBENDAZOLE	1120
MEBENDAZOLE, TABLETS	1120
MEBENDAZOLE, ORAL SUSPENSION	1122
MOVING AVERAGES	346
HENBANE	1123
CULTURE MEDIUM AND DILUENTS FOR SAMPLING AND MEASUREMENT OF VIABLE PARTICLES	335
MELAMINE (REAGENTS AND REAGENT SOLUTIONS)	471
MELISSA	1127
MERBROMINE	1135
MERCURY SRA - 1 MG/ML (REAGENTS AND REAGENT SOLUTIONS)	471
MEROPENEM	1136
MEROPENEM, TRIHYDRATE, POWDER FOR INJECTABLE SOLUTION	1138
METABISULFITE, SODIUM	1140
METABISULPHITE, SODIUM (REAGENTS AND REAGENT SOLUTIONS)	471
METAPHOSPHATE, POTASSIUM	1140
METHANOL (REAGENTS AND REAGENT SOLUTIONS)	471
METHENAMINE (REAGENTS AND REAGENT SOLUTIONS)	471
METHYLBROMIDE, HOMATROPINE	1141
METHYLCELLULOSE 450 (REAGENTS AND REAGENT SOLUTIONS)	471
METHYLDOPA	1143
METHYLDOPA, TABLETS	1144
METHYLENEBISACRYLAMIDE (REAGENTS AND REAGENT SOLUTIONS)	472
METHYL ETHYL KETONE (REAGENTS AND REAGENT SOLUTIONS)	472
METHYL ISOBUTYL KETONE (REAGENTS AND REAGENT SOLUTIONS)	472

METHYLPARABEN	1145
METHYLPARABEN (REAGENTS AND REAGENT SOLUTIONS)	472
METHOD, AZEOTROPIC DISTILLATION	125
METHOD, COMBUSTION	182
METHOD, GAS CHROMATOGRAPHY	190
METHOD, DISTILLATION	189
METHOD, CHEMICAL	324
METHOD, VOLUMETRIC	123
METHODS, BIOLOGICAL	207
METHODS AND TESTS, BIOLOGICAL AND MICROBIOLOGICAL	207
METHODS, ANALYSIS OF VEGETABLE DRUGS	196
METHODS, ANALYSIS OF VEGETABLE EXTRACTS	206
METHODS, STERILIZATION	321
METHODS, PHARMACOGNOSY	192
METHODS, PREPARATION OF VEGETABLE EXTRACTS	204
METHODS, PREPARATION AND ANALYSIS OF VEGETABLE EXTRACTS	204
METHODS AND EQUIPMENT FOR MONITORING ENVIRONMENT	335
METHODS AND EQUIPMENT FOR MONITORING VIABLE PARTICLES ON SURFACES	335
METHODS, PHYSICAL	322
METHODS, PHYSICAL, APPLIED TO SURGICAL AND HOSPITAL MATERIALS	279
METHODS, PHYSICAL AND PHYSICAL-CHEMICAL	81
METHODS, GENERAL	59
METHODS, GENERAL, APPLIED TO DRUGS	59
METHODS, IMMUNOCHEMICAL	278
METHODS, CHEMICAL	162
METHOXY AZOBENZENE (REAGENTS AND REAGENT SOLUTIONS)	472
METHOXY AZOBENZENE SR (REAGENTS AND REAGENT SOLUTIONS)	472
METHOXIDE, LITHIUM 0.1 M SV (VOLUMETRIC SOLUTIONS)	504
METHOXIDE, POTASSIUM (REAGENTS AND REAGENT SOLUTIONS)	472
METHOXIDE, SODIUM (REAGENTS AND REAGENT SOLUTIONS)	472
METHOXIDE, SODIUM 0.1 M SV (VOLUMETRIC SOLUTIONS)	504
METHOXYETHANOL (REAGENTS AND REAGENT SOLUTIONS)	472
METRONIDAZOLE	1145
METRONIDAZOLE, TABLETS	1146
METRONIDAZOLE, INJECTABLE SOLUTION	1148
MICRO-ORGANISMS APPLIED TO TESTS	276
MYRISTATE, METHYL (REAGENTS AND REAGENT SOLUTIONS)	472
MIXTURE, ERIOCHROME BLACK T (REAGENTS AND REAGENT SOLUTIONS)	473
MIXTURE, REDUCING (REAGENTS AND REAGENT SOLUTIONS)	473
MIXTURE, SULFO-CHROMIC (REAGENTS AND REAGENT SOLUTIONS)	473
MIXTURES, SURPLUS HUMAN PLASMA, VIRUS INACTIVATION TREATMENT	1148
MIXTURES, HUMAN PLASMA, VIRUS INACTIVATION TREATMENT	1150
MITOTANE	1153
MITOTANE, TABLETS	1153
MOLYBDATE, AMMONIUM (REAGENTS AND REAGENT SOLUTIONS)	473
MOLYBDATE, AMMONIUM AT 1% (W/V) IN SULFURIC ACID M (REAGENTS AND REAGENT SOLUTIONS)	473
MOLYBDATE, AMMONIUM SR (REAGENTS AND REAGENT SOLUTIONS)	473
MOLYBDATE, AMMONIUM, ACID SOLUTION (REAGENTS AND REAGENT SOLUTIONS)	473
MOLYBDATE, SODIUM (REAGENTS AND REAGENT SOLUTIONS)	473
MOLYBDATE, VANADIUM SR (REAGENTS AND REAGENT SOLUTIONS)	473
MORPHOLINE (REAGENTS AND REAGENT SOLUTIONS)	473

MORINA (REAGENTS AND REAGENT SOLUTIONS)	473

N

N, N'- DIISOPROPYLETHYLENEDIAMINE (REAGENTS AND REAGENT SOLUTIONS)	453
N, N- DIETHYL ETHYLENEDIAMINE (REAGENTS AND REAGENT SOLUTIONS)	452
N, N- DIMETHYLANILINE (REAGENTS AND REAGENT SOLUTIONS)	453
NAPHTHALENE (REAGENTS AND REAGENT SOLUTIONS)	474
NAPHTHALENEDIOL, REAGENT (REAGENTS AND REAGENT SOLUTIONS)	474
NARINGIN (REAGENTS AND REAGENT SOLUTIONS)	474
STARCH BLACK 10B (REAGENTS AND REAGENT SOLUTIONS)	475
STARCH BLACK 10B SR (REAGENTS AND REAGENT SOLUTIONS)	475
ERIOCHROME BLACK T (CI 14645) (INDICATORS AND INDICATOR SOLUTIONS)	418
ERIOCHROME BLACK T SI (INDICATORS AND INDICATOR SOLUTIONS)	418
N-HEPTANE (REAGENTS AND REAGENT SOLUTIONS)	463
N-HEXANE (REAGENTS AND REAGENT SOLUTIONS)	464
NIFEDIPINE	1155
NIFEDIPINE, CAPSULES	1156
NIMESULIDE	1157
NIMESULIDE, TABLETS	1159
NINHYDRIN (REAGENTS AND REAGENT SOLUTIONS)	475
NINHYDRIN, ACETIC ETHANOLIC SR (REAGENTS AND REAGENT SOLUTIONS)	475
NINHYDRIN SR (REAGENTS AND REAGENT SOLUTIONS)	475
NYSTATIN	1160
NYSTATIN, VAGINAIL TABLETS	1161
NYSTATIN, CREME VAGINAL	1162
NYSTATIN, ORAL SUSPENSION	1162
NITRATE, CERIUM AMMONIUM (REAGENTS AND REAGENT SOLUTIONS)	475
NITRATE, CERIUM AMMONIUM 0.01 M SV (VOLUMETRIC SOLUTIONS)	504
NITRATE, CERIUM AMMONIUM 0.1 M SV (VOLUMETRIC SOLUTIONS)	504
NITRATE, ALUMINUM, NONAHYDRATE (REAGENTS AND REAGENT SOLUTIONS)	475
NITRATE, AMMONIUM (REAGENTS AND REAGENT SOLUTIONS)	475
NITRATE, AMMONIUM SR (REAGENTS AND REAGENT SOLUTIONS)	475
NITRATE, AMMONIUM, SATURATED SOLUTION (REAGENTS AND REAGENT SOLUTIONS)	475
NITRATE, BARIUM (REAGENTS AND REAGENT SOLUTIONS)	475
NITRATE, BARIUM 0.01 M SV (VOLUMETRIC SOLUTIONS)	505
NITRATE, CADMIUM (REAGENTS AND REAGENT SOLUTIONS)	475
NITRATE, LEAD (REAGENTS AND REAGENT SOLUTIONS)	475
NITRATE, LEAD 0.1 M SV (VOLUMETRIC SOLUTIONS)	505
NITRATE, COBALT (II) (REAGENTS AND REAGENT SOLUTIONS)	476
NITRATE, COBALT (II) SR (REAGENTS AND REAGENT SOLUTIONS)	476
NITRATE, LANTHANUM (REAGENTS AND REAGENT SOLUTIONS)	476
NITRATE, LANTHANUM SR (REAGENTS AND REAGENT SOLUTIONS)	476
NITRATE, MAGNESIUM (REAGENTS AND REAGENT SOLUTIONS)	476
NITRATE, MERCURY (I) (REAGENTS AND REAGENT SOLUTIONS)	476
NITRATE, MERCURY (I) SR (REAGENTS AND REAGENT SOLUTIONS)	476
NITRATE, MERCURY (II) (REAGENTS AND REAGENT SOLUTIONS)	476
NITRATE, MERCURY (II) 0.1 M SV (VOLUMETRIC SOLUTIONS)	505
NITRATE, MICONAZOLE	1163
NITRATE, POTASSIUM (REAGENTS AND REAGENT SOLUTIONS)	476
NITRATE, SILVER	1164
NITRATE, SILVER (REAGENTS AND REAGENT SOLUTIONS)	476

NITRATE, SILVER 0.1 M (REAGENTS AND REAGENT SOLUTIONS)	476
NITRATE, SILVER, OPHTHALMIC SOLUTION	1165
NITRATE, SILVER SR (REAGENTS AND REAGENT SOLUTIONS)	476
NITRATE, SILVER SR1 (REAGENTS AND REAGENT SOLUTIONS)	477
NITRATE, SODIUM (REAGENTS AND REAGENT SOLUTIONS)	477
NITRATE, SODIUM SR (REAGENTS AND REAGENT SOLUTIONS)	477
NITRATE, THORIUM (REAGENTS AND REAGENT SOLUTIONS)	477
NITRATE, THORIUM 0.005 M SV (VOLUMETRIC SOLUTIONS)	505
NITRATE, ZIRCONYL (REAGENTS AND REAGENT SOLUTIONS)	477
NITRATE, ZIRCONYL SR (REAGENTS AND REAGENT SOLUTIONS)	477
NITRATE, PHENYLMERCURIC (REAGENTS AND REAGENT SOLUTIONS)	477
NITRAZEPAM (REAGENTS AND REAGENT SOLUTIONS)	477
NITRITE, SODIUM	1165
NITRITE, SODIUM (REAGENTS AND REAGENT SOLUTIONS)	477
NITRITE, SODIUM 0.1 M SV (VOLUMETRIC SOLUTIONS)	505
NITRITE, SODIUM SR (REAGENTS AND REAGENT SOLUTIONS)	477
NITROBENZENE (REAGENTS AND REAGENT SOLUTIONS)	478
NITROFURANTOIN	1166
NITROFURANTOIN, TABLETS	1167
NITROMETHANE (REAGENTS AND REAGENT SOLUTIONS)	478
NITROPRUSSIDE, SODIUM (REAGENTS AND REAGENT SOLUTIONS)	478
NITROPRUSSIDE, SODIUM AND PIPERAZINE SR (REAGENTS AND REAGENT SOLUTIONS)	478
KOLA NUT	1168

O

O-CRESOL (REAGENTS AND REAGENT SOLUTIONS)	450
OCTA HYDROCHLORIDE, ALUMINUM AND ZIRCONIUM	1173
OCTA HYDROCHLORIDE, ALUMINUM AND ZIRCONIUM, SOLUTION	1174
OCTYL SULFATE, SODIUM (REAGENTS AND REAGENT SOLUTIONS)	478
OCTOXYNOL 10 (REAGENTS AND REAGENT SOLUTIONS)	478
OFLOXACIN	1176
OFLOXACIN, TABLETS	1177
OFLOXACIN, OPHTHALMIC SOLUTION	1178
OIL, PEANUT	1179
OIL, SESAME	1180
OIL, OLIVE (REAGENTS AND REAGENT SOLUTIONS)	478
OILS, UNUSUAL IN FIXED OILS BY GAS CHROMATOGRAPHY	156
OILS, UNUSUAL IN VEGETABLE OILS BY THIN-LAYER CHROMATOGRAPHY	155
OMEPRazole	1181
OXALATE, AMMONIUM (INDICATORS AND INDICATOR SOLUTIONS)	418
OXALATE, AMMONIUM (REAGENTS AND REAGENT SOLUTIONS)	478
OXALATE, AMMONIUM SI (INDICATORS AND INDICATOR SOLUTIONS)	418
OXALATE, AMMONIUM SR (REAGENTS AND REAGENT SOLUTIONS)	478
OXALATE, POTASSIUM (REAGENTS AND REAGENT SOLUTIONS)	479
OXALATE, SODIUM (REAGENTS AND REAGENT SOLUTIONS)	479
OXALATE, MALACHITE GREEN (REAGENTS AND REAGENT SOLUTIONS)	479
OXIDE, ALUMINUM (REAGENTS AND REAGENT SOLUTIONS)	479
OXIDE, HOLMIUM (REAGENTS AND REAGENT SOLUTIONS)	479
OXIDE, MAGNESIUM	1183
OXIDE, MAGNESIUM (REAGENTS AND REAGENT SOLUTIONS)	479
OXIDE, SILVER (REAGENTS AND REAGENT SOLUTIONS)	479

OXIDE, ZINC	1184
OXIDE, MERCURY (REAGENTS AND REAGENT SOLUTIONS)	479
P	
PALLADIUM SRA - 1 MG/ML (REAGENTS AND REAGENT SOLUTIONS)	479
PALMITATE, METHYL (REAGENTS AND REAGENT SOLUTIONS)	479
PANTOPRAZOLE, SODIUM	1187
PANTOTHENATE, CALCIUM	1188
PAPER, SILVER-MANGANESE (REAGENTS AND REAGENT SOLUTIONS)	479
PARACETAMOL	1189
PARACETAMOL, TABLETS	1190
PARACETAMOL, ORAL SOLUTION	1192
PARAFFIN, LIQUID (REAGENTS AND REAGENT SOLUTIONS)	480
PARA-AMINO BENZOATE, POTASSIUM	1193
PARTICLES, SUB-VISIBLE	77
PARTICLES, VISIBLE	79
P-CHLOROACETANILIDE (REAGENTS AND REAGENT SOLUTIONS)	449
P-DIMETHYLAMINO BENZALDEHYDE (REAGENTS AND REAGENT SOLUTIONS)	453
P-DIMETHYLAMINO BENZALDEHYDE SR (REAGENTS AND REAGENT SOLUTIONS)	453
P-DIMETHYLAMINO BENZALDEHYDE SR1 (REAGENTS AND REAGENT SOLUTIONS)	453
P-DIMETHYLAMINO BENZALDEHYDE SR2 (REAGENTS AND REAGENT SOLUTIONS)	453
PENTOXIDE, PHOSPHORUS (REAGENTS AND REAGENT SOLUTIONS)	480
PENTOXIDE, VANADIUM (REAGENTS AND REAGENT SOLUTIONS)	480
PEPSIN, PURIFIED (REAGENTS AND REAGENT SOLUTIONS)	480
PEPTONE (REAGENTS AND REAGENT SOLUTIONS)	480
PERCHLORATE, POTASSIUM	1194
PERCHLORATE, SODIUM (REAGENTS AND REAGENT SOLUTIONS)	480
PERIODATE, POTASSIUM (REAGENTS AND REAGENT SOLUTIONS)	480
PERIODATE, SODIUM (REAGENTS AND REAGENT SOLUTIONS)	480
PERIODATE, POTASSIUM FERRIC SR (REAGENTS AND REAGENT SOLUTIONS)	480
PERMANGANATE, POTASSIUM	1196
PERMANGANATE, POTASSIUM (REAGENTS AND REAGENT SOLUTIONS)	481
PERMANGANATE, POTASSIUM 0.02 M SV (VOLUMETRIC SOLUTIONS)	505
PERMANGANATE, POTASSIUM SR (APPROXIMATELY 0.2M) (REAGENTS AND REAGENT SOLUTIONS)	481
PEROXIDE, CARBAMIDE (REAGENTS AND REAGENT SOLUTIONS)	481
PEROXIDE, HYDROGEN AT 3% (W/V) (REAGENTS AND REAGENT SOLUTIONS)	481
PEROXIDE, CONCENTRATED HYDROGEN (REAGENTS AND REAGENT SOLUTIONS)	481
PEROXIDE, METHANOLIC HYDROGEN (REAGENTS AND REAGENT SOLUTIONS)	481
PEROXIDE, HYDROGEN, 30 VOLUMES, SR (REAGENTS AND REAGENT SOLUTIONS)	481
PEROXIDE, SODIUM (REAGENTS AND REAGENT SOLUTIONS)	481
PERSULFATE, AMMONIUM (REAGENTS AND REAGENT SOLUTIONS)	482
PERSULFATE, POTASSIUM (REAGENTS AND REAGENT SOLUTIONS)	482
PERSULFATE, SODIUM (REAGENTS AND REAGENT SOLUTIONS)	482
RESEARCH OF IMPURITIES RELATED TO PHENOTHIAZINES IN THIN-LAYER CHROMATOGRAPHY	168
RESEARCH OF PATHOGENIC MICRO-ORGANISMS	243
RESEARCH OF SUBSTANCES RELATED TO SULFONAMIDES IN THIN-LAYER CHROMATOGRAPHY	168
RESEARCH OF UNUSUAL STEROIDS IN THIN-LAYER CHROMATOGRAPHY	167
PETROLATUM, WHITE	1196
PETROLATUM, LIQUID	1197
PICRATE, SODIUM, ALKALINE SR (REAGENTS AND REAGENT SOLUTIONS)	482

PIPERAZINE	1198
PIPERAZINE (REAGENTS AND REAGENT SOLUTIONS)	482
PYRAZINAMIDE	1199
PYRAZINAMIDE, TABLETS	1200
PYRIDINE (REAGENTS AND REAGENT SOLUTIONS)	482
PYRIDINE, ANHYDROUS (REAGENTS AND REAGENT SOLUTIONS)	482
PYRIMETHAMINE	1202
PYRIMETHAMINE, TABLETS	1203
PYROPHOSPHATE, SODIUM (REAGENTS AND REAGENT SOLUTIONS)	482
PYROGALLOL (REAGENTS AND REAGENT SOLUTIONS)	482
PYROGENS	229
PIROXICAM, CAPSULES	1204
PIROXICAM, GEL	1204
SURINAM CHERRY	1206
PLAN AND SAMPLING SITES	334
PLASMA, HUMAN, FRACTIONATING	1211
P-NITROANILINE (REAGENTS AND REAGENT SOLUTIONS)	477
P-NITROANILINE AND SODIUM NITRITE SR (REAGENTS AND REAGENT SOLUTIONS)	478
POWDER FOR INJECTABLE SOLUTION	
AMOXICILLIN AND CLAVULANATE POTASSIUM	620
AMPICILLIN, SODIUM	632
CEFALOTIN, SODIUM	765
CEFOXITIN, SODIUM	767
SULFATE, STREPTOMYCIN	1313
POWDER FOR ORAL SUSPENSION	
AMOXICILLIN AND CLAVULANATE POTASSIUM	621
AMOXYCILLIN, TRIHYDRATE	624
AMPICILLIN	629
AMPICILLIN, TRIHYDRATE	636
AZITHROMYCIN	667
CEFADROXIL	759
CEPHALEXIN	764
CLARITHROMYCIN	793
POLAROGRAPHY	117
POLYACRYLAMIDE (REAGENTS AND REAGENT SOLUTIONS)	482
POLYGALA	1213
POLYSORBATE 20	1217
POLYSORBATE 20 (REAGENTS AND REAGENT SOLUTIONS)	483
POLYSORBATE 40	1218
POLYSORBATE 60	1218
POLYSORBATE 80	1219
POLYSORBATE 80 (REAGENTS AND REAGENT SOLUTIONS)	483
OINTMENT	
HYDROCHLORIDE, LIDOCAINE	841
THIABENDAZOLE	1341
POTASSIUM SRA - 600 ML/ML (REAGENTS AND REAGENT SOLUTIONS)	483
POTENCY, WEIGHTED AVERAGE AND CONFIDENCE LIMITS	347
PRAZIQUANTEL	1220
PRAZIQUANTEL, TABLETS	1221
PREDNISOLONE (REAGENTS AND REAGENT SOLUTIONS)	483
PREDNISONE	1222
PREDNISONE (REAGENTS AND REAGENT SOLUTIONS)	483

PREDNISONE, TABLETS	1224
PREFACE	7
PREPARATION OF SPORE SUSPENSION	329
PREPARATION OF STERILE PRODUCTS	321
PREPARATION OF BIOLOGICAL INDICATOR	327
PREPARATION OF MICROSCOPIC ANALYSIS MATERIAL	193
SHINY BLACK BN (REAGENTS AND REAGENT SOLUTIONS)	483
PROCEDURES FOR RELEASE	336
PROCEDURES, STATISTICAL, FOR BIOLOGICAL TESTS	338
PROCESS, ASEPTIC	329
PROGESTERONE	1225
PROGRAM, MICROBIOLOGICAL EVALUATION, RELATED CLEAN ROOMS AND CONTROLLED ENVIRONMENTS	331
PROJECT AND IMPLEMENTATION OF MICROBIOLOGICAL ENVIRONMENTAL CONTROL PROGRAM	334
PROPYLENE GLYCOL (REAGENTS AND REAGENT SOLUTIONS)	483
PROPYLPARABEN	1226
PROPYLPARABEN (REAGENTS AND REAGENT SOLUTIONS)	483
PROPIONATE, TESTOSTERONE	1227
P-TOLUALDEHYDE (REAGENTS AND REAGENT SOLUTIONS)	498
P-TOLUIDINE (REAGENTS AND REAGENT SOLUTIONS)	498
PURPLE, BROMOCRESOL (INDICATORS AND INDICATOR SOLUTIONS)	418
PURPLE, BROMOCRESOL SI (INDICATORS AND INDICATOR SOLUTIONS)	418
PURPLE, BROMOCRESOL, REAGENT (INDICATORS AND INDICATOR SOLUTIONS)	418
PURPLE, PHTHALEIN (REAGENTS AND REAGENT SOLUTIONS)	483
PURPLE, METACRESOL (INDICATORS AND INDICATOR SOLUTIONS)	418
PURPLE, METACRESOL SI (INDICATORS AND INDICATOR SOLUTIONS)	418

Q

QUALIFICATION OF PERFORMANCE	325
QUALIFICATION OF INSTALLATION	325
QUALIFICATION OF OPERATION	325
SEED-UNDER-LEAF	1229
SEED-UNDER-LEAF	1235
QUILLAY	1241
QUINA-AMARELA	1244
QUINALIZARIN (CI 58500) (REAGENTS AND REAGENT SOLUTIONS)	483
QUINIDINE (REAGENTS AND REAGENT SOLUTIONS)	484
QUINHYDRONE (REAGENTS AND REAGENT SOLUTIONS)	484
QUININE (REAGENTS AND REAGENT SOLUTIONS)	484

R

RADIOPHARMACEUTICALS	373
RHAPONTICIN (REAGENTS AND REAGENT SOLUTIONS)	484
RHATANY	1248
RHATANY, TINCTURE	1252
RAUVOLFIA	1253
REACTIONS OF IDENTIFICATION	162
REAGENT, ALUMINON (REAGENTS AND REAGENT SOLUTIONS)	484
REAGENT, COLOR (REAGENTS AND REAGENT SOLUTIONS)	484

REAGENT, MODIFIED ERLICH (REAGENTS AND REAGENT SOLUTIONS)	484
REAGENT, FOLIN-DENIS (REAGENTS AND REAGENT SOLUTIONS)	484
REAGENT, HANTZACH (REAGENTS AND REAGENT SOLUTIONS)	484
REAGENT, JONES (REAGENTS AND REAGENT SOLUTIONS)	484
REAGENT, MARQUIS (REAGENTS AND REAGENT SOLUTIONS)	484
REAGENT, XANTHYDROL (REAGENTS AND REAGENT SOLUTIONS)	484
REAGENT, PHOSPHOTUNGSTIC MOLYBDATE (REAGENTS AND REAGENT SOLUTIONS)	484
REAGENT, IODINE PLATINUM (REAGENTS AND REAGENT SOLUTIONS)	485
REAGENT, SULPHO-MOLYBDIC (REAGENTS AND REAGENT SOLUTIONS)	485
REAGENTS	413
REAGENTS AND REAGENT SOLUTIONS	421
ACETAL	421
ACETALDEHYDE	421
ACETANILIDE	421
ACETATE, AMMONIUM	421
ACETATE, AMMONIUM SR	422
ACETATE, BORNYL	422
ACETATE, BUTYL	422
ACETATE, CELLULOSE	422
ACETATE, LEAD, TRIHYDRATE	422
ACETATE, LEAD, PAPER	422
ACETATE, LEAD SR (APPROXIMATELY 0.25 M)	422
ACETATE, LEAD, SATURATED SOLUTION	422
ACETATE, CHLORHEXIDINE	422
ACETATE, CHLORHEXIDINE AT 0.1% (W/V)	422
ACETATE, COPPER	422
ACETATE, CORTISONE	422
ACETATE, CORTISONE, INJECTABLE	423
ACETATE, DESOXYCORTONE	423
ACETATE, ETHYL	423
ACETATE, PHENYLMERCURIC	423
ACETATE, INDOPHENOL SR	423
ACETATE, MAGNESIUM	423
ACETATE, MENTHYL	423
ACETATE, MERCURY	423
ACETATE, MERCURY SR	423
ACETATE, METHYL	423
ACETATE, POTASSIUM	424
ACETATE, POTASSIUM SR	424
ACETATE, PREDNISOLONE	424
ACETATE, SODIUM	424
ACETATE, SODIUM SR (APPROXIMATELY 0.02 M)	424
ACETATE, URANYL	424
ACETATE, URANYL AND ZINC SR	424
ACETATE, ZINC	424
ACETYLACETONE	424
ACETONE	424
ACETONE, DEHYDRATED	424
ACETONE, BUFFERED SR	424
ACETONITRILE	425
ACETIC ACID M	425
ACETIC ACID 6 M	425

ACETIC ACID, DILUTED	425
ACETIC ACID SR	425
ACETIC ACID, GLACIAL	425
ACID, 7-AMINODESACETOXYCEPHALOSPORANIC	425
ACID, ASCORBIC	425
ACID, BENZOIC	425
ACID, BORIC	425
ACID, BORIC, SATURATED SOLUTION	425
ACID, HYDROBROMIC	425
ACID, CAFFEIC	426
ACID, CALCON CARBOXYLIC	426
ACID, CYCLOBUTANE-1,1-DICARBOXYLIC	426
ACID, 1,2- CYCLOHEXYLENE DINITRILE TETRAACETIC	426
ACID, CINNAMIC	426
ACID, CITRIC, MONOHYDRATE	426
ACID, HYDROCHLORIC	426
ACID, BROMINATED HYDROCHLORIC SR	426
ACID, DILUTED HYDROCHLORIC	426
ACID, HYDROCHLORIC M	426
ACID, HYDROCHLORIC SR	426
ACID, METHANOLIC HYDROCHLORIC 0.01 M	426
ACID, TIN HYDROCHLORIC SR	426
ACID, CHLOROGENIC	427
ACID, CHLOROPLATINIC	427
ACID, CHROMIC	427
ACID, 3,5- DINITRO-BENZOIC	427
ACID, EDETIC	427
ACID, PHENOL DISULFONIC SR	427
ACID, PHENOXYACETIC	427
ACID, HYDROFLUORIC	427
ACID, FORMIC	427
ACID, PHOSPHOMOLYBDIC	427
ACID, PHOSPHOMOLYBDIC SR	427
ACID, PHOSPHORIC	428
ACID, PHOSPHORIC SR	428
ACID, PHOSPHOTUNGSTIC SR	428
ACID, PHTHALIC	428
ACID, GALLIC	428
ACID, P-HYDROXYBENZOIC	428
ACID, HYPOPHOSPHOROUS	428
ACID, HYDRIODIC	428
ACID, LACTIC	428
ACID, METAPHOSPHORIC	428
ACID, METAPHOSPHORIC-ACETIC SR	428
ACID, METHANESULFONIC	428
ACID, NITRIC	429
ACID, NITRIC, FUMING	429
ACID, NITRIC SR	429
ACID, OXALIC	429
ACID, OXALIC SR	429
ACID, PERCHLORIC	429
ACID, PERCHLORIC M	429

ACID, PERCHLORIC SR	429
ACID, PERFORMIC	429
ACID, PERIODIC	429
ACID, PICRIC	429
ACID, PICRIC SR	429
ACID, PICRIC SR1	429
ACID, ROSMARINIC	430
ACID, SALICYLIC	430
ACID, SELENIOS	430
ACID, SULPHAMIC	430
ACID, SULFANILIC	430
ACID, SULFANILIC, DIAZOTIZED SR	430
ACID, SULFANILIC SR	430
ACID, SULFURIC	430
ACID, SULFURIC, DILUTED	430
ACID, SULFURIC, NITROGEN-FREE	430
ACID, SULFURIC, METHANOLIC 0.1 M	430
ACID, SULFURIC / METHANOL SR	430
ACID, SULFURIC, METHANOLIC SR	430
ACID, SULFURIC, ETHANOLIC SOLUTION	431
ACID, SULFURIC SR	431
ACID, SULFUROS	431
ACID, TARTARIC	431
ACID, THIOGLYCOLIC	431
ACID, P-TOLUENESULFONIC	431
ACID, TRICHLOROACETIC	431
ACID, TRICHLOROACETIC - CHLORAMINE - T SR	431
ACID, TRIFLUOROACETIC	431
ACRYLAMIDE	432
ACRYLAMIDE / BISACRYLAMIDE (29:1) AT 30% (W/V) SR	432
AGAR	432
AGAROSE, GEL	432
AGAROSE - DEAE FOR ION EXCHANGE CHROMATOGRAPHY	432
BROMINE WATER SR	432
CHLORINE WATER SR	432
CARBON DIOXIDE-FREE WATER	432
AMMONIA-FREE WATER	432
NITRATE-FREE WATER	432
PARTICLE-FREE WATER	432
ALBUMIN, BOVINE	432
ALBUMIN, HUMAN	432
ALBUMIN, HUMAN, REAGENT SOLUTION	432
ALCOHOL, ISOAMYL	432
ALCOHOL, ISOBUTYL	433
ALCOHOL, ISOPROPYL	433
ALCOHOL, N-AMYL	433
ALCOHOL, N-PROPYL	433
ALCOHOL, POLYVINYL	433
ALCOHOL, TERT-AMYL	433
ALCOHOL, TERT-BUTYL	433
ALUMINUM, METALLIC	433
ALUMINON	433

AMARANTH (CI 16185)	434
STARCH, IODINE SR	434
STARCH, IODINE SR1	434
STARCH, IODIDE-FREE SR	434
STARCH, SOLUBLE	434
STARCH SR	434
STARCHES	434
4- AMINOANTIPYRINE	434
AMINO BUTANOL	434
2- AMINO HEPTANE	434
4- AMINOPHENOL	434
2- AMINOPYRIDINE	434
AMMONIA SR	434
AMMONIA 6 M	434
AMMONIA 10 M	434
AMMONIA, CONCENTRATED SOLUTION	435
ANETWELL	435
ANHYDRIDE, ACETIC	435
ANHYDRIDE, ACETIC - PYRIDINE SR	435
ANHYDRIDE, PHTHALIC	435
ANHYDRIDE, PROPIONIC	435
ANISALDEHYDE	435
ANISALDEHYDE, SOLUTION	435
ANISALDEHYDE SR	435
ANISALDEHYDE SR1	435
ANTITHROMBIN III	435
ANTITHROMBIN III SR	435
APROTININ	435
ASIATICOSIDE	436
ASPARAGINE	436
AZIDE, SODIUM	436
ACID, BLUE 83	436
ACID, BLUE 90	436
ASTRA, BLUE	436
COOMASSIE, BLUE SR	436
SULFANE, BLUE (CI 42045)	436
TETRAZOLIUM, BLUE	436
BALSAM, CANADA	436
BARBALOINE	436
BARBITAL	436
BARBITAL, SODIUM	437
BARIUM SRA - 1 MG/ML	437
BENZENE	437
BENZENESULFONAMIDE	437
BENZYL	437
BENZOATE, BENZYL	437
BENZOATE, CWELLSTERYL	437
BENZOATE, METHYL	437
BENZOPHENONE	437
BENZOIN	437
BICARBONATE, SODIUM	437
BICINCHONINATE, DISODIUM	438

BIPHThALATE, POTASSIUM	438
BIPHThALATE, POTASSIUM 0.05 M	438
BISULFATE, POTASSIUM	438
BISULFATE, SODIUM	438
BISULFITE, SODIUM	438
BITARTRATE, SODIUM	438
BITARTRATE, SODIUM SR	438
BIURET	438
BIURET, REAGENT	438
BOLDINE	438
BORNEOL	438
BROMATE, POTASSIUM	439
BROMELAIN	439
BROMELAIN SR	439
BROMIDE, DIMIDIUM	439
BROMIDE, DIMIDIUM - SULFANE BLUE SR	439
BROMIDE, HEXADIMETHRINE	439
BROMIDE, IODINE	439
BROMIDE, IODINE SR	439
BROMIDE, POTASSIUM	439
BROMIDE, TETRABUTYLAMMONIUM	439
BROMIDE, TETRA HEPTYL AMMONIUM	439
BROMIDE, MERCURY	439
BROMINE	440
BROMINE 0.2 M IN GLACIAL ACETIC ACID	440
BROMINE SR	440
1-BUTANOL	440
BUTANE SULPHONATE, SODIUM	440
BUTYL, HYDROXYANISOLE	440
BUTYLAMINE	440
BUTYLPARABEN	440
CALCIFEROL	440
CALCIUM SRA - 400 ML/ML	440
CAMPHENE	440
CAMPHOR	441
KAOLIN, LIGHT	441
CARBONATE, AMMONIUM	441
CARBONATE, AMMONIUM SR	441
CARBONATE, CALCIUM	441
CARBONATE, STRONTIUM	441
CARBONATE, LITHIUM	441
CARBONATE, LITHIUM, ANHYDROUS	441
CARBONATE, POTASSIUM, SESQUIHYDRATE	441
CARBONATE, SODIUM, ANHYDROUS	442
CARBONATE, SODIUM, DECAHYDRATE	442
CARBONATE, SODIUM, MONOHYDRATE	442
CARBONATE, SODIUM SR	442
CARVONE	442
CATECHIN	442
CEPHALIN	442
CEPHALIN SR	442
CELLULOSE, CHROMATOGRAPHIC	442

LEAD SRA - 100 MG/ML	442
CYANIDE, POTASSIUM	442
CYANIDE, POTASSIUM SR	443
CYANIDE, AMMONIA SR	443
CYANOACETATE, ETHYL	443
CYCLOHEXANE	443
CINNAMATE, BENZYL	443
CINNAMATE, METHYL	443
CINCHONINE	443
1,8-CINEOL	443
CITRAL	443
CITRATE, AMMONIUM SR	443
CITRATE, ALKALINE CUPRIC SR	443
CITRATE, SODIUM	444
CITRONELLAL	444
CITRONELLOL	444
CHLORAMINE-T	444
CHLORATE, POTASSIUM	444
CHLORIDE, COBALTOUS	444
CHLORIDE, COBALTOUS SR	444
CHLORIDE, ACETYL	444
CHLORIDE, ALUMINIUM, HEXAHYDRATE	444
CHLORIDE, ALUMINUM SR	444
CHLORIDE, AMMONIUM	445
CHLORIDE, AMMONIUM SR	445
CHLORIDE, AMMONIUM - AMMONIUM HYDROXIDE SR	445
CHLORIDE, BARIUM	445
CHLORIDE, BARIUM SR	445
CHLORIDE, BENZALKONIUM	445
CHLORIDE, BENZETHONIUM	445
CHLORIDE, BENZYL	445
CHLORIDE, CALCIUM	445
CHLORIDE, CALCIUM, ANHYDROUS	445
CHLORIDE, CALCIUM SR	446
CHLORIDE, CAESIUM	446
CHLORIDE, TIN (II) SR	446
CHLORIDE, MAGNESIUM	446
CHLORIDE, MERCURY (II)	446
CHLORIDE, METHYLENE	446
CHLORIDE, METHYLENE, SATURATED WITH AMMONIA	446
CHLORIDE, METHYLTHIONIUM	446
CHLORIDE, METHYLTHIONIUM SR	446
CHLORIDE, METHYLTHIONIUM SR1	446
CHLORIDE, NICKEL (II)	446
CHLORIDE, NITROBENZOYL	446
CHLORIDE, GOLD	446
CHLORIDE, GOLD SR	447
CHLORIDE, PALLADIUM	447
CHLORIDE, POTASSIUM	447
CHLORIDE, POTASSIUM, SATURATED SOLUTION	447
CHLORIDE, SODIUM	447
CHLORIDE, SODIUM AT 0.9% (W/V)	447

CHLORIDE, STANNOUS	447
CHLORIDE, STANNOUS SR	447
CHLORIDE, STANNOUS SR1	447
CHLORIDE, FERRIC	447
CHLORIDE, FERRIC SI (APPROXIMATELY 0.4 M)	447
CHLORIDE, FERRIC, ACID SR	447
CHLORIDE, FERRIC, METHANOLIC	447
CHLORIDE, MERCURY SR (APPROXIMATELY 0.2 M)	447
CHLORIDE, PLATINUM SR	448
HYDROCHLORIDE, BENZOYL	448
HYDROCHLORIDE, (2-CHLOROETHYL), DIETHYLAMINE	448
HYDROCHLORIDE, DIMETHYL-P-PHENYLENEDIAMINE	448
HYDROCHLORIDE, O-PHENYLENEDIAMINE	448
HYDROCHLORIDE, P-PHENYLENEDIAMINE	448
HYDROCHLORIDE, PHENYLHYDRAZINE	448
HYDROCHLORIDE, PHENYLHYDRAZINE SR	448
HYDROCHLORIDE, HYDRASTINE	448
HYDROCHLORIDE, HYDROXYLAMINE	448
HYDROCHLORIDE, HYDROXYLAMINE SR	448
HYDROCHLORIDE, HYDROXYLAMINE SR1	448
CHLORINE SR	449
P-CHLOROACETANILIDE	449
CHLOROBENZENE	449
1-CHLORINE-2,4-DINITROBENZENE	449
CHLOROFORM	449
CHLOROFORM, ALCOHOL-FREE	449
CHLOROTHIAZIDE	449
COBALTINITRITE, SODIUM	449
COBALTINITRITE, SODIUM SR	449
COPPER	449
COPPER SRA - 1 MG/ML	449
O-CRESOL	450
CHROMATE, POTASSIUM	450
CHROMATE, POTASSIUM SR	450
CHROMOTROPATE, DISODIUM	450
DEOXYCHOLATE, SODIUM	450
DEXTROSE	450
DEXTROSE 0.1% (W/V)	450
DIACETATE, CHLORHEXIDINE	450
1,8-DIAMINONAPHTHALENE	450
DIAVERIDINE	450
2,6-DIBROMOQUINONE-4-CHLORIMIDE	450
DIBUTYLAMINE	450
DICHLORIDE, ETHYLENE	450
DIHYDROCHLORIDE, N-(1-NAPHTHYL) ETHYLENEDIAMINE	451
DIHYDROCHLORIDE, N-(1-NAPHTHYL) ETHYLENEDIAMINE SR	451
2,6-DICHLOROQUINONE-4-CHLORIMIDE	451
1 - (2,6-DICHLOROPHENYL) -1,3-DIHYDRO-2H-INDOL-2-ONE (IMPURITY A OF DICLOFENAC)	451
2,6- DICHLOROINDOPHENOL, SODIUM	451
DICHROMATE, POTASSIUM	451
DICHROMATE, POTASSIUM SR	451

DIETHYLAMINE	451
DIETHYLAMINOETHYL DEXTRAN	451
DIETHYLDITHIOCARBAMATE, SILVER	451
DIETHYLDITHIOCARBAMATE, SODIUM	451
N, N- DIETHYL ETHYLENEDIAMINE	452
DIETHYLPHTALATE	452
DIPHENYLAMINE SR	452
DIPHENYL BENZIDINE	452
DIPHENYL BORATE, AMINOETHANOL SR	452
DIPHENYL CARBAZIDE	452
DIPHENYL CARBAZIDE SR	452
DIPHENYL CARBAZONE	452
DIPHENYL CARBAZONE, BROMOPHENOL BLUE SR	452
DIPHENYL CARBAZONE, MERCURY SR	452
N, N'-DIISOPROPYLETHYLENEDIAMINE	453
DIMETHYLACETAMIDE	453
P-DIMETHYLAMINO BENZALDEHYDE	453
P-DIMETHYLAMINO BENZALDEHYDE SR	453
P-DIMETHYLAMINO BENZALDEHYDE SR1	453
P-DIMETHYLAMINO BENZALDEHYDE SR2	453
4-DIMETHYLAMINOCINNAMALDEHYDE	453
2,6-DIMETHYLANILINE	453
N, N-DIMETHYLANILINE	453
1,1-DIMETHYLETHYL AMINE	453
2,5-DIMETHYLPHENOL	453
DIMETHYLFORMAMIDE	454
DIMETHYLSULFOXIDE	454
1,3-DINITROBENZENE	454
1,3-DINITROBENZENE SR	454
DIOXANE	454
DIOXIDE, SULPHUR	454
DIOXIDE, MANGANESE	454
DIPROPYLENEGLYCOL	454
DISULFIDE, CARBON	454
DITHIOL	454
DITHIOL SR	455
DITHIOTHREITOL	455
DITHIZONE	455
DITHIZONE SR	455
DITHIZONE, CONCENTRATED SOLUTION	455
DITHIZONE, DILUTED SOLUTION	455
DITHIZONE, EXTRACTING SOLUTION	455
EDETATE, DISODIUM	455
EDETATE, DISODIUM, SOLUTION 0.05 M	455
EMODIN	455
SULPHUR	455
ESCIN	455
TIN, METAL	456
STEARATE, METHYL	456
ETHYL ESTER, TETRABROMO PHENOLPHTHALEIN	456
ESTOLATE, ERYTHROMYCIN	456
STRONTIUM SRA - 1 MG/ML	456

ETHANOL	456
ETHANOL, ABSOLUTE	456
ETHANOL, GLYCERIN	456
ETHER, PETROLEUM	456
ETHER, ETHYL	456
ETHER, ISOPROPYL	457
ETHYLENE GLYCOL	457
ETHYLPARABEN	457
EUGENOL	457
FAST GREEN (CI 42053)	457
FACTOR XA OF BOVINE BLOOD CLOTTING	457
FACTOR XA, BOVINE, SOLUTION	457
1,10-PHENANTHROLINE	457
DL-PHENYLALANINE	457
PHENOL	457
PHENOLPHTHALEIN	458
PHENOLPHTHALEIN AT 0.1% (W/V)	458
2-PHENOXYETHANOL	458
FERRICYANIDE, POTASSIUM	458
FERRICYANIDE, AMMONIACAL POTASSIUM	458
FERROCYANIDE, POTASSIUM	458
FERROCYANIDE, POTASSIUM SR	458
FIBRINOGEN	458
PHOROGLUCINE SR	458
PHLOROGLUCINOL	458
SIMULATED GASTRIC FLUID	458
SIMULATED GASTRIC FLUID (NO ENZYME)	459
SIMULATED INTESTINAL FLUID (NO PANCREATIN) PH 7.5	459
FLUORIDE, AMMONIUM	459
FLUORIDE, CALCIUM	459
FLUORIDE, SODIUM	459
FLUORIDE, SODIUM SR	459
FORMALDEHYDE, SOLUTION	459
FORMAMIDE	459
FORMATE, AMMONIUM	459
PHOSPHATASE, ALKALINE, SOLUTION	459
PHOSPHATE, DIBASIC AMMONIUM	459
PHOSPHATE, MONOBASIC AMMONIUM	460
PHOSPHATE, CODEIN	460
PHOSPHATE, POTASSIUM	460
PHOSPHATE, MONOBASIC POTASSIUM	460
PHOSPHATE, DIBASIC POTASSIUM	460
PHOSPHATE, DIBASIC SODIUM, DIHYDRATE	460
PHOSPHATE, DIBASIC SODIUM, DODECAHYDRATE	460
PHOSPHATE, DIBASIC SODIUM, DODECAHYDRATE SR	460
PHOSPHATE, DIBASIC SODIUM, HEPTAHYDRATE	460
PHOSPHATE, DIBASIC SODIUM, HEPTAHYDRATE SR	460
PHOSPHATE, MONOBASIC SODIUM	460
PHOSPHATE, MONOBASIC SODIUM, MONOHYDRATE	461
PHOSPHATE, MONOBASIC SODIUM, DIHYDRATE	461
PHOSPHATE, TRIBASIC SODIUM, DODECAHYDRATE	461
PHOSPHATE, TETRABUTYLAMMONIUM	461

PHOSPHATE, TRIBUTYL	461
PHOSPHATE, EQUIMOLAR 0.05 M	461
PHOSPHATE, BROMOCRESOL PURPLE SR	461
PHOSPHORUS, RED	461
FRUCTOSE	461
FRUCTOSE AT 0.1% (W/V)	461
PHTHALALDEHYDE	461
PHTHALATE, DIBUTYL	462
PHTHALAZINE	462
FUCHSIN, BASIC (CI 42510)	462
FUCHSIN, BLEACHED SR	462
GALACTOSE	462
GALACTOSE AT 0.1% (W/V) IN PYRIDINE	462
GELATIN	462
GELATIN, GLYCERINATED	462
GELATIN SR	462
GLYCEROL	462
GLYCINE	462
GLUCOSE	463
GLUCOSE AT 0.1% (W/V) IN PYRIDINE	463
GLUTARALDEHYDE	463
GUAIACOL	463
GUANINE	463
HEPARIN, SODIUM	463
HEPTANE	463
N-HEPTANE	463
HEPTANE SULPHONATE, SODIUM	463
HEXANE	464
N-HEXANE	464
SODIUM 1-HEXANE SULFONATE	464
HEXYLAMINE	464
HYDRATE, CHLORAL	464
HYDRAZINE, HYDRATE	464
HYDROXIDE, AMMONIUM	464
HYDROXIDE, BARIUM	464
HYDROXIDE, CALCIUM	464
HYDROXIDE, CALCIUM, SATURATED SOLUTION	464
HYDROXIDE, CALCIUM SR	464
HYDROXIDE, LITHIUM	464
HYDROXIDE, POTASSIUM	465
HYDROXIDE, ETHANOLIC POTASSIUM SR (APPROXIMATELY 0.5 M)	465
HYDROXIDE, ETHANOLIC POTASSIUM 2 M	465
HYDROXIDE, SODIUM	465
HYDROXIDE, SODIUM SR	465
HYDROXIDE, SODIUM M	465
HYDROXIDE, SODIUM, CONCENTRATED SOLUTION SR (APPROXIMATELY 10 M)	465
HYDROXIDE, TETRABUTYLAMMONIUM	465
HYDROXIDE, TETRAMETHYLAMMONIUM	465
D-A-4 -HYDROXYPHENYL GLYCINE	465
HYDROXYQUINOLINE	466
HYDROXYTOLUENE, BUTYLATED	466
HYPEROSIDE	466

HYPOCHLORITE, SODIUM	466
HYPOCHLORITE, SODIUM SR	466
HYPOPHOSPHITE, SODIUM	466
HYPOPHOSPHITE, SODIUM SR	466
IMIDAZOLE	466
IMINO DIBENZYL	466
IODATE, POTASSIUM	466
IODIDE, MERCURY (II)	466
IODIDE, POTASSIUM	467
IODIDE, POTASSIUM, APPROXIMATELY M	467
IODIDE, POTASSIUM SR	467
IODIDE, POTASSIUM, ALKALINE MERCURY SR1	467
IODIDE, MERCURY POTASSIUM SR	467
IODIDE, SODIUM	467
IODIDE, SODIUM, IN ACETIC ACID	467
IODIDE, TETRABUTYLAMMONIUM	467
INDIGO CARMINE	467
INDIGO CARMINE SR	467
IODINE	468
IODINE SR	468
IODINE 0.05 M	468
IODINE AT 0.5% (W/V) IN CHLOROFORM	468
IODINE 1% (W/V) IN ETHANOL	468
IODOBISMUTHATE, POTASSIUM	468
IODOBISMUTHATE, POTASSIUM, AQUEOUS ACETIC	468
IODOBISMUTHATE, POTASSIUM, DILUTED SR	468
IODIDE, POTASSIUM AND BISMUTH SUBNITRATE SR	468
LODOBISMUTHATE, POTASSIUM SR	468
IODOBISMUTHATE, POTASSIUM SR1	468
LODOBISMUTHATE, POTASSIUM SR2	468
IRGANOX 1010	469
IRGANOX 1076	469
IRGANOX PS 800	469
ISOOCTANE	469
ISOTHIOCYANATE, FLUORESCEIN	469
LACTOSE	469
LACTOSE AT 0.1% (W/V) IN PYRIDINE	469
LAURATE, METHYL	469
LAURYL SULFATE, SODIUM	470
LAURYL SULFATE, SODIUM SR	470
LECITHIN	470
ALLOY, NICKEL-ALUMINUM	470
LINALOOL	470
LITHIUM	470
LITHIUM SRA - 2 MG/ML	470
MACROGOL 300	470
MACROGOL 1000	470
MAGNESIUM SRA - 1 MG/ML	470
MAGNESON	470
MELAMINE	471
2- MERCAPTOETHANOL	

MERCURY SRA - 1 MG/ML	471
METABISULFITE, SODIUM	471
METHANOL	471
METHENAMINE	471
METHYLCELLULOSE 450	471
4,4-METHYLENEBIS- N,N-DIMETHYLANILINE	471
METHYLENEBISACRYLAMIDE	472
METHYL ETHYL KETONE	472
METHYL ISOBUTYL KETONE	472
METHYLPARABEN	472
4- METHYLPENTAN-2-OL	472
4- METHYL-2- PENTANONE	472
METHOXY AZOBENZENE	472
METHOXY AZOBENZENE SR	472
METHOXIDE, POTASSIUM	472
METHOXIDE, SODIUM	472
METHOXYETHANOL	472
MYRISTATE, METHYL	472
MIXTURE, ERIOCHROME BLACK T	473
MIXTURE, REDUCING	473
MIXTURE, SULFO-CHROMIC	473
MOLYBDATE, AMMONIUM	473
MOLYBDATE, AMMONIUM SR	473
MOLYBDATE, AMMONIUM, ACID SOLUTION	473
MOLYBDATE, AMMONIUM AT 1% (W/V) IN SULFURIC ACID M	473
MOLYBDATE, SODIUM	473
MOLYBDATE, VANADIUM SR	473
MORPHOLINE	473
MORINA	473
NAPHTHALENE	474
1,3-NAPHTHALENEDIOL	474
2,7-NAPHTHALENEDIOL	474
NAPHTHALENEDIOL, REAGENT	474
1-NAPHTHYLAMINE	474
1-NAPHTHOL	474
1-NAPHTHOL SR	474
2-NAPHTHOL	474
2-NAPHTHOL SR	474
2-NAPHTHOL SR1	474
NARINGIN	474
STARCH BLACK 10B	475
STARCH BLACK 10B SR	475
NINHYDRIN	475
NINHYDRIN, ACETIC ETHANOLIC SR	475
NINHYDRIN SR	475
NITRATE, CERIUM AMMONIUM	475
NITRATE, ALUMINUM, NONAHYDRATE	475
NITRATE, AMMONIUM	475
NITRATE, AMMONIUM SR	475
NITRATE, AMMONIUM, SATURATED SOLUTION	475
NITRATE, BARIUM	475
NITRATE, CADMIUM	475

NITRATE, LEAD	475
NITRATE, COBALT (II)	476
NITRATE, COBALT (II) SR	476
NITRATE, LANTHANUM	476
NITRATE, LANTHANUM SR	476
NITRATE, MAGNESIUM	476
NITRATE, MERCURY (I)	476
NITRATE, MERCURY (I) SR	476
NITRATE, MERCURY (II)	476
NITRATE, POTASSIUM	476
NITRATE, SILVER	476
NITRATE, SILVER 0.1 M	476
NITRATE, SILVER SR	476
NITRATE, SILVER SR1	477
NITRATE, SODIUM	477
NITRATE, SODIUM SR	477
NITRATE, THORIUM	477
NITRATE, ZIRCONYL	477
NITRATE, ZIRCONYL SR	477
NITRATE, PHENYLMERCURIC	477
NITRAZEPAM	477
NITRITE, SODIUM	477
NITRITE, SODIUM SR	477
P-NITROANILINE	477
P-NITROANILINE AND SODIUM NITRITE SR	478
2-NITROBENZALDEHYDE	478
NITROBENZENE	478
NITROMETHANE	478
NITROPRUSSIDE, SODIUM	478
NITROPRUSSIDE, SODIUM AND PIPERAZINE SR	478
SODIUM 1-OCTANE SULFONATE	478
OCTYL SULFATE, SODIUM	478
OCTOXYNOL 10	478
OLIVE OIL	478
OXALATE, AMMONIUM	478
OXALATE, AMMONIUM SR	478
OXALATE, POTASSIUM	479
OXALATE, SODIUM	479
OXALATE, MALACHITE GREEN	479
OXIDE, ALUMINUM	479
OXIDE, HOLMIUM	479
OXIDE, MAGNESIUM	479
OXIDE, SILVER	479
OXIDE, MERCURY	479
PALLADIUM SRA - 1 MG/ML	479
PALMITATE, METHYL	479
PAPER, SILVER-MANGANESE	479
PARAFFIN, LIQUID	480
SODIUM 1-PENTANE SULFONATE	480
PENTOXIDE, PHOSPHORUS	480
PENTOXIDE, VANADIUM	480
PEPSIN, PURIFIED	480

PEPTONE	480
PERCHLORATE, SODIUM	480
PERIODATE, POTASSIUM	480
PERIODATE, POTASSIUM FERRIC SR	480
PERIODATE, SODIUM	480
PERMANGANATE, POTASSIUM	481
PERMANGANATE, POTASSIUM SR (APPROXIMATELY 0.2 M)	481
PEROXIDE, CARBAMIDE	481
PEROXIDE, CONCENTRATED HYDROGEN	481
PEROXIDE, HYDROGEN, 30 VOLUMES, SR	481
PEROXIDE, HYDROGEN AT 3% (W/V)	481
PEROXIDE, METHANOLIC HYDROGEN	481
PEROXIDE, SODIUM	481
PERSULFATE, AMMONIUM	482
PERSULFATE, POTASSIUM	482
PERSULFATE, SODIUM	482
PICRATE, SODIUM, ALKALINE SR	482
PIPERAZINE	482
PYRIDINE	482
PYRIDINE, ANHYDROUS	482
PYROPHOSPHATE, SODIUM	482
PYROGALLOL	482
POLYACRYLAMIDE	482
POLYSORBATE 20	483
POLYSORBATE 80	483
POTASSIUM SRA - 600 P	483
PREDNISONE	483
SHINY BLACK BN	483
PROPYLENE GLYCOL	483
PROPYLPARABEN	483
PURPLE, PHTHALEIN	483
QUINALIZARIN (CI 58500)	483
QUINIDINE	484
QUINHYDRONE	484
QUININE	484
RHAPONTICIN	484
REAGENT, ALUMINON	484
REAGENT, COLOR	484
REAGENT, MODIFIED ERLICH	484
REAGENT, FOLIN-DENIS	484
REAGENT, HANTZACH	484
REAGENT, JONES	484
REAGENT, MARQUIS	484
REAGENT, XANTHYDROL	484
REAGENT, PHOSPHOTUNGSTIC MOLYBDATE	484
REAGENT, IODINE PLATINUM	485
REAGENT, SULPHO-MOLYBDIC	485
REINECKATO, AMMONIUM	485
REINECKATO, AMMONIUM SR	485
RESAZURIN	485
RESORCINOL	485
RISTOCETIN	485

RHODAMINE B	485
RUTIN	485
SUCROSE	485
SUCROSE 0.1% (W/V) IN PYRIDINE	485
SAFRANIN O	485
SALICYLATE, SODIUM	486
SANTONIN	486
SAPONINS	486
SILICA, DESICCATED	486
SILICA, GEL "G"	486
SILICA, GEL "GF254"	486
SILICA, GEL "H"	486
SILICA, GEL "HF254"	486
SILICA, KIESELGUHR	486
SILICA, KIESELGUHR "G"	486
SODIUM SRA - 200 MG/ML	487
SOLUTION, STANNOUS CHLORIDE AND NINHYDRIN	487
SOLUTION, JEFFREY	487
SOLUTION, KARL-FISCHER	487
SOLUTION, CHROMIC ACID, FOR CLEANING	487
SOLUTION, ACETALDEHYDE, STANDARD (100 PPM C ₂ H ₄ O)	487
SOLUTION, AMMONIUM, STANDARD (1 PPM NH ₄)	487
SOLUTION, BARIUM, STANDARD (10 PPM BA)	487
SOLUTION, CADMIUM, STANDARD (0.1% CD)	487
SOLUTION, CADMIUM, STANDARD (5 PPM CD)	487
SOLUTION, CALCIUM, STANDARD (10 PPM CA)	487
SOLUTION, LEAD, STANDARD (0.1% PB)	487
SOLUTION, COPPER, STANDARD (10 PPM CU)	487
SOLUTION, CHLORIDE, STANDARD (8 PPM CL)	487
SOLUTION, CHLORIDE, STANDARD (5 PPM CL)	487
SOLUTION, DITHIZONE, STANDARD	488
SOLUTION, TIN, STANDARD (5 PPM SN)	488
SOLUTION, MAGNESIUM, STANDARD (10 PPM MG)	488
SOLUTION, NITRATE, STANDARD (100 PPM NO ₃)	488
SOLUTION, NITRATE, STANDARD (2 PPM NO ₃)	488
SOLUTION, SILVER, STANDARD (5 PPM AG)	488
SOLUTION, SELENIUM, STANDARD (100 PPM SE)	488
SOLUTION, SODIUM, STANDARD (200 PPM NA)	488
SOLUTION, SULFATE, STANDARD (10 PPM SO ₄)	488
SOLUTION, ZINC, STANDARD (100 PPM ZN)	488
SOLUTION, ZINC, STANDARD (10 PPM ZN)	488
SOLUTION, BROWN, STANDARD	488
SOLUTION, REDUCING	488
SUBNITRATE, BISMUTH	488
SUBSTITUTE, PLATELETS	488
SUBSTRATE, PLASMA	488
SUBSTRATE, PLASMA 1	489
SUBSTRATE, PLASMA 2	489
SUBSTRATE, PLASMA, FACTOR V DEFICIENT	489
SUDAN III	489
SUDAN III SR	489
SUDAN IV SR	489

SULFAMATE, AMMONIUM	490
SULFANILAMIDE	490
SULFATE, CERIUM	490
SULFATE, AMMONIUM CERIUM	490
SULFATE, CUPRIC, PENTAHYDRATE	490
SULFATE, CUPRIC SR	490
SULFATE, CUPRIC, AMMONIACAL SR	490
SULFATE, ALUMINUM AND POTASSIUM, DODECAHYDRATE	490
SULFATE, AMMONIUM	490
SULFATE, BARIUM	490
SULFATE, CADMIUM	491
SULFATE, CALCIUM, HEMIHYDRATE	491
SULFATE, CALCIUM SR	491
SULFATE, N, N-DIMETHYL-P-PHENYLENEDIAMINE	491
SULFATE, DIMETHYL	491
SULFATE, HYDRAZINE	491
SULFATE, LITHIUM	491
SULFATE, MAGNESIUM, HEPTAHYDRATE	491
SULFATE, MANGANESE	491
SULFATE, 4- METHYLAMINO PHENOL	491
SULFATE, 4- METHYLAMINO PHENOL SR	492
SULFATE, POTASSIUM	492
SULFATE, PROTAMINE	492
SULFATE, SODIUM, ANHYDROUS	492
SULFATE, SODIUM, DECAHYDRATE	492
SULFATE, TETRABUTYLAMMONIUM	492
SULFATE, ZINC, HEPTAHYDRATE	492
SULFATE, ZINC 0.1 M	492
SULFATE, FERRIC	492
SULFATE, AMMONIACAL FERRIC	492
SULFATE, AMMONIACAL FERRIC, ACID SR	493
SULFATE, AMMONIACAL FERRIC SR	493
SULFATE, AMMONIACAL FERRIC SR1	493
SULFATE, AMMONIACAL FERRIC SR2	493
SULFATE, FERRIC - POTASSIUM FERRICYANIDE SR	493
SULFATE, ACIDIFIED FERROUS SR	493
SULFATE, AMMONIACAL FERROUS	493
SULFATE, FERROUS, HEPTAHYDRATE	493
SULFATE, FERROUS SR	493
SULFATE, AMMONIUM SR	493
SULFATE, HYDROGEN	493
SULFATE, HYDROGEN SR	494
SULFATE, SODIUM	494
SULFATE, SODIUM SR	494
SULFATE, SODIUM SR1	494
SULFITE, SODIUM	494
TANNIN	494
TARTRATE, EPINEPHRINE ACID	494
TARTRATE, ALKALINE CUPRIC SR	494
TARTRATE, ANTIMONY AND POTASSIUM	494
TARTRATE, SODIUM	494
TARTRATE, SODIUM AND POTASSIUM	495

TARTRATE, SODIUM AND POTASSIUM SR	495
TARTRATE, FERROUS SR	495
TETRABORATE, SODIUM	495
TETRACHLORIDE, CARBON	495
TETRADECANE	495
TETRAPHENYLBORATE, SODIUM	495
TETRAHYDROBORATE, SODIUM	495
3,3 '- DIAMINO BENZIDINE TETRAHYDROCHLORIDE	495
3,3 '- DIAMINO BENZIDINE TETRAHYDROCHLORIDE SR	495
TETRAHYDROFURAN	496
1, 1,3,3- TETRAMETHYLBUTYLAMINE	496
TETRAMETHYLETHYLENEDIAMINE	496
TETRA OXALATE, POTASSIUM	496
TETROXIDE, OSMIUM	496
TETROXIDE, OSMIUM SR	496
THYMIDINE	496
THYMINE	496
THYMOL	496
THIOACETAMIDE	496
THIOACETAMIDE SR	497
THIOCYANATE, AMMONIUM	497
THIOCYANATE, AMMONIUM SR	497
THIOCYANATE, MERCURY	497
THIOCYANATE, MERCURY SR	497
THIOCYANATE, POTASSIUM	497
THIOGLYCOLATE, SODIUM	497
THIONINE (CI 52000)	497
THIONINE SR	497
THIOSULFATE, SODIUM	497
THIOSULFATE, SODIUM 0.1 M	497
THIOUREA	498
TYROSINE	498
P-TOLUALDEHYDE	498
TOLUENE	498
P-TOLUIDINE	498
TORINA	498
TORINA SR	498
TRICINE	498
1,1,1-TRICHLOROETHANE	498
TRICHLOROETHYLENE	498
TRIETHANOLAMINE	498
TRIETHYLAMINE	499
TRIPHENYLMETHANOL	499
TRIFLUORIDE, BORON	499
TRIFLUORIDE, BORON, METHANOL SOLUTION	499
TRINITROPHENOL SR	499
TRIOXIDE, ARSENIC	499
TRIOXIDE, CHROME	499
THROMBIN, BOVINE	499
THROMBIN, HUMAN	499
THROMBOPLASTIN	499
THROMBOPLASTIN, REAGENT	499

TROMETHAMINE	500
TUNGSTATE, SODIUM	500
UREA	500
VANADATE, AMMONIUM	500
VANILLIN	500
VANILLIN SR	500
VANILLIN, SULFURIC SR	500
WARFARIN, SODIUM	500
BROMOCRESOL GREEN SR	500
PHENOL RED SR	500
VITEXIN	501
XANTHYDROL	501
XYLENE	501
ZINC, ACTIVATED	501
ZINC, GRANULATED	501
ZINC SRA - 5 MG/ML	501
VIALS FOR LIQUIDS, MULTIPLE DOSE AND SINGLE DOSE	302
VIALS FOR TABLETS AND CAPSULES, MULTIPLE UNITS	300
VIALS, PLASTIC - PERFORMANCE TESTS	299
VIALS, POLY (ETHYLENE TEREPHTHALATE) AND POLY (ETHYLENE GLYCOL TEREPHTHALATE)	291
VIALS, POLYETHYLENE	289
VIALS, POLYPROPYLENE	290
VIALS FOR TABLETS AND CAPSULES, SINGLE UNIT AND SINGLE DOSE	301
VIALS, GLASS	285
VIALS AND PLASTIC-RELATED	289
VIALS, DRUGS AND RELATED	285
VIALS, PLASTIC	288
VIALS, PLASTIC AND ELASTOMER LIDS	304
REINECKATO, AMMONIUM (REAGENTS AND REAGENT SOLUTIONS)	485
REINECKATO, AMMONIUM SR (REAGENTS AND REAGENT SOLUTIONS)	485
RESAZURIN (INDICATORS AND INDICATOR SOLUTIONS)	418
RESAZURIN (REAGENTS AND REAGENT SOLUTIONS)	485
RESAZURIN SI (INDICATORS AND INDICATOR SOLUTIONS)	419
TENSILE STRENGTH	279
RESISTANCE TO NEEDLE ENCASEMENT	282
RESISTANCE, HYDROLYTIC OR ALKALINITY	285
RESORCINOL (INDICATORS AND INDICATOR SOLUTIONS)	419
RESORCINOL (REAGENTS AND REAGENT SOLUTIONS)	485
RESORCINOL SI (INDICATORS AND INDICATOR SOLUTIONS)	419
MANUFACTURER'S RESPONSIBILITY	328
USER'S RESPONSIBILITY	328
REVIEW AND APPROVAL OF VALIDATION	326
RIFAMPICIN	1257
RIFAMPICIN, CAPSULAS	1258
RIFAMPICIN, ORAL SUSPENSION	1259
RISTOCETIN (REAGENTS AND REAGENT SOLUTIONS)	485
RITONAVIR, CAPSULES	1261
RHODAMINE B (REAGENTS AND REAGENT SOLUTIONS)	485
RHUBARB	1261
RUTIN (REAGENTS AND REAGENT SOLUTIONS)	485

S

ELDERBERRY	1265
ELDERBERRY, BRAZILIAN	1271
SUCROSE	1277
SUCROSE (REAGENTS AND REAGENT SOLUTIONS)	485
SUCROSE AT 0.1% (W/V) IN PYRIDINE (REAGENTS AND REAGENT SOLUTIONS)	485
SAFRANIN O (REAGENTS AND REAGENT SOLUTIONS)	485
SALTS, ORAL REHYDRATION	1278
CLEAN ROOMS AND CONTROLLED RELATED ENVIRONMENTS	330
WILLOW, WHITE	1279
SALICYLATE, SODIUM (REAGENTS AND REAGENT SOLUTIONS)	486
SANTONIN (REAGENTS AND REAGENT SOLUTIONS)	486
SAPONINS (REAGENTS AND REAGENT SOLUTIONS)	486
SELECTION OF BIOLOGICAL INDICATOR FOR STERILIZATION PROCESS	327
SEMI-MICRO DETERMINATION (METHOD II)	181
SENE	1284
SILICA, KIESELGUHR (REAGENTS AND REAGENT SOLUTIONS)	486
SILICA, KIESELGUHR "G" (REAGENTS AND REAGENT SOLUTIONS)	486
SILICA, DESICCATED (REAGENTS AND REAGENT SOLUTIONS)	486
SILICA, GEL "G" (REAGENTS AND REAGENT SOLUTIONS)	486
SILICA, GEL "GF254" (REAGENTS AND REAGENT SOLUTIONS)	486
SILICA, GEL "H" (REAGENTS AND REAGENT SOLUTIONS)	486
SILICA, GEL "HF254" (REAGENTS AND REAGENT SOLUTIONS)	486
SODIUM SRA - 200 MG/ML (REAGENTS AND REAGENT SOLUTIONS)	487
SOLUTION, OCTA HYDROCHLORIDE, ALUMINUM AND ZIRCONIUM	1174
SOLUTION, STANNOUS CHLORIDE AND NINHYDRIN (REAGENTS AND REAGENT SOLUTIONS)	487
SOLUTION, JEFFREY (REAGENTS AND REAGENT SOLUTIONS)	487
SOLUTION, KARL-FISCHER (REAGENTS AND REAGENT SOLUTIONS)	487
SOLUTION, CHROMIC ACID, FOR CLEANING (REAGENTS AND REAGENT SOLUTIONS)	487
INJECTABLE SOLUTION	
ACID, ASCORBIC	572
ANTIMONIATE, MEGLUMINE	648
ARTEMETHER	656
BUTYLBROMIDE, SCOPOLAMINE	710
CARBOPLATIN	739
CYANOCOBALAMIN	780
CIMETIDINE	784
CIPROFLOXACIN	785
CISPLATIN	787
CHLORIDE, SODIUM	804
BUPIVACAINE HYDROCHLORIDE AND GLUCOSE	813
HYDROCHLORIDE, HYDRALAZINE	835
HYDROCHLORIDE, LIDOCAINE	842
HYDROCHLORIDE, METOCLOPRAMIDE	848
HYDROCHLORIDE, PROMETHAZINE	856
HYDROCHLORIDE, THIAMIN	870
HYDROCHLORIDE, TRAMADOL	871
HYDROCHLORIDE, VERAPAMIL	875
DIAZEPAM	903
PHENYTOIN, SODIUM	956
FLUNITRAZEPAM	969

FUROSEMIDE	990
GLUCOSE	1008
HALOPERIDOL	1015
HYDROXOCOBALAMIN	1039
METRONIDAZOLE	1148
SULFATE, ATROPINE	1309
SULFATE, EPHEDRINE	1313
SULFATE, MORPHINE	1319
ZIDOVUDINE	1380
OPHTHALMIC SOLUTION	
HYDROCHLORIDE, CIPROFLOXACIN	818
NITRATE, SILVER	1165
OFLOXACIN	1178
ORAL SOLUTION	
BROMOPRIDE	707
CLONAZEPAM	798
DIPHENHYDRAMINE HYDROCHLORIDE	822
HYDROCHLORIDE, METOCLOPRAMIDE	849
DIPYRONE	914
PHENOBARBITAL	960
FLUORIDE, SODIUM	972
PHOSPHATE, SODIUM	984
HALOPERIDOL	1016
LORATADINE	1100
LORATADINE AND PSEUDOEPHEDRINE SULFATE	1100
MALEATE, DEXCHLORPHENIRAMINE	1109
PARACETAMOL	1192
SULPHATE, SALBUTAMOL	1324
SULFATE, FERROUS	1328
ZIDOVUDINE	1380
SOLUTION, ACETALDEHYDE, STANDARD (100 PPM C ₂ H ₄ O) (REAGENTS AND REAGENT SOLUTIONS) _487	
SOLUTION, AMMONIUM, STANDARD (1 PPM NH ₄) (REAGENTS AND REAGENT SOLUTIONS)	487
SOLUTION, BARIUM, STANDARD (10 PPM BA) (REAGENTS AND REAGENT SOLUTIONS)	487
SOLUTION, CADMIUM, STANDARD (0.1% CD) (REAGENTS AND REAGENT SOLUTIONS)	487
SOLUTION, CADMIUM, STANDARD (5 PPM CD) (REAGENTS AND REAGENT SOLUTIONS)	487
SOLUTION, CALCIUM, STANDARD (10 PPM CA) (REAGENTS AND REAGENT SOLUTIONS)	487
SOLUTION, LEAD, STANDARD (0.1% PB) (REAGENTS AND REAGENT SOLUTIONS)	487
SOLUTION, CHLORIDE, STANDARD (5 PPM CL) (REAGENTS AND REAGENT SOLUTIONS)	487
SOLUTION, CHLORIDE, STANDARD (8 PPM CL) (REAGENTS AND REAGENT SOLUTIONS)	487
SOLUTION, COPPER, STANDARD (10 PPM CU) (REAGENTS AND REAGENT SOLUTIONS)	487
SOLUTION, DITHIZONE, STANDARD (REAGENTS AND REAGENT SOLUTIONS)	488
SOLUTION, TIN, STANDARD (5 PPM SN) (REAGENTS AND REAGENT SOLUTIONS)	488
SOLUTION, MAGNESIUM, STANDARD (10 PPM MG) (REAGENTS AND REAGENT SOLUTIONS)	488
SOLUTION, NITRATE, STANDARD (100 PPM NO ₃) (REAGENTS AND REAGENT SOLUTIONS)	488
SOLUTION, NITRATE, STANDARD (2 PPM NO ₃) (REAGENTS AND REAGENT SOLUTIONS)	488
SOLUTION, SILVER, STANDARD (5 PPM AG) (REAGENTS AND REAGENT SOLUTIONS)	488
SOLUTION, SELENIUM, STANDARD (100 PPM SE) (REAGENTS AND REAGENT SOLUTIONS)	488
SOLUTION, SODIUM, STANDARD (200 PPM NA) (REAGENTS AND REAGENT SOLUTIONS)	488
SOLUTION, SULFATE, STANDARD (10 PPM SO ₄) (REAGENTS AND REAGENT SOLUTIONS)	488
SOLUTION, ZINC, STANDARD (10 PPM ZN) (REAGENTS AND REAGENT SOLUTIONS)	488
SOLUTION, ZINC, STANDARD (100 PPM ZN) (REAGENTS AND REAGENT SOLUTIONS)	488
SOLUTION, BROWN, STANDARD (REAGENTS AND REAGENT SOLUTIONS)	488

SOLUTION, REDUCING (REAGENTS AND REAGENT SOLUTIONS)	488
TOPICAL SOLUTION	
CICLOPIROX OLAMINE	781
VOLUMETRIC SOLUTIONS	501
ACID, HYDROCHLORIC M SV	501
ACID, OXALIC 0.05 M SV	501
ACID, PERCHLORIC 0.1 M SV	502
ACID, SULFURIC M SV	502
BROMATE, POTASSIUM 0.1 M SV	502
BROMINE 0.05 M SV	502
CHLORIDE, BARIUM 0.1 M SV	502
CHLORIDE, BENZETHONIUM 0.004 M SV	502
DICHLOROPHENOL-INDOPHENOL, STANDARD SOLUTION	502
EDETATE, DISODIUM 0.05 M SV	502
EDETATE, DISODIUM 0.1 M SV	503
HYDROXIDE, ETHANOLIC POTASSIUM 0.5 M SV	503
HYDROXIDE, POTASSIUM M SV	503
HYDROXIDE, SODIUM M SV	503
HYDROXIDE, ETHANOLIC SODIUM 0.1 M SV	503
HYDROXIDE, TETRABUTYLAMMONIUM 0.1 M SV	503
INDIGO CARMINE SV	504
IODATE, POTASSIUM 0.02 M SV	504
IODATE, POTASSIUM 0.1 M SV	504
IODINE 0.05 M SV	504
IODINE 0.1 M SV	504
METHOXIDE, LITHIUM 0.1 M SV	504
METHOXIDE, SODIUM 0.1 M SV	504
NITRATE, CERIUM AMMONIUM 0.01 M SV	504
NITRATE, CERIUM AMMONIUM 0.1 M SV	504
NITRATE, BARIUM 0.01 M SV	505
NITRATE, LEAD 0.1 M SV	505
NITRATE, MERCURY (II) 0.1 M SV	505
NITRATE, THORIUM 0.005 M SV	505
NITRITE, SODIUM 0.1 M SV	505
PERMANGANATE, POTASSIUM 0.02 M SV	505
SULFATE, CERIUM 0.05 M SV	505
SULFATE, AMMONIUM CERIUM 0.1 M SV	506
SULFATE, ZINC 0.1 M SV	506
TETRAPHENYLBORATE, SODIUM 0.02 M SV	506
THIOCYANATE, AMMONIUM 0.1 M SV	506
THIOSULFATE, SODIUM 0.1 M SV	506
SOLVENTS, CHROMATOGRAPHY (ANNEX C)	523
SERUM, ANTIBOTHRIC, PENTAVALENT	1289
SERUM, ANTIBOTHRIC, PENTAVALENT AND ANTICROTALIC	1290
SERUM, ANTIBOTHRIC, PENTAVALENT AND ANTILAQUETIC	1290
SERUM, ANTIBOTHRIC, PENTAVALENT, ANTICROTALIC AND ANTILAQUETIC	1291
SERUM, ANTIBOTULINIC, TRIVALENT	1291
SORUM, ANTICROTALIC	1292
SERUM, DIPHTHERIA	1293
SERUM, ANTI-ELAPIDIC, BIVALENT	1294
SERUM, ANTI-SCORPION	1294
SERUM, ANTILONOMIC	1295

SERUM, ANTI-LOXOSCELIC, TRIVALENT	1296
SERUM, ANTI-RABIES	1297
SERUM, ANTI-TETANUS	1298
SERUM, HYPERIMMUNE, HUMAN USE	1299
SPECTROMETRY, ATOMIC	94
SUBNITRATE, BISMUTH (REAGENTS AND REAGENT SOLUTIONS)	488
SUBSTANCES, DYE	401
SUBSTANCES, REFERENCE CHEMICAL	399
SUBSTANCES, VASODEPRESSIVE	236
SUBSTANCES, VASOPRESSIVE	234
SUBSTITUTE, PLATELETS (REAGENTS AND REAGENT SOLUTIONS)	488
SUBSTRATE, PLASMA (REAGENTS AND REAGENT SOLUTIONS)	488
SUBSTRATE, PLASMA, FACTOR V DEFICIENT (REAGENTS AND REAGENT SOLUTIONS)	489
SUBSTRATE, PLASMA 1 (REAGENTS AND REAGENT SOLUTIONS)	489
SUBSTRATE, PLASMA 2 (REAGENTS AND REAGENT SOLUTIONS)	489
SUDAN III (REAGENTS AND REAGENT SOLUTIONS)	489
SUDAN III SR (REAGENTS AND REAGENT SOLUTIONS)	489
SUDAN IV SR (REAGENTS AND REAGENT SOLUTIONS)	489
SULFADIAZINE	1301
SULFADIAZINE, TABLETS	1302
SULFAMATE, AMMONIUM (REAGENTS AND REAGENT SOLUTIONS)	490
SULFAMETHOXAZOLE	1304
SULFAMETHOXAZOLE AND TRIMETHOPRIM, TABLETS	1305
SULFAMETHOXYPYRIDAZINE	1306
SULFANILAMIDE	1307
SULFANILAMIDE (REAGENTS AND REAGENT SOLUTIONS)	490
SULFATE, CERIUM (REAGENTS AND REAGENT SOLUTIONS)	490
SULFATE, CERIUM 0.05 M SV (VOLUMETRIC SOLUTIONS)	505
SULFATE, AMMONIUM CERIUM (REAGENTS AND REAGENT SOLUTIONS)	490
SULFATE, AMMONIUM CERIUM 0.1 M SV (VOLUMETRIC SOLUTIONS)	506
SULFATE, CUPRIC, AMMONIACAL SR (REAGENTS AND REAGENT SOLUTIONS)	490
SULFATE, CUPRIC SR (REAGENTS AND REAGENT SOLUTIONS)	490
SULFATE, CUPRIC, PENTAHYDRATE (REAGENTS AND REAGENT SOLUTIONS)	490
SULFATE, 4- METHYLAMINO PHENOL (REAGENTS AND REAGENT SOLUTIONS)	491
SULFATE, 4- METHYLAMINO PHENOL SR (REAGENTS AND REAGENT SOLUTIONS)	492
SULFATE, ALUMINUM AND POTASSIUM, DODECAHYDRATE (REAGENTS AND REAGENT SOLUTIONS)	490
SULFATE, AMMONIUM (REAGENTS AND REAGENT SOLUTIONS)	490
SULFATE, ATROPINE	1308
SULFATE, ATROPINE, INJECTABLE SOLUTION	1309
SULFATE, BARIUM	1310
SULFATE, BARIUM (REAGENTS AND REAGENT SOLUTIONS)	490
SULFATE, CADMIUM (REAGENTS AND REAGENT SOLUTIONS)	491
SULFATE, CALCIUM	1311
SULFATE, CALCIUM SR (REAGENTS AND REAGENT SOLUTIONS)	491
SULFATE, CALCIUM, HEMIHYDRATE (REAGENTS AND REAGENT SOLUTIONS)	491
SULFATE, DIMETHYL (REAGENTS AND REAGENT SOLUTIONS)	491
SULFATE, EPHEDRINE	1312
SULFATE, EPHEDRINE, INJECTABLE SOLUTION	1313
SULFATE, STREPTOMYCIN, POWDER FOR INJECTABLE SOLUTION	1313
SULFATE, HYDRAZINE (REAGENTS AND REAGENT SOLUTIONS)	491
SULFATE, INDINAVIR	1314
SULFATE, LITHIUM (REAGENTS AND REAGENT SOLUTIONS)	491

SULFATE, MAGNESIUM, HEPTAHYDRATE _____	1316
SULFATE, MAGNESIUM, HEPTAHYDRATE (REAGENTS AND REAGENT SOLUTIONS) _____	491
SULFATE, MANGANESE (REAGENTS AND REAGENT SOLUTIONS) _____	491
SULFATE, MORPHINE _____	1317
SULFATE, MORPHINE, TABLETS _____	1318
SULFATE, MORPHINE, INJECTABLE SOLUTION _____	1319
SULPHATE, N, N-DIMETHYL-P-PHENYLENEDIAMINE (REAGENTS AND REAGENT SOLUTIONS) _____	491
SULFATE, NEOMYCIN _____	1320
SULFATE, POTASSIUM (REAGENTS AND REAGENT SOLUTIONS) _____	492
SULFATE, PROTAMINE (REAGENTS AND REAGENT SOLUTIONS) _____	492
SULFATE, PSEUDOEPHEDRINE _____	1322
SULFATE, SALBUTAMOL _____	1323
SULFATE, SALBUTAMOL, ORAL SOLUTION _____	1324
SULFATE, SODIUM _____	1324
SULFATE, SODIUM, ANHYDROUS (REAGENTS AND REAGENT SOLUTIONS) _____	492
SULFATE, SODIUM, DECAHYDRATE (REAGENTS AND REAGENT SOLUTIONS) _____	492
SULFATE, TETRABUTYLAMMONIUM (REAGENTS AND REAGENT SOLUTIONS) _____	492
SULFATE, ZINC 0.1 M (REAGENTS AND REAGENT SOLUTIONS) _____	492
SULFATE, ZINC 0.1 M SV (VOLUMETRIC SOLUTIONS) _____	506
SULFATE, ZINC, HEPTAHYDRATE (REAGENTS AND REAGENT SOLUTIONS) _____	492
SULFATE, FERRIC (REAGENTS AND REAGENT SOLUTIONS) _____	492
SULFATE, AMMONIACAL FERRIC (REAGENTS AND REAGENT SOLUTIONS) _____	492
SULFATE, AMMONIACAL FERRIC, ACID SR (REAGENTS AND REAGENT SOLUTIONS) _____	493
SULFATE, AMMONIACAL FERRIC SR (REAGENTS AND REAGENT SOLUTIONS) _____	493
SULFATE, AMMONIACAL FERRIC SR1 (REAGENTS AND REAGENT SOLUTIONS) _____	493
SULFATE, AMMONIACAL FERRIC SR2 (REAGENTS AND REAGENT SOLUTIONS) _____	493
SULFATE, FERRIC - POTASSIUM FERRICYANIDE SR (REAGENTS AND REAGENT SOLUTIONS) _____	493
SULFATE, FERROUS _____	1325
SULFATE, FERROUS, ACIDIFIED SR (REAGENTS AND REAGENT SOLUTIONS) _____	493
SULFATE, AMMONIACAL FERROUS (REAGENTS AND REAGENT SOLUTIONS) _____	493
SULFATE, FERROUS, TABLETS _____	1326
SULFATE, FERROUS, HEPTAHYDRATE _____	1326
SULFATE, FERROUS, ORAL SOLUTION _____	1328
SULFATE, FERROUS SR (REAGENTS AND REAGENT SOLUTIONS) _____	493
SULFATE, FERROUS, HEPTAHYDRATE (REAGENTS AND REAGENT SOLUTIONS) _____	493
SULFATE, AMMONIUM SR (REAGENTS AND REAGENT SOLUTIONS) _____	493
SULFATE, HYDROGEN (REAGENTS AND REAGENT SOLUTIONS) _____	493
SULFATE, HYDROGEN SR (REAGENTS AND REAGENT SOLUTIONS) _____	494
SULFATE, SELENIUM _____	1328
SULFATE, SODIUM (REAGENTS AND REAGENT SOLUTIONS) _____	494
SULFATE, SODIUM SR (REAGENTS AND REAGENT SOLUTIONS) _____	494
SULFATE, SODIUM SR1 (REAGENTS AND REAGENT SOLUTIONS) _____	494
SULFITE, SODIUM _____	1329
SULFITE, SODIUM (REAGENTS AND REAGENT SOLUTIONS) _____	494
SUPPOSITORIES	
BISACODYL _____	691
GLYCEROL _____	1002
INDOMETHACIN _____	1068
ORAL SUSPENSION	
ACID, NALIDIXIC _____	582
ALBENDAZOLE _____	592
BENZOYL METRONIDAZOLE _____	686

CEFACLOR	754
ESTOLATE, ERYTHROMYCIN	936
MEBENDAZOLE	1122
NYSTATIN	1162
RIFAMPICIN	1259
THIABENDAZOLE	1342

T

TABLE, PERIODIC, CHEMICAL ELEMENTS - NAMES, SYMBOLS, AND ATOMIC MASSES (ANNEX A)	511
TABLES, STATISTICAL	349
BUFFER, CUPRIC SULFATE (BUFFERS)	510
ELASTOMER LID	294
BUFFERS	506
ACETATE 0.05 M PH 4.5	507
ACETATE, AMMONIUM PH 8.5	509
ACETATE, SODIUM 0.1 M PH 5.0	507
ACETATE, SODIUM PH 4.5	507
ACETATE PH 3.0	506
ACETATE PH 3.5	506
ACETATE PH 4.0	507
ACETATE PH 4.4	507
ACETATE PH 6.0	507
ACETATE PH 7.0	508
ACETIC ACID, AMMONIUM ACETATE	509
ACID, HYDROCHLORIC PH 2.0	506
ALBUMIN -PHOSPHATE PH 7.2	508
BARBITAL PH 7.4	508
BARBITAL PH 8.4	508
BARBITAL PH 8.6	509
BIPHTHALATE PH 4.4	507
BORATE PH 8.0	508
BORATE PH 9.0	509
BORATE PH 9.6	509
CARBONATE / BICARBONATE, SODIUM PH 9.6	509
CITRATE, PHOSPHATE PH 5.0	507
CITRO, PHOSPHATE PH 6.0	507
CITRO, PHOSPHATE PH 7.0	508
CHLORIDE, AMMONIUM PH 10.0	509
CHLORIDE, AMMONIUM PH 10.7	509
CONCENTRATE FOR DSS-EGPA SAMPLES AT REDUCING CONDITIONS	509
CONCENTRATE FOR DSS-EGPA SAMPLES	509
ELECTROPHORESIS, DSS-EGPA	509
PHOSPHATE 0.025M PH 6.86	507
PHOSPHATE, POTASSIUM PH 7.4 WITH POLYSORBATE 80 AT 2% (V/V)	508
PHOSPHATE M/L5 PH 7.0	508
PHOSPHATE PH 2.2	506
PHOSPHATE PH 5.5	507
PHOSPHATE PH 5.8	507
PHOSPHATE PH 6.0	507
PHOSPHATE PH 6.5	507
PHOSPHATE PH 6.8	507

PHOSPHATE PH 7.0	508
PHOSPHATE PH 7.1	508
PHOSPHATE PH 7.2	508
PHOSPHATE PH 7.3	508
PHOSPHATE PH 8.5	509
PHOSPHATE PH 8.6	509
PHOSPHATE, SODIUM LAURYL SULFATE PH 11.0	509
PHOSPHATE, SODIUM LAURYL SULFATE PH 6.8	507
PHOSPHATE-SALINE (PBS)	510
IMIDAZOLE PH 7.4	508
SULFATE, CUPRIC	510
TRIS 0.05 M PH 9.0	509
TRIS, SODIUM CHLORIDE PH 7.5	508
TRIS, HYDROCHLORIDE 1.5 M PH 8.8	509
TRIS, HYDROCHLORIDE PH 6.8	507
TROMETHAMINE, SODIUM CHLORIDE PH 7.4	508
TROMETHAMINE-EDTA PH 8.4	508
TANNIN (REAGENTS AND REAGENT SOLUTIONS)	494
TARTRATE, EPINEPHRINE ACID (REAGENTS AND REAGENT SOLUTIONS)	494
TARTRATE, ALKALINE CUPRIC SR (REAGENTS AND REAGENT SOLUTIONS)	494
TARTRATE, ANTIMONY AND POTASSIUM	1331
TARTRATE, ANTIMONY AND POTASSIUM (REAGENTS AND REAGENT SOLUTIONS)	494
TARTRATE, ANTIMONY AND SODIUM	1331
TARTRATE, METOPROLOL, TABLETS	1332
TARTRATE, POTASSIUM AND SODIUM	1333
TARTRATE, SODIUM (REAGENTS AND REAGENT SOLUTIONS)	494
TARTRATE, SODIUM AND POTASSIUM (REAGENTS AND REAGENT SOLUTIONS)	495
TARTRATE, SODIUM AND POTASSIUM SR (REAGENTS AND REAGENT SOLUTIONS)	495
TARTRATE, FERROUS SR (REAGENTS AND REAGENT SOLUTIONS)	495
TARTRAZINE	1334
GAUZE, PURIFIED HYDROPHILIC MATERIAL	1335
TECHNIQUES, NUCLEIC ACID AMPLIFICATION	214
TECHNOLOGIES, ALTERNATIVES, ASEPTIC PROCESS	332
TERCONAZOLE	1337
TERCONAZOLE, CREAM	1339
TEST, DISINTEGRATION OF SUPPOSITORIES, OVA, AND VAGINAL TABLETS	65
TEST, DISINTEGRATION OF TABLETS AND CAPSULES	63
TEST, DISSOLUTION	66
TEST, HARDNESS	62
TEST, ANTIMICROBIAL EFFECTIVENESS	273
TEST, STERILITY	253
TEST, FRIABILITY	62
TEST, DRIP	80
TEST, LIGHT TRANSMISSION	303
TESTS, DISINTEGRATION	63
TESTS, BIOLOGICAL REACTIVITY IN VITRO	312
TESTS, BIOLOGICAL REACTIVITY IN VIVO	314
TEST, VALIDITY	343
TESTS, IN VITRO, IN VIVO, AND DESIGNATION OF CLASS FOR PLASTICS AND OTHERS POLYMERS	304
TETRABORATE, SODIUM (REAGENTS AND REAGENT SOLUTIONS)	495
TETRACHLORIDE, CARBON (REAGENTS AND REAGENT SOLUTIONS)	495
TETRADECANE (REAGENTS AND REAGENT SOLUTIONS)	495

TETRAPHENYLBORATE, SODIUM (REAGENTS AND REAGENT SOLUTIONS)	495
TETRAPHENYLBORATE, SODIUM 0.02 M SV (VOLUMETRIC SOLUTIONS)	506
TETRAHYDROBORATE, SODIUM (REAGENTS AND REAGENT SOLUTIONS)	495
TETRAHYDROFURAN (REAGENTS AND REAGENT SOLUTIONS)	496
TETRAMETHYLETHYLENEDIAMINE (REAGENTS AND REAGENT SOLUTIONS)	496
TETRA OXALATE, POTASSIUM (REAGENTS AND REAGENT SOLUTIONS)	496
TETROXIDE, OSMIUM (REAGENTS AND REAGENT SOLUTIONS)	496
TETROXIDE, OSMIUM SR (REAGENTS AND REAGENT SOLUTIONS)	496
THIABENDAZOLE	1339
THIABENDAZOLE, TABLETS	1340
THIABENDAZOLE, OINTMENT	1341
THIABENDAZOLE, ORAL SUSPENSION	1342
THYMIDINE (REAGENTS AND REAGENT SOLUTIONS)	496
THYMININE (REAGENTS AND REAGENT SOLUTIONS)	496
THYMOL (REAGENTS AND REAGENT SOLUTIONS)	496
THYMOLPHTHALEIN (INDICATORS AND INDICATOR SOLUTIONS)	419
THYMOLPHTHALEIN SI (INDICATORS AND INDICATOR SOLUTIONS)	419
TINCTURE RHATANY_ 1252	
TINCTURE, STRONG IODINE	1343
TINCTURE, LOW IODINE	1343
THIOACETAMIDE (REAGENTS AND REAGENT SOLUTIONS)	496
THIOACETAMIDE SR (REAGENTS AND REAGENT SOLUTIONS)	497
THIOCYANATE, AMMONIUM (INDICATORS AND INDICATOR SOLUTIONS)	419
THIOCYANATE, AMMONIUM (REAGENTS AND REAGENT SOLUTIONS)	497
THIOCYANATE, AMMONIUM 0.1 M SV (VOLUMETRIC SOLUTIONS)	506
THIOCYANATE, AMMONIUM SI (INDICATORS AND INDICATOR SOLUTIONS)	419
THIOCYANATE, AMMONIUM SR (REAGENTS AND REAGENT SOLUTIONS)	497
THIOCYANATE, MERCURY (REAGENTS AND REAGENT SOLUTIONS)	497
THIOCYANATE, MERCURY SR (REAGENTS AND REAGENT SOLUTIONS)	497
THIOCYANATE, POTASSIUM (REAGENTS AND REAGENT SOLUTIONS)	497
THIOGLYCOLATE, SODIUM (REAGENTS AND REAGENT SOLUTIONS)	497
THIONINE (CI 52000) (REAGENTS AND REAGENT SOLUTIONS)	497
THIONINE SR (REAGENTS AND REAGENT SOLUTIONS)	497
THIOSULFATE, SODIUM (REAGENTS AND REAGENT SOLUTIONS)	497
THIOSULFATE, SODIUM 0.1 M (REAGENTS AND REAGENT SOLUTIONS)	497
THIOSULFATE, SODIUM 0.1 M SV (VOLUMETRIC SOLUTIONS)	506
THIOUREA (REAGENTS AND REAGENT SOLUTIONS)	498
TYPES OF DELINEATION	342
TYPES OF BIOLOGICAL INDICATORS	326
TYROSINE (REAGENTS AND REAGENT SOLUTIONS)	498
TITRATION, COMPLEXOMETRIC	183
TITRATION, NON-AQUEOUS MEDIUM	184
TITRATIONS, DIAZOTIZATION	180
TOLMETIN, SODIUM	1344
TOLUENE (REAGENTS AND REAGENT SOLUTIONS)	498
TORINA (REAGENTS AND REAGENT SOLUTIONS)	498
TORINA SR (REAGENTS AND REAGENT SOLUTIONS)	498
LITMUS (INDICATORS AND INDICATOR SOLUTIONS)	419
LITMUS, BLUE, PAPER (INDICATORS AND INDICATOR SOLUTIONS)	419
LITMUS SI (INDICATORS AND INDICATOR SOLUTIONS)	419
LITMUS, RED, PAPER (INDICATORS AND INDICATOR SOLUTIONS)	419
TOXICITY	234

TOXOID, ADSORBED TETANUS	1345
TRAINING OF STAFF	333
TRETINOIN, CREAM	1347
TRETINOIN, GEL	1348
TRICINE (REAGENTS AND REAGENT SOLUTIONS)	498
TRICHLOROETHYLENE (REAGENTS AND REAGENT SOLUTIONS)	498
TRIETHANOLAMINE (REAGENTS AND REAGENT SOLUTIONS)	498
TRIETHYLAMINE (REAGENTS AND REAGENT SOLUTIONS)	499
TRIPHENYLMETHANOL (REAGENTS AND REAGENT SOLUTIONS)	499
TRIFLUORIDE, BORON (REAGENTS AND REAGENT SOLUTIONS)	499
TRIFLUORIDE, BORON, METHANOL SOLUTION (REAGENTS AND REAGENT SOLUTIONS)	499
TRIMETHOPRIM	1349
TRINITROPHENOL SR (REAGENTS AND REAGENT SOLUTIONS)	499
TRIOXIDE, ARSENIC (REAGENTS AND REAGENT SOLUTIONS)	499
TRIOXIDE, CHROME (REAGENTS AND REAGENT SOLUTIONS)	499
TRIS 0.05 M PH 9.0 (BUFFERS)	509
TRIS-SODIUM CHLORIDE PH 7.5 (BUFFERS)	508
TRIS, HYDROCHLORIDE 1.5 M PH 8.8 (BUFFERS)	509
THROMBIN, BOVINE (REAGENTS AND REAGENT SOLUTIONS)	499
THROMBIN, HUMAN (REAGENTS AND REAGENT SOLUTIONS)	499
THROMBOPLASTIN (REAGENTS AND REAGENT SOLUTIONS)	499
THROMBOPLASTIN, REAGENT (REAGENTS AND REAGENT SOLUTIONS)	499
TROMETHAMINE (REAGENTS AND REAGENT SOLUTIONS)	500
TROMETHAMINE-SODIUM CHLORIDE 7.4 PH (BUFFERS)	508
TROMETHAMINE-EDTA PH 8.4 (BUFFERS)	508
TROPEOLIN O (CI 14270) (INDICATORS AND INDICATOR SOLUTIONS)	419
TROPEOLIN O (CI 14270) (INDICATORS AND INDICATOR SOLUTIONS)	419
TROPEOLIN OO (CI 13080) (INDICATORS AND INDICATOR SOLUTIONS)	420
TUNGSTATE, SODIUM (REAGENTS AND REAGENT SOLUTIONS)	500
TURBIDIMETRY AND NEPHELOMETRY	104

U

INTERNATIONAL SYSTEM OF UNITS (SI) USED IN PHARMACOPEIA AND EQUIVALENTS WITH OTHER UNITS (ANNEX B)	517
UNIFORMITY OF DOSAGE UNIT	73
UREA	1351
UREA (REAGENTS AND REAGENT SOLUTIONS)	500
USE OF BIOLOGICAL INDICATOR FOR VALIDATION	329

V

VACCINE, ADSORBED, DIPHTHERIA AND TETANUS, ADULT	1355
VACCINE, ADSORBED, DIPHTHERIA AND TETANUS, INFANTILE	1356
VACCINE, ADSORBED, DIPHTHERIA, TETANUS, AND PERTUSSIS	1359
VACCINE, BCG	1361
VACCINE, ATTENUATED MUMPS	1362
VACCINE, ATTENUATED YELLOW FEVER	1363
VACCINE, ATTENUATED 1, 2, AND 3 POLIO	1364
VACCINE, INACTIVATED 1, 2, AND 3 POLIO	1366
VACCINE, RABIES (INACTIVATED)	1366
VACCINE, ATTENUATED RUBELLA	1368

VACCINE, ATTENUATED MEASLES	1369
VACCINE, MEASLES, MUMPS, RUBELLA*	1370
VACCINE, MEASLES, RUBELLA*	1371
VACCINE, ATTENUATED VARICELLA	1372
VACCINES, HUMAN USE	1353
VALIDATION, STERILIZATION PROCESS	324
VALUES, ATYPICAL	340
VANADATE, AMMONIUM (REAGENTS AND REAGENT SOLUTIONS)	500
VANILLIN (REAGENTS AND REAGENT SOLUTIONS)	500
VANILLIN SR (REAGENTS AND REAGENT SOLUTIONS)	500
VANILLIN, SULFURIC SR (REAGENTS AND REAGENT SOLUTIONS)	500
WARFARIN, SODIUM	1373
WARFARIN, SODIUM (REAGENTS AND REAGENT SOLUTIONS)	500
GREEN, BROMOCRESOL (INDICATORS AND INDICATOR SOLUTIONS)	420
GREEN, BROMOCRESOL SI (INDICATORS AND INDICATOR SOLUTIONS)	420
GREEN, BROMOCRESOL SR (REAGENTS AND REAGENT SOLUTIONS)	500
GREEN, MALACHITE SI (INDICATORS AND INDICATOR SOLUTIONS)	420
GREEN, MALACHITE, OXALATE (INDICATORS AND INDICATOR SOLUTIONS)	420
GREEN, METHYL (CI 42590) (INDICATORS AND INDICATOR SOLUTIONS)	420
GREEN, METHYL SI (INDICATORS AND INDICATOR SOLUTIONS)	420
CRESOL RED (INDICATORS AND INDICATOR SOLUTIONS)	420
CRESOL RED SI (INDICATORS AND INDICATOR SOLUTIONS)	420
CONGO RED (CI 22120) (INDICATORS AND INDICATOR SOLUTIONS)	420
CONGO RED SI (INDICATORS AND INDICATOR SOLUTIONS)	420
CONGO RED, PAPER (INDICATORS AND INDICATOR SOLUTIONS)	420
PHENOL RED (INDICATORS AND INDICATOR SOLUTIONS)	421
PHENOL RED SI (INDICATORS AND INDICATOR SOLUTIONS)	421
PHENOL RED SR (REAGENTS AND REAGENT SOLUTIONS)	500
METHYL RED (CI 13020) (INDICATORS AND INDICATOR SOLUTIONS)	421
METHYL RED SI (INDICATORS AND INDICATOR SOLUTIONS)	421
QUINALDINE RED (INDICATORS AND INDICATOR SOLUTIONS)	421
QUINALDINE RED SI (INDICATORS AND INDICATOR SOLUTIONS)	421
PONCEAU 4R	1374
PONCEAU 4R, ALUMINUM LAKE	1375
VITEXIN (REAGENTS AND REAGENT SOLUTIONS)	501

X

SHAMPOO	
KETOCONAZOLE	772
XANTHYDROL (REAGENTS AND REAGENT SOLUTIONS)	501
XYLENE (REAGENTS AND REAGENT SOLUTIONS)	501

Z

ZIDOVUDINE	1377
ZIDOVUDINE, CAPSULES	1378
ZIDOVUDINE AND LAMIVUDINE, TABLETS	1381
ZIDOVUDINE, INJECTABLE SOLUTION	1380
ZIDOVUDINE, ORAL SOLUTION	1380
ZINC SRA - 5 MG/ML (REAGENTS AND REAGENT SOLUTIONS)	501
ZINC, ACTIVATED (REAGENTS AND REAGENT SOLUTIONS)	501
ZINC, GRANULATED (REAGENTS AND REAGENT SOLUTIONS)	501
