Brazilian Health Surveillance Agency

Brazilian Pharmacopoeia

Volume 1

Esta tradução é um produto de termo de cooperação entre a Agência Nacional de Vigilância Sanitária (ANVISA) e a Organização Pan-Americana de Saúde (OPAS), e não substitui a versão em português.

This translation is a product of a cooperation agreement between Brazilian Health Surveillance Agency (ANVISA) and Pan American Health Organization (PAHO), and does not replace the portuguese version.

Esta traducción es un producto del acuerdo de cooperación entre la Agencia Brasileña de Vigilancia Sanitaria (ANVISA) y la Organización Panamericana de Salud (OPAS), y no sustituye la versión en portugués.

5th edition

Brasilia
2010

5th edition

ANVISA
Agência Nacional de Vigilância Sanitária

President of the Republic

Luiz Inácio Lula da Silva

Minister of Health
José Gomes Temporão

Chairman
Dirceu Raposo de Mello

Deputy Director-President
Pedro Ivo Sebba Ramalho

Directors
Dirceu Aparecido Brás Barbano
José Agenor Álvares da Silva
Maria Cecília Martins Brito

Deputy Directors
Luiz Roberto da Silva Klassmann
Neilton Araujo de Oliveira
Luiz Armando Erthal

Chief of Staff
Iliana Alves Canoff

Drafting and editing
BRAZILIAN SANITARY SURVEILLANCE AGENCY
SIA Trecho 5, Área Especial 57, Lote 200
71205-050, Brasília – DF
Tel.: (61.3462-6000
Home page: www.anvisa.gov.br

Ministério da Saúde

FIOCRUZ
Fundação Oswaldo Cruz

President
Paulo Gadelha

Vice President of Education, Information, and Communication
Maria do Carmo Leal

EDITORA FIOCRUZ

Director
Maria do Carmo Leal

Executive Editor
João Carlos Canossa Mendes

Science Editors
Nísia Trindade Lima e Ricardo Ventura Santos

Editorial Board
Ana Lúcia Teles Rabello
Armando de Oliveira Schubach
Carlos E. A. Coimbra Jr.
Gerson Oliveira Penna
Gilberto Hochman
Joseli Lannes Vieira
Lígia Vieira da Silva
Maria Cecília de Souza Minayo

Brasil. Farmacopeia Brasileira, volume 1 / Agência Nacional de Vigilância Sanitária. Brasília: Anvisa, 2010.
548p., 1v/il.

1. Substâncias farmacêuticas químicas, vegetais e biológicas. 2. Medicamentos e correlatos. 3. Especificações e métodos de análise. I Título.

ISBN 978-85-88233-40-9

RESOLUTION OF THE COLLEGIATE BOARD OF OFFICERS – RDC No. 49, NOVEMBER 23RD, 2010

It approves Brazilian Pharmacopoeia, 5th edition and provides further remedies.

The Collegiate Board of Officers of the Brazilian Health Surveillance Agency, by using their attributions conferred by item IV of the art. 11 of the Regulation approved by Decree no. 3.029, April 16th, 1999, and in view of the provision in item II and paragraphs one and three of art. 53 of the Internal Regulations approved under Attachment I of the Ordinance No. 354 of ANVISA, August 11th, 2006, republished on the Official Gazette of August 21st, 2006 and what further appears in art. 7, item XIX of Law no. 9.782, January 26th, 1999, at a meeting held on November 11th, 2010, adopts the following Resolution of the Collegiate Board of Officers and I, CEO, determine its publication:

Art. 1 It is hereby approved the Brazilian Pharmacopoeia, 5th edition, comprised of Volume 1 – General Methods and texts and Volume 2 - Monographs.

Art. 2 The pharmaceutical inputs, the medicines and other products subject to health surveillance must fulfill the standards and specifications established in the Brazilian Pharmacopoeia.

Sole paragraph. In the absence of the official monograph on raw material, pharmaceutical forms, correlates and general methods in the fifth edition of the Brazilian Pharmacopoeia, for the control of pharmaceutical inputs and products, the adoption of the latest edition of the official monograph, and foreign pharmaceutical codes in the manner provided in specific standards will be admitted.

Art. 3 Printing, distributing, reproducing or selling the Brazilian Pharmacopoeia, 5th edition, with no previous and express consent from ANVISA is forbidden.

Sole paragraph. Without prejudice of the provision in the caput of this article, ANVISA will make available, for free, on its website, the copy of the fifth edition and its updates.

Art. 4 Fundação Oswaldo Cruz, by means of Editora Fiocruz, is authorized to market the copies of the fifth edition of the Brazilian Pharmacopoeia.

Art. 5 All the monographs and general methods from previous editions of the Brazilian Pharmacopoeia are revoked.

Art. 6 This Resolution will be in effect ninety (90) days after its publication

Brasília, November 24, 2010

DIRCEU RAPOSO DE MELLO
CEO of the Brazilian Health Surveillance Agency

Published in the Daily Official Gazette No. 224, November 24, 2010

SUMMARY

Volume 1

Volume 2

MONOGRAPHS

1 PREFACE

To preface such a significant work of a national pharmacopoeia is not an easy task. When presenting a work, in which you are involved in a whole constructive process, you must take care to issue an opinion as exempt as highly possible.

However, it is a huge pride to make it external, on behalf of a Commission and several Committees, the final impressions of a technical and scientific work, to be instrument for public health actions intended to protect the population of your country by means of health risk prevention. You cannot be negligent to the political force which Brazilian Pharmacopoeia, 5thedition (FB 5) brings to the country.

We were called by Brazilian Health Surveillance Agency (Anvisa) in order to preside the works which would be the body of the fifth edition of the Brazilian Pharmacopoeia, and we did not hesitate a single minute because we knew the level of competence, commitment and responsibilities of the members of the Brazilian Pharmacopoeia Commission (CFB). Accordingly, CFB indicated the coordinators of the Thematic Technical Committees (CTT) and they formed their teams by using the same criterion.

We have now a work which is a milestone of this and future editions, as well as in professional relationships between the technical-scientific staff, administrative staff and the society.

Previous editions of the Brazilian Pharmacopoeia were not then revoked and, therefore, they were lawfully in force. In addition to the scientific prejudice, due to the gap of the described methods, they provided a certain hindrance to the health actions which are based on the pharmacopoeia descriptions, either in registration area and quality control area, as well as concerning surveillance.

In virtue of superior determination, CFB deployed a hard work in reviewing one thousand, seven hundred and twenty-seven monographs inserted into the four previous editions and proposed exclusions, text reassessments, methodological reassessments, updates for safer procedures, inclusions of new texts, *inter alia*.

Projects financed by Anvisa were started with participation of conceptualized Education and Research Institutions in a pioneer work which involved two hundred professionals from the healthcare area, among them is a great part of the academic area, providing the country with qualified workforce to address the Brazilian pharmaceutical sector.

The involvement with the Academy, as expected, led to the production of a hundred pieces of technical and scientific information by publishing articles, work presentations in congresses, discussions in round tables, lectures at national and international events, preparation of doctorate thesis, master's degree dissertation and specialization monographs.

The scientific productions from the fifth edition took the Brazilian Pharmacopoeia 5 into a high technical-scientific degree with acknowledgement of international congeners.

All of this work was accomplished mainly due to the structure constructed by the Permanent Commission for the Review of the Brazilian Pharmacopoeia, responsible for the fourth edition, which has the merit of having established the necessary dynamics to prepare such a significant document and, mainly, having consolidated the mentality on the actual significance of this compendium for a constantly evolving society.

Thus, CFB and its Committees, in consonance with Anvisa, may bring to Brazilian society a new code totally revitalized, presented in two volumes, divided into General Methods and Monographs. One hundred and seventy-six general methods and five hundred and ninety-nine monographs were included, of which two hundred and seventy-seven are on pharmaceutical inputs, two hundred and ten on specialty inputs, fifty-seven on medicinal plants, six on correlates, thirty on biological products and nineteen from hemocorresponents and hemoderivatives.

In the General Provisions (4) chapter, an update of the definitions and the inclusion of several other definitions were performed, by meeting the specifications of each Committee.

In Technical Procedures applied to medicines **(5.1)**, the full review of the uniformity method of unit doses **(5.1.6)** is the highlight, harmonized now with new methods published by the main international pharmacopoeia. Other highlight is the inclusion of the drip test **(5.1.8)**, having great significance for the pharmaceutical equivalence study of the liquid pharmaceutical forms of oral usage.

In Physical and physical-chemical methods **(5.2)**, the full review of the Atomic spectrometry methods **(5.2.13**) was performed, as well as the chromatography methods **(5.2.17)** which were rewritten and are found to be more complete. A text on the capillary electrophoresis methods **(5.2.22.1)** was included, as well as several other methods which underwent full review of text modification.

For Chemical methods **(5.3)**, the full review of the limit testing was performed, focusing on the elimination of the use of hydrogen sulfide and the inclusion of the atomic spectrometry into the Limit testing for heavy metals **(5.3.2.3)**. The Iodinemetric testing of antibiotics **(5.3.3.10)** was included, whose procedure was previously described in each monograph and now it is comprised as its own general method.

Pharmacopoeia methods **(5.4)** were reviewed and extended, with highlight to the Vegetable extract preparation and analysis methods **(5.4.3)**, with the addition of six new general methods.

The biological methods, biological and microbiological testing **(5.5)** are presented with the inclusion of a number of Biological methods **(5.5.1)**, such as dosing of human blood coagulation factors, totaling sixteen new methods. The work of reorganization, review and extension of the biological testing **(5.5.2)** and microbiological testing **(5.5.3)** was performed.

In Vessels for medicines and correlates **(6)**, as well as Sterile product preparation methods **(7)**, the full review of reorganization and extension of texts for medicines and correlates was performed.

New examples of testing were inserted and those appearing in the fourth edition of the Brazilian Pharmacopoeia were review in the Statistical procedures applicable to biological testing **(8)**.

Three new chapters were included: Pharmaceutical Equivalence and Bioequivalance (10); Water for pharmaceutical use (11) and Reference Chemical Substances (12). The inclusion of new chapters was an initiative of the said Committees and brings to light literature information in line with professional experiences of their respective members. Dyes chapter (13) was reviewed and extended.

The consolidation of the Reagents chapter (14) was a special work. In this chapter, all of the indicators, indicator solutions, reagents, reagent solutions, volumetric solutions and buffers, described in the monographs from the volume 2 of FB 5, were congregated. Therewith, the description of the reagent in the monograph itself was eliminated, providing then a more dynamic reading by means of using any specific chapter. Currently, one thousand and fifty-one titles are described, representing an addition higher than one hundred percent in relation to the previous edition.

All of the attachments were reviewed and the Attachment C was included, with regards to the solvents commonly deployed in chromatographic analyses.

We cannot be naïve thinking we made a flawless pharmacopoeia. Despite all of the texts have been submitted to public consultation and a discerning review; in case any improper information has been included which may lead to difficulties in final understanding, there will be a procedure to solve this doubt and arrange the quick replacement, as required, in the Coordination of the Brazilian Pharmacopoeia. New texts or corrections will be electronically available in the pharmacopoeia, a novelty of this edition.

We would not be delivering FB 5 if we did not have the extreme dedication of all of the members of CFB, CTT, COFAR, and all of the employees. Without their technical-scientific knowledge and without the firm guidance of the Officer Maria Cecília Brito Martins, our path would have been more tortuous.

In spite of insisting in the acknowledgements, we understand that these people took part in the whole process, by identifying themselves with the healthcare issues, imbued in a civic attitude, since they are volunteers at most.

We reiterate that the whole process which resulted into the publication of the FB 5 will be lost if an actual State policy is not implemented, ensuring us the continuity of the works of the Commission of the Brazilian Pharmacopoeia and CTT, responsible for further products: Homeopathic Pharmacopoeia, National Form, Common Brazilian Denominations, Reference Chemical Substances and Phytotherapic Form.

Gerson Antônio Pianetti

CFB President

PREFACE OF THE PHARMACOPOEIA OF THE UNITED STATES OF BRAZIL, 1ST EDITION

Up to the Independence Day in Brazil – September 7th, 1822 – an official pharmaceutical code was in force, the "General Pharmacopoeia for the Kingdom and lands of Portugal", by Dr. Francisco Tavares, professor of the Universidade de coimbra and published in 1794 by order of the queen Lady Mary I.

From this day on, in spite of our political emancipation, not only the same pharmacopoeia continued on being adopted, also French "Codex medicamentarius", after 1837.

The Regulations of the Board of Public Hygiene, caused to be executed by means of the Decree no. 828, September 29th, 1851, without explicitly determining which pharmacopoeia should be followed, a list of books which pharmacies would have to own was established, and it is the following: "French Codex, Pharmacopoeia Conspect, by Joudan; Medical matter, Bouchardat's form; General Pharmacopoeia, Foy's Pharmacopoeia; Pharmaceutical Code and Pharmacography by Agostino Albano da Silveira Pinto (latest edition) ".

The first legal mention establishing in a mandatory manner French Codex as official Brazilian Pharmacopoeia is the one appearing in the article 58 of the Regulation which appeared with Decree no. 8.387, January 19th, 1882, whose contents is the following: "for the preparation of the officinae medicines, it will follow the French pharmacopoeia, until it is composed of a Brazilian pharmacopoeia, to which the Government will appoint a Commission composed of competent people. After publishing by authorization of the Government the Brazilian pharmacopoeia, the pharmacists will have the medicines made pursuant to the formulas of this pharmacopoeia, which will not inhibit them to be in accordance with the formulas of other ones, in order to meet the prescriptions of the physicians, who may prescribe as they desire.

Written, however, for a country so different from ours in any aspect, as France, "Codex medicamentarius gallicus" could not meet our needs, which all agreed to enact, without our directors, deaf and indifferent at all times to the claims of the pharmaceutical class, taking any initiative to provide Brazil with its own pharmaceutical code.

In view of such negligence of the public power, the pharmaceutical and medical associations sought for carrying forward the organization of our pharmacopoeia more than once, having, however, failed in all the attempts by lack of official support and due to obstacles from any nature.

Brazil, however, which was able to be leveled with further civilized nations regarding every area of the sciences, arts, etc., could not continue on being governed, as far as Pharmacy is concerned, by a foreign code, which, even though it is optimal for their country, did not absolutely meet our needs. Thereby, although we acknowledge the boldness of this initiative, we resolved to bear the arduous task and the high responsibility of writing our future pharmaceutical code, believing that our strong love towards the occupation would win all of the hindrances, surpass all of the obstacles. After two more patient works of polishing, we were able to present our project of Brazilian Pharmacopoeia to the Hon. Mr. Dr. Carlos Chagas,

the general director of the Brazilian Department of Public Health, requesting from His Excellency to appoint a commission to judge it, which was constituted as follows: Professors Drs. Antonio Pacheco Leão, Renato de Souza Lopes and Artidonio Pamplona and the Pharmacists Alfredo da Silva Moreira, Malhado Filho and Isaac Werneck da Silva Santos.

After a discerning examination of this work, this commission resolved to accept it, requesting from the Government its officialization as National Pharmaceutical Code, suppressing, however, the following articles, by day considered for restrict use to be officialized: Pineapple – Basic Copper Acetate – Sodium acetyrlasanilate – Chrysophanic acid – Dipropylbarbituric acid – Dilute iohydric acid – Agaric of oak – Artificial Carlsbad water – Imperial water – Vulneary Alcoholature – Liquid aloe – Rice amylase – Apocynum – Bitter, brayera, promegranate tree, semen-contra, stomachic, purgative and sudorific apozems – Strontium bromate – Lithium bromate – Cassia fistula – Strontium carbonate – Blessed thistle – Oak – Cassava flour cataplasm – Potato starch cataplasm – Vienna caustic – Spermaceti cerate – Nutmeg cerate – Black cherry – Red cherry – Chloralformamide – Gold and sodium chloride – Mercury amide chloride – Effervescent citrate of caffeine – Iron and quinine citrate – Amylose cluster – Camomila Cluster – Laxative cluster – Cantharidal collodion – Iodineform collodion – Canned cassia fistula – Cotton – Compound carbona, compound copaiba and senna electuaries – Adjuvant elixir – Anise elixir – Antipyrine elixir – Bucco elixir – Gentian elixir – Ferric phosphate elixir – Sucupira elixir – Dentifrice elixir – Camphorated soap plaster – Salicylate soap plaster – Oxycroccum plaster – Tupertine essence emulsion – Compound spirit of juniper – Pepper essence – Staphysagria – Diquinine ethylcarbonate – Quinine ethylsalicylate – Bistort extract – Blessed thistle extract – Common erythraea – Cicuta extract – Bugbane extract – Brazilian nightshade extract – Angelica glowing shield extract –Aromatic stem fluid extract – Chinese cinnamon fluid extract – Blessed thistle fluid extract – oak fluid extract – Cotton fluid extract – Staphysagria fluid extract – Mercuric fluid extract – Mercuric mellitus – Mercuric – Hexamethylenetetramine methylenecitrate – White mustard – Basic nithrate of bismuth – Paratoluolsulfurdichloride – Tolu balsam tablets – sodium bicarbonate tablets, sodium borate tablets, coal tablets, potassium chlorate tablets, cocaine hydrochloride tablets, codein tablets, sulfur tablets, peppermint tablets, ipecacuanha tablets, opium ipecacuanha tablets, kermes tablets, opium kermes tablets, phenolphthalein tablets, santonin tablets, compound santonin and tannin tablets – Sinapized pediluvium – Effervescent sodium phospate – Powders of apocynum, Brazilian nightshade and quebracho – Purified cassia fistula pulp – Mercury chlorine-amide pomade – Tannin pomade – Kayapo purge – Quebracho – Artificial Carlsbad Salt – Compound

sodium phosphate and basic ferric sulphate solute – Pineapple juice – Cherry juice – Cotton dye – Zinc valerate – Aromatic wine – Cocoa wine – Ipecacuanha wine – Syrup from pineapple, iohydric acid, cherry, cipó azougue, Brazilian nightshade, espelina, ginger, manacá, majoram, muirapuama and squaw mint.

PREFACE OF THE PHARMACOPOEIA OF THE UNITED STATES OF BRAZIL, 2ND EDITION

Thirty years had been elapsed since the first edition of the BRAZILIAN Pharmacopoeia had been in force, edited that it had been made in 1929. In this board time elapse, Brazilian Pharmaceutical Code had become outdated, in virtue of the huge progress medical and pharmaceutical sciences had reached worldwide.

It must be stood out, as well, that since 1950, the work had been totally used up, creating, thus, severe difficulties to the new Pharmacies and Industrial Pharmaceutical Laboratories, which cannot operate without this Official Code. Consequently, constant warnings pointing out the need for a new edition of the Pharmacopoeia was submitted to the public power from all the locations of the Country, which had increased during the nine yeards of its total using.

Those were the strong reasons so that the preparation of the second edition was activated. However, difficulties of any aspect were appearing, precluding it to be led to the purpose more quickly of the desired work, in spite of the effort and will of our health authorities.

The full review and updating of the entire contents of the first edition had been imposed and this task had undoubtedly been one of the most difficult and delicate tasks, maximum in a such large country, such as Brazil, when a collaboration or contribution at a national level, as the case might had been, is fundamental.

Soon after the publication of the Pharmacopoeia and its use in the pharmaceutical laboratories, critiques and notes started to appear, which were being collected and coordinated by BRAZILIAN ASSOCIATION OF PHARMACISTS**,** headquartered in the Capital of the Republic, since there was no Official Commission for its study and review.

In THE HISTORY of the BRAZILIAN Pharmacopoeia, inserted in this edition, there is detailed news on the activities developed for full review and updating of this second edition of the Brazilian Pharmaceutical Code. It must be added that a paritary Commission, constituted of members from the Capital of the Republic and São Paulo, was liable for the final review of the work to be printed, with powers to solve doubts and verified faults, as well as to provide the necessary uniformization to the pharmacopoeia language adopted by the Official Review Commission.

In the preparation of this edition, the REVIEW COMMISSION of the Pharmacopoeia followed at first the same guidance adopted by the NORTH AMERICAN Pharmacopoeia and, partly, the INTERNATIONAL Pharmacopoeia, concerning the distribution of the matter and study of the monographs; as far as the latter Pharmacopoeia is concerned, it took into consideration that Brazil was one of the first countries which had adopted that International-like Code.

The monographs appearing in the first edition and which will continue on the second edition underwent full review process, in a way to be updated, as for the processes of testing, dosing and other requirements, in order to correspond well to the modern technique requirements.

The nomenclature of the medicines was maintained in Portuguese, in alphabetic order, such as in the previous edition, as well as the synonyms, and the current official nomenclature in Latin was corrected.

For the patented or registered products, the names were adopted by which they are known, being highlighted with a classic asterisk (*).

In the 1st Edition of the Brazilian Pharmacopoeia, although Brazil had not signed Bussels Protocol in 1906 and 1929, relating to the Unification of the Heroic Medicine Formula, the presceiptions contained therein had been accepted at most, as per it may be vierfied in the comparative tables included in the Pharmacopoeia.

By the new Protocol, of May 20th, 1952, the previous ones were derogated, and the prescriptions corresponding to the International Pharmacopoeua of the World Health Organization had been adopted in replacement. The Internacional Pharmacopoeia had been successfully adopted in Brazil, as it had been well translated its delegation to the 2nd Panamerican Congress of Pharmacy and Biochemistry, the 2nd edition of the Brazilian Pharmacopoeia had met the said prescriptions as much as possible,

The Review Commission, after a discerning study, has deliberated that a large number of various official drugs and galenic preparations, currently being few used, was suppressed from the 2nd edition, being included, at most, in the National Form, to be published soon, as the Pharmacopoeia complement.

The Commision, in view of the nullity of the therapeutical action of many drugs and medicines, as well as the current complete non-use of many others, deliberated, after long inquiries with all of the members of the Official Commission and State Sub-Commissions, the exclusion of monographs, whose relation goes further.

Taking into consideration the great progress reached in the latest three decades in the field of Medicine and Pharmacy, several monographs on valuable medicines were included in this edition, which currently dominate the morden therapeutics, such as: antibiotics, sulpha drugs, hormones, vitamins, barbiturates, etc., as per the relation which is discussed thereafter.

At last, there is the transcription of the full list of all the personalities who, by using so much effort and devotion, provided their valuable collaboration, so that the new edition becomes a brightful reality. At this point, it would be unfair not to highlight particularly the PHARMACEUTICAL

STANDARDIZATION COMMISSION OF SÃO PAULO, which, patriotically. provided a precious and constant contribution, deploying their maximum effort so that the new edition reached its finish point, following the most up-to-date Pharmacopoeias in the World.

The Review Commission of the Pharmacopoeia will be rewarded by their great effort, if the new edition may correspond to, as expected, its high pratical purpose, which is the perfect selection of the raw materials of medicinal use and its standardization, a major condition of the activity and efficiency of the medicines, providing thus the public healthcare in Brazil with relevant services.

Rio de Janeiro, February 22nd, 1959

Luis Salgado Lima Filho
President of the Review Commission
of the Pharmacopoeia

PRESENTATION OF THE BRAZILIAN PHARMACOPOEIA, 3RD EDITION

In the context of the several events which has been signaling the execution of the National Plans of Development, as milestones in the global growth of the country, Health sector could not absent and, among its basic accomplishments, the launching of the Brazilian Pharmacopoeia, in reviewed and updated edition, for current times.

Hence, the making process of some non-frutified arrangements, as of 1962, which just bodied and had culmination in the current management of the Minister Paulo Almeida Machado, upon new initiative, materialized through the National Ministry of Health and its specific agency, the National Service of Medicine and Pharmacy Inspection.

The Minister assigned a new Review Commission of the Pharmacopoeia, as per Ordinance 276/75, and this collegiate fulfilled its mission, satisfactorily on time, by having the valuable collaboration of the Federal Council of Pharmacy.

Upon approving this 3rd edition of the Pharmacopoeia, with the issuance of the Decree 78.840/76, by referendum of the Minister of Health, Hon. Mr. President of the Republic contemplated the Ministry of Health and the medical and pharmaceutical class of the country, also with this important technical and normative pharmaceutical instrument of broad range, in sequence of other operationalization measures which has been implemented in this highlighted Sector of Brazilian life.

Upon submitting the originals of this 3rd edition to approval of the President Ernesto Geisel, the Minister not only obtained it, but he could, and it is a result of his effort, celebrate the fiftieth anniversary of the 1st edition, launched exactly on November 25th, 1927, day and month coinciding with this publication.

The review preformed on the previous edition was laborious and discerning, in a way that the interested media could count on the most reliable and safe instrument of standards and consultations, which will be evidenced when examining the big modifications added to the current text.

It was highly considered that the internacional experience obtained stronger convictions that the pharmaceutical substances used and the means of their identification and control were becoming more and more generalized. Povided that it its legit to strengthen the grounds of the regionalized therapeutic values, it stands out by evidencing the uniformization of the controls. Apart from the therapeutic resources arisen out of the flora – with lesser representativity – to respond for local distinctions, chemotherapics are growing, in volume and quantity, favoring the uniformization of the methods of identification and control. European and Internacional Pharmacopoeias are result thereof, the latter obtaining the status of parameter to be respected and accepted.

The routine of the Commission was not different. To the possible extent, the standards from the World Health Organization prevailed. Thus, the Latin nomenclature preceding the national one, checmical name and molecular formula, and even the general analysis methods.

The laboratory resources within the national reality were not lost. For this reason, and as musch as possible and required, various, simple and sophisticated methods were adopted for the same examination. On the other hand, by having the accurate identification of the therapeutical agents appearing in the phytopharmaceuticals as an uncontrollable need, only those to which we already have efficient methods of identification and dosing were included in the Pharmacopoeia. Subsequent editions published as Supplements, and the National Form itself – which will be certainly edited – will fill in existing gaps.

The broad listing of new therapeutical agents necessarily obliges to file its effective need at a national level.

The pharmaceutical industry was invited to manifest, offering subsidies by means of the representative entities, a contribution which deserved a judicious selection of the Commission.

It must also be added that, regarding this list and subsidies, it also became legit to make a survey of the most representative medicinies in the Brazilian prescriptions and consumption.

This list plus the reviewed monographs remaining of the 2nd edition constitute in full the archive of monographs of the 3rd edition of the Brazilian Pharmacopoeia.

There will certainly remain other monographs to be added, such as some existing or persisting ones, perhaps they may be susceptible to suspension. This evidence just favors the Pharmacopoeia itself, dynamic as the therapeutic one, and outstanding with more frequent updates, as the Pharmacopoeia itself may sound to be.

Taking into account that the 2nd edition of the Brazilian Pharmacopoeia is used up and many monographs appearing in it, not reviewed, still represent a bibliopgrahic source

of merit and under legal force, the Commission decided that the 1st Supplement of the 3rd edition will represent as a whole and exclusively the contents of that archive to be published immediately in sequence to this new edition.

Critiques, corrections and repairs, as expected, will be comprehensively accepted. It is appealed, since now, that they are made in a clear and objective manner, for higher facility of the editions which will succeed. All of them, when constructive, will represent the valuable subsidy for the enhancement of the Brazilian Pharmacopoeia, to the extent that this work intends to be, in the natural comparison with the previous edition.

PREFACE OF THE BRAZILIAN PHARMACOPOEIA, 4TH EDITION

In compliance with the provisions of the Federal Decree no. 78.840.11/25/1976, the new edition of the Brazilian Pharmacopoeia meets the desires of the Brazilian technical-scientific community, expressly interested in the review of the previous edition.

The Permanent Review Commission of the Brazilian Pharmacopoeia, constituted by the Ordinance no. 151/82 of the Hon. Minister of Health, could only perform his work thanks to the decisive support of the National Secretariat of Health Surveillance – SNVS – of the Ministry of Health. Arrangements and agreements entered into between SNVC, Central of Medicines – CEME – of the Ministry of Social Security and Social Assistance – MPAS – and the Brazilian Council of Scientific and Technological Development – CNPq-, ensured the Commission with indispensable financial resources, including scholarships for the performance of the works.

The preparation of the monographs was trusted to effectively experienced professionals of the area; these monographs were reviewed by other professionals of the same area of activity. In spite, eventual imperfections, mistakes or omissions are exclusively the responsibility of the Permanent Review Commission of the Brazilian Pharmacopoeia, whom was responsible for the final text approval.

The 4th Edition of the Brazilian Pharmacopoeia sets the beginning of a new wera. It is an edition in which the new presentation system is adopted. The quick advancement of the technology and the growing complexity of the medicinal substances determine the need for frequent reviews of the Pharmacopoeia. In order to facilitate these reviews and enable the introduction of new required monographs and methods of analysis, the Commission adopted this new manner of presentation.

This volume constitutes the Part I of the Pharmacopoeia and comprises the general provisions, general methods of analysis. Part II will be constituted of monographs on raw materials and pharmaceutical specifications, published in fascicles. A table of contents will indicate the title of the monographs, their reference numbers and the date that they will be in force.

Brazilian Pharmacopoeia in its 4th edition is effective in whole Brazilian Territory. The nomenclature, the methods of identification and analysis and all further data contained therein prevail over any other highlighted data in several pharmaceutical codes. In the omitted cases, International Pharmacopoeia, European Pharmacopoeia and other pharmaceutical codes may be used in the latest editions. *

The monographs of the 4th edition of the Brazilian Pharmacopoeia establish parameters that the product shall meet at any time throughout its period of use and not to be interpreted only as specifications for manufacturer's release.

The non-inclusion of a pharmaceutical or adjuvant in the 4th edition of the Brazilian Pharmacopoeia does not release these substances of the analysis, pursuant to other official codes; such as the presence of impurity not specifically described in the Pharmacopoeia does not mean the substance may be used by the simple fact that the Pharmacopoeia does not specify it. In these acases, the decision must be made based on the good technical sense and in the good manufacturing practices.

The Pharmacopoeia is not a work for duly qualified and trained professionaks. For this reason, it does not provide didactic explanations, presenting the monographs with clear, succinct writing, devoid of details judged as unnecessary by the Commission.

The Permanent Review Commission of the Brazilian Pharmacopoeia makes its acknowledgments public to all of those who collaborated in the preparation of this edition, particularly, the Federal Council of Pharmacy for its support, which enabled the official publication of the Brazilian Pharmacopoeia IV.

* National Extra-phmarcopoeia Standards shall obtain previously approval of the Permanent Review Commission of the Brazilian Pharmacopoeia of the National Health Council.

2 HISTORY

BRIEF HISTORY UPDATE OF THE BRAZILIAN PHARMACOPOEIA, 5TH EDITION

The almost centenarian Brazilian Pharmacopoeia outlines a highly significant cycle for the country. As of its first edition, result of the laborious work of a single pharmacist, it went through eight decades seeking for its space, under factual and legal grounds, as fundamental instrument of supporting to the national health policies emanated from governments with serious projects for Brazilian citizen protection.

In case the provisions of the decrees and resolutions indicating the review at every fifty years had been respected, its seventeenth edition would be being published, which unfortunately is not occurring, certainly due to problems occurred, but with no demerit to its past, since our purpose is to look ahead at all times.

In the beginning of the 20th Century, the apothecary's shops are the main locations of health practice and the country experiences the convivence with their young republic. Rodolpho Albino Dias da Silvia totally involved himself in a Herculean effort to transfer into a book an entire life of researching on vegetable and animal drugs, description of chemicals and officinal preparations. Thus, it is born the first edition of the Brazilian Pharmacopoeia, officialized by the federal government by means of the decree No. 17.509, November 4th, 1926, however, mandatory since August, 15th, 1929.

A big war devastated the planet in the 40's and then a great world change takes place in all of the developed and developing countries, and our first edition does not meet its role any longer. The apothecary's shops are gradually replaced with drugstores which do not perform the art of magistral manipulation any longer, and the start-up of the end of a huge service rended by the pharmacy professional to the population is decreed. The country is invaded by multinational industries which, little by little, were able to eliminate all of the small Brazilian companies of the area.

In parallel, the access of modern medicines which require the differentiated quality control is started due to the large scale production and to the quantity of synthetized pharmaceuticals, as well as those originated from several sources. The Pharmacopoeia did not escape from the modernist movement impressed by President Juscelino Kubitschek who, in 1959, signed the Decree No. 45.502 approving the second edition of the Brazilian Pharmacopoeia.

Yet, in other reality, that edition is presented oriented to the pharmaceutical inputs and specialties seeking for national quality standards of the health assets to be made available to the society. The officinal formulations were, then, submitted for a future publication which intended to publish it as National Form, which just took place in the 80's.

The third edition of the Brazilian Pharmacopoeia was published seventeen years later by means of the Decree No. 78.840, November 25th, 1976 and reinforces the previous edition by extending and modernizing its contents.

In the same way as the previous editions, the fourth edition of the Brazilian Pharmacopoeia was prepared from the initiative of selfless healthcare professionals. The works were started in 1982 with the creation of the Review Commision of the Brazilian Pharmacopoeia (CPRFB) appointed by the Director of the National Secretariat of Health Surveillance, Dr. Antônio Carlos Zanini.

Only in 1988, the launching of the Part I of the fourth edition was possible, containing general methods, and started the prepatation of the Part II, containing the monographs of pharmaceuticals and specialties. The delivery of the first fascicle was started in 1996. The dedication, persistence and unstoppable work of Dr. Celso F. Bittencourt, the President of CPRFB at that time, contributed to the creation and maintenance of the required infrastructure for the development of the fascicles of the fourth edition until its conclusion, by reinforcing the bases for the progression of the work up to this moment. The participation of the academic area, by means of public universities, was and continue on being intense and indispensable.

With the creation of Anvisa, in 1999, the permanent review of the Brazilian Pharmacopoeia starts to be the agency's administrative, technical and scientific responsibility. The sound support of the Collegiate Board of Officers, since then, particularly by its first CEO, Dr. Gonzalo Vecina Neto, led the Permanent Review Commission of the Brazilian Pharmacopoeia to reach the maturity of its works.

Work methodologies were created based on the most modern and updated world references in consonance with the publication of pharmaceutical codes performed by congeners of very respectful international pharmacopoeias in pharmaceutical area worldwide.

By means of contracts and agreements, it was possible to finance laboratory studies and, thus, it was able to launch the fascicles 2 (2000); 3 (2002); 4 (2003); 5 (2004) and 6 (2005) the latter was already under the management of the CEO Dr. Dirceu Raposo de Mello, contemplating thus, the fourth edition of the Brazilian Pharmacopoeia.

On this interim, fascicle 1 of the Brazilian Homeopathic Pharmacopoeia 2nd edition and the National Form were further published. 67 batches of reference chemical substances of the Brazilian Pharmacopoeia were certified, and other 58 batches were monitored.

The fact that a new edition of the Brazilian Pharmacopoeia does not revoke previous editions has always been a hindrance for the regulatory actions of health Surveillance. It was, therefore, decided to work on the fifth edition in order to carry out an exhausting survey of all the texts published in the fourth editions, assess needs for permanence, text replacement and procedures with or without laboratory assessment and exclusion of obsolete monographs.

Thus, the fifth edition revokes all of the other editions and intends to serve as central nucleus of future editions in a continuous process of review seeking at all times for the insertion into a new international reality, placing it in highlight among the best pharmacopoeias. It will also serve to guide the proposal of a joint pharmacopoeia with countries of the South-American continent. Currently, the Commission of the Brazilian Pharmacopoeia has a place as observer of the European and Internacional Pharmacopoeias and mutural acknowledgement with the Argentinian Pharmacopoeia.

The Commission of the Brazilian Pharmacopoeia and all of its committees have strong allies nowadays within the Brazilian Health Surveillance Agency with special highlight for Dr. Maria Cecília Martins Brito, stoppable fighter so that all of the actions proposed by the collegiate of the Commission and Committees come true. We have not been lacking favorable positioning, either in conducting the approval processes of the projects inherent to our activities or in the several logistic needs for facilitating the works of the Commission and Committees comprised of high-class professionals and who would hold the title by means of volunteering.

Having a pharmacopoeia is a matter of national security, technical and scientific development, insertion into a worldwide acknowledgment level and it is not on the simple area of Government policy any longer, but on the State policy. This fact eases CFB in knowing that it executes a national interest project and with agenda to be fulfilled within the health policy practiced by the regulatory agency and by the Ministry of Health.

We cannot be naïve in not assuming that some faults in this fifth edition will be quickly identified, however, it is under creation phase at the Coordination of the Brazilian Pharmacopoeia, structure which aims at quickly addressing the questions of the users, supplying quick and objective responses which may clarify doubts on the published texts. In the end of 2011, the first supplement providing modifications, corrections and inclusions is intended to be launched.

It is required to inform that all the texts published in the fifth edition underwent public consultation for access of the citizen and free manifestation, therefore, it is a work whose construction was collective with the participation of the stakeholders. All of the external manifestations were considered.

The history of our pharmacopoeia has been accounted for, with perfection, by our precedessors and contains very important data for the understanding of its entire evolution. We chose to reproduce them, except for the history not contemplated in the 1st edition, with no review to safeguard the authenticity and provide the reader with the impression of being participating in this history as well.

As President of the Commission of the Brazilian Pharmacopoeia, at last, we would like to use this space to express our sincere acknowledgments to those who helped to construct this work and be ascertain that we will continue on working as partners to finally be able to maintain the BRAZILIAN PHARMACOPOEIA modern and updated.

Gerson Antônio Pianetti

CFB President

HISTORY OF THE BRAZILIAN PHARMACOPOEIA, 2ND EDITION

The first legal mention establishing in a mandatory manner the Franch Codex as official Pharmacopoeia in Brazil is the one which appears in Decree no. 828, September 29th, 1851, in its article 45, whose contents is the following:

was in force, the "General Pharmacopoeia for the Kingdom and lands of Portugal", by Dr. Francisco Tavares, professor of the Universidade de coimbra and published in 1794 by order of the queen Lady Mary I.

From this day on, in spite of our political emancipation, not only the same pharmacopoeia continued on being adopted, also French "Codex Medicamentarius", after 1837.

"For the composition of the officinal medicines, the French Pharmacopoeia will be followed, until the Brazilian Pharmacopoeia is organized, so that the Government will appoint a Commission comprised of competent people. After publishing the Brazilian Pharmacopoeia, which will take place by authorization of the Government, the Pharmacists shall have the medicines prepared in accordance with the formulations of this Pharmacopoeia, which does not inhibit that they may have them in accordance with other Pharmacopoeias to meet the prescriptions of the physicians, who may prescribe, as desired".

In the attachment to the Regulation containing the "Table of medicines, vessels, instruments, utensils and books, organized for apothecary's shops of the Empire", it came the list of books which the apothecary's shops should have: "French Codex, Pharmacopoeia Conspect, by Joudan; Medical matter, Bouchardat's form; General Pharmacopoeia, Foy's Pharmacopoeia; Pharmaceutical Code and Pharmacography by Agostino Albano da Silveira Pinto (latest edition) ".

The article 58 of the Decree no. 8387, January 19th, 1882, reproduced the determinations of the article 45 of the Decree no. 828, of 1851; there was just a modification of some words and the list of books, of which the latest edition, the pharmacist should only have one specimen. In addition to the French Codex, the form by Dorvault, Bouchardat. Fosagrives, Jeannel, Réveil, Gallois, Chernoviz, Langaard, Pratical Pharmacy by Deschamps (d'Avallon), Méhu

Yearbook, Practical Guide by Le Page and Patrouillaud, Pharmacy Treaty by Soubeiran, Dictionary of alterations and forfeits by Chevalier and Baudrimont, Vademecum by Ferrand were also required.

During the long period from 1851 to 1929, the French Codex was mandatory "for the composition of the officinal preparations, until the Brazilian Pharmaceutical Code is organized". Thus, all of the health regulations are determined, among them, the Decrees no. 169 of 1890; no. 1.172, of 1892; no. 1.647, of 1894; no. 2.449, of 1897, whose table of books was reduced to the French Cidez and Dorvault, Bouchardat, Chernoviz and Langaard's Form; no. 2458 of 1897.5.156 of 1904.14.189 and 14.354, of 1920 (D. N. S. P.); 15.003, of 1921 and 16.300, of 12-31-1923.

However, the desire of the Brazilian pharmacists to have their own National Code was manifested in many opportunities by scientific agencies of the class. Several commissions were appointed for its preparation, with no result.

The efforts made by Ezequiel Corrêa dos Santos, Silva Costa, Corrêa Dutra, Oliveira Fausto, Almeida Rego, Eugênio Marques de Hollanda, Eduardo Julio Janvrot and other was in vain.

Only in 1887, in compliance with the requisition of the national scientific centers, the Imperial Government tried to resolve the problem by instituting a commission which was composed, among others, of Ezequiel Corrêa dos Santos Filho, Agostinho José de Souza Lima and Marques de Hollanda.

From this commission, however, there was no practical result, after ten years, in 1897, the Minister of Inland and Justice, Amaro Cavalcanti, appointed other commission with the same purpose and which was composed of the professors Agostinho de Souza Lima, César Diogo and Orlando Rangel. This new attempt failed as well.

"Brazil, however, which was able to be leveled with further civilized nations regarding every area of the sciences, arts, etc., could not continue on being governed, as far as Pharmacy is concerned, by a foreign code, which, even though it is optimal for their country, did not absolutely meet our needs". "Thereby, although we acknowledge the boldness of this initiative, we resolved to bear the arduous task and the high responsibility of writing our future pharmaceutical code, believing that our strong love towards the occupation would win all of the hindrances, surpass all of the obstacles. " (*) The Pharmacist, little known at that time, Rodolpho Albino Dias da Silva, in 1924, after ten more years of patient work, could present his project of the Brazilian Pharmacopoeia to the Hon. Mr. Dr. Carlos Chagas, the general director of the Brazilian Department of Public Health, appointed a commission constituted by Professors Drs. Antonio Pacheco Leão, Renato de Souza Lopes and Artidonio Pamplona and the Pharmacists Alfredo da Silva Moreira, Malhado Filho and Isaac Werneck da Silva Santos.

After a discerning examination of the work, this Commission resolved to accept it, requesting from the Government its officialization as National Pharmaceutical Code, however, with the suppression of certain articles deemed as resitricted use to be officialized, which are listed in the preface of the first edition.

On November 4th, 1926, by Decree no. 17.509, signed by the President of the Republic, Dr. Arthur da Silva Bernardes and by the Minister of Inland and Justice, Dr. Affonso Penna Junior, under article 252 of the Decree no. 16.300, of December 31st, 1923, the Brazilian Pharmacopoeia prepared by the Pharmacist Rodolpho Albino Dias da Silva was approved and adopted as Brazilian Pharmaceutical Code, with the amendments of the review commission. The Code would be in force 60 days after the publication of the first official edition, being its execution the liability of the National Department of Public Health, by means of the Inspection Agency of Practice of Medicine.

At last, Brazil had its Pharmacopoeia, a one man's work, which, under the judgment of eminent pharmacologists in the world, was onde of the most advanced and updated pharmaceutical codes at that time.

Rodolpho Albino, born in the State of Rio de Janeiro, in the city of Cantagalo, passed away prematurely in Rio de Janeiro, at the age of 42, on October 7th, 1931.

All of the pharmaceutical code are reviewd on a periodical basis; and, thus, in order to codify, coordinate and study suggestions, in order to provide facilities for a future review, in 1932, by means of proposal made in one of the sessions of the Brazilian Association of Pharmacists, a commission was appointed for that purpose, the responsibility of being the president was given to Prof. João Vicente de Souza Martins, who prepared internal regulations, creating several sections. This commission worked up to 1938, when the present of the Brazilian Association of Pharmacists, Prof. Virgílio Lucas, directed to the Minister of Education and Health the requisition for the appointment of an official commission to proceed with the review of our Code, since there is enough matter to be studied and deliberated.

This Commission, appointed by the Ordinance no. 1.21-A, June 23rd, 1938, by the Minister Gustavo Capanema, was constituted by the following seven members: Professors Renato Guimarães de Souza Lopes, Oswaldo de Almeida Costa, Virgílio Lucas and Abel Elias de Oliveira; Pharmacists Antônio Caetano de Azevedo Coutinho and Oswaldo de Lazzarini Peckolt and the physician Sebastião Duarte de Barros. By the ordinance no. 141, April 22nd, 1939, the commission was added with two more members, Prof. Artidônio Pamplona and Pharmacist José Eduardo Alves Filho.

This Commission, in spite of the difficulties found, performed something useful, proposing exclusions of the obsolete drugs and inclusions of other ones which represent a higher interest, as per the report presented by the Pharmacist Oswaldo Peckolt to the Third Brazilian Congress of Pharmacy, held in Belo Horizonte, on April 14th to 21st, 1939.

The Decree no. 810, July 1st, 1942, which approved the Regulations of the Nacional Service of Medicine Inspection, printing a new character to this agency, considered them as linked therein, under the presidency of the respective officer, the Biopharmacy and Review Commissions of the Pharmacopoeias; it started, then, to be constituted of a professor of the National

Pharmacy School or other institution compared to it, a general practicioner, a biologist assigned at Instituto Oswaldo Cruz, a chemist, a pharmaceutical industry technician and a pharmacist assigned at S. N. F. M. F.

As a result, the General Director of the National Department of Health, by the Ordinance no. 136, July 11th of the same year, assigned for these functions the Prof. Oswaldo de Almeida Costa, the physician Dr. Sebastião Duarte de Barros, the biologist Dr. Gilberto Guimarães Vilela; the chemical Pharmacist Oswaldo de Lazzarini Peckolt, the technician Prof. Virgilio Lucas and the assistant Pharmacist Antônio Caetano de Azevedo Coutinho, working at the presidency the Service Officer, Dr. Roberval Cordeiro de Farias, and as Work Coordinator, Dr. Sebastião de Barros; thereafter, Dr. Gilberto Vilela was substituted, upon requisition, with the biologist Dr. Tito Arcoverde de Albuquerque Cavalcanti, and the Pharmacist Caetano Coutinho, who retired from Public Service, was substituted with its section colleague, Pharmacist Flávio Frota, who started to hold the title of secretary, in accordance with the regulation provisions.

The new Commission, with the experience collected from the previous commissions and the experience from its own activity, published the First Supplement of the Pharmacopoeia, put into force by Ordinance no. 42, March 2nd, 1943.

They proceeded with the studies, well coordinated and with good yield, constituting a good evidence for the appearance of the approved Second and Third Supplements, respectively, by Ordinances no. 24 of April 14th, 1945 and no. 39 of June 13th, 1950.

These publications presented themselves as interesting pieces of work, under various aspects, among them the inclusion of national drugs as substitutes of similar imported drugs, the registration of new formulas and the substitution, in others, of foreign substances with national ones, with no compromising of the respective therapeutical actions.

The Internal Regulations proposed with the Decree no. 21.339, June 20th, 1946, and amended by Decree no. 29.828, July 30th, 1951, aiming at the organization and the competence of the various public health agencies, did not substantially altered the provisions which had been previously established, and the Commission continued on working on a regular basis.

Ordinance no. 147, November 6th, 1951, approving the Instructions suggested by the National Service of Medicine Inspection, in accordance with the said Regulations, and amending the guidance followed then, determined the appointment for te Review Commission of the Pharmacopoeia and its subcommissions of scientist from the whole country, specialized in matters being studied and incumbent of reediting on a ten year basis the Pharmacopoeia; it also transformed the primitive agency into an Executive Commission, coordinator and main responsible for all of the works.

The former components of the Review Commission were thus confirmed in the capacity of members of the Executive Commission, thereafter the president, Dr. Roberrval Cordeiro de Farias, was replaced with Dr. Vasco Barcelos† and afterwards

with Dr. Benoni Laurindo Ribas, who have been succeeded in the Service Board; furthermore, in the occasional hindrances of the respective titulars, Dr. Luiz Salgado Lima Filho held the office of president, who afterwards was the actual president.

Then, the members of the technical subcommissions were chosen; priority was given to professionals from Rio and States, pharmacists, physicians, chemists, professors, increasing the number thereafter, in virtue of the ulterior assignments.

The subcommissions, counting 10, were organized as follows: Inclusions, Exclusions and Posology; Pharmacognosy; Organic Chemistry; Inorganic Chemistry; Galenic Pharmacy; Biological Testing, Hormones and Vitamins; Sera, Vaccines, Antibiotics and Sterilization; General Provisions, Testing, Reagents and Tables; General Planning; Writing; having as coordinators, respectively, Dr. Sebastião de Barros, in the first, seventh, ninth and tenth subcommissions; Prof. Oswaldo Costa, in the second and fourth subcommissions; Pharmacist Oswaldo Peckolt, in the third and eighth subcommissions; Prof. Virgílio Lucas, in the fifth subcommission and Dr. Tito Cavalcanti, in the sixth subcommission.

In this same opportunity, Regional Commissions were created in the States of Paraná, Minas Gerais, Rio Grande do Sul and São Paulo.

In this State, the works had a big boost because a Commission of Pharmaceutical Standardization was installed in the Capital for identical purposes, by mututal agreement between Instituto Aldolfo Lutz, Universidade de São Paulo, Professional Exercice Inspection and the state associations representative of the Pharmacy industry and commerce, integrating it to the more evident figures in the scientific areas of that State, having Dr. Ariosto Büller Souto and Pharmacist Júlio Sauerbronn de Toleto as president and secretary, respectively.

When the V Brazilian Congress of Pharmacy was held together in the city of São Paulo, jointly with the III Panamerican Pharmaceutical and Biochemical Congress, December 1st to 8th, 1954, São Paulo's contribution was materialized into a draft of the Pharmacopoeia, presented to that process, and new directions were given to the works.

The Congress, ratifying the motion approved in the III Brazilian Congress of Pharmacy, held in Belo Horizonte, April 14th to 21st, 1939, and the vote expressed in the II Panamerican Pharmaceutical and Biochemical Congress, took into effect in Lima, December 1st to 8th, 1951, recommended the organization of a National Form, as a complement, in which usual drugs and medicines would appear, which were not appearing in the Pharmacopoeia yet.

The discussions resulted into the the deliberation of joint examination, made by the commissions from Rio de Janeiro and São Paulo, of the entire study material gathered so far, in order to enable the short-term finishing of the review.

Upon finishing the V Congress, the technical subcommission of Planning and Review was organized, constituted as follows: Antônio Caetano de Azeredo Coutinho, Flávio Frota, Militino Cesário Rosa, Oswaldo de Almeida Costa, Oswaldo de Lazzarini Peckolt, Tito Arcoverde de Albuquerque Cavalcanti, Virgílio Lucas and Sebastião Duarte de Barros, from Rio; Ariosto Büller Souto, Cendy de Castro Guimarães, Germínio Nazário, Henrique Tastaldi, Hércules Vieira de Campos, Quintino Mingoja, Richard Wasicky and Júlio Sauerbronn de Toledo, from São Paulo, Dr. Sebastião Duarte de Barros was the coordinator and Dr. Benoni Ribas was still the president. Months later, Drs. Oswaldo de Almeida Costa and Tito Arcoverde de Albuquerque Cavalcanti assigned their offices to Professors Jayme Pecegueiro Gomes da Cruz and Raymundo Moniz de Aragão, temporarily substituted with the Admiral Vicente de Paulo Castilho.

With the return of Dr. Moniz de Aragão, it coincided the inclusion of four more elements in the paritary agency: Prof. Carlos Henrique R. Liberalli and the Pharmacist Vicente Ferreira Greco†, from São Paulo, and Prof. Abel Elias de Oliveira and the Pharmacist Admiral Vicente de Paulo Castilho, from Rio.

At that time, Dr. Sebastilio de Barros, who continued on part of the group from Rio, left his office of coordinator, being succeeded by the Pharmacist Oswaldo de Lazzarini Peckolt and, at last, by the Pharmacist Flávio Frota.

Out of the works of this subcommission, performed in Rio and São Paulo, the 2nd edition of the Pharmacopoeia, in its originals, resulted to be possible to present, on September 1st, 1955, to the Minister of Health, Dr. Aramis Athayde, being on this date signed by the President of the Republic, Dr. João Café Filho, the Decree no. 37.843, September 1st, 1955, who officialized it.

Through Dr. Luiz Salgado Lima Filho, Minister of Health Mano Pinotti, it was presented to the President of the Republic, Dr. Juscelino Kubitschek, a decree with new inclusions and modifications and which made the Pharmacopoeia mandatory at drugstores, laboratories, pharmaceutical industries and congener enterprises. This decree took the no. 45.502, February 27th, 1959.

In this document, there is included the codification of the current pharmaceuticals and formulations, the normalization of the techniques deployed in the several pharmaceutical practices, the standardization of the methods, testing, reagents and tables, required for the professional exercise.

From the first edition, most of the contents was used, in virtue of its wise writing; several of its monographs will appear in the *National Form*, whose making process is under conclusion, expecting to be soon published, as the second volume of the Brazilian Pharmaceutical Code.

(*) Rodolpho Albino Dias da Silva – Pharmacopoeia of the United States of Brazil – Preface, page VIII, 1st edition, 1929.

† Deceased.

HISTORY OF THE BRAZILIAN PHARMACOPOEIA, 3RD EDITION

The significance of the pharmacopoeias – considered then the official codes, or those which are officially acknowledged, where the identification and quality standards of the substances deployed in pharmacology are established – grows in the proportion of the cultural development of the Pharmacy and Medicine.

Consigned in its first existence in the 3rd century of our Age, it was since the middle of the last century that the pharmacopoeias started to gain clear characteristics of national need, embodying the effort for adjusting the resources of identification and control of therapeutical substances to the regional nature of the pharmaceuticals themselves, and, at most, come from the flora, usually native and local,animal organs and minerals admitted as proper for therapeutical purposes.

Submissive to Portugal as far as sciences and technique are concerned, our Country was subject, when it was a Colony, to the General Pharmacopoeia for the Kingdom and Lands of Portugal, amended in 1794.

With the Independence of Brazil, in 1822, there were openings for other cultural influences and Brazil easily followed to French guidance, prevailing at the time for Western world. With such regards, in 1851, by Decree, the obligatoriness of the French Pharmacopoeia as the official code for Brazil was established.

From 1851 to 1929, the whole Brazilian health legislation sustained the same obligatoriness “for the elaboration of officinal preparations, until the Brazilian Pharmaceutical Code is organized”.

August 15th, 1929 was the milestone of this redemption, since the Pharmacopoeia of the United States of Brazil took effect as of that date, in the whole Brazilian territory, a conquer very ceebrated, even because it also praised the person in charge for this work, the extraordinary pharmacist Rodolpho Albino Dias da Silva, who “spent twelve years in a silent and Benedictine labor, in the composition of the

pages full of knowledge which would have to be build in breviary for those in his congregation, so harmonious that it was required to be included as one of the best among the contemporary, although, due to the competence of unique artifice, a hard-to-repeat feat".

As for the history of the pharmaceutical codes, the 2nd edition of the Brazilian Pharmacopoeia constitutes an irrefutable repository, until the time of that edition, a plausible reason for us not to raise up previously known details.

It is suitable for the Phamacopoeias, not matter how well they are prepared, a periodical review, natural characteristics arising out of the evolution of the Pharmacopoeia.

Hence, because the Federal Decree no. 45.502, February 27th, 1959, when approving the Second Edition of the Brazilian Pharmacopoeia, fixed its review on a ten year basis, regardless of the intermediate editions of Supplements.

With regards to that, on June 13th, 1962, as per Ordinance no. 82 of the National Department of Health; the first commission was constituted for the review works, appointing Drs. Fernando Luz Filho, Lauro Sollero, Maria Alzira Ferreira Nobrega, Laerte Manhães de Andrade, Anésio Faria e Souza, Mário Victor de Assis Pacheco, Nilson dos Reis Rodrigues and Elza Magalhães Pêcego, as Secretary. The works of this commission was linked to the preliminary provisions, considering that on 4.16.68, by the Ordinance no. 28 of the National Department of Health, a new commission was constituted, appearing in it Drs. Lúcio Costa, Maria Alriza Ferreira Nobrega, Lauro Sollero, Gobert de Araújo Costa, Emílio Diniz da Silva, João Haikal Helou and Aníbal da Rocha Nogueira Júnior, and Josepha Paul as Secretary, with the development of work similar to the previous commission.

The Ministry Ordinance no. 112, March 20th, 1972, created a work group, composed of Drs. Evaldo de Oliveira, Moacir Nogueira, Caio Romero Cavalcante and Lt. Col. Pharm. Ex. Júlio Fernandes Silva, and this group fixed some work bases, discontinued in face of random and contingent reasons.

Finally, on June 25th, 1975, by force of the Ministry Ordinance no. 266, a new Review Commission of the Pharmacopoeia was constituted, taking part of it Drs. Fernando Ayres da Cunha, National Service Officer of Medicine and Phamacy Inspection, and President of the Commission, Ítalo Suassuna, Maria Alzira Ferreira Nobrega, Evaldo de Oliveira, José Aleixo Prates a Silva, Lauro Sollero, Paulo Dias da Costa and, as Secretary, Dora Alves Gonçalves Cruz.

Willing to conclude its mission in short-term since the beginning of the works, the Commission gathered for the first time on August 5th, 1975, decided to promote weekly meetings at the head office of the National Service of Medicine and Pharmacy Inspection, Rio de Janeiro.

At first, it abstained of constituting subcomissions, choosing to request for special collaborators for the matters in which the Commission judges itself unable or insufficiently safe to decide.

Based on this guidance, the steps were suspended on a gradual basis and earning celerity as soon as the problems became clearer.

In case the material and technique are not able to solve all of the problems, the Commission used the experience from other commissions and Technical Agencies, and from Pharmacopoeias, notably in which the guidance of the World Health Organization is respected. It also accepted, on a timely basis, the support of the Federal Council of Pharmacy which, for a more integrated collaboration, set a whole technical provision of permanent service, facilitating thus the various work steps. Since the reassessment of the primitive listing of monographs, in order to update them, to the exhaustive effort of reaching writing and technical unity of the collaborations arisen out of the reporters from all the corners of the Country, translations are outside.

It is very pertinent to point out the factual meaning that the professional collaboration to report monographs represented a national movement, running adjesions from all of the quadrants in Brazil.

Based on the joint effort – Ministry of Health (by the National Service of Medicine and Pharmacy Inspection), Review Commission of the Pharmacopoeia (by the team work present in all of the work stages), Federal Council of Pharmacy (which favoed the material and human infrastructure to impress celerity to the work) and reporters – it was possible to win the initial challenge of obtaining the approval of the originals in the event of the fiftieth anniversary of the 1st edition of the Brazilian Pharmacopoeia.

The fixation of this date, because it is a fair tribute, started to configure a term which is impossible to be extended, providing the whole work, as a result, with a favorable and dynamic climate, requiring objectivity.

Out of the 770 monographs appearing in the 2nd edition, at last, 280 subsisted, upon the review of their text, because they valued the repairs published by Prof. Dr. João Haikal Helou. 205 new monographs were incorporated, naturally those which represent new therapeutical agents, by meeting the laws fixed by the Commission and referred to in the Preface of this edition.

It is admitted that, perhaps, other monographs were applicable; in general, it is equally admitted that some of them were not applicable any longer. However, it is required to have it, since the Commission adopted their own criteria, subject to the nosologic reality and national therapeutics. These criteria and not the monographs may or may not lead to pertinences. Naturally, the Commission is the sole and exclusive responsible for them, without sharing the merits or demerits of their guidance with others.

The fact that, pursuant to the terms of the act which approved the 3rtd edition, previous monographs not expressly cancelled in this Pharmacopoeia subsist with validity for all legal effects is considered as a maximum relevance.

It is finally admitted that the universal trend of embodying the pharmacopoeia and form in a single text is not ignored.

Tempted to this since the beginning, the Commission decided, however, to choose for a divided work, willing to prepare, afterwards, the National Form. Still, this second provision will no longer represent otherwise the development of a work aiming at unifying the preparation of the Form in the subsequent step, and that intends to effectively fullfil it.

HISTORY OF THE BRAZILIAN PHARMACOPOEIA, 4TH EDITION

"The books of this type are ephemeral in nature, assigned to spread one of the sides of the pharmacology, sciencie which will currently go through the most accelerate stage of its evolution". SOUZA MARTINS, in Introduction Report of the 3rd edition of the Portuguese Phmaracopoeia, 1876.

The term Pharmacopoeia arises out of the agglutination of two Greek terms, namely, φαρμακον = medicine or poison, and ποιοζ = manufacturer and manufacturing. The Pharmacopoeias constitute official or officially adopted pharmaceutical codes, in which the identification, quality standards and analysis methods of the pharmaceuticals in use are established. Existing since the 3rd century, the first compendia were regional in nature, because those pharmaceuticals are from animal organs, minerals, and, above all, from local and native flora. Some of them were officialized, even though in regional character, such as, for example, Escola de Salermo's form – *Regimen Sanitatis*, of 1066, adopted in 1240 by Ferdinand II, King of the Two Sicilies. The attempts invidually made by several authors in order to unify the description and identification of the most import pharmaceuticals are dated on late 17th Century and 18th Century. Among other works, the following ones deserve a citation: *Pharmacopeia Internationalis* by Lémery (1690), The Pharmacopoeias by James (1747), De Quincy (1758), Triller (1764) and, particularly, *Pharmacopeia Universalis*, by Jourdan (1828), which complied data of almost 50 different Pharmacopoeias and compendia. None of these works, however, had any official character.

National pharmacopoeias, those with official character and mandatory adoption, started to appear in late 18th century and early 19th century. Thus, the first editions of the Portuguese (1794), Dutch (1805), French (1818) and American (1820) were published.

Colonial Brazil adopted the *General Phamracopoeia for the Kingdom and Lands of Portugal*, of 1794, whose authorship is attributed to Francisco Tavares, professor of Universidade de Coimbra.

With the Independence, Brazil is guided to the French culture and, in the field of Pharmacy, French *Codex Medicamentarius* acquires legal force. The Regulation of the Public Hygiene Board, caused to be executed by Decree no. 828 of 09/29/1851, without specifying which Pharmacopoeia is to be fulfilled, establishes the list of books that drugstores must have, appearing in it, among others, the Portuguese Pharmacopoeia og 1794, *French Codex* and *Portuguese Pharmaceutical Code*, by Agostinho Albano da Silveira Pinto, whose first edition was published in 1835 and considered today as the 2nd edition of the *Portuguese Pharmacopoeia*.

The Decree no. 8.837.01/19/1882, establishes textually: "for the preparation of official medicines, the French Pharmacopoeia will be followed until the Brazilian Pharmacopoeia is composed…", and this situation will endure until 1926, when the Decree no. 17.509.11/04/1926 approved the first Brazilian Pharmacopoeia, by Rodolpho Albino Dias da Silva, made mandatory as of August 15th, 1929.

The first edition of the Brazilian Pharmacopoeia leveled with the Pharmacopoeias of that time, from more developed countries, revaling to be notable in virtue of the monographs and, above all, by the great number of inclusions of the pharmaceuticals obtained in the Brazilian flora, not existing in any other Pharmacopoeia.

The constant evolution of the pharmacology, the introduction of new pharmaceuticals into the therapeutics, the appearance of new analysis methods, more modern and accurate and the need for updated specifications for the raw material and pharmaceutical product control are fundamental factors determining the obsolence of the pharmaceutical codes and the need for reviewing and updating them on a periodical basis. The Decree which approved the first edition of the Brazilian Pharmacopoeia was omissive as for the reviews; thus, the second edition came up after 30 years after the first one and represented five years of work of ten specialized subcomissions. The 2nd edition incorporated the acquisitions arising out of the pharmacology updating itself. It could not, however, be richer and more accurate than the first edition, a result of a single author. The Federal Decree no. 45.502 of 02/27/1959, when approving the 2nd edition of the Brazilian Pharmacopoeia, fixed its review on a ten year basis. Unfortunately, several hindrances did not allow the accomplishment of this Decree. More than 15 years elapsed until a new edition was suggested.

On November 25th, 1976, the third edition of the Brazilian Pharmacopoeia was officialized by the Decree no. 78.840. The same Decree fixed the term of its review in five years. Performed in a determined and very short time, task possible to pursue only thanks to the support of the Federal Council of Pharmacy, the work awaken sensitive manifestations of the technical-scientific community to recommend the quick review of its text, regardless of the legal provision.

Thus, the 4th edition arises delayed. It sought, in this edition, to solve the deficiencies of the previous one. It also sought to adopt modern analysis methods, compatible, however, with the national reality. The publication of this part and the adoption of a new presentation systematic which enables its continuous updating through the permanent reviews is the prioritary goals that the Permanent Review Commission of the Brazilian Pharmacopoeia proposes to achieve.

3 BRAZILIAN PHARMACOPOEIA

PRESIDENTS OF THE PREVIOUS EDITIONS OF THE BRAZILIAN PHARMACOPOEIA

RODOLPHO ALBINO DIAS DA SILVA	1st edition
LUIZ SALGADO LIMA FILHO	2ndedition
FERNANDO AYRES CUNHA	3rd edition
JOÃO GILVAN ROCHA	4th edition – Part I
CELSO FIGUEIREDO BITTENCOURT	4th edition – Part II

COMMISSION OF THE BRAZILIAN PHARMACOPOEIA – CFB

PRESIDENT
GERSON ANTÔNIO PIANETTI

VICE-PRESIDENT
MIRACY MUNIZ DE ALBUQUERQUE

MEMBERS

ADRIANO ANTUNES DE SOUZA ARAÚJO
Universidade Federal de Sergipe – UFS

ANTONIO CARLOS DA COSTA BEZERRA
Brazilian Health Surveillance Agency – Anvisa

CLÉVIA FERREIRA DUARTE GARROTE
Universidade Federal de Goiás – UFG

EDUARDO CHAVES LEAL
National Institute of Health Quality Control – INCQS / FIOCRUZ

ELFRIDES EVA SCHERMAN SCHAPOVAL
Universidade Federal do Rio Grande do Sul – UFRGS

ÉRICO MARLON DE MORAES FLORES
Universidade Federal de Santa Maria – UFSM

GERSON ANTÔNIO PIANETTI
Universidade Federal de Minas Gerais – UFMG

JOÃO CARLOS PALAZZO DE MELLO
Federal Council of Pharmacy – CFF

JOSÉ CARLOS TAVARES
Universidade Federal do Amapá – UNIFAP

KÁTIA REGINA TORRES
Ministry of Health – MS

LAURO DOMINGOS MORETTO
Syndicate of the Pharmaceutical Product Industry in the State of São Paulo – Sindusfarma

LEANDRO MACHADO ROCHA
Universidade Federal Fluminense – UFF

3

LUIZ ALBERTO LIRA SOARES
Universidade Federal do Rio Grande do Norte – UFRN

MIRACY MUNIZ DE ALBUQUERQUE
Universidade Federal de Pernambuco – UFPE

ONÉSIMO ÁZARA PEREIRA
Brazilian Association of the Pharmacochemistry and Pharmaceutical Input Industry – ABIQUIFI

SILVANA TERESA LACERDA JALES
Association of Official Pharmaceutical Laboratories in Brazil – ALFOB

VLADI OLGA CONSIGLIERI
Universidade de São Paulo – USP

COORDINATION OF THE BRAZILIAN PHARMACOPOEIA

BRAZILIAN HEALTH SURVEILLANCE AGENCY – Anvisa

ANTONIO CARLOS DA COSTA BEZERRA – Coordinator

Experts in Regulation and Health surveillance
ANDREA REZENDE DE OLIVEIRA JAIMARA AZEVEDO OLIVEIRA
MARIA LÚCIA SILVEIRA MALTA DE ALENCAR
SILVÂNIA VAZ DE MELO MATTOS

THEMATIC TECHNICAL COMMITTEES OF THE COMMISSION OF THE BRAZILIAN PHARMACOPOEIA – CTT

SUPPORT TO THE NATIONAL POLICY OF MEDICINAL PLANTS AND PHYTOTHERAPICS

JOSÉ CARLOS TAVARES CARVALHO – Coordenador
Universidade Federal do Amapá – UNIFAP

ANA CECÍLIA BEZERRA CARVALHO
Brazilian Health Surveillance Agency – Anvisa

ANA CLAUDIA FERNANDES AMARAL
Institute of Technology of Pharmaceuticals – Farmanguinhos / FIOCRUZ

ANA MARIA SOARES PEREIRA
Universidade de Ribeirão Preto – UNAERP

BERTA MARIA HEINZMANN
Universidade Federal de Santa Maria – UFSM

ELFRIEDE MARIANNE BACCHI
Universidade de São Paulo – USP

EMIDIO VASCONCELOS LEITÃO DA CUNHA
Universidade Estadual de Campina Grande – UECG

LUIZ ANTÔNIO BATISTA DA COSTA
Excellence Center of Integral Health of Paraná – CESIP

NILTON LUZ NETTO JÚNIOR
Universidade Católica de Brasília – UCB

ROSANE MARIA SILVA ALVES
Ministry of Health – MS

WAGNER LUIZ RAMOS BARBOSA
Universidade Federal do Pará – UFPA

MEDICINE CORRELATES

TEREZINHA DE JESUS ANDREOLI PINTO
Coordinator
Universidade de São Paulo – USP

ADRIANA BUGNO
Instituto Adolfo Lutz – IAL

ALBA VALÉRIA DOS SANTOS
Baxter Hospitalar Ltda

DHALIA GUTEMBERG
Brazilian Chamber of Laboratory Diagnosis – CBDL

IRENE SATIKO KIKUCHI
Universidade de São Paulo – USP

MICHELE FEITOSA SILVA
National Institute of Health Quality
Control – INCQS / FIOCRUZ

RENATA ARACELLI PIRES
Baxter Hospitalar Ltda

WALFREDO DA SILVA CALMON
Brazilian Health Surveillance Agency – Anvisa

BRAZILIAN COMMON NAMES

AULUS CONRADO BASILE – Coordinator
Universidade de São Paulo – USP

CARLOS CÉZAR FLORES VIDOTTI Federal Council of Pharmacy – CFF

ELFRIEDE MARIANNE BACCHI Universidade de São Paulo – USP

ONÉSIMO ÁZARA PEREIRA
Brazilian Association of the Pharmacochemistry
and Pharmaceutical Input Industry – ABIQUIFI

PAULO CHANEL DEODATO DE FREITAS
Universidade de São Paulo – USP

RICARDO CHIAPPA
União Educacional do Planalto Central – UNIPLAC

ROSANA MIGUEL MESSIAS MASTELARO
Syndicate of the Pharmaceutical Product Industry
in the State of São Paulo – Sindusfarma

SILVÂNIA VAZ DE MELO MATTOS
Brazilian Health Surveillance Agency – Anvisa

PHARMACEUTICAL EQUIVALENCE AND MEDICINE BIOEQUIVALENCE

SÍLVIA STORPIRTIS – Coordinator
Universidade de São Paulo – USP

CHANG CHIANN
Universidade de São Paulo – USP

GERSON ANTÔNIO PIANETTI
Universidade Federal Minas Gerais – UFMG

JACQUELINE DE SOUZA
Universidade Federal de Ouro Preto – UFOP

LEONARDO DE SOUZA TEIXEIRA
Instituto de Ciências Farmacêuticas
de Estudos e Pesquisas– ICF

RAQUEL LIMA E SILVA
Brazilian Health Surveillance Agency – Anvisa

RODRIGO CRISTOFOLETTI
Brazilian Health Surveillance Agency – Anvisa

SOLANGE MARIA COUTINHO BRANDÃO
National Institute of Health Quality
Control – INCQS / FIOCRUZ

TERESA CRISTINA TAVARES DALLA COSTA
Universidade Federal do Rio Grande do Sul – UFRGS

PHARMACEUTICAL SPECIALTIES

ELFRIDES EVA SCHERMAN SCHAPOVAL
Coordinator
Universidade Federal do Rio Grande do Sul – UFRGS

ANIL KUMAR SINGH
Universidade de São Paulo – USP

HÉRIDA REGINA NUNES SALGADO
Universidade Estadual Paulista Júlio
de Mesquita Filho – UNESP

JAIR CALIXTO
Syndicate of the Pharmaceutical Product Industry
in the State of São Paulo – Sindusfarma

LUCIANE VARINI LAPORTA
Centro Universitário Franciscano – UNIFRA

MÔNICA DA LUZ CARVALHO SOARES
Brazilian Health Surveillance Agency – Anvisa

NADIA MARIA VOLPATO
Universidade Federal do Rio Grande do Sul – UFRGS

RUTH RIESINGER STRATTMANN
Universidade Federal de Pernambuco – UFPE

EXCIPIENTS AND ADJUVANTS

PEDRO JOSÉ ROLIM NETO – Coordinator
Universidade Federal de Pernambuco – UFPE

ÁDLEY ANTONINI NEVES DE LIMA
Universidade Federal do Amazonas – UFAM

FABIANA CREMASCHI PALMA
Brazilian Association of Distributors and Importers of Pharmaceutical Inputs, Cosmetics, Veterinaries, Food and Additives – ABRIFAR

FABRICIO CARNEIRO DE OLIVEIRA
Brazilian Health Surveillance Agency – Anvisa

GABRIELA GONÇALVES DA SILVA
Brazilian Association of Distributors and Importers of Pharmaceutical Inputs, Cosmetics, Veterinaries, Food and Additives – ABRIFAR

GEISIANE MARIA ALVES PRESMICH
Industrial Pharmaceutical Laboratory of Alagoas – LIFAL

JOSÉ LAMARTINE SOARES SOBRINHO
Universidade Federal do Piauí – UFPI

ROSALI MARIA FERREIRA DA SILVA
Universidade Federal do Pará – UFPA

PHARMACOGNOSY

AMÉLIA TERESINHA HENRIQUES – Coordinator
Universidade Federal do Rio Grande do Sul – UFRGS

CID AIMBIRÉ DE MORAES SANTOS
Universidade Federal do Paraná – UFPR

EVELIN ELFRIEDE BALBINO
Brazilian Health Surveillance Agency – Anvisa

JOSÉ ANGELO SILVEIRA ZUANAZZI (*Ad hoc*)
Universidade Federal do Rio Grande do Sul – UFRGS

LILIAN AULER MENTZ
Universidade Federal do Rio Grande do Sul – UFRGS

LUIZ ALBERTO LIRA SOARES
Universidade Federal do Rio Grande do Norte – UFRN

MARIA DAS GRAÇAS LINS BRANDÃO
Universidade Federal de Minas Gerais – UFMG

RODNEY ALEXANDRE FERREIRA RODRIGUES
Universidade Estadual de Campinas – UNICAMP

TATIANE PEREIRA DE SOUZA
Universidade Federal do Amazonas – UFAM

MEDICINAL GASES

ALAÍDE ALINE XAVIER LEAL – Coordinator
Institute of Technology in Immunobiologicals – Bio Manguinhos FIOCRUZ

CRISTIANE RODRIGUES AUGUSTO
National Institute of Metrology, Standardization and Industrial Quality – INMETRO

DESIRÉE MICHELS CORTEZ
Linde Gases Ltda.

HEITOR CONRADO
Air Liquide do Brasil Ltda

JOÃO PAULO SILVÉRIO PERFEITO
Brazilian Health Surveillance Agency – Anvisa

KOICHI MIZUTA
Institute of Technological Researches of the State of São Paulo – IPTSP

SÁLVIO FILGUEIRAS
Linde Gases Ltda.

HEMOCOMPONENTS AND HEMODERIVATES

JÚLIO CÉSAR CARESTIATO – Coordinator
Universidade Federal Fluminense – UFF

DENISE FERREIRA LEITE
Brazilian Health Surveillance Agency – Anvisa

HELDER TEIXEIRA MELO
Ministry of Health – MS

JANAÍNA DUQUE DE SOUZA
Bio Manguinhos – FIOCRUZ

MARISA COELHO ADATI
National Institute of Health Quality Control – INCQS / FIOCRUZ

MELÂNIA DE FÁTIMA CORDELINO
Baxter Hospitalar Ltda

NEEMIAS SILVA DE ANDRADE
Brazilian Health Surveillance Agency – Anvisa

SEVERINO BARBOSA
Universidade Federal do Pernambuco – UFPE

HOMEOPATHY

LEANDRO MACHADO ROCHA – Coordinator
Universidade Federal Fluminense – UFF

BIANCA RODRIGUES DE OLIVEIRA (*Ad hoc*)
Universidade do Vale do Itajaí – UNIVALI

CARLA HOLANDINO QUARESMA
Universidade Federal do Rio de Janeiro – UFRJ

EZEQUIEL PAULO VIRIATO
Laboratório Homeopático Almeida Prado Ltda

FRANCISCO JOSÉ DE FREITAS
Universidade Federal do Rio de Janeiro – UFRJ

MARCELO CAMILO MORERA
Brazilian Health Surveillance Agency – Anvisa

MARIA DIANA CERQUEIRA SALES
Faculdade Brasileira – UNIVIX

RICARDO CHIAPPA
União Educacional do Planalto Central – UNIPLAC

RINALDO FERREIRA
Universidade do Vale do Itajaí – UNIVALI

ACTIVE PHARMACEUTICAL INGREDIENTS

MIRACY MUNIZ DE ALBUQUERQUE – Coordinator
Universidade Federal de Pernambuco – UFPE

ADRIANO ANTUNES SOUZA DE ARAÚJO
Universidade Federal de Sergipe – UFS

ANDRÉ AUGUSTO GOMES FARACO
Universidade Federal de Minas Gerais – UFMG

DANIEL KARL RESENDE
Brazilian Health Surveillance Agency – Anvisa

LÚCIA DE FÁTIMA FRANCELINO DA SILVA
Central Laboratory of Public Health
of Pernambuco -LACEN

SAID GONÇALVES DA CRUZ FONSECA
Universidade Federal do Ceará – UFC

SEVERINO GRANJEIRO JÚNIOR
Pharmaceutical Laboratory of the State
of Pernambuco – LAFEPE

TÉRCIO PASCHKE OPPE
Universidade Federal do Rio Grande do Sul – UFRGS

MARKERS FOR PHYTOTHERAPICS

JOÃO CARLOS PALAZZO DE MELLO – Coordinator
Universidade Estadual de Maringá – UEM

ALBERTO JOSÉ CAVALHEIRO
Universidade Estadual Paulista Júlio
de Mesquita Filho – UNESP

CECÍLIA ELENA DE FIGUEIREDO OGNIBENE
Association of National Pharmaceutical
Laboratories –ALANAC

FERNÃO CASTRO BRAGA
Universidade Federal de Minas Gerais – UFMG

MARIA DO CARMO VASQUES GARCIA
National Institute of Health Quality
Control – INCQS / FIOCRUZ

VALQUIRIA LINCK BASSANI
Universidade Federal do Rio Grande do Sul – UFRGS

VALDIR FLORÊNCIO DA VEIGA JUNIOR
Universidade Federal Amazonas – UFAM

SILVIA PAREDES FONTES
Brazilian Health Surveillance Agency – Anvisa

MICROBIOLOGY

CLÉVIA FERREIRA DUARTE GARROTE-Coordinator
Universidade Federal de Goiás – UFG

ANA CRISTINA REGIS DE BARROS CORREIA
Universidade Federal de Pernambuco – UFPE

CLAUDIO KIYOSHI HIRAI
Biolab Sanus Farmacêutica Ltda

MARTIN STEPPE
Universidade Federal do Rio Grande do Sul – UFRGS

MIRIAM DE FÁTIMA VIANNA LEONEL Universidade
Federal de Minas Gerais – UFMG

ROSEMARIE APARECIDA DE ARAÚJO BONATTO
Merck Sharp & Dohme Farmacêutica Ltda

SILÉSIA DE SOUZA AMORIM
Brazilian Health Surveillance Agency – Anvisa

TEREZINHA DE JESUS ANDREOLI PINTO
Universidade de São Paulo – USP

STANDARDIZATION OF TEXTS AND VISUAL IDENTITY OF THE BRAZILIAN PHARMACOPOEIA

ANTÔNIO BASÍLIO PEREIRA – Coordinator
Universidade Federal de Minas Gerais – UFMG

FERNANDO HENRIQUE ANDRADE NOGUEIRA
Universidade Federal de Minas Gerais – UFMG

ISABELA DA COSTA CÉSAR
Instituto de Ciências Farmacêuticas
de Estudos e Pesquisas
– ICF

JOSÉ ANTÔNIO DE AQUINO RIBEIRO
Brazilian Company of Agriculture Research – EMBRAPA

LAÍS SANTANA DANTAS
Brazilian Health Surveillance Agency – Anvisa

PAULA CRISTINA REZENDE ENÉAS
Universidade Federal de Minas Gerais – UFMG

BIOLOGICAL PRODUCTS

EDUARDO CHAVES LEAL – Coordinator
National Institute of Health Quality
Control – INCQS / FIOCRUZ

DANIELA MARRECO CERQUEIRA
Brazilian Health Surveillance Agency – Anvisa

DARCY AKEMI HOKAMA
Institute of Technology in Immunobiologicals – Bio
Manguinhos FIOCRUZ

HISAKO GONDO HIGASHI
Universidade Estadual Paulista Júlio
de Mesquita Filho – UNESP

LILIA RIBEIRO SERÔDIO
Instituto Vital Brazil – IVB

MARA EL-CORAB MOREIRA DE OLIVEIRA
Ministry of Health – MS

MARCO ANTONIO STEPHANO
Universidade de São Paulo – USP

ORLANDO SILVA
Serono Produtos Farmacêuticos Ltda.

MAGISTRAL AND OFFICINAL PRODUCTS

VLADI OLGA CONSIGLIERI – Coordinator
Universidade de São Paulo – USP

ELISABETE PEREIRA DOS SANTOS
Universidade Federal do Rio de Janeiro – UFRJ

JOSÉ ANTONIO DE OLIVEIRA BATISTUZZO
Faculdades Oswaldo Cruz

LETÍCIA NORMA CARPENTIERI RODRIGUES
Universidade Federal de São Paulo – UNIFESP

GUILHERME DINIZ TAVARES
Universidade de São Paulo – USP

MÁRCIA MACIEL ANTUNES
Farmácia de Manipulação de Cosméticos Ltda – FACIAL

PATRICIA HAUSCHILDT DE OLIVEIRA MENDES
Brazilian Health Surveillance Agency – Anvisa

PAULA RENATA APARECIDA NIGRO RIVERA CARAZZATTO
National Association of Magistral
Pharmaceuticals – ANFARMAG

ROBERTO PONTAROLO
Universidade Federal do Paraná – UFPR

RADIOPHARMACEUTICALS

ELOY JULIUS GARCIA – Coordinator
Universidade Estadual do Rio Grande do Sul – UERGS

ANA MARIA SILVEIRA BRAGHIROLLI
Institute of Nuclear Engineering – IEN-CNEN

ELAINE BORTOLETI DE ARAÚJO
Institute of Energetic and Nuclear Researches – IPEN

LUIZ CLÁUDIO MARTINS ALEIXO Institute of Nuclear Engineering – IEN-CNEN

MARYANGELA REZENDE MASCARENHAS
Brazilian Health Surveillance Agency – Anvisa

MARYCEL ROSA FELISA FIGOLS DE BARBOSA
Institute of Energetic and Nuclear Researches – IPEN

NEUZA TAEKO OKASAKI FUKUMORI
Institute of Energetic and Nuclear Researches – IPEN

RALPH SANTOS OLIVEIRA
Institute of Nuclear Engineering – IEN-CNEN

SUBSTÂNCIA QUÍMICA DE REFERÊNCIA

PEDRO EDUARDO FRÖEHLICH – Coordenador
Universidade Federal do Rio Grande do Sul – UFRGS

ÉRICO MARLON DE MORAES FLORES
Universidade Federal de Santa Maria – UFSM

JAIMARA AZEVEDO OLIVEIRA
Brazilian Health Surveillance Agency – Anvisa

MARIA ALICE BÖCKELMANN
Cristália Produtos Químicos Farmacêuticos Ltda

MARIA DO CARMO VASQUES GARCIA
National Institute of Health Quality Control – INCQS / FIOCRUZ

RENATA BARBOSA DE OLIVEIRA
Universidade Federal de Minas Gerais – UFMG

VALÉRIA PEREIRA DE SOUSA
Universidade Federal do Rio de Janeiro – UFRJ

COLLABORATORS OF THE 5TH EDITION OF THE BRAZILIAN PHARMACOPOEIA

ADILSON SARTORATTO
Universidade Estadual de Campinas – UNICAMP

ÁDLEY ANTONINI NEVES DE LIMA
Universidade Federal do Amazonas – UFAM

ADRIANA BUGNO Instituto Adolfo Lutz – IAL

ADRIANA DA SILVA SANTOS DE OLIVEIRA
Universidade Estadual de Campinas – UNICAMP

ADRIANA PASSOS OLIVEIRA
Universidade Federal Fluminense – UFF

ADRIANO ANTUNES SOUZA DE ARAÚJO
Universidade Federal de Sergipe – UFS

ALAÍDE ALINE XAVIER LEAL
Institute of Technology in Immunobiologicals – Bio- Manguinhos / FIOCRUZ

ALBA VALÉRIA SANTOS
Baxter Hospitalar Ltda

ALBERTO JOSÉ CAVALHEIRO
Universidade Estadual Paulista Júlio de Mesquita Filho – UNESP

ALICE SIMON
Universidade Federal do Rio de Janeiro – UFRJ

ALINE LIMA HERMES MÜLLER
Universidade Federal de Santa Maria – UFSM

ALLAN WEBERLING MATOS
Universidade Federal de Minas Gerais – UFMG

AMADEU CARDOSO JUNIOR
Universidade Federal Fluminense – UFF

AMANDA THOMAS BARDEN
Universidade Federal do Rio Grande do Sul – UFRGS

AMARILIS SCREMIN PAULINO
Universidade Federal de Santa Catarina – UFSC

AMÉLIA TERESINHA HENRIQUES
Universidade Federal do Rio Grande do Sul – UFRGS

ANA CAROLINA ZAVAREZI
Universidade Federal Fluminense – UFF

ANA CECÍLIA BEZERRA CARVALHO
Brazilian Health Surveillance Agency – Anvisa

ANA CLAUDIA FERNANDES AMARAL
Institute of Technology of Pharmaceuticals – Farmanguinhos / FIOCRUZ

ANA CRISTINA REGIS DE BARROS CORREIA
Universidade Federal de Pernambuco – UFPE

ANA ELISA DE OLIVEIRA
Universidade do Vale do Itajaí – UNIVALI

ANA GABRIELA REIS SOLANO
Universidade Federal de São João Del Rei – UFSJ

ANA LAURA ESCARRONE
Universidade Federal de Santa Maria – UFSM

ANA LÚCIA ABOY
Universidade Federal do Rio Grande do Sul – UFRGS

ANA MARIA BERGOLD
Universidade Federal do Rio Grande do Sul – UFRGS

ANA MARIA SILVEIRA BRAGHIROLLI Institute of Nuclear Engineering – IEN-CNEN

ANA MARIA SOARES PEREIRA
Universidade de Ribeirão Preto – UNAERP

ANDRÉ AUGUSTO GOMES FARACO
Universidade Federal de Minas Gerais – UFMG

ANDRÉ LIMA DE OLIVEIRA COSTA
Universidade Federal de Minas Gerais – UFMG

ANDRÉ LUIS GEMAL
National Institute of Health Quality Control – INCQS / FIOCRUZ

ANDREA REZENDE DE OLIVEIRA
Brazilian Health Surveillance Agency – Anvisa

ANDREJUS KOROLKOVAS (*In memoriam*)
Permanent Review Commission of the
Brazilian Pharmacopoeia – 4th edition

ANDRESSA BLAINSKI
Universidade Estadual de Maringá – UEM

ANDRESSA DALMAS NARVAEZ
Universidade Federal do Rio Grande do Sul – UFRGS

ANGELA CRISTINA LEAL BADARÓ TRINDADE
Universidade Federal do Paraná – UFPR

ANGELICA GARCIA COUTO
Universidade do Vale do Itajaí – UNIVALI

ANGELO JOSÉ COLOMBO
Permanent Review Commission of the
Brazilian Pharmacopoeia – 4th edition

ANIL KUMAR SINGH
Universidade de São Paulo – USP

ANNA KAROLINA PASTOREK
Universidade Estadual de Campinas – UNICAMP

ANTÔNIA MARIA CAVALCANTI DE OLIVEIRA
Universidade Federal Fluminense – UFF

ANTÔNIO BASÍLIO PEREIRA
Universidade Federal de Minas Gerais – UFMG

ANTÔNIO CARLOS DA COSTA BEZERRA Brazilian Health Surveillance Agency – Anvisa

ARMANDO DA SILVA CUNHA JÚNIOR
Universidade Federal de Minas Gerais – UFMG

ARTHUR LUIZ CORRÊA
Universidade Federal Fluminense – UFF

ATANA MPALANTINOS DA SILVA
Universidade Federal Fluminense – UFF

AULUS CONRADO BASILE
Universidade de São Paulo – USP

ÁUREA SILVEIRA CRUZ Instituto Adolfo Lutz – IAL

BERTA MARIA HEINZMANN
Universidade Federal de Santa Maria – UFSM

BEATRIZ PINHEIRO BEZERRA
Universidade Federal do Ceará – UFC

BIANCA FERNANDES GLAUSER
Universidade Federal do Rio de Janeiro – UFRJ

BIANCA RODRIGUES DE OLIVEIRA
Universidade do Vale do Itajaí – UNIVALI

BRUNA TRINDADE DE CARVALHO
Universidade Federal de Minas Gerais – UFMG

BRUNO VALENTE
Universidade Federal de Santa Catarina – UFSC

CAIO PINHO FERNANDES
Universidade Federal Fluminense – UFF

CAMILA ADOLFO GONÇALVES
Centro Universitário Franciscano – UNIFRA

CARLA HOLANDINO QUARESMA
Universidade Federal do Rio de Janeiro – UFRJ

CARLOS CÉZAR FLORES VIDOTTI Federal Council of Pharmacy – CFF

CARLOS EDUARDO DE OLIVEIRA PEREIRA
Universidade Federal de Minas Gerais – UFMG

CAROLINA DOS SANTOS PASSOS
Universidade Federal do Rio Grande do Sul – UFRGS

CAROLINA LUPI DIAS
Universidade Federal do Rio Grande do Sul – UFRGS

CÁSSIA VIRGINIA GARCIA
Universidade Federal do Rio Grande do Sul – UFRGS

CECÍLIA ELENA DE FIGUEIREDO OGNIBENE
Association of National Pharmaceutical
Laboratories -ALANAC

CÉLIA DE FREITAS GUIMARÃES PRAÇA
Universidade Federal do Ceará – UFC

CELINA ROCHA FILGUEIRAS
Universidade Federal Fluminense – UFF

CELSO FIGUEIREDO BITTENCOURT
Permanent Review Commission of the
Brazilian Pharmacopoeia – 4th edition

CÉSAR ALEXANDRE JUNQUEIRA
Universidade Federal do Rio Grande do Sul – UFRGS

CHANG CHIANN
Universidade de São Paulo – USP

CHARISE DALLAZEM BERTOL
Universidade Federal de Santa Catarina – UFSC

CHRISTIANE SOUTO MORAES
Brazilian Health Surveillance Agency – Anvisa

CID AIMBIRÉ DE MORAES SANTOS
Universidade Federal do Paraná – UFPR

CLARICE MITIE SANO YUI
Medley S. A. Indústria Farmacêutica

CLARISSA MARQUES MOREIRA DOS SANTOS
Universidade Federal de Santa Maria – UFSM

CLARISSE MADALENA BUENO ROLIM
Universidade Federal de Santa Maria – UFSM

CLÁUDIA MARIA OLIVEIRA SIMÕES
Universidade Federal de Santa Catarina – UFSC

CLAUDIA SEIDL
Universidade Federal do Paraná – UFPR

CLAUDIO KIYOSHI HIRAI
Biolab Sanus Farmacêutica Ltda

CLÉSIO SOLDATELI PAIM
Universidade Federal do Rio Grande do Sul – UFRGS

CLÉVIA FERREIRA DUARTE GARROTE
Universidade Federal de Goiás – UFG

CRISTIANE DA SILVA
Universidade do Vale do Itajaí – UNIVALI

CRISTIANE DE BONA DA SILVA
Universidade Federal de Santa Maria – UFSM

CRISTIANE RODRIGUES AUGUSTO
National Institute of Metrology, Standardization and Industrial Quality – INMETRO

CRISTIANNE DA SILVA GONÇALVES
Brazilian Health Surveillance Agency – Anvisa

CRISTINA DUARTE VIANNA SOARES
Universidade Federal de Minas Gerais – UFMG

CYPRIANO CARDOSO FILHO
Permanent Review Commission of the Brazilian Pharmacopoeia – 4th edition

DANIEL KARL RESENDE
Brazilian Health Surveillance Agency – Anvisa

DANIELA MARRECO CERQUEIRA
Brazilian Health Surveillance Agency – Anvisa

DANIELE DE SOUZA TEIXEIRA
Universidade Federal de Minas Gerais – UFMG

DANILE RUBERT PEREIRA
Universidade Federal de Santa Maria – UFSM

DARCY AKEMI HOKAMA
Institute of Technology in Immunobiologicals – Bio Manguinhos FIOCRUZ

DEISE CRISTINA DA SILVA
Universidade do Vale do Itajaí – UNIVALI

DENILSON DA SILVA SANTOS
Brazilian Health Surveillance Agency – Anvisa

DENISE FERREIRA LEITE
Brazilian Health Surveillance Agency – Anvisa

DESIRÉE MICHELS CORTEZ Linde Gases Ltda.

DHALIA GUTEMBERG
Brazilian Chamber of Laboratory Diagnosis – CBDL

DIEGO LEONEL DA COSTA VIEIRA
Universidade Federal de Minas Gerais – UFMG

DIOGO POMPÉU DE MORAES
Universidade Federal de Santa Maria – UFSM

ECIO GEOVANI NETO
Universidade Federal de Minas Gerais – UFMG

ÉDER LISANDRO DE MORAES FLORES
Universidade Federal de Santa Maria – UFSM

EDSON IRINEU MÜLLER
Universidade Federal de Santa Maria – UFSM

EDUARDO AUGUSTO MOREIRA
Permanent Review Commission of the Brazilian Pharmacopoeia – 4th edition

EDUARDO CHAVES LEAL
National Institute of Health Quality Control – INCQS / FIOCRUZ

EDUARDO SCHMIDT DE SOUZA
Universidade Federal de Santa Maria – UFSM

ELAINE BORTOLETI DE ARAUJO
Institute of Energetic and Nuclear Researches – IPEN

ELDA AZEVEDO GUERRA
Universidade Federal de Pernambuco – UFPE

ELFRIDES EVA SCHERMAN SCHAPOVAL
Universidade Federal do Rio Grande do Sul – UFRGS

ELFRIEDE MARIANNE BACCHI
Universidade de São Paulo – USP

ELIANA NUNES
Universidade Federal do Rio Grande do Sul – UFRGS

ELIANE COUTINHO DE MEDEIROS
Universidade Federal do Rio de Janeiro – UFRJ

ELISABETE PEREIRA DOS SANTOS
Universidade Federal do Rio de Janeiro – UFRJ

ELIZABETH IGNE FERREIRA
Comissão Permanente de Revisão da Farmacopeia Brasileira – 4ª edição

ELIZABETH MARIA ROCHA LOBO
Universidade Federal Fluminense – UFF

ELIZABETH VALVERDE DOS SANTOS
Universidade Federal Fluminense – UFF

ELOY JULIUS GARCIA
Universidade Estadual do Rio Grande do Sul – UERGS

ELZA ANDERS SAAD
Comissão Permanente de Revisão da Farmacopeia Brasileira – 4ª edição

ELZÍRIA DE AGUIAR NUNAN
Universidade Federal de Minas Gerais – UFMG

EMANUELA ANSELMO VIEIRA
Brazilian Health Surveillance Agency – Anvisa

EMIDIO VASCONCELOS LEITÃO DA CUNHA
Universidade Estadual de Paraíba – UEPB

ÉRICO MARLON DE MORAES FLORES
Universidade Federal de Santa Maria – UFSM

EVELIN ELFRIEDE BALBINO
Brazilian Health Surveillance Agency – Anvisa

EZEQUIEL PAULO VIRIATO
Laboratório Homeopático Almeida Prado Ltda

FABIANA CREMASCHI PALMA
Brazilian Association of Distributors and Importers of Pharmaceutical Inputs, Cosmetics, Veterinaries, Food and Additives – ABRIFAR

FABIANA ERNESTINA BARCELLOS DA SILVA
Universidade Federal do Pampa – UNIPAMPA

FABIANE GOLDCHMIDT ANTES
Universidade Federal de Santa Maria – UFSM

FÁBIO ANDREI DUARTE
Universidade Federal do Rio Grande – FURG

FÁBIO SEIGI MURAKAMI
Universidade Federal de Santa Catarina – UFSC

FABRICIO CARNEIRO DE OLIVEIRA
Brazilian Health Surveillance Agency – Anvisa

FÁVERO REISDORFER PAULA
Universidade Federal do Pampa – UNIPAMPA

FELIPE DA ROSA NOBRE
Universidade Federal de Santa Maria – UFSM

FELIPE REBELLO LOURENÇO
Universidade de São Paulo – USP

FERNANDA HERMSDORFF DAS NEVES
Universidade Federal Fluminense – UFF

FERNANDO HENRIQUE ANDRADE NOGUEIRA
Universidade Federal de Minas Gerais – UFMG

FERNÃO CASTRO BRAGA
Universidade Federal de Minas Gerais – UFMG

FLÁBIO DA ROSA PONS JÚNIOR
Centro Universitário Franciscano – UNIFRA

FLÁVIA DIAS MARQUES MARINHO
Universidade Federal de Minas Gerais – UFMG

FLÁVIA NEVES ROCHA ALVES
Brazilian Health Surveillance Agency – Anvisa

FRANCINETE RAMOS CAMPOS
Universidade Federal do Paraná – UFPR

FRANCISCA MARIA BARROS SOUSA
Universidade Federal do Ceará – UFC

FRANCISCO JOSÉ DE FREITAS
Universidade Federal do Rio de Janeiro – UFRJ

GABRIELA GONÇALVES DA SILVA
Brazilian Association of Distributors and Importers of Pharmaceutical Inputs, Cosmetics, Veterinaries, Food and Additives – ABRIFAR

GEISIANE MARIA ALVES PRESMICH
Laboratório Industrial Farmacêutico de Alagoas – LIFAL

GERALDO FENERICH
Comissão Permanente de Revisão da Farmacopeia Brasileira – 4ª edição

GERSON ANTÔNIO PIANETTI
Universidade Federal Minas Gerais – UFMG

GILBERTO DOLEJAL ZANETTI
Universidade Federal de Santa Maria – UFSM

GILSON ANDRADE RAMALDES
Universidade Federal de Minas Gerais – UFMG

GIOVANA SECRETTI VENDRUSCOLO
Universidade Comunitária da Região
de Chapecó – UNOCHAPECÓ

GISELE DA SILVA BOTAS
Universidade Federal Fluminense – UFF

GISELY CRISTINY LOPES
Universidade Estadual de Maringá – UEM

GISLAINE CARMO ROESCH
Universidade Federal do Rio Grande do Sul – UFRGS

GISLAINE KUMINEK
Universidade Federal de Santa Catarina – UFSC

GIZELE SCOTTI DO CANTO
Universidade Federal de Santa Maria – UFSM

GLYN MARA FIGUEIRA
Universidade Estadual de Campinas – UNICAMP

GUILHERME DINIZ TAVARES
Universidade de São Paulo – USP

GUSTAVO RODRIGUES DE REZENDE
Universidade Federal de Minas Gerais – UFMG

HÉRIDA REGINA NUNES SALGADO
Universidade Estadual Paulista Júlio
de Mesquita Filho – UNESP

HIDELGARDO SEIBERT FRANÇA
Universidade Federal Fluminense – UFF

HISAKO GONDO HIGASHI
Universidade Estadual Paulista Júlio
de Mesquita Filho – UNESP

IARA COUTINHO DESMARAIS
Universidade Federal Fluminense – UFF

ILIO MONTANARI JÚNIOR
Universidade Estadual de Campinas – UNICAMP

INGRID BEZERRA
Universidade Federal do Ceará – UFC

IRENE SATIKO KIKUCHI
Universidade de São Paulo – USP

ISABEL CRISTINA FRAÇÃO DIEFENBACH
Universidade Federal de Santa Maria – UFSM

ISABELA DA COSTA CÉSAR
Instituto de Ciências Farmacêuticas
de Estudos e Pesquisas
- ICF

ISABELLA PIAZZA
Universidade Federal Fluminense – UFF

JACQUELINE DE SOUZA
Universidade Federal de Ouro Preto – UFOP

JAIMARA AZEVEDO OLIVEIRA
Brazilian Health Surveillance Agency – Anvisa

JAIR CALIXTO
Syndicate of the Pharmaceutical
Industry Products in the State of
São Paulo – Sindusfarma

JAIR CÁSSIO FARIA
Independent Collaborator

JANAÍNA DUQUE DE SOUZA
Bio Manguinhos – FIOCRUZ

JARBAS FARIAS LEAL
Universidade Federal Fluminense – UFF

JOÃO BATISTA DA SILVA JÚNIOR
Brazilian Health Surveillance Agency – Anvisa

JOÃO CARLOS PALAZZO DE MELLO
Universidade Estadual de Maringá – UEM

JOÃO CLEVERSON GASPARETTO
Universidade Federal do Paraná – UFPR

JOÃO DIMAS RIBEIRO
Brazilian Health Surveillance Agency – Anvisa

JOÃO FERREIRA MARTINS
National Institute of Health Quality
Control – INCQS / FIOCRUZ

JOÃO MÁXIMO DE SIQUEIRA
Universidade Federal do Mato Grosso do Sul – UFMS

JOÃO PAULO SILVÉRIO PERFEITO
Brazilian Health Surveillance Agency – Anvisa

JONATHAS FELIPE REVOREDO LOBO
Universidade Federal Fluminense – UFF

JOSÉ ALEIXO PRATES E SILVA
Permanent Review Commission of the
Brazilian Pharmacopoeia – 4th edition

JOSÉ ANGELO SILVEIRA ZUANAZZI
Universidade Federal do Rio Grande do Sul – UFRGS

JOSÉ ANTÔNIO DE AQUINO RIBEIRO
Brazilian Company of Agriculture Research – EMBRAPA

JOSÉ ANTONIO DE OLIVEIRA BATISTUZZO
Faculdades Oswaldo Cruz

JOSÉ CARLOS BARBÉRIO
Genese Produtos Diagnósticos Ltda.

JOSÉ CARLOS TAVARES CARVALHO
Universidade Federal do Amapá – UNIFAP

JOSÉ LAMARTINE SOARES SOBRINHO
Universidade Federal do Piauí – UFPI

JOSÉ LOURENÇO DE FREITAS NETO
Universidade Federal de Pernambuco – UFPE

JOSÉ MARIA BARBOSA FILHO
Universidade Federal da Paraíba – UFPB

JOSÉ MURADIAN FILHO
Pall do Brasil Ltda.

JOSEANE MARIA BEZERRA DE MENEZES
Health surveillance – RN

JOSIANE DE CARVALHO VITORINO
Universidade do Vale do Itajaí – UNIVALI

JULIA APARECIDA LOURENÇO DE SOUZA
Universidade Federal de Pernambuco – UFPE

JULIANA ASSUMPÇÃO DA SILVA
Universidade Federal do Rio de Janeiro – UFRJ

JULIANA SEVERO FAGUNDES PEREIRA
Universidade Federal de Santa Maria – UFSM

JULIANO DE MORAIS FERREIRA SILVA
Universidade de São Paulo – USP

JULIANO SMANIOTO BARIN
Universidade Federal de Santa Maria – UFSM

JÚLIO CÉSAR CARESTIATO
Universidade Federal Fluminense – UFF

KARINA ALVES DA SILVA
Universidade Estadual de Campinas – UNICAMP

KATIA ANDREA DOMINGOS DE MORAIS Brazilian Health Surveillance Agency – Anvisa

KATIA REGINA TORRES Ministry of Health – MS

KATIA SUZI DA SILVEIRA SILVA
Institute of Energetic and Nuclear Researches – IPEN

KOICHI MIZUTA
Institute of Technological Researches of the State of São Paulo – IPTSP

LAÍS SANTANA DANTAS
Brazilian Health Surveillance Agency – Anvisa

LARISSA SAKIS BERNARDIS
Universidade Federal de Santa Catarina – UFSC

LAURA JANE MOREIRA SANTIAGO
Universidade Estadual do Norte Fluminense – UENF

LAURA TERUMI UEDA HERNANDES MELERO
Institute of Energetic and Nuclear Researches – IPEN

LAUREN ROSA CROSSETTI VAUCHER
Universidade Federal de Santa Maria – UFSM

LAURO DOMINGOS MORETTO
Syndicate of the Pharmaceutical Product Industry in the State of São Paulo – Sindusfarma

LEANDRO MACHADO ROCHA
Universidade Federal Fluminense – UFF

LEANDRO SOARES PINHEIRO
Universidade Federal Fluminense – UFF

LEILA BRASIL FERREIRA
Universidade Federal do Rio Grande do Sul – UFRGS

LEILA SCHREINER DELGADO
Universidade Federal de Santa Maria – UFSM

LENISE DE LIMA SILVA
Universidade Federal de Santa Maria – UFSM

LEONARDO BAHIA TAVARES
Universidade Federal de Minas Gerais – UFMG

LEONARDO DE SOUZA TEIXEIRA
Instituto de Ciências Farmacêuticas
de Estudos e Pesquisas
- ICF

LEONARDO PAES CINELLI
Universidade Federal do Rio de Janeiro – UFRJ

LEOPOLDO CLEMENTE BARATTO
Universidade Federal do Paraná – UFPR

LETÍCIA LENZ SFAIR
Universidade Federal do Rio Grande do Sul – UFRGS

LETICIA NORMA CARPENTIERI RODRIGUES
Universidade Federal de São Paulo – UNIFESP

LETÍCIA SCHERER KOESTER
Universidade Federal do Rio Grande do Sul – UFRGS

LIDIANE BUENO DE MORAES
Brazilian Health Surveillance Agency – Anvisa

LÍGIA MARIA MOREIRA DE CAMPOS
Universidade Federal de Minas Gerais – UFMG

LILIA RIBEIRO SERÔDIO
Instituto Vital Brazil – IVB

LILIAN AULER MENTZ
Universidade Federal do Rio Grande do Sul – UFRGS

LIVIA DUARTE PEREIRA
Universidade Federal Fluminense – UFF

LORENA FRATINI
Universidade Federal do Rio Grande do Sul – UFRGS

LUANA LENZI
Universidade Federal do Paraná – UFPR

LUANA MARTINS DA LUZ
Brazilian Health Surveillance Agency – Anvisa

LUCÉLIA MAGALHÃES DA SILVA
Universidade Federal de Santa Maria – UFSM

LÚCIA DE FÁTIMA FRANCELINO DA SILVA
Central Laboratory of Public Health
of Pernambuco -LACEN

LUCIANA CATIA BLOCK
Universidade do Vale do Itajaí – UNIVALI

LUCIANA CRISTINA MOTA
Brazilian Health Surveillance Agency – Anvisa

LUCIANE VARINI LAPORTA
Centro Universitário Franciscano – UNIFRA

LUÍS CARLOS BRÍGIDO MOURA
Universidade Federal do Ceará – UFC

LUIS CARLOS DE ALENCAR SAMPAIO FILHO
Universidade Federal de Pernambuco – UFPE

LUIZ ALBERTO LIRA SOARES
Universidade Federal do Rio Grande do Norte – UFRN

LUIZ ANTÔNIO BATISTA DA COSTA
Excellence Center of Integral Health of Paraná – CESIP

LUIZ ARMANDO ERTHAL
Brazilian Health Surveillance Agency – Anvisa

LUIZ CLÁUDIO MARTINS ALEIXO
Institute of Nuclear Engineering – IEN-CNEN

LUIZ FERNANDO SECIOSO CHIAVEGATTO
Universidade Federal do Rio de Janeiro – UFRJ

LUIZ GUSTAVO TORRES
Naturofarma Produtos Naturais Ltda.

LUIZA DE CASTRO MENEZES CANDIDO
Universidade Federal de Minas Gerais – UFMG

LUMA WOSCH
Universidade Federal do Paraná – UFPR

LUZIA DE FÁTIMA PEREIRA
Alko do Brasil Ind e Com LTDA

MAGDA RHAYANNY ASSUNÇÃO FERREIRA
Universidade Federal do Rio Grande do Norte – UFRN

MAIQUE WEBER BIAVATTI
Universidade do Vale do Itajaí – UNIVALI

MANUELA DA COSTA SOLIZ
Universidade Federal de Santa Maria – UFSM

MARA EL CORAB MOREIRA DE OLIVEIRA
Ministry of Health – MS

MARCELA ZART AREND
Universidade Federal de Santa Maria – UFSM

MARCELE GIACOMIN GONÇALVES
Universidade Federal do Rio Grande do Sul – UFRGS

MARCELO CAMILO MORERA
Brazilian Health Surveillance Agency – Anvisa

MÁRCIA FOSTER MESKO
Universidade Federal de Pelotas – UFPel

MÁRCIA VIGNOLI DA SILVA
Universidade Federal de Ciências da Saúde de Porto
Alegre – UFCSPA

MÁRCIO LABASTIE (*In memorian*)
National Institute of Health Quality
Control – INCQS / FIOCRUZ

MARCO ANDRÉ CARDOSO
Universidade Federal do Paraná – UFPR

MARCO ANTONIO STEPHANO
Universidade de São Paulo – USP

MARCOS ANTONIO SEGATTO SILVA
Universidade Federal de Santa Catarina – UFSC

MARCOS ROBERTO DOS SANTOS
Centro Universitário Franciscano – UNIFRA

MARGARETH LINDE ATHAYDE
Universidade Federal de Santa Maria – UFSM

MARGARETH MIE NAKAMURA MATSUDA
Institute of Energetic and Nuclear Researches – IPEN

MARIA ALICE BÖCKELMANN
Cristália Produtos Químicos Farmacêuticos Ltda.

MARIA AUGUSTA TRAVASSOS LEMOS
Universidade Federal Fluminense – UFF

MARIA AUXILIADORA MILANEZE GUTIERRE
Universidade Estadual de Maringá – UEM

MARIA CRISTINA DICIAULA
Universidade Estadual de Maringá – UEM

MARIA DAS GRAÇAS LINS BRANDÃO
Universidade Federal de Minas Gerais – UFMG

MARIA DIANA CERQUEIRA SALES
Faculdade Brasileira – UNIVIX

MARIA DO CARMO ESTANISLAU DO AMARAL
Universidade Estadual de Campinas – UNICAMP

MARIA DO CARMO VASQUES GARCIA
National Institute of Health Quality
Control – INCQS / FIOCRUZ

MARIA DO ROSÁRIO SILVEIRA BRITTO
Universidade Federal de Pernambuco – UFPE

MARIA ELISA GIRÃO MOREIRA
Brazilian Health Surveillance Agency – Anvisa

MARIA ELISABETE AMARAL DE MORAES
Universidade Federal do Ceará – UFC

MARIA ELIZABETE DALMORA
Universidade Federal de Santa Maria – UFSM

MARIA JOSÉ CARNEIRO DO NASCIMENTO
Brazilian Company of Hemoderivates and
Biotechnology – HEMOBRAS

MARIA LÚCIA SILVEIRA MALTA DE ALENCAR
Brazilian Health Surveillance Agency – Anvisa

MARIA TERESINHA KREINEKER DRESH
Universidade Federal do Rio Grande do Sul – UFRGS

MARIANE GEHLEN PERIN
Universidade Federal de Santa Maria – UFSM

MARIÂNGELA TORCHIA DO NASCIMENTO
Brazilian Health Surveillance Agency – Anvisa

MARIBETE HOMRICH HOLZSCHUH
Universidade Federal do Rio Grande do Sul – UFRGS

MARILI VILLA NOVA RODRIGUES
Universidade Estadual de Campinas – UNICAMP

MARILIA DE MORAES CASTRO
Universidade Estadual de Campinas – UNICAMP

MARINA DAUMAS NEVES DUARTE
Universidade Federal Fluminense – UFF

MARINA SCOPEL
Universidade Federal do Rio Grande do Sul – UFRGS

MARINÊS JOST E SOUZA
Brazilian Health Surveillance Agency – Anvisa

MÁRIO LUIS RIBEIRO MOURA
Universidade Federal do Ceará – UFC

MÁRIO SÉRGIO PIANTAVINI
Universidade Federal do Paraná – UFPR

MARISA COELHO ADATI
National Institute of Health Quality
Control – INCQS / FIOCRUZ

MARISA DE MOURA SOUZA DA LUZ
Laboratório de Controle de Qualidade
e Pesquisa – LCQPq

MARISA KEIKO UEMA
Eurofarma Comercial e Importadora Ltda

MARTA CRISTINA TEIXEIRA DUARTE
Universidade Estadual de Campinas – UNICAMP

MARTHA ANA GATTUSO
Universidade Nacional de Rosário, Argentina

MARTIN STEPPE
Universidade Federal do Rio Grande do Sul – UFRGS

MARY ANN FOGLIO
Universidade Estadual de Campinas – UNICAMP

MARYANGELA REZENDE MASCARENHAS
Brazilian Health Surveillance Agency – Anvisa

MARYCEL ROSA FELISA F. BARBOSA
Institute of Energetic and Nuclear Researches – IPEN

MAURO FERREIRA WITZEL
Blanver Farmoquimica Ltda

MAX WEBER PEREIRA
Brazilian Health Surveillance Agency – Anvisa

MELÂNIA DE FÁTIMA CORDELINO
Baxter Hospitalar Ltda

MELÂNIA PALERMO MANFRON
Universidade Federal de Santa Maria – UFSM

MERIANE PIRES CARVALHO
Universidade Federal Fluminense – UFF

MICHELE FEITOSA SILVA
National Institute of Health Quality Control – INCQS / FIOCRUZ

MICHELI WRASSE SANGÓI
Universidade Federal de Santa Maria – UFSM

MIGUEL DE LUCCA NETO
Medley S. A. Industria Farmacêutica

MILENA BANDEIRA BARROZO
Universidade Federal do Ceará – UFC

MIRACY MUNIZ DE ALBUQUERQUE
Universidade Federal de Pernambuco – UFPE

MIRIAM ANDERS APEL
Universidade Federal do Rio Grande do Sul – UFRGS

MIRIAM DE FÁTIMA VIANNA LEONEL
Universidade Federal de Minas Gerais – UFMG

MIRIAN PARENTE PINHEIRO
Universidade Federal do Ceará – UFC

MÔNICA DA LUZ CARVALHO SOARES
Brazilian Health Surveillance Agency – Anvisa

MONIKA TAGLIARI
Universidade Federal de Santa Catarina – UFSC

NADIA LIMA DIAS CABRAL
Brazilian Health Surveillance Agency – Anvisa

NADIA MARIA VOLPATO
Universidade Federal do Rio Grande do Sul – UFRGS

NAIALY FERNANDES ARAÚJO REIS
Universidade Federal de Minas Gerais – UFMG

NARA DEITOS BITTENCOURT
Universidade Federal de Santa Maria – UFSM

NEEMIAS SILVA DE ANDRADE
Brazilian Health Surveillance Agency – Anvisa

NELIO DE BASTOS MORAIS
Brazilian Health Surveillance Agency – Anvisa

NELISE GONÇALVES DUARTE E DUARTE
Universidade Federal Fluminense – UFF

NEUZA TAEKO OKASAKI FUKUMORI
Institute of Energetic and Nuclear Researches – IPEN

NIKOLAI SHARAPIN (*In memoriam*)
Permanent Review Commission of the Brazilian Pharmacopoeia - 4th edition

NILTON LUZ NETTO JÚNIOR
Universidade Católica de Brasília – UCB

NIRLA RODRIGUES ROMERO
Universidade Federal do Ceará – UFC

ONÉSIMO ÁZARA PEREIRA
Associação Brasileira da Indústria Farmoquímica e de Insumos Farmacêuticos – ABIQUIFI

ORLANDO FATIBELLO FILHO
Universidade Federal de São Carlos – UFSCar

ORLANDO SILVA
Serono Produtos Farmacêuticos Ltda

PAOLA DE AZEVEDO MELLO
Universidade Federal de Santa Maria – UFSM

PATRÍCIA DE ANDRADE MARTINS
Institute of Energetic and Nuclear Researches – IPEN

PATRICIA HAUSCHILDT DE OLIVEIRA MENDES B
razilian Health Surveillance Agency – Anvisa

PAULA CRISTINA REZENDE ENÉAS
Universidade Federal de Minas Gerais – UFMG

PAULA REGINA ALVES DE SIQUEIRA
Universidade do Vale do Itajaí – UNIVALI

PAULA RENATA APARECIDA NIGRO RIVERA CARAZZATTO
National Association of Magistral Pharmaceuticals – ANFARMAG

PAULA ROCHA CHELLINI
Universidade Federal de Minas Gerais – UFMG

PAULO ANTONIO DE SOUZA MOURÃO
Universidade Federal do Rio de Janeiro – UFRJ

PAULO CHANEL DEODATO DE FREITAS
Universidade de São Paulo – USP

PAULO EDUARDO MAYORGA BORGES
Universidade Federal do Rio Grande do Sul – UFRGS

PAULO RENATO DE OLIVEIRA
Universidade Federal de Santa Catarina – UFSC

PAULO VICTOR PIRES DOS SANTOS
Universidade Estadual de Maringá – UEM

PEDRO EDUARDO FRÖEHLICH
Universidade Federal do Rio Grande do Sul – UFRGS

PEDRO JOSÉ ROLIM NETO
Universidade Federal de Pernambuco – UFPE

PEDRO MELILO DE MAGALHÃES
Universidade Estadual de Campinas – UNICAMP

POLIANA BERNARDES GONÇALVES
Brazilian Health Surveillance Agency – Anvisa

POLIANA DE FÁTIMA HILÁRIO
Brazilian Health Surveillance Agency – Anvisa

PRISCILLA STUDART
Universidade Federal do Ceará – UFC

RAFAEL DEITOS BEGNIS
Universidade Federal de Santa Maria – UFSM

RAFAEL NICOLAY PEREIRA
Universidade Federal de Santa Catarina – UFSC

RAFAELA MARIN
Universidade Federal do Rio Grande do Sul – UFRGS

RAFAELLA GOMES BEZERRA
Universidade Federal do Ceará – UFC

RALPH SANTOS OLIVEIRA
Institute of Nuclear Engineering – IEN-CNEN

RAPHAEL DE ANDRADE LUCAS E SILVA
Brazilian Health Surveillance Agency – Anvisa

RAQUEL LIMA E SILVA
Brazilian Health Surveillance Agency – Anvisa

REBECA RIBEIRO DE MOURA
Universidade Federal do Ceará – UFC

RENATA APARECIDA DIAS
Sindicato da Indústria de Produtos Farmacêuticos no Estado de São Paulo – Sindusfarma

RENATA ARACELLI PIRES
Baxter Hospitalar Ltda

RENATA BARBOSA DE OLIVEIRA
Universidade Federal de Minas Gerais – UFMG

RENIERE HENRIQUE DA SILVA
Universidade Federal de Pernambuco – UFPE

RICARDO CHIAPPA
União Educacional do Planalto Central – UNIPLAC

RICARDO PEREIRA LOURO
Universidade Federal do Rio de Janeiro – UFRJ

RINALDO FERREIRA
Universidade do Vale do Itajaí – UNIVALI

ROBERTO PONTAROLO
Universidade Federal do Paraná – UFPR

ROCHELE CASSANTA ROSSI
Universidade Federal do Rio Grande do Sul – UFRGS

ROCHELE SOGARI PICOLOTO
Universidade Federal de Santa Maria – UFSM

RODNEY ALEXANDRE FERREIRA RODRIGUES
Universidade Estadual de Campinas – UNICAMP

RODRIGO ALVES SOARES CRUZ
Universidade Federal Fluminense – UFF

RODRIGO CRISTOFOLETTI
Brazilian Health Surveillance Agency – Anvisa

RODRIGO FERNANDES ALEXANDRE
Ministry of Health – MS

RONALDO FERREIRA DA SILVA
Universidade Federal Fluminense – UFF

ROSA NORIKO YAMAMOTO
Universidade de São Paulo – USP

ROSALI MARIA FERREIRA DA SILVA
Universidade Federal do Pará – UFPA

ROSANA MIGUEL MESSIAS MASTELARO
Syndicate of the Pharmaceutical Product Industry in the State of São Paulo – Sindusfarma

ROSANE MARIA SILVA ALVES
Ministry of Health – MS

ROSANGELA ANDRADE Linde Gases Ltda.

ROSANGELA BOLZAN
Brazilian Health Surveillance Agency – Anvisa.

ROSE VANESSA BANDEIRA
Universidade Federal de Santa Maria – UFSM

ROSEMARIE APARECIDA DE ARAÚJO BONATTO
Merck Sharp & Dohme Farmacêutica Ltda

ROSILENE RODRIGUES SANTIAGO
Universidade Federal do Rio Grande do Norte – UFRN

ROSIMAR LEITENBERG DA SILVEIRA
Centro Universitário Franciscano – UNIFRA

ROSIMEIRE PEREIRA ALVES DA CRUZ
Brazilian Health Surveillance Agency – Anvisa

RUBENS RAPHAEL SIMÕES DE OLIVEIRA
Institute of Energetic and Nuclear Researches – IPEN

RUTH RIESINGER STRATTMANN
Universidade Federal de Pernambuco – UFPE

SAID GONÇALVES DA CRUZ FONSECA
Universidade Federal do Ceará – UFC

SALVADOR ALVES PEREIRA (*In memoriam*)
Comissão Permanente de Revisão da Farmacopeia Brasileira – 4ª edição

SÁLVIO FILGUEIRAS
Linde Gases Ltda.

SAMANTA CARDOSO MOURÃO
Universidade do Vale do Itajaí – UNIVALI

SAULO FERNANDES DE ANDRADE
Universidade Federal de Minas Gerais – UFMG

SEBASTIÃO BAETA HENRIQUE
Permanent Review Commission of the Brazilian Pharmacopoeia – 4th edition

SERGIO LUIZ DALMORA
Universidade Federal de Santa Maria – UFSM

SERGIO ROBERTO MORTARI
Centro Universitário Franciscano – UNIFRA

SEVERINO BARBOSA
Universidade Federal do Pernambuco – UFPE

SEVERINO GRANJEIRO JÚNIOR
Pharmaceutical Laboratory of the State of Pernambuco – LAFEPE

SILAS GOUVEIA
Fundação Ezequiel Dias – FUNED

SILÉSIA DE SOUZA AMORIM
Brazilian Health Surveillance Agency – Anvisa

SILVANA TERESA LACERDA JALES
Associação dos Laboratórios Oficiais Brasileiros – ALFOB

SILVÂNIA VAZ DE MELO MATTOS
Brazilian Health Surveillance Agency – Anvisa

SILVIA DEBENEDETTI
Universidade Nacional Argentina

SILVIA HELENA MIOLLO BORGMANN
Universidade Federal de Santa Catarina – UFSC

SILVIA PAREDES FONTES
Brazilian Health Surveillance Agency – Anvisa

SÍLVIA STORPIRTIS
Universidade de São Paulo – USP

SIMONE CRISTINA BENOVIT
Universidade Federal de Santa Maria – UFSM

SIMONE GONÇALVES CARDOSO
Universidade Federal de Santa Catarina – UFSC

SOLANGE MARIA COUTINHO BRANDÃO
National Institute of Health Quality Control – INCQS / FIOCRUZ

SUSANA JULIA GATTUSO BITTEL
Universidade Nacional de Rosário, Argentina

SUZANA MACHADO DE ÁVILA
Permanent Review Commission of the Brazilian Pharmacopoeia – 4th edition

TAILANE SANT'ANNA MOREIRA
Universidade Federal do Rio de Janeiro – UFRJ

TALITA GESSER CESCA
Universidade do Vale do Itajaí – UNIVALI

TANIA MARI BELLÉ BRESOLIN
Universidade do Vale do Itajaí – UNIVALI

TATIANE PEREIRA DE SOUZA
Universidade Federal do Amazonas – UFAM

TAYOMARA DE SOUSA NASCIMENTO
Brazilian Health Surveillance Agency – Anvisa

TELMA MARY KANEKO
Universidade de São Paulo – USP

TÉRCIO PASCHKE OPPE
Universidade Federal do Rio Grande do Sul – UFRGS

TERESA CRISTINA TAVARES DALLA COSTA
Universidade Federal do Rio Grande do Sul – UFRGS

TEREZINHA DE JESUS ANDREOLI PINTO
Universidade de São Paulo – USP

THAIS MARTINS GUIMARÃES DE FRANCISCO
Universidade Federal do Paraná – UFPR

THAIS MESQUITA DO COUTO ARAUJO
Brazilian Health Surveillance Agency – Anvisa

THELMA DE BARROS MACHADO
Universidade Federal Fluminense – UFF

THEREZA CRISTINA VESSONI PENNA
Universidade de São Paulo – USP

THEREZINHA COELHO BARBOSA TOMASSINI
Permanent Review Commission of the Brazilian Pharmacopoeia – 4th edition

THIELE FACCIN DE BRUM
Centro Universitário Franciscano – UNIFRA

TIAGO ASSIS MIRANDA
Universidade Federal de Minas Gerais – UFMG

VALDIR FLORÊNCIO DA VEIGA JUNIOR
Universidade Federal Amazonas – UFAM

VALÉRIA PEREIRA DE SOUSA
Universidade Federal do Rio de Janeiro – UFRJ

VALQUIRIA LINCK BASSANI
Universidade Federal do Rio Grande do Sul – UFRGS

VERA LÚCIA GARCIA REHDER
Universidade Estadual de Campinas – UNICAMP

VIVIANE DE OLIVEIRA GARCIA
Universidade Federal de Santa Maria – UFSM

VLADI OLGA CONSIGLIERI
Universidade de São Paulo – USP

VOLKER BITTRICH
Universidade Estadual de Campinas – UNICAMP

WAGNER LUIZ RAMOS BARBOSA
Universidade Federal do Pará – UFPA

WALFREDO DA SILVA CALMON
Brazilian Health Surveillance Agency – Anvisa

WILSON CAMARGO
National Institute of Health Quality Control – INCQS / FIOCRUZ

WILSON REINHARDT FILHO
Permanent Review Commission of the Brazilian Pharmacopoeia – 4th edition

SMIN LOURENZO FIGUEIREDO
Health and Environmental Surveillance – BA

YEDO ALQUINI
Universidade Federal do Paraná – UFPR

4 GENERAL PROVISIONS

TITLE

The full title of this work is "Pharmacopoeia of the Federative Republic of Brazil, 5th edition". It may also be named as "Brazilian Pharmacopoeia, 5th edition" or FB 5.

DEFINITIONS

Action, use and doses

Those are the ones appearing in the report for product registration with the health agency, updated upon reviewing national and international bibliography, if any.

Where indicated in the monographs, the doses represent the quantity of medicine usually prescribed, which has therapeutic efficiency for adult patients. The licensed prescriber, at his discretion and under his exclusive responsibility, taking into consideration the pharmacokinetics and the pharmacodynamics, may vary the quantity and the frequency of the administration of any medicine. However, the prescription of doses very higher than the usual ones, established in literature, obliges the pharmacist to confirm with the prescriber the established doses.

Acidity and alknalinity – quick testing

A solution is considered as *neutral* when it does not modify the color of blue and red litmus papers, or when the universal indicator paper acquires the neutral scale colors, or when 1 mL of the same solutions turns into green with a drop of IS bromothymol blue (pH 7.0).

It is considered as *acidic* when the blue litmus paper turns into red or 1 mL turns into yellow with a drop of IS phenol red (pH 1.0 to 6.6).

It is considered as *weakly acidic* when the blue litmus paper turns slightly into red or 1 mL turns into orange with a drop of IS methyl red (pH 4.0 to 6.6).

It is considered as *strongly acidic* when the congo red paper turns into blue or 1 mL turns into red by adding a drop of IS methyl orange (pH 1.0 to 4.0).

It is considered as *alkaline* when the red litmus paper turns into blue or 1 mL turns into blue with a drop of IS bromothymol blue (pH 7.6 to 13.0).

It is considered as *weakly alkaline* when the red litmus paper turns into blue or 1 mL turns into pink with a drop of IS cresol red (pH 7.6 to 8.8).

It is considered as *strongly alkaline* when it turns blue with the drop of IS thymolphtalein (pH 9.3 to 10.5*)* or into red with a drop of phenolphtalein (pH 10.0 to 13.0).

Adhesive

It is the adhesive assigned to produce a systemic effect by diffusion of the active ingredient (s) in a constant speed for an extended period of time.

Water for injectables

Water for injectables is the input used in the preparation of the medicines for parenteral administration, as vehicle or in the dissolution of substances or preparations.

Water for pharmaceutical use

It is considered as water for pharmaceutical use the several types of water deployed in the pharmaceuticals synthesis, in the formulation and manufacturing of medicines, in testing laboratories, diagnoses and further applications related to the healthcare area, including as main component when cleaning utensils, equipment and systems.

Purified water

Purified water is the potable water which underwent some type of treatment to remove the possible contaminants and meet the purity requirements established in the monographs.

Ultrapurified water

Ultrapurified water is the purified water which underwent additional treatment to remove the possible contaminants and meet the purity requirements established in the monographs.

Aromatic waters

Those are saturated solutions of essential oils or other aromatic substances in water. They have the characteristic odor of the drugs with which they are prepared, receiving their names as well.

Water bath and vapor bath

It is a bath with boiling water, unless the monograph specifies other temperature. The expressions *hot water* and *very hot water* indicate approximate temperatures between 60°C and 70°C and between 85°C and 95°C, respectively.

Vapor bath means the exposure to the fluent vapor or any other form of heat, corresponding to the fluent vapor in temperature.

Bioavailability

It indicates the speed and extension of absorption of an active ingredient in a dosage form, from its concentration/ time curve in the systemic circulation and its excretion in the urine.

Bioequivalence

It consists of the proof of pharmaceutical equivalence among products presented under the same pharmaceutical form, containing identical qualitative and quantitative composition of active ingredient (s) and having comparable bioavailability, when studied under the same experimental design.

Capsule

It is the solid pharmaceutical form in which the active ingredient and the excipients are contained in a hard or soft soluble casing, in variable shapes and sizes, usually containing a single dose of the active ingredient. Normally, it is formed by gelatin, but it may also be formed by amide or other substances. Abbreviation: cap.

Hard capsule

It is the capsule consisting of two pre-manufactured cylindric sections (body and cap) which fit and whose ends are rounded. It is typically fillied in with solid active ingredients and excipients. It is normally formbed by gelatin, but it may also be formed by other substances. Abbreviation: hard cap.

Extended release hard capsule

It is the capsule consisting of two pre-manufactured cylindric sections (body and cap) which fit and whose ends are rounded. It is typically filled in with solid active ingredients and excipients. Normally, it is formed by gelatin, but it also may be formed by other substances. See general definition of extended release. Abbreviation: ext. rel. hard cap.

Delayed release hard capsule

It is the capsule consisting of two pre-manufactured cylindric sections (body and cap) which fit and whose ends are rounded. It is typically filled in with solid active ingredients and excipients. Normally, it is formed by gelatin, but it also may be formed by other substances. See general definition of delayed release. Abbreviation: del. rel. hard cap.

Soft capsule

It is the capsule consistiuted of a gelatin casing, in various shapes, more malleable than the hard capsules. Normally, they are filled in with liquid or quasi-solid contents, but they may also be filled in with powders and other dry solids. Abbreviation: soft cap.

Extended release soft capsule

It is the capsule consistiuted of a gelatin casing, in various shapes, more malleable than the hard capsules. Normally, they are filled in with liquid or quasi-solid contents, but they may also be filled in with powders and other dry solids. See the general definition of extended release Abbreviation: ext. rel. soft cap.

Delayed release soft capsule

It is the capsule consistiuted of a gelatin casing, in various shapes, more malleable than the hard capsules. Normally, they are filled in with liquid or quasi-solid contents, but they may also be filled in with powders and other dry solids. See the general definition of delayed release Abbreviation: del. rel. soft cap.

Gas cylinder

It is the metallic vessel, perfectly closed, with resistant walls, intended to contain gas under pressure, plugged by regulating valve, able to maintain the gas output under determined flow rate.

Collyrium

It is the pharmaceutical liquid preparation intended to the application over the ocular mucosa. Abbreviation: col.

Total lyophilized human prothrombin complex

It is a fraction of plasmatic proteins containing, in a mandatory manner, the Factors II, VII, IX and X of the human coagulation.

Tablet

It is the solid pharmaceutical form containing a single dose of one or more active ingredients, with or without excipients, obtained by compressing uniform volumes of particles. It may be from broad variety in sizes, shapes, present markings in the surface and be coated or not. Abbreviation: tab.

Modified release tablet

It is the Tablet which has modified release. It must be classified as modified release only when the classifications “delayed release” and “extended release” are not proper. Abbreviation: mod. rel. tab.

Extended release tablet

It is the tablet whose excipients are intended to specifically modify the release of the active ingredient into the digestive fluids. See definition of extended release. Abbreviation: ext. rel. tab.

Effervescent tablet

It is the tablet containing, in addition to the active ingredients, acidic substances and carbonates or bicarbonates, which release carbon dioxide when the tablet is dissolved into the water. It is intended to be dissolved or dispersed into water before the administration. Abbreviation: eff. tab..

Chewable tablet

It is the tablet formulated so that it may be chewed, producing a pleasing residual flavor in the oral cavity. Abbreviation: chew. tab.

Orodispersible tablet

It is the tablet which disintegrates or dissolves, quickly, when it is placed onto the water. Abbreviation: orodis. tab.

Mouthwash tablet

It is the tablet which must be dissolved into water for the preparation of the mouthwash, which is a liquid intended to wash the mouth, acting over the gums and mucosas of the mouth and throat. It must not be swallowed. Abbreviation: mout. tab.

Solution tablet

It is the tablet intended to be dissolved into water before the administration. The produced solution may be slightly milky due to the excipients used in the manufacturing of the tablets. Abbreviation: sol. tab.

Tablet for suspension

It is the tablet that, when in contact with a liquid, quicly produces a homogeneous dispersion (suspension). It is intended to be dispersed before the administration. Abbreviation: tab. susp.

Coated tablet

It is the tablet which has one or more fine coating layers, normally, polymeric, intended to protect the pharmaceutical against air or umidity; for pharmaceuticals with unpleasant odor and flavor; to improve the appearance of the tablets or for any other property which is not to change the speed or extension of the active ingredient release. Abbreviation: coat. tab.

Extended release coated tablet

It is the tablet which has one or more fine coating layers, normally polymeric, intended to change the speed or extension of the active ingredient release. See the definition of extended release. Abbreviation: ext. rel. coat. tab.

Delayed release coated tablet

It is the tablet which has one or more fine coating layers, normally polymeric, intended to change the speed or extension of the active ingredient release, presenting a delayed release of the active ingredient. See the definition of delayed release. Abbreviation: del. rel. coat. tab.

Uncoated tablet

It is the tablet in which the excipients used are not specifically intended to change the release of the active ingredient in the digestive fluids. Abbreviation: unc. tab.

Quality Control

It is the set of measures intended to ensure, at any time, the manufacturing of medicine and further product batches, which meet the standards for identity, activity, contents, purity, efficiency and innocuity.

Dyes

They are additional substances to the medicines, products, dietaries, cosmetics, parfums, hygiene products and similar, sanitizing products, disinfecting products and similar, with the effect of providing color and, in certain types of cosmetics, transfer it to the cutaneous surface and skin attachments. For their use, observe the Federal legislation and the resolutions edited by Anvisa.

Correlative

Healthcare product, such as equipments, device, material, article or system of medical, dentistry or laboratory use or application, intended to the prevention, diagnosis, treatment, rehabilitation or contraception and which does not use pharmaceutical, immunological or metabolic medium to perform its main function in human beings and it may, however, be assisted in their functions by those media.

Cosmetics

Those are products for external use, intended to the protection or gilding of any of the different body parts, such as face powders; talcs; beauty creams; hand creams and similar; face masks; beauty lotions; milky, creamy and astringent solutions; hand lotions; make-up foundations and cosmetic oils; rouges; blushes; lipsticks; lip pencils; sunblock preparations; tanning lotions and simulatories; mascaras; eye shadows; eyeliner; hair dyes; hair lightening hair; preparations to crimp and straighten hair; hairsprays;

hair oil and similar; hair lotions; hair removal products; preparations for fingernails and others.

Cream

It is the quasi-solid pharmaceutical form consisting of a emulsion, formed by a lyophilic phase and a hydrophilic phase. It contains one or more active ingredients dissolved or dispersed into a proper base and it is normally used for external application on skin or mucosal membranes.

Cryoprecipitates of the fresh human plasma

They are constituted of the cold insoluble fractions containing particularly the Factors I (140 to 250 mg) and VIII (70 to 120 UI) of the human coagulation per unit of human blood collection. Others factors of coagulation are also found in smaller concentrations together with the cryoprecipitate, such as Von Willbrand Factor (40 to 70%) and Factor XIII (20 to 30%).

Common Brazilian Names (DCB)

It is the names of the pharmaceutical or pharmacologically active ingredient, approved in the federal agency responsible for health surveillance.

Internacional Common Names (DCI)

It is the names of the pharmaceutical or pharmacologically active ingredient, recommended at the World Health Organization.

Mass density and relative density

Mass density (ρ) of a substance is the ratio of its mass by its volume at 20°C.

Pills

They are tablets coated with layers constituted of mixtures of various substances, such as resins, either natural or synthetic, gums, gelatins, inactive and insoluble materials, surgars, plasticizers, polyols, waxes, authorized dyes and, at times, aromatizers and active ingredients. Abbreviation: pill.

Elixir

It is the pharmaceutical preparation, liquid, limpid, hydroalcoholic, sweet flavored, pleasant, presenting alcoholic contents in the range from 20% to 50%.

The elixirs will be prepared by simple dissolution and they must be packed in ambar colored vials and maintained at fresh place and in a dark place.

Package

It is the casing, vessel or any other manner of packaging, removable or not, intended to cover, package, protect or maintain, particularly or not, the medicines, drugs, pharmaceutical inputs and correlatives, cosmetics, sanitizing products and other products. The packaging conditions are described in the individual monographs by using the terms listed as follows.

Primary package

This is the one which is in direct contact with its contents during the whole time. It is considered as primary package material: ampoule, tube, envelope, case, flacon, glass or plastic vial, vial-ampoule, cartdrige, can, pot, paper bag and others.

The relative density usually adopted (d^{20}_{20}) is defined as the relation between the mass of a substance at 20°C air and the same of equal volume of water at the same temperature.

Disinfectants

They are products intended to destroy, wildly or selectively, microorganisms when they are applied into inanimate or ambient objects.

Detergents

They are products intended to dissolve fats; to the hygiene of vessels and receptacles and to applications of domestic use.

Blood donors

They are healthy and carefully selected individuals who, after medical examinations, laboratory blood tests and study of their medical history, lacking of transmittable infectious agents which may be accepted and used for collecting their total blood or cell or plasma fractions for profilatic, curative or fractioning purposes.

There must not be any interaction between the primary package material and its contents which is able to change the concentration, quality or purity of the packaged material.

Secondary package

This is the one which enables the total protection of the packaging material under the usual conditions of transportation, storage and distribution. It is considered as secondary package: cartons, cartridge of pasteboard, wood or plastic material or cases of pasteboard and others.

Plaster

It is the quasi-solid pharmaceutical form for external application. It consists of a adhesive foundation containing one ore more active ingredients distributed in a uniform

layer over a proper support made of synthetic or natural material. It is intended to maintain the active ingredient in contact with the skin, acting as a protector or keratolytic agent. Abbreviation: plast.

Emulsion

It is the liquid pharmaceutical form of one and more active ingredients consisting of a two-phase systems involving at least two immiscible liquids and in which one liquid is disperse in the form of small drops (internal or disperse phase) through another liquid (external or continuous phase). Normally, it is stabilized by means of one or more emulsifying agents. Abbreviation: emu.

Aerosol emulsion

It is the emulsion packaged under pressure contained one propelling gas and therapeutically active ingredients which are released after the activation of a proper valve system. Abbreviation: aer. emu.

Drop emulsion

It is the emulsion intended to the administration in the form of drops. Abbreviation: dr. emu.

Injectable emulsion

It is the sterile emulsion. Abbreviation: inj. emu.

Emulsion for infusion

It is the sterile emulsion with water, as the continuous phase, normally, isotonic with the blood and particularly used for administration in big volumes. Abbreviation: emu. inf.

Spray emulsion

It is the emulsion administered as a liquid finally divided by air or vapor blast. Abbreviation: spray emu.

Biological testing

These are procedures intended to assess the power of active ingredients contained in the raw materials and pharmacopoeia preparations by using biological reagents, such as microorganisms, animals, fluids and isolated animal organs.

Spirit

It is the liquid alcoholic or hydroalcoholic pharmaceutical form containing aromatic or medicinal ingredients and classified as simple or compound. Abbreviation: spir.

The spirits are obtained by dissolving aromatic substances into ethanol, generally at the ratio of 5% (p/v).

Sterility

Sterility is the absence of viable microorganisms.

Extract

It is the preparation in liquid, solid or intermediate consistency, obtained from animal or vegetable material. The material used in the preparation of extracts may undergo preliminary treatment, such as enzyme inactivation, milling or degreasing. Abbreviation: ext.

The extract is prepared by percolation, maceration or other proper and validated method, using, as solvent, ethyl alcohol, water or other proper solvent. After the extraction, undesirable materials may be eliminated.

Fluid extract

It is the liquid preparation obtained from vegetable or animal drugs by extraction with proper liquid or dissolution of the corresponding dry extract in which, except when otherwise indicated, a part of the extract, in mass or volume, corresponds to a part, in mass, of the drug dried used in its preparation. As required, the fluid extracts may be standardized in terms of concentration of the solvent; contents of the constituents or dry residue. As required, preservatives inhibitors of the microbial growth. They must present contents of active ingredients and dry residues prescribed in their respective monographs. Abbreviation: flu. ext.

Soft extract

It is the pasty preparation obtained by partial evaporation of solvent used in its preparation. The following are used as solvent only: ethyl alcohol, water or ethyl alcohol/water mixtures in the proper propotion. It presents, a minimum, 70% of dry residue (w/w). As required, preservatives inhibitors of microbial growth may be added. Abbreviation: soft ext.

Dry extract

It is the solid preparation, obtained by evaportation of the solvent used in its preparation. It presents, as a minimum, 95% of dry residue, calculated as a mass percentage. Proper inert materials may be added. Abbreviation: dry ext.

The standardized dry extracts have the content of their constituents adjuted by adding proper inert materials or adding dry extracts obtained with the same pharmaceutical used in the preparation.

Manufacturing

All of those are operations which are required for obtaining healthcare products.

Distillation range

The distillation range is the interval of the temperature corrected into the pressure 101.3 kPa (760 mmHg), in which the liquid or specific fraction of the liquid distillates entirely.

Fusion range

Fusion range of a substance is the interval of temperature comprised between the beginning (in which the substance starts to fluidify) and the end of the fusion (which is evidenced by the disappearance of the solid phase).

Pharmaceutical

See *active pharmaceutical input*.

Pharmacopoeic

The expression pharmacopoeic supersedes the expressions: official and officinal, used in previous editions, being equivalent to these expressions for all of the effects.

Factor VII of the human blood coagulation, lyophilized

It is the proteic fraction of the plasma containing the Factor VII (a simple chain glycoprotein derivate), and it may equally contain small quantities of its activated form (2-chain derivate or Factor VIIa).

Factor VIII of the human origin blood coagulation, lyophilized

It is the proteic fraction of the plasma containing a glycoprotein named as Factor VIII of the coagulation, and in virtue of the purification method, variable quantities of the Von Willebrand method. It is prepared from a mixture of human plasma for fractionation obtained from healthy donors.

Human fibrinogen, lyophilized

It is the soluble fraction of the human plasma, obtained from the *Human plasma for fractionation*, which by adding the thrombin, it turns into fibrin. The preparation may contain addititives (salts, buffers or stabilizers) and when resconstructed (addition of diluent), it must contain, as a minimum, 10 g/L of fibrinogen.

Pharmaceutical form

It is the final status of the presentation of the pharmaceutical active ingredients after one or more pharmaceutical operations executed by adding or not proper excipients in order to facilitate its use and obtain the desired therapeutical effect, with proper characteristics to a certain administration route.

Gel

It is the quasi-solid pharmaceutical form of one ore more active ingredients containing a gelling agent to provide hardness to a colloidal solution or dispersion (a system in which the colloidally sized particles – typically between 1 nm and 1μm – are distributed in a uniform manner through the liquid). A gel may contain suspended particles.

Hydrophobic gel

It is the gel containing, usually, liquid paraffin with polyethylene or fatty oils with colloidal silica or aluminum or zinc soaps.

Lipophilic gel

It is the gel resulting from the preparation obtained by the incorporation of gelling agents – goat's thorn, amide, cellulose derivates, carboxyvinylic polymers and double magnesium and aluminum silicates to water, glycerol or propylene glycol.

Globule

It is the solid pharmaceutical form presented as small spheres constituted of sucrose or the mixture of sucrose and lactose. They are impregnated by the desired power and with alcohol higher than 70%.

Chewing gum

It is the solid single dose pharmaceutical form containing one or more active ingredients, consisting of insoluble, sweet and tasty plastic material. When it is chewed, it releases the active ingredient.

Granulate

It is the solid pharmaceutical form containing a single dose of one or more active ingredients, with or without excipients. It consists of solid and dry aggregates of uniform volume of powder particles resistant to the handling. Abbreviation: granu

Effeverscent granulate

It is the granulate containing, in addition to the active ingredients, acidic substances and carbonates or bicarbonates, which release carbon dioxide when the granulate is dissolved into water. It is intended to be dissolved or dispersed into water before the administration. Abbreviation: eff. granu.

Granulate for solution

It is the granulate intended to be dissolved into water before the administration. The solution produced may be slightly milky due to the excipients used in the manufacturing of the granulate. Abbreviation: granu. sol.

Granulate for suspension

It is the granulate which, in contact with a liquid, quickly produces a homogeneous dispersion (suspension). It is intended to be dispersed before the administration. Abbreviation: gran. susp.

Coated granulate

It is the granulate which has one ore more fine layers of coating, normally polymeric, intended to protect the pharmaceutical against air or umidity, for pharmaceuticals with unpleasant odor and flavor, to improve the appearance of the granulates or for some other property which is not to change the speed or extension of the active ingredient release extension. Abbreviation: coat. granu.

Extended release coated granulate

It is the granulate which has one or more fine layers of coating, normally polymeric, intended to change the speed or extension of the active ingredient release. See definition of extended release. Abbreviation: ext. rel. coat. gran.

Delayed release coated granulate

It is the granulate which has one or more fine layers of coating, normally polymeric, intended to change the speed or extension of the active ingredient release, presenting a delayed release of the active ingredient. See general definition of delayed release. Abbreviation: del. rel. coat. granu.

Human hepatitis A immunoglobulin

It is a sterile, liquid or lyophilized preparation containing the immunoglobulin, particularly immunoglobulin G.

Human hepatitis B immunoglobulin

It is a sterile, liquid or lyophilized preparation containing the immunoglobulins, human hepatitis B immunoglobulin, particularly immunoglobulin G.

Human hepatitis B immunoglobulin for intravenous use

It is a sterile, liquid or lyophilized preparation containing the immunoglobulin, particularly immunoglobulin G.

Human rabies immunoglobulin

It is a sterile, liquid or lyophilized preparation containing the immunoglobulin, particularly immunoglobulin G

Human rubella immunoglobulin

It is a sterile, liquid or lyophilized preparation containing the immunoglobulin, particularly immunoglobulin G.

Human chickenpox immunoglobulin

It is a sterile, liquid or lyophilized preparation containing the immunoglobulin, particularly immunoglobulin G.

Human chickenpox immunoglobulin for intravenous use

It is a sterile, liquid or lyophilized preparation containing the immunoglobulin, particularly immunoglobulin G

Human anti-D immunoglobulin

It is a sterile, liquid or lyophilized preparation containing the immunoglobulin, particularly immunoglobulin G

Human measles immunoglobulin

It is a sterile, liquid or lyophilized preparation containing the immunoglobulin, particularly immunoglobulin G.

Human tetanus immunoglobulin

It is a sterile, liquid or lyophilized preparation containing the immunoglobulin, particularly immunoglobulin G. It is obtained from the plasma containing specific antibodies against the toxin of *Clostridium tetani*.

Normal human immunoglobulin

It is a sterile, liquid or liophylized preparation containing particularly IgG. Other proteins may also be present.

Normal human immunoglobulin for intravenous administration

It is a sterile, liquid or liophylized preparation containing particularly immunoglobulin G (IgG). Other proteins may also be present. It contains IgG antibodies from normal individuals.

Biological indicator

It is a preparation characterized by specific microorganism which has defined and stable resistance to a certain process os sterilization.

Refractve index

The refractive index (n) of a substance is the relation between the speed of light in vacuum and its speed within a substance.

For practical purposes, the regraction is measured with reference to the air and the substance and not with reference to the vacuum and the substance.

The refractive index may be defined as the relation between the sine of the angle of incidence and the sines of the angle of refraction, that is, n = *sine I / sine r.*

Injectable

It is the sterile preparation intended to the parenteral administration. It is presented as solution, suspension or emulsion. Abbreviation: inj.

Insecticides

They are products for external uses, intended to the prevention and control of insects, at houses, places and public places and its surroundings

Insulin

Insulin is a protein which affects the glycose metabolism. It is obtained from healthy bovine and swine pancreas, or both of them, used as food by human beings.

Human insulin

Human insulin is a protein corresponding to an active ingredient elaborated in the human pancreas which affects the carbohydrates (particularly glycose), lipids and proteins.

Human insulin isophane suspension

Human insolun isophane suspension is a steril suspension of human insulin crystals of zinc and protamine sulfate into the buffered water for the injection, combined in a way that the solid phase of the suspension is composed of human insulin crystals, protamine and zinc.

Human insulin isophane suspension and human insulin injection

Human insulin isophane suspension and human insulin injection is a sterile buffered suspension of human insulin, complexed with protamine sulfate in a human insulin solution.

Human insulin zinc suspension

It is a sterile human insulin suspension in buffered water for inhection, changed by adding a proper zinc salt in a way that the solid phase of the suspension is constituted of a mixture of crystalline insulin and amorphous insulin in a ratio of about 7 parts of crystals and 3 parts of amorphous material.

Extended human insulin zinc suspension

It is a steril human insulin suspeion in buffered water for injection, changed by adding a proper zinc salt in a way that the solid phase of the suspension is prominently crystalline.

Injectable insulin

Injectable insulin is an isotonic and steril solution of insulin.

Insulin lispro

It has an identical structure as the human insolun, except that it has lysine and proline in the positions 28 and 29, respectively,

of the chain B, while this sequence is inverted into human insulin. Insulin lispro is produced by microbial synthesis through a DNA recombination process.

Active pharmaceutical input

It is an active chemical substance, pharmaceutical, drug or raw materials which has pharmacological properties with medicinal purposes, used for diagnosis, relief or treatment, deployed to change or explore physiologic systems or pathologic status in benefit of the person under administration.

When it is intended to the deployment in medicines, they must meet the requirements set forth in the individual monographs.

Isolators

Equipment deploying technology used for double purpose: to protect the product against contamination by the environment and personnel when packaging and closing and to protect the personnel from toxic or deleterious products which are produced.

Conventional release

It is the type of release of pharmaceutical forms which are not intentionally changed by a special formulation design and/or manufacturing method.

Parametric release

It is defined as the release of cargo or product batches submitted to terminal sterilization, by meeting the critical parameters of the sterilization process, without performing the sterility test.

Extended release

It is the type of changed release of pharmaceutical forms which enables at least a reduction in the dose frequency, when compared to the medicine presented as conventional release. It is obtained by means of a special formulation design and/or manufacturing method.

Delayed release

It is the type of changed release of pharmaceutical forms which presents a delayed release of the active ingredient. It is obtained by means of a special formulation design and/or manufacturing method. The gastro-resistant preparations are considered as delayed release forms, because they are not intended to resist the gastric fluid and release the active ingredient into the instestinal fluid.

Lotion

It is the aqueous liquid or hydroalcoholic preparation, with variable viscosity, for application onto the skin, including the scalp. It may be a solution, emulsion or suspension containing one or more active or adjuvant ingredients.

Batch or departure

It is the quantity of a medicine or other product which is produced in a manufacturing cycle and whose essential characteristic is the homogeneity.

Packaging material

It is understood as packaging material the vessel, bundle, casing or any other protective form, removable or not, used to package, protect, maintain or cover, specifically or not, the raw materials, reagents and medicines. Abbreviation: pack. mat.

Raw materials

Active or inactive substances which are deployed in the manufacturing of medicines and other products, either those which remain unchanged or those which are subject to be modified.

Media fill

It is a test for simulation of the aseptic operations in which the product is substituted by a culture medium and serves to ensure that the products used are able to produce sterile products.

Medicine

It is the pharmaceutical product, technically obtained or prepared, containing one or more pharmaceuticals and other substances, with profilatic, curative, palliative purpose or intended for diagnosis.

Reference medicine

It is the innovative product registered with the federal Brazilian agency, responsible for the health surveillance and marketed in Brazil, whose efficiency, securitu and quality were scientifically proven in the competent federal agency, at the time of the registration.

Generic medicine

It is the medicine similar to a reference or innovative product, which intents to be interchangeable with this, generally produced after the expiration or renunciation of the patentary protection or other exclusivity rights, upon proving its efficiency, security and quality, and designated by DCB or, in its absence, by DCI.

Interchangeable medicine

It is the equivalent therapeutical medicine of a reference medicine, upon proving, essentially, the same effects of efficiency and security.

Magistral medicine

It is every medicine whose prescription details the composition, pharmaceutical form and posology. It is prepared at the drugstore, by a licensed pharmacist or under his direct supervision.

Pressurized medicine

It is the medicine packaged in vials maintained under pressure, containing a propelling gas and ingredients, therapeutically active which is released after activating the proper valve system.

Similar medicine

This is the one containing the same active ingredient (s), presents the same concentration, pharmaceutical form, route of administration, posology and therapeutical indication, and which is equivalent to the medicine registered with the federal agency, responsible for the health surveillance, and it may differ only in characteristics related to the size and shape of the product, expiration date, package, labeling, excipients and vechicle, and it must be at all times be identified by its commercial name or brand.

Biological half-life

It is the time required for a organism to remove, by biological elimination, half of the quantity of an administered substance.

Effective half-life

It is the time required that a radionuclide in an organism reduces its activity in half, as a combined result of the biological elimination and radioactive decay. The effective half-life is important for the optimal dose calculation of the radiopharmaceutical to be administered and when monitoring the quantity of exposure to radiation.

Immunochemical methods

Those are methods based on a selective, reversible and non-covalent bond between antigens and antibodies.

Exceeding human plasma mixtures treated by viral inactivation

Frozen or lyophilized preparation, sterile, apyrogenic, obtained from exceeding human plasma from donors of the same blood group ABO and Rh (D_u). The preparation is thawed or reconstituted before its use, in order to obtain an injectable solution. Human plasma used must meet the requirements of the monograph Human plasma for fractionation.

Warranty level of sterility

It is the warranty degree which the process sterilizes a population of items, being expressed as the probability of a non-sterile item in that population.

Chemical name

It is the name of the pharmacopoeic substance, in accordance with the nomenclature stated by the International Union of Pure and Applied Chemistry (IUPAC).

Batch number

Name printed in the label of a medicine and other products which allows identifying the batch or departure to which they belong and, as required, locating and reviewing all of the manufacturing and inspection operations practiced during the manufacturing.

Nutriments

They are substances constituents of the nutritional value of food, including proteins, fats, carbohydrates, water, mineral elements and vitamins.

Osmolality

It is a pratical form which provides a total measure of the contribution of several solutes into a solution by the osmotic pressure of the solution. The osmolality unit is the osmol per kilogram (osmol/kg), but the submultiple milliosmol per kilograms (mosmol/kg) is normally used.

Ovule

It is a solid pharmaceutical form, single dose, containing one or more active ingredients dispersed or dissolved into a proper base which has several shapes, usually, ovoid. They fuse at the body temperature

Reference standards of the Brazilian Pharmacopoeia

In accordance with the WHO definition, pharmacopoeic reference standards (PRef) are products of acknowledged uniformity, intended to be used in testing where one ore more of their properties will be compared to the ones of the testing substance. They have a purity grade suitable for the use to which they are intended.

PRef is established and distributed by pharmacopoeic authorities, whose value attributed to one ore more of its properties is accepted without the necessity of comparison with other standard, intended to be used in specific testing described in the pharmacopoeic monographs. They include reference chemical substances, biological products, vegetable extracts and powders, radiopharmaceuticals, among others. The most used related expression is: Chemical Substance of Pharmacopoeic Reference.

Paste

It is a pomade containing a large quantity of solids in dispersion (at least 25%). They shall meet the specifications established for pomades.

Pastille

It is the solid pharmaceutical form containing one ore more active ingredients, usually, in a sweetened and flavored base. It is used for dissolution or slow disintegration in the mouth. It may be prepared by modeling or compression. Abbreviation: pas.

Hard pastille

Rigid pastille to be slowly dissolved. Abbreviation: hard pas.

Gummy pastille

Flexible and soft pastille of mixtures containing synthetic or natural polymers. Abbreviation: gu. pas.

Parfum

It is the product of aromatic composition obtained on the basis of natural or synthetic substances which, in proper concentrations and vehicles, have as main purpose the odorization of people and environments, including extracts, scented water, creamy parfums, preparations for bath and environment odorizer, presented in liquid, gellied, pasty or solid form.

Fresh frozen plasma

It is the liquid part of a total blood unit obtained after centrifuging and separating its cell fractions which shall be totally frozen until 4 hours after the total blood collection, which originated it, ensuring the maintenance of the integrity and concentration of the leaving factors of the coagulation.

Human plasma for fractionation

It is the liquid part remaining of the total blood after separating the blood cell fractions upon using the proper closed systems of colletion or centrifugation, which contains the leaving factors of the coagulation. It contains the anticoagulant, conservative and preservative solution,

being stored at a temperature of -30°C or less. It is intended to the preparation of the hemoderivate, in accordance with the Good Manufacturing Practices of Medicines.

Powder Injectable solution powder

It is a solid pharmaceutical form containing one or more dry active ingredients with reduced particle size, with or without excipients.

Aerosol powder

It is the poweder packaged under pressure, containing a propelling gas and therapeutically active ingredients which are released after the activation of a proper valve system. Abbreviation: aer. powder.

Effervescent powder

It is the powder containing, in addition to the active ingredients, acidic substances and carbonates or bicarbonates, which release carbon dioxide when the powder is dissolved into water. It is itended to be dissolved or dispersed into water before the administration. Abbreviation: eff. powder.

Lyophilized powder for injectable solution

It is the sterile powder intended to the subsequent addition of liquid to form a solution. Prepared by lyophilization, a process which involves the removal of water from products by freezing the extremely low pressures. Abbreviation: lyo. pow. inj. sol.

Lyophilized powder for injectable suspension

It is the sterile powder intended to the subsequent addition of liquid to form a suspension. Prepared by lyophilization, a process which involves the removal of water from products by freezing the extremely low pressures. Abbreviation: lyo. pow. inj. sus.

Lyophilized powder for injectable suspension of extended release

It is the sterile powder intended to the subsequent addition of liquid to form a suspension. Prepared by lyophilization, a process which involves the removal of water from products by freezing the extremely low pressures. See general definition of extended release. Abbreviation: lyo. pow. inj. sus. ext. rel.

Mouthwash powder

It is the powder which must be dissolved into water before being used for the preparation of the mouthwash, which is a liquid intended to wash the mouth, in order to act on the gums and mucosas of the mouth and throat. It must not be swollen. Abbreviation: mout. pow.

Solution powder

It is the powder intended to be reconstituted to form a solution. Abbreviation: sol. pow.

It is the stetile powder intended to the subsequent addition of liquid to form a solution. Abbreviation: inj. sol. pow.

Infusion solution powder

It is the sterile powder intended to the reconstitution to form a solution for infusion. This solution is normally isotonic with the blood and mainly used for administration in big volumes. Abbreviation: inf. sol. pow.

Suspesion powder

It is the powder intended to be reconstituted to form a suspension Abbrevion: sus. pow.

Injectable solution powder 4

It is the powder intended to the subsequent addition of liquid to form a suspension. Abbreviation: inj. sus. pow.

Injectable suspension powder of extended release

It is the sterile powder intended to the subsequent addition of liquid to form a suspension. See definition of extended release. Abbreviation: inj. sus. pow. ext. rel.

Pomade

It is the quasi-solid pharmaceutical form, for application onto the skin or in mucuous membrane, which consists of a solution or dispersion of one or more active ingredients in low proportions in a proper, usually non-aqueous, base. Abbreviation: pom.

Expiration date

It is the time through which the product may be used, characterized as the shelf life period and based on the specific stability studies. Abbreviation: exp.

The expiration date shall be indicated in the primary and secondary packages. When month and year are indicated, the expiration date is construed as the last day of this month. The conditions speficied by the manufacturer as for the storage and transportation must be maintained.

Quasi-solid topical preparation

It is the preparation expected for the application onto the skin or certain mucosas for local action or percutaneos penetration of medicines, or also for its moisturizing or protective action.

Aseptic process

This is the one designed to prevent the contamination of the sterile components by viable microorganisms or still in the intermediate manufacturing phase.

Hygiene product

It is the product for external use; antiseptic or not; intended to the body cleanliness or disinfection, comprised of the soap, shampoo, dentrifice, mouthwash, antiperspirant, deodorant, shaving products and post shave products, styptics and others. Abbreviation: hyg. pro.

Dietetic product

It is the product technically prepared to meet the dietetic needs of people under special physiological conditions. Abbreviation: diet. pro.

Semi-elaborated product

It is every substance or mixture of substances under the process of manufacturing.

Purity

Grade in which a permaceutical, raw material contains others foreign materials.

Raticide

It is the preparation intended to fight rats, mice and other rodents, at houses, ships, places and public use places, containing active, isolated or associated substances, which do not jeopardize men and useful hot blooded animal's life or health, when applied in compliance with the recommendations contained in its presentation.

Chemical reactions of identification

Those are reactions used in assisting the characterization of a substance. Although they are specific, they will only be sufficient to establish or confirm the identity of the substance, when they are considered together with other tests and specifications appearing in the monograph.

Unless the monograph specifies otherwise, the chemical reactions are made in approximately 15 mm internal diameter test tubes. 5 mL of the liquid or solution to be examined will be used, adding three drops of the reagent or of each reagent. The examination of the test tube contents must be made over the entire liquid layer, observing top-down, in the direction of the longitudinal axis of the tubes, after a five-minute rest.

Usually, the preference order of the identification tests is presented in the monograph. When the order does not appear, all of the identification tests must be performed.

Reagents

Those are substances used in tests, reactions, pharmacopoeic testing and dosing, as such or in solutions.

Well-closed vessel

This is the one which protects its contents from loss and contamination by foreign solids, under the usual conditions of manipulation, storage, distribution and transportation.

Hermetic vessel

This is the one which it air-tight, or any other gas-tight, under the usual conditions of manipulation, storage, distribution and transportation.

Opaque vessel

This is the one which precludes the view of the contents, comprehending all of the colors. It constitutes the protective barrier for luminosity.

Single dose vessel

This is the hermetic vessel which contains certain quantity of medicine intended to be administered in a single time and which, after it is open, may not be closed with sterility warranty.

Multiple dose vessel

This is the hermetic vessel which allows the removal of successive portions of its contents, without modifying the concentration, purity and sterility of the remaning portion.

Prefectly closed vessel

This is the one which protects its contents from loss and contamination of foreign solids, liquids and vapors, efflorescence, deliquescence or evaporation under the usual conditions of manipulation, storage, distribution and transportation.

Translucent vessel

This is the one which allows the partial view of the contents, comprehending all of the colors, except for amber.

Transparent vessel

This is the one which allows the total view of the contents, comprehending all of the colors, except for amber.

Registration

It is the registration, in own book after the concessive dispatch by the director of the Ministry of Health's agency, under the order number, of the products, with the indication

of name, manufacturer, origin, purpose and other elements characterizing it.

Hydrolytic resistance or alkalinity

It is the testing which quantifies the intensity of the chemical reaction between the water and the alkalin elements existing in,

the glass, particularly, sodium and potassium. This resistance determines the classification of the type of glass

Label

It is the printed or lithographed identification, as well as the wording painted or recorded by fire, pressure or autoadhesive, directly applied over the vessels, casings, bundles, cartridges or any other package protector, external or internal, and it may not be removed or changed during the use of the product and during its transportation or storage.

The manufacturing of the labels shall meet the standards in effect from the Federal Health Surveillance Agency.

Clean room

It is a room in which the concentration of particles in suspension in the air is controlled. It is built and used in order to minimize the introduction, genetation and retainment of particles within it, in which other relevant parameters, such as temperature, umidity and pressure are controlled as required.

Disinfecting product

This is the substance or preparation intended to the household hygienization, disinfection or disinfestations, as well as, collective environments, private or public, at common places and water treatment.

Human blood

It is a living, circulating, conjunctive, cellular, plasmatic or proteic origin tissue which is contained within the cardiovascular system, performing multiple and complex functions ensuring the human organism with life maintenance.

Transfusional human blood

It is the total human blood *in vitro* from healthy donors, collected in packaging systems for collection, storage and processing of the human blood containing anticoagulant, conservative and preservative solution.

Closed system

It is a system of parenteral solution administration which, throughout the prepration and administration, does not allow the contact of the solution with the environment.

Packaging systems for collection, storage and processing of the human blood or closed systems of human blood collection

They are vessels known or named as plastic bags containing or not an anticoagulant, conservative and preservative solution, intended to the collection, storage, fractionation and administration of the human blood or its derivates. They are atoxic, sterile, apyrogenic and disposable, and it may be manufacted from one or several polymers and, as may be the case of certain additives and are validated by their respective analytical methods.

Solution – pharmaceutical form

It is the liquid, limpid and homogenous pharmaceutical form, which contains one or more active ingredients dissolved into a proper solvent or in a mixture of miscible solvents. Abbreviation: sol.

Colorimetric solution

It is the solution used in the prepration of colorimetric standards for purposes of comparison. They are designated by “SC”.

Human albumin solution

Human albumin solution is a proteic, sterile and apyrogenic solutions obtained from human plasma which is in accordance with the requirements of the monograph *Human Plasma for Fractioning*.

Molal solution

It is the solution containing one mol of solute per kilogram of solvent.

Molar solution

It is the solution containing one molecule-gram of solute in 1000 mL of the solution. The multiples and submultiples of the molar solution are also designated by intergers or decimal fractions, such as: 2 *M*; *M*; 0.5 *M*; 0.1 *M*; etc.

Normal solution

It is the solution containing one equivalent gram of the solute in 1000 mL of the solution. The multiples and submultiples of the normal solution are also designated by intergers or decimal fractions, such as, 2 N, N; 0.5 N, 0.1 N, etc.

Volumetric solution

It is the solution of reagents, known concentration, intended to be used in certain quantitative. In FB 5, the concentrations of the volumetric solutions are expressed in molarity. They are designated by "SV".

Anticoagulant, conservative and preservative solutions of the human blood

Those are solutions intended to the collection of the human blood, aiming at not only making it incoagulable, but also ensuring the morphofunctional and proteic maintenance and integrity of its cell and plasmatic constituents.

Indicator solutions

They are solutions of indicators in specific solvents and defined concentrations. They are designated by "SI".

Reagent solutions

They are reagent solutions in specific solvents and defined concentrations. They are designated by "SR".

Hyperimmune sera for human use

The hyperimmune sera are preprations containing purified immunoglobulin, of animal origin, neutralizing specifically bacterial toxins, bacterias, viruses or toxic components of the posion of one or more species of poisonous animals.

Adjuvant substance

It is the substance with the specific purpose added to the injectable preparations. This substance must be selected in view of the increase of the product stability; not the interference in the therapeutic efficiency nor in the active ingredient dosing; nor even to cause toxicity in the quantity administered to the patient. The adjuvant substance may be solubilizing, antioxidant, chelating agent, buffer, antibacterial agent, antifungal agent, antifoam agent and others, when specified in the individual monograph. Abbreviation: adj. subs.

The presence of the adjuvant substance must be clearly indicated in the labels of the primary and secondary packages, in which the product is delivered to the consumption. In case there is no expressed contraindication, the air of the vessels may be replaced with carbon dioxide or nitrogen. The addition of dye is not allowed.

The maximum limites for some adjuvants are listed as follows, unless the monograph specifies otherwise:

a) for agents containing mercury or cationic tensoactive compounds – 0.01%;

b) for agents of the chlorobutanol, cresol and phenol type – 0.5%;

c) for sulfur dioxide or equivalent quantity of sulfite, bisulfite or metabisulfite of potassium or sodium – 0.2%.

Characterized chemical substance

SQR used in the inexistence of a Pharmacopoeic SQR. This SQR must be characterized by means of proper testing and the values obtained must be duly documented.

Reference Chemical Substance of the Brazilian Pharmacopoeia (SQR. FB)

It is established and made available by the Board of Officers of the Brazilian Pharmacopoeia, following WHO principles, and officialized by Anvisa; its use is mandatory throughout Brazilian territory. In the absence of a SQR FB, the use of SQR established by other acknowledged pharmacopoeias is allowed, as per the effective law.

The standards for Spectophotometry of the Atomic Absorption are identified by means of the naming of the metal, followed by the SRA (Reagent Solution for Atomic Absorption) accronym.

Working chemical substance

It is established by comparison with a Pharmacopoeic SQR, by means of pharmacopoeic testing, or duly validated and registered by the very laboratory which will use it. At this situation, the analytic records shall be maintained and periodic controls shall be perfofmed, deploying a Pharmacopoeic SQR.

Unsaponifiable substances

Unsaponifiable substances are the ones remaining to the reaction of the saponification, not volatile at 100 – 105°C and which were carried in the extraction process of the substance to be tested.

Suppository

It is the solid pharmaceutical form in several sizes and shapes adapted for introduction into the anal, vaginal or urethral orifice of the human body, containing one or more active ingredients dissolved into a proper base. They usually fuse themselves or dissolve at the body temperature. Abbreviation: supp.

Suspension

It is the liquid pharmaceutical form which constains solid particles dispersed into a liquid vechicle, in which the particles are not soluble. Abbreviation: sus.

Aerosol suspension

It is the suspension packaged under pressure, containing a propelling gas and therapeutically active ingredients which

are released after the activation of a proper valve system. Abbreviation: aer. sus.

Extended release suspension

It is the liquid pharmaceutical form which contains solid particles dispersed into a liquid vehicle, in which the particles are not soluble. See definition of extended release. Abbreviation: ext. rel. sus.

Delayed release suspension

It is the liquid pharmaceutical form which contains solid particles dispersed into a liquid vehicle, in which the particles are not soluble. See definition of delayed release. Abbreviation: del. rel. sus.

Drop suspension

It is the suspension intended to the administration in the form of drops. Abbreviation: dr. sus.

Injectable suspension

It is the sterile suspension. Abbreviation: inj. sus.

Extended release injectable suspension

It is the sterile suspension. See definition of extended release. Abbreviation: ext. rel. inj. sus.

Spray suspension

It is the suspension administered in the form of liquid finely divided by air or vapor blast. Abbreviation: spray sus.

Bar

It is the solid pharmaceutical form from any mass made with hydroalcoholic solution, the active ingredient and lactose, or from the wet milling itself into alcoholic solution. It is molded on trays and it is fragile and friable.

Buffer

It is the preparation based on salts which are able to support variations in the hydrogen ion activity.

Freezing temperature or point

Freezing temperature or point of cast liquid or solid is the highest temperature at which it solidifies.

For pure substances melting without decomposition, the freezing point of the liquid is equal to its melting point.

Boiling temperature or point

Boiling temperature or point of a liquid is the corrected temperature at which the liquid boils under vapor pressure of 101.3 kPa (760 mmHg).

Melting temperature or point

Melting temperature or point of a substance is the temperature at which it is completely molten.

Dye

It is the alcoholic or hydroalcoholic prepration resulting from the extraction of vegetable or animal drugs or the dilution of the respective extracts. It is classified as simple and compound, as prepared with one or more raw materials. Abbreviation: dye.

Unless it is otherwise indicated in the individual monograph, 10 mL of simple dye correspond to 1 g of dry drug.

Vaccines

They are biological products which contain one or more antigenic substances which, when they are inoculated, are able to induce specific active immunity and protect against disease caused by the infectious agent which originated the antigen.

D Value (decimal reducing time)

It is the time, in minutes, required to reduce the microbial population in 90% or a logarithmic cycle.

F_o Value 4

It is a measure of the sterilization efficiency, that is, the number of minutes of thermal sterilization by vapor to the certain temperature provided at a vessel or product unit, in a certain Z value.

Z value

It is the elevation of temperature, in degrees, required to recude *D Value* in 90% or produce a reduction of a logarithmic cycle in the thermal resistance curve.

Routes of administration

It is the place of the organism by which the medicine is administered.

Viscosity

It is the expression of the liquid resistance to the runoff, that its, to the displacement of part of its molecules over the neighbor molecules. The viscosity of the liquids comes from the internal friction, that is, from the cohesion forces between the relatively close molecules. By increasing the

temperature, the average kinectic energy of the molecues increases, the interval of time in which the molecues passes close together decreases (in average), the intermolecular forces and the viscosity become less effectives.

The dynamic unit, CGS system, of viscosity is the poise. CGS system of units is a unit system of physical measures, or dimensional system, of LMT typology (length, mass, time), whose base units are the centimeter for the length, gram for the mass and the second for the time.

Syrup

It is the aqueous pharmaceutical form characterized by the high viscosity which presets not less than 45% (w/w) of sucrose or other sugars in its composition. The syrups usually contain flavoring agents. Abbreviation: syr.

When it is not intended to the immediate consumption, it must be added with authorized antimicrobial preservatives.

GENERAL INFORMATION

Water

The water mentioned in the tests, reactions and testing is purified water. For injectable preparations, *water for injectables* must be used, described in individual monograph. When the use of *carbon dioxide-free water* is prescribed, used purified water boiled during, as a minimum, five minutes and protected from the atmospheric air during the cooling and storage.

Volumetric devices

The volumetric devices are deployed in the volume measurements in the tests, in testing and in pharmacopoeic dosing, and they must be checked for the temperature of 25°C. In case the volumetric device is not checked for 25°C, the volume measurements must be performed at the temperature indicated in it.

In the volume measurements, the lower level of the liquid meniscus contained in the volumetric devices must be tangent to the upper part of the reference line, with the view line at the same plan. In the cases of strongly colored or opaque liquids, the upper edge of the meniscus is used as reference, in the horizontal view plan.

The volumetric devices for liquid transfer (pipettes or burettes), because they have been checked with water, will only be able to provide exactly the indicated volume when the liquids to be measured have approximately the viscosity, surface tension and density of the water.

Conservation

The pharmacopoeic substances must be conserved under conditions, such as those which avoid their contamination or deterioration. The conservation conditions of pharmacopoeic substances appear in the respective monographs.

Protecting from the light means that the substance must be conserved in opaque vessel, or vessel which is able to prevent the action of the light.

Protecting from the dust means that the substance must be kept in corked vial and use protective hood

In the monograph, there may be defined the conditions of temperature in which the substance must be conserved, by using the terms described as follows:

In freezer – At temperature between -20 ºC and 0 ºC.

In refrigerator – At temperature between 2 ºC and 8 ºC.

Fresh place – Environment in which the temperature remains between 8 ºC and 15 ºC.

Cold place – Environment in which the temperature does not exceed 8 ºC.

Room temperature – Temperature, normally found at the working environment, between 15 ºC amd 30 ºC.

Hot place – Environment in which the temperature remains between 30 ºC and 40 ºC.

Excessive heat – It indicates temperatures higher than 40 ºC.

When it is required to conserve a pharmaceutical at fresh place, it may be conserved in refrigerator, unless it is otherwise indicated in the individual monograph.

When, in the monograph, the conditions of conservation are not specified, they include the protection against umidity, freezing and excessive heat.

Substance description

The information referring to the description of a substance is generic and intended to the preliminary assessment of its integrity. The description itself does not indicate purity, and it must be associated to other pharmacopoeic tests, in order to ensure that the substance is in accordance with the monograph.

Desiccation until constant weight

This expression means that the drying must be proceeded until two consecutive weighing do nto differ in more than 0.5 mg per gram of the testing substance, and the second weighing must be performed after one hour of the additional drying, under the specified conditions.

Desiccator

The diseccator is described as a vessel which may be perfectly closed, in proper shape and dimensions which enable keeping the low umidity atmosphere by means

of diseccating agents introduced into it, such as: silica gel, anhydrous calcium chloride, phosphorus pentoxide, sulfuric acid, among others.

Reduced pressure diseccator is the one which enables keeping the low umidity atmosphere to the reduced pressure not higher than 6.7 kPa (approximately 50 mm of mercury), or to the pressure indicated in the monograph.

Potency dosage and determination

When the result of a testing or dosage is expressed in relation to the dry substance; in relation to the substance'or any other specific basis, the determination of the loss by drying, contents of water or other designated property is performed in accordance with the method described in the respective testing in the monograph of the substance in cause, or pursuant to the description in the labeling.

Identification testing

The identification testing enables verifying, with an acceptable certainty level, that the material identity under examination is in

accordance with its package label. Yet specific, they are not necessarily sufficient to establish absolute evidence of identity. However, the non-compliance with the requirements of an identification testing may mean the mis-labeling of the material. Other tests and specifications in the monograph contribute to the confirmation of the identity of the examined article..

Some identification testing must be considered as conclusive, such as: infrared; spectrophotometry with specific absorption and high performance liquid chromatography coupled to the spectophotometry. This testing must be performed as a complement to the ion testing, when applicable.

Monograph structure

The raw material monographs are identified by their common Brazilian names (DCB), written in capital letters and centered. In addition, the following are also included:

- whenever possible, the Latin name proposed by INN – International Non-proprietary Names from World Health Organization;
- the structural formula of the substance;
- molecular formula followed by molar mass;
- Common Brazilian Name and its respective number;
- chemical name, as per ACS – American Chemical Society;
- CAS – Chemical Abstracts Service registration;
- monograph text.

The monographs of the pharmaceutical preprations are identified by the corresponding raw material ane, followed by the name of the pharmaceutical form.

Expression of the concentrations

The concentrations in percentage are expressed as follows.

W/w (weight on weight) percent or % w/w – It expresses the number of grams of a component in 100g of mixture

W/v (weight on volume) percent or % w/v – It expresses the number of grams of a component in 100 mL of solution.

V/v (volume on volume) percent or % v/v – It expresses the number of mL of a component in 100 mL of solution.

V/w (volume on weight) percent or % v/w – It expresses the number of mL of a component in 100 g of misture.

The expression percent, used without any other attribution, means: solids and quasi-solids mixture, w/w percent; for solutions or suspensions of solids into liquids, w/v percent; for solutions of liquids, v/v percent; for solutions of gases into liquids, w/v percent; in order to express the contents of essential oils into vegetable drugs, v/w percent.

Impurities

The tests described in the monograph limit the impurities to quantities ensuring the quality to the pharmaceutical. The fact of the testing does not include any less frequent impurity does not mean it may be tolerated.

Incineration until constant weight

This expression means that the incineration must proceed at 800 + 25°C, or at any other temperature indicated in the monograph, until two consecutive weighings do not differ in more than 0.5 mg per gram of the testing substance, and the second weighing must be performed fifteen minutes after the additional incineration.

Interpretation of the accuracy of the numeric data and tolerance limits

The accuracy desired in the tests, reactions and pharmacopoeic testing is indicated by the number of decimals which is presented in the text. For example, the numeric number 20 indicates that values not lower than 19.5 and not higher than 20.5; the numeric value 2.0 indicates values not lower than 1.95 and not higher than 2.05; the numeric value 0.20 indicates values not lower than 0.195 and not higher than 0.205.

The tolerance limits, numerically expressed by a maximum and minimum value, indicate the purity of a pharmacopoeic substance. These values may be expressed as percentage or absolute numbers.

The variation range must be strictly observed, and values out of the maximum and minimum limits are not tolerated.

Primary and secondary packaging material

Packaging material is understood as the vessel, bundle, casing or any other form of protection, whether it is removable or not, used to package, protect, maintain, or cover particularly or not raw materials, reagents and medicines.

Primary packaging material is the ine which is in direct contact with its contents throughout the time. It is considered as primary packaging material: ampoule; tube; envelope; case; flacon; glass or plastic vial; vial-ampoule; cartridge; can; pot; paper bag and others.

Secondary packaging is the one which is intended to the full protection of the packaging material under the usual conditions of transportation, storage and distribution. It is considered as secondary package: cartons, cartridge of pasteboard, wood or plastic material or cases of pasteboard and others. There must not be any interaction between the primary packaging material and its contents able to change the concentration, quality or purity of the packaged material. The packaging conditions are described in the individual monographs, by using the terms listed as follows

Well-closed vessel – It is the one which protects its contents of loss and contamination by foreign solids, under the usual conditions of manipulation, storage, distribution and transportation.

Perfectly closed vessel – It is the one which protects its contents of losses and contamination by foreign solids, liquids and vapors, efflorescence, deliquescence or evaporation under the usual conditions of manipulation, storage, distribution and transportation.

Hermetic vessel – It is the one airtight, or any another vapor-tight, under the usual conditions of manipulation, storage, distribution and transportation.

Gas cylinder – It is the metallic vessel, perfectly closed, with resistant walls, intended to contain gas under pressure, plugged by regulating valve, able to maintain the gas output under determined flow rate..

Single dose vessel – This is the hermetic vessel which contains certain quantity of medicine intended to be administered in a single time and which, after it is open, may not be closed with sterility warranty.

Multiple dose vessel – This is the hermetic vessel which allows the removal of successive portions of its contents, without modifying the concentration, purity and sterility of the remaning portion.

Pressure measurements

The pascal expression (Pa), used for pressure measurements, such as the arterial, atmospheric or internal measurements of a device refers to the use of manometers or barometers calibrated in relation to the pressure exercised by the force of 1 Newton distributed in an uniform manner over a flat surface of $1m^2$ in perpendicular area towards the force; 1 pascal is equivalent to 7.5×10^{-3} mmHg.

Odor

The expressions: *inodorous; practically inodorous; slight characteristic odor*; or their variations are used by examining the sample after being exposed to air for fifteen minutes, when they are 25g packages recently opened. In case of bigger packages, transfer samples of approximately 25g for 100 mL capsule.

The odor characterization is just descriptive and it may be considered as purity standard, except for cases in which a particular unallowed odor is indicated in the individual monograph.

Preparation of solutions

All of the solutions used in tests, testing and reactions are prepared with purified water, unless it is otherwise indicated in the individual monograph.

The expression *recently prepared*, referring to the preparation of solutions used in tests, testing and reactions, indicates that the solution must be prepared, as a maximum, 24 horas before the preparation of the testing.

Reduced pressure

The expression *reduced pressure* means a pressure lower than or equal to 6.7 kPa (approximately 50 mmHg), unless it is otherwise indicated in the monograph. When *diseccation under reduced pressure over diseccating agent* is indicated in the monograph, the operation must be made under reduced pressure in diseccator or other proper device.

Manufacturing process

Whatever is the method used, the final product must correspond to the specifications included in the Brazilian Pharmacopoeia, 5th edition.

When manufacturing injectable products, tablets, capsules or other pharmacopoeic preparations, the use of adjuvant substances is allowed, described in the monographs and added with specific purpose. They must be innocuous and they must not have any adverse influence on the therapeutic effieciency of the active substance contained in the preparation, or even interfere in testing and determinations.

Blank solution

The expressions: *execute parallel blank; perform blank solution;* or *perform blank testing* mean to repeat the determination under identical conditions and with identical quantities of reagents, omitting only the testing substance.

Vessels for injectables

The vessels for injectable preparations must be manufactured with materials which do not cause any interaction with the contents or have sufficient transparency to allow visual inspection. The caps, when used, may not influence the composition or conservation of the medicine, offering perfect sealing, even after being pierced several times.

The vessels for injectable preparations are classified as:

- single dose vessels;
- multiple dose vessels;
- perfusion vessels.

The single dose vessels, ampoules and dentistry cartridge are proper glass or plastic vials, closed by melting glass or using fixed or movable operculum. The contents must only be used in a single dose, and it may not be reused.

The multiple dose vessels are glass vials with resistant walls which, after being filled with liquid preparations or solids to be dissolved or suspended, are seals with other material cap. The contents of these vials may be removed for administration in a single dose or in multiple ones.

The perfusion vessels are vials with more than 50 mL in capacity and they may reach 1000 mL, either sealed with other material cap or not, manufactured from glass or plastic. The medicines packaged in these types of vessels must be administered in a single time, by using sterile equipment and they may not contain bactericide or antifungal agents. The use of other types of adjuvants must be carefully considered.

Solubility

The indicated solubility must not be taken in the strict sense of physical constant, however, it complements and confirms further testing, being possible to have a definite value, in case the substance does not present the minimum required solubility, particularly in the solvent water.

The indications about the solubility refer to the determinations made to the 25°C temperature. The expression *solvent* refers to the water, unless it is otherwise indicated in the individual monograph.

The expression *parts* refers to the dissolution of 1 g of a solid into the number of millimeters of the solvent established in the number of parts.

The approximate solubilities appearing in the monographs are assigned by descriptive terms, whose meanings are listed in **Table 1**.

Table 1 – Descriptive terms of solubility and their meanings

Solvent	*Descriptive term*
Very soluble	less than 1 part
Easily soluble	1 to 10 parts
Soluble	10 to 30 parts
Slightly soluble	30 to 100 parts
Little soluble	100 to 1000 parts
Hardly soluble	1000 to 1.000 parts
Pratically insoluble or insoluble	more than 1.000 parts

Temperature

All of the temperatures appearing in the FB 5 are expressed in Celsius scale, and the measurements are made at 25°C, except for the density measurement and unless it is otherwise indicated in the individual monograph.

Measurement units

The units appearing in the International System of Units (IS) are adopted in this Pharmacopoeia, as listed in **Attachment B.**

Aqueous

Water for injectables is generally sued as vehicle for aqueous injectabes. *Sodium chloride* solutions or *Ringer's solution*, or other proper solutions prepared with *water for injectables* may be used in part or full instead of only *water for injectables*, unless the monograph specifies otherwise.

Nonaqueous vehicles

Nonaqueous vehicles partially or totally used in the obtainment of injectable preparations may be miscible or immiscible with water. Among the vehicles miscible with water, the most used vehicles are the polyalcohols and polymers of the ethylene oxide. Among the vehicles immiscible with water, the most used vehicles are the fixed vegetable oils and the mono- and diglycerides of fatty acids.

The fixed oils are inodorous or almost inodours and their odor and flavor must remember rank smell. They must meet the requirements specified in the monographs and present the characteristics described as follows.

a) cooling test – transfer quantity of fixed oil, previously diseccated at 105°C for two hours and cooled at room temperature in diseccator containing silica gel, to incolor cylindric glass vessel, with internal diameter of approximately 25 mm. Close the vessel and dip it for four hours into water maintained at 10°C. The liquid must remain sufficiently limpid, so that a 0.5 mm thick black line may be seen when it is vertically maintained behind the cylinder and agains a white background;

b) saponification index – between 185 and 200 **(5.5.29.8);**

c) iodine index – between 79 and 128 **(5.5.29.10**);

d) unsaponifiable substances – reflux 10 mL of the oilin bain- marie with 15 mL of sodium hydroxide (1:16) and 30 mL of ethyl alcohol, occasionally shaking until the mixture is clear. Tranfer the mixture to the china capsule, evaporate the ethyl alcohol in bain-marie and mix the residue with 10 mL of water. The solution must be the result;

e) free fatty acids – the free fatty acids in 10 g of the oil must consume, as a maximum, 2 mL of the 0.02 *M* sodium hydroxide

Synthetic mono or diglycerides of fatty acids must fulfill the following requirements:

a) they are liquids and remain limpid when cooled at 10 ºC;

b) iodine index – not higher than 140 **(5.5.29.10).**

The nonaqueous vehicles must be selected with special care, because they cannot be irritating, toxic or sensitizing and they must not interfere on the therapeutic efficiency of the prepration. In exceptional cases, very known names, other than those adopted by Common Brazilian Names for Pharmaceuticals, may be quoted as “other names”.

5 GENERAL METHODS

5.1 GENERAL METHODS APPLIED TO DRUGS

5.1.1 WEIGHT DETERMINATION

This assay is applied to solid pharmaceutical unit dosage forms (uncoated tablets, coated tablets, hard and soft shell capsules and suppositories), solid dosage forms stored in unit dose vessels (sterile talc powders, lyophilized powders, powders for injection and reconstituted powder for oral administration) and solid and semisolid dosage dosage forms stored in multiple-dose vessels (granules, powders, gels, creams, ointments and powders for reconstitution).

The weighing is performed in scales with suitable sensibility.

PROCEDURE FOR unit dose PRODUCTS

For unit dose produts, the assay allows checking if the units of the same batch features the same weight. In order to carry out the assay, it is required to previously establish the weighted mean of the batch units.

Uncoated tablets or film-coated tablets

Individually weigh 20 tablets and establish the weighted mean. In regard to the weighted mean, only two units outside the limits set out in **Table 1** can be tolerated, however, no unit can be more or less than twice the percentages indicated.

Sugar-coating tablets (dragees)

Individually weigh 20 dragees and establish the weighted mean. In regard to the weighted mean, only five units outside the limits set out in **Table 1** can be tolerated, however, no unit can be more or less than twice the percentages indicated.

Hard capsules

Individually weigh 20 units, remove the content of each one, clean and weight again. Establish the weight of the content of each capsule by

the weight difference between the empty capsule and the full capsule. Establish the weighted mean of the content using the values obtained. In regard to the weighted mean, only two units outside the limits set out in **Table 1** can be tolerated, however, no unit can be more or less than twice the percentages indicated.

Soft capsules

Proceed as described in the *Hard capsules* paragraph. To establish the weighted mean of the content, cut the capsules which were previously weighted and wash them with ethyl ether or other suitable solvent. Leave the shells exposed to air, in a room temperature, until the solvent is totally evaporated. Weigh again.

Suppositories and ovules

Individually weigh 20 suppositories and ovules and establish the weighted mean. In regard to the weighted mean, only two units outside the limits set out in **Table 1** can be tolerated, however, no unit can be more or less than twice the percentages indicated.

Sterile powders, lyophilized powders and powders for injection

Carry out the assay with 20 units. Remove the metal seals, in case of injection vials. Remove the labels which may be damaged during the assay. Dry, if necessary, the external surface of the vessels. Individually weigh the 20 units with the respective seals. Remove the content and wash the respective vessels using water and then ethanol. Dry in oven at 105 ° C for 1 hour, or at lower temperatures, depending on the nature of the material, until the constant weight. Cool at room temperature, put the seal again and reweigh. The difference between the two weighings represents the content weight. Establish the weighted mean of the content of the 20 units. In regard to the weighted mean, only two units outside the limits set out in **Table 1** can be tolerated, however, no unit can be more or less than twice the percentages indicated.

Powders for reconstitution (oral administration)

Proceed as described in the *Sterile powders, lyophilized powders and powders for injection* paragraph. In regard to the weighted mean, only two units outside the limits set out in **Table 1** can be tolerated, however, no unit can be more or less than twice the percentages indicated.

Table 1 – Evaluation criteria for the weight establishment ofsolid pharmaceutical unit dosage forms.

Pharmaceutical unit dosage forms	*Weighted mean*	*Limits of variation*
Uncoated tablets or film-coated tablets, effervescent tablets, sublingual tablets, vaginal tablets and lozenges	80mg or lower higher than 80 mg and lower than 250 g 250 mg or more	± 10.0% ± 7.5% ± 5.0%
Sugar-coating tablets (dragees)	25 mg or lower more than 25 mg and up to 150 mg more than 150 mg and ower than 300 mg 300 mg or more	±15.0% ± 10.0% ± 7.5% ± 5,0%
Hard capsules and soft capsules, vaginal capsules	lower than 300 mg 300 mg or more	± 10.0% ± 7.5%
Suppositories and ovules	Independent from the weighted mean	± 5,0 %
Sterile powders, lyophilized powders and powders for injection	more than 40 mg*	± 10.0%
Powders for reconstitution (oral administration)	lower than 300 mg	± 10.0%
	300 mg or more	7,5%

(*) If the weighted mean is 40 mg or lower, submit to the assay *for uniformity of dosage units* **(5.1.6)**.

5

PROCEDURE FOR MULTIPLE-DOSE PRODUCTS

For products stored in vessels for multiple doses, the assay allows checking the homogeneity in the vessel.

Powders for reconstitution (oral and parenteral administration)

Individually weigh 10 units. Remove the content and wash the respective vessels using a suitable solvent. Dry, cool to room temperature and weigh again. The difference between the two weighings represents the content weight.

Establish the weighted mean of the content of the 10 units. The individual values do not differ ±10% in relation to the weighted mean.

Granules, powders, gels, creams and ointments

Note:it is required to know the nominal amount of the vessel before performing the assay.

Individually weigh 10 units. Remove the content and wash the respective vessels using a suitable solvent. Dry, cool to room temperature and weigh again. The difference between the two weighings represents the content weight.

Establish the weighted mean of the content of the 10 units. The weighted mean of the contents is not lower than the weight stated and the individual weight of any of the tested units is lower than the percentage indicated in **Table 2**, in relation to the weight stated.

In case this requirement is not complied, establish the content individual weight of the 20 additional units. The content weighted mean of the 30 units is not lower than the weight stated, and the individual weight of one unit in 30 is lower than the percentage indicated in **Table 2**, in relation to the weight stated.

Table 2 – Evaluation criteria for the weight establishment of pharmaceutical multiple-dose forms.

Pharmaceutical multiple-dose forms	*Stated weight*	*Minimal percentage in relation to the stated weight*
Granules, powders, gels, creams and ointments	up to 60 g more than 60 g and up to 50 g more than 150.0 g	90.0% 92.5% 95.0%

5.1.2 WEIGHT DETERMINATION

The volume determination assay is required for liquid products in multiple-dose vessels and single-dose vessels. The assay is applied to liquid preparations and also to liquid preparations obtained from powders for reconstitution. The assay is not required for liquid products in single-dose vessels when the requirement for *Uniformity of dosage units* **(5.1.6)** is present in the individual monograph.

PROCEDURE

Liquid products in multiple-dose vessels (except injections)

Separate 10 units. Remove the metal seals, if necessary. Remove the labels which may be damaged during the assay. Individually weigh each vessel with the respective seals. Mix thoroughly, remove and gather the contents and reserve to determine the mass density. Wash the vessels and seals with water and then, with ethanol. Dry in oven at 105 °C for 1 hour, or at temperatures compatible with the vessel material, until the constant weight. Cool to room temperature, put the seal and the other parts and weigh again. The difference between the two weighings represents the content weight. Establish the corresponding individual volumes (V), in mL, using the expression:

$$V = \frac{m}{p}$$

where as

m = content weight, ing;
ρ = product mass density, in g/mL, established at 20 ºC, as described in*Establishment of the mass density and relative density* **(5.2.5).**

Liquid products in single-dose vessels (except injections)

Separate 10 units. Separately pour the content of each unit on dry graduated measuring cylinders, with a capacity which does not exceed 2.5 times the volume to be measured, taking care to avoid the bubble formation. The liquid must drain for 5 seconds, unless otherwise indicated in the individual monograph. Perform the measurement.

From the values obtained, calculate the average volume of the tested units. The average volume is not less than the volume stated, and the individual volume of any unit tested is not less than 95.0% or more than 110.0% of the volume stated.

Liquid injections

The assay is applied to liquid injections stored in vessels such as ampoules, vials, plastic bags, plastic vials, carpules or prefilled syringes. The vessels are filled with small excess volume, according to the characteristics of the product, to allow administration of the volume stated. Minimum excess volume recommended in **Table 1** is usually enough to allow the removal and administration of the volume stated.

Table 1 – Volume excess recommend ed for liquid injections.

Volume declarado (mL)	*Excesso mínimo de volume recomendado*	
	móveis / mL	*viscosos / mL*
0,5	0,10	0,12
1,0	0,10	0,15
2,0	0,15	0,25
3,0	0,20	0,35
4,0	0,25	0,45
5,0	0,30	0,50
10,0	0,50	0,70
20,0	0,60	0,90
30,0	0,80	1,20
50,0 ou mais	2%	3%

From the values obtained, calculate the average volume of the tested units. The average volume is not less than the volume stated, and the individual volume of any unit tested is not less than 95.0% of the volume stated.

Liquid products in vessels for multiple doses obtained from powders for reconstitution (except injections)

Separate 10 units. Re-establish each unit as indicated in the label. Proceed as described in *products in vessels for multiple doses obtained from powders for reconstitution (except injections).*

From the values obtained, calculate the average volume of the tested units. The average volume is not less than the volume stated, and the individual volume of any unit tested is not less than 95.0% or more than 110.0% of the volume state.

Suspensions and emulsions must be agitated before the removing the content and the density establishment. Oily preparations or highly viscous preparations can be heated, if necessary, according to the label directions or at 37 °C and vigorously agitated before removing the content. The contents are then cooled from 20 °C to 25 ° C before measuring the volume.

For injections in single-dose vessels, assay 6 units if the volume stated is equal to or higher than 10 mL, 10 units if the volume stated is higher than 3 mL but lower than 10 ml, or 12 units if the stated volume is equal to or lower than 3 mL. Remove all the contents of each unit with the aid of a syringe with a capacity not exceeding three times the volume to be measured, provided with a needle

#21 with at least 2.5cm in length. Eliminate any bubbles in the needle and syringe and transfer the contents of the

syringe, without emptying the needle, to a dry graduated measuring cylinder, with a capacity which does not exceed 2.5 times the volume to be measured. Alternatively, the syringe may be transferred to dry and tared beaker, the volume calculated by the weight of the liquid, in grams, divided by its density. For vessels with a stated volume of 2 mL or less, the contents of the vessels may be gathered to obtain the volume required for the measurement and dry syringes and needles must be used separately for each vessel. The content of vessels with a stated volume of 10 mL or more can be determined by emptying the contents of each vessel directly into tared beakers or graduated measuring cylinders.

The volume of each examined vessel is not lower than the volume stated. In case of vessels with a stated volume of 2 mL or less, the volume of the contents together is not less than the sum of the volumes declared of the vessels used for the assay.

For injections in multiple-dose vessels labeled to contain a specific number of doses of a given volume, select an unit and proceed as described for injection in injections in single-dose vessels, using separate syringes and needles equal to the number of doses specified on the label. The volume dispensed by each syringe volume is not less than the dose stated.

For injections in cartridges or prefilled syringes, assay one unit if the volume stated is equal to or higher than 10 mL, 3 units if the volume stated is higher than 3 mL but lower than 10 ml, or 5 units if the stated volume is equal to or lower than 3 mL. Adjust to the vessels the accessories required for the use (needle, plunger, syringe barrel), if applicable, and transfer the content of each vessel, without emptying the needle, to the dry and tared beaker, slow and regularly pushing the plunger. Calculate the volume in milliliters dividing the liquid weight, in grams, by its density. The volume of each vessel is not lower than the volume stated.

For large volume injections (parenteral infusions), select two units and transfer the contents of each vessel to dry graduated measuring cylinders, with a capacity which does not exceed 2.5 times the volume to be measured. The volume of each vessel is not lower than the volume stated.

5.1.3 DETERMINATION OF THE MECHANICAL STRENGTH OF TABLETS

Mechanical strength tests, as hardness and friability, are considered as official tests according to the legal context of this Pharmacopeia, becoming useful elements in the overall quality evaluation of tablets. The purpose of these tests is to show the tablet strength to the rupture caused by falls or friction.

5.1.3.1 HARDNESS ASSAY

The hardness assay establishes the tablet resistance to crushing or rupture, under radial stress. The hardness of a tablet is proportional to the compression strength and inversely proportional to its porosity. The assay is mainly applied to uncoated tablets.

The assay consists of subjecting the tablet to the action of a device that measures the diametrically applied force necessary to crush. The strength is measured in newtons (N).

APPARATUS

Several types of apparatus may be used, they are basically different in relation to the mechanism applied to put the pressure on. The strength may be manual or mechanical. As the pressure increases, a plunger, a plate or a piston applies a certain force to the tablet, withstanded in the fixed base. The apparatus is calibrated with precision of 1 N.

PROCEDURE

The assay is carried out with 10 tablets, removing any superficial residue before every establishment. The tablets are individually tested always according to the same matrix (consider the shape, presence of groove and recording). Show the result as the average mean of the values obtained from the establishments. The assay result is only for information purposes.

5.1.3.2 FRIABILITY ASSAY

The friability assay evaluates the ability of the tablet to withstand abrasion in mechanical actions of a specific apparatus. The assay is only applied to uncoated tablets.

The assay weighes a certain number of tablets, which are placed in the apparatus and removed after 100 revolutions. After removing any powder residue from the tablets, they are weighed again. The difference between the initial and the final weight is the friability, the value is expressed as a percentage of the lost powder.

APPARATUS

The apparatus (**Image 1**) is a rotating cylinder, with 287.0 ± 4.0 mm of diameter and 38.0 ± 2.0 of depth, formed by a transparent synthetic polymer provided with polished internal faces with a low static activity, which rotates around its own axis at a speed of 25 ± 1 rotations per minute. One of the cylinder faces is removable. The tablets are collected every turn of the cylinder by a curved projection with the inner radius of 80.5 ± 5.0 mm extending from the center to the outer wall of the cylinder, and are lifted to a height of 156.0 ± 2.0 mm, from where they fall repeatedly.

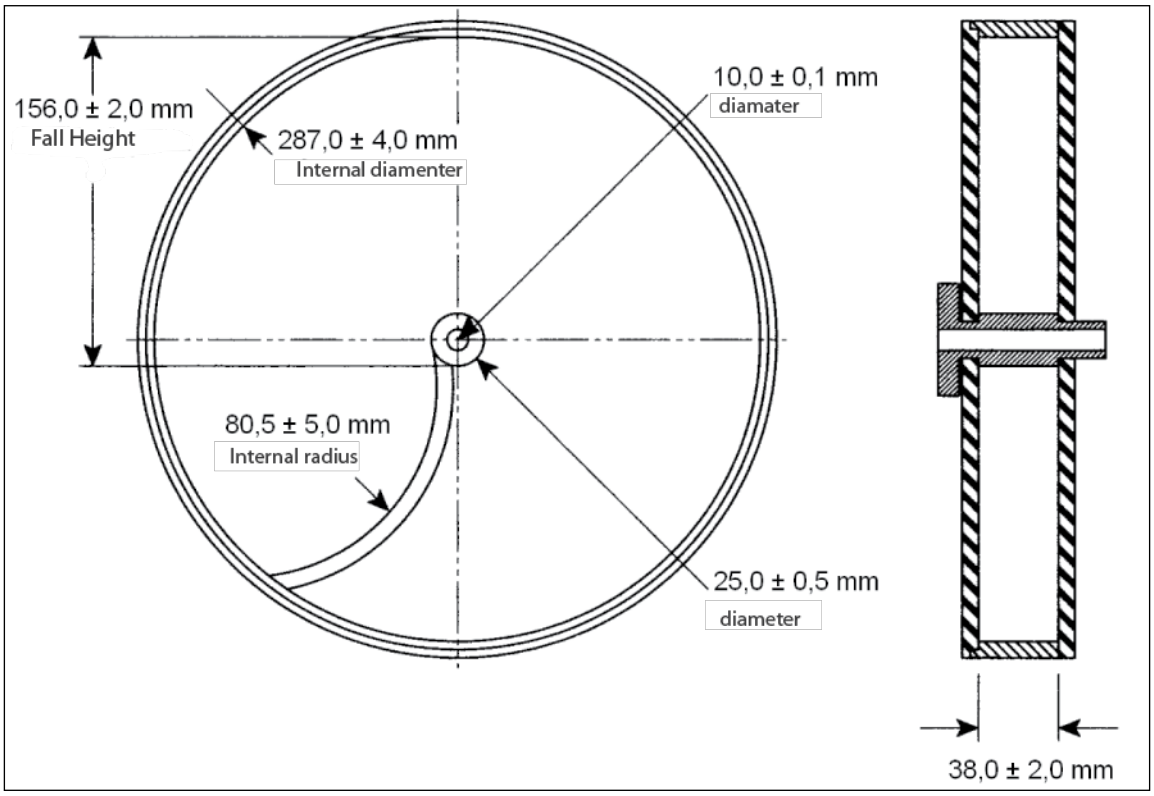

Image 1 – Apparatus for the friability assay (friabilometer).

PROCEDURE

For tablets with weighted mean equal to or less than 0.65g, use 20 tablets. For tablets with weighted mean higher than 0.65g, use 10 tablets. Precisely weigh the tablets and put them into the apparatus. Ajust the speed to 25 rotations per minute and the assay time for 4 minutes. After the 4 minutes, remove any power residue of the tablet surface and weigh again. By the end of the assay, no tablet can be broken, splited, cracked or shattered. Tablets with loss equal to or less than 1.5% of the weight or the percentage established in the monograph are acceptable. If the result is questionable or if the loss is higher than the limit specified, carry out the assay twice, considering the average result of the three determinations obtained from the evaluation.

5.1.4 DESINTEGRATION TESTS

5.1.4.1 DESINTEGRATION ASSAY FOR TABLETS AND CAPSULES

The desintegration tests are performed to find out if tablets and capsules completely desintegrate within the specified time, when six units of the batch are subject to the action of the specific apparatus under the defined experimental conditions.

The assay is applied to uncoated tablets, film-coated tablets, sugar-coating tablets (dragees), enteric-coated tablets, sublingual tablets, soluble tablets, dispersible tablets, hard capsules and soft capsules. The assay can also be applied to chewable tablets. In this case, the conditions and criteria for evaluation will be shown in the individual monograph. The assay is not applied to tablets and pills or controlled release capsules (extended).

For the purposes of this assay the disintegration is defined as the state in which any residue of the tested units (capsules or tablets) remains on the wire mesh of the disintegration apparatus, except insoluble fragments of the coating of tablets or capsule shells. Units are considered desintegrated when they become a doughy mass during the assay, since they do not show a palpable core.

APPARATUS

The apparatus consists of a basket and tube system (**Image 1**), provided with a suitable vessel for immersion liquid (a beaker with a capacity of 1 liter), a thermostat to keep the fluid at 37 ± 1 ° C and a mechanism to vertically move the basket and tubes in the immersion liquid, with constant frequency and specific route. The volume of the immersion liquid should be enough, by reaching the highest point of the route,for the bottom of the basket to be at least 25 mm below the liquid surface, and that in the lowest point, at least 25 mm from the bottom of the beaker. The upward

and downward movements should have the same speed and the change of the movement direction should be smooth.

The basket consists of six tubes made of glass or transparent acrylic, open on both sides. The tube dimensions are:length 77.5 ± 2.5mm, internal diameter between 20.7mm and 23.0mm and a wall thickness of approximately 2mm.

The tubes are held vertically, adapting the at each end of the basket a suitable transparent disk, with a diameter between 88.0mm and 92.0mm and thickness between 5.0mm and 8.5mm, with six holes in which the tubes are inserted. The six holes are equidistant from the center of each disk, equally spaced from one another. On the external face of the lower disk, there is a stainless steel wire mesh (diameter: 0.635.0.030mm) with an opening of 1.8 mm and 2.2 mm, fastened by three screws.

To assay the disintegration of capsules, a stainless steel wire mesh similar to the one adapted to the lower disc of the basket or other suitable device, may be adapted to the external face of the upper disc to prevent the capsules from escaping from the tubes during the assay.

The parts of the basket are assembled and firmly held together by a metallic central shaft with a diameter of about 5mm. The upper end of the central shaft must have a device for securing the basket to the mechanism that produces the vertical movement of the system.

When indicated, a suitable transparent cylindric disk should be added to each tube of the basket, with a relative density between 1.18 and 1.20, diameter of 20.70 ± 0.15 mm, and a thickness of 9.50 ± 0,15 mm. Each disk has five holes, with 2mm in diameter each, with one hole in the cylinder axis and the other four are equidistant, arranged on a circle of radius of 6 mm relative to the center of the disk. The side surface of the disk has four equidistant V-shape indentations, with a depth of 2.6 ± 0.1 mm, which, on the upper side of the disc, have 9.4 ± 0.2 mm width, and on the lower side, 1.6 mm. All disc surfaces are smooth. The design and assembly of basket may vary since the specifications for the tubes and the mesh openings are maintained.

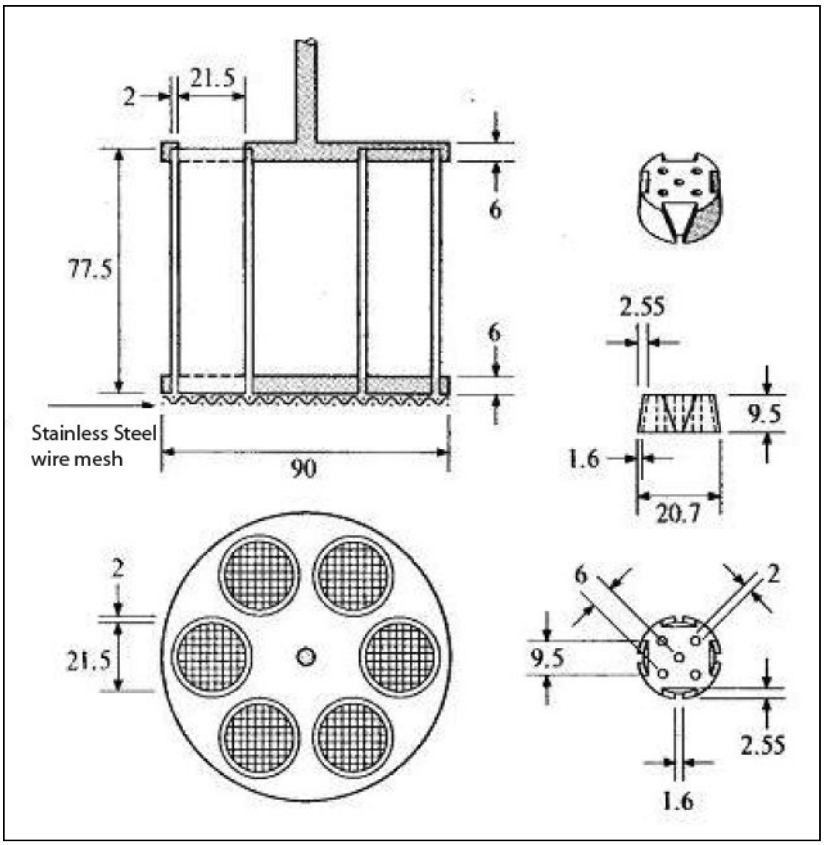

Fig 1 – Apparatus for testing the disintegration of tablets and capsules (dimensions in mm).

PROCEDURE

Uncoated tablets

Use six tablets in the assay. Place a tablet in each of the six tubes of the basket, add a disc to each tube and start the device, using water maintained at 37 ± 1 °C as the immersion liquid, unless another liquid is specified in the drug monograph.

At the end of the specified time interval, stop the basket movement and observe the material in each tube. All tablets should be completely disintegrated. If the tablets do not disintegrate due to the adherence to the disks, repeat the assay with other six tablets, omitting the disks. All tablets should be completely disintegrated by the end of the assay. The time limit established as a general criterion for the disintegration of uncoated

tablets is 30 minutes, unless otherwise stated in the individual monograph.

Sugar-coating tablets (dragees) or film-coated tablets

Use six tablets in the assay. Place a tablet in each of the six tubes of the basket. Add a disc to each tube and start the device, using water maintained at 37 ± 1 °C as the immersion liquid. At the end of the specified time interval, stop the basket movement and observe the material in each tube. If the tablets do not disintegrate due to the adherence to the disks, repeat the assay with other six tablets, omitting the disks, replacing the water for hydrochloric acid 0,1 *M*, maintained at 37 ± 1 °C as the immersion liquid. At the end of the specified time interval, stop the basket movement and observe the material in each tube. All tablets should be completely disintegrated. If the tablets do not disintegrate due to the adherence to the disks, repeat the assay with other six tablets, omitting the disks. All tablets should be completely disintegrated by the end of the assay. The time limit established as a general criterion for disintegrating film-coated tablets is 30 minutes, and sugar-coating tablets (dragees) is 60 minutes, unless otherwise stated in the individual monograph.

Enteric-coated tablets or capsules (gastro-resistant)

Use six units in the assay. Place an unit in each of the six tubes of the basket. Start the apparatus, without starting the disks, using hydrochloric acid 0,1 *M*, maintained at 37 ± 1 °C as the immersion liquid for 60 minutes or during the time stated in the individual monograph. Stop the basket movement and observe the tablets or capsules. No unit can feature any sign of disintegration, cracking, or softening which allows the extravasation of its contents. Add a disc to each tube and start the device, using phosphate buffer solution pH 6.8 maintained at 37 ± 1 °C as the immersion liquid. After 45 minutes or at the end of the specified time stated in the monopgraph, stop the basket movement and observe the material in each tube. All tablets or capsules should be completely disintegrated, and only fragments of insoluble coating should remain. If the tablets or capsules do not disintegrate due to the adherence to the disks, repeat the assay with other six tablets, omitting the disks. All tablets or capsules should be completely disintegrated by the end of the assay. The assay is not applied to uncoated capsules containing enteric-release preparation.

Sublingual tablets

Carry out the assay as described for Uncoated tablets, omitting the use of disks. After 5 minutes, all tablets should be completely disintegrated.

Soluble tablets and dispersible tablets

Carry out the assay as described for *Uncoated tablets* using water maintained at 15 ºC and 25 ºC, as immersion liquid. After 3 minutes, all tablets should be completely disintegrated.

Gelatin capsules (hard)

Carry out the assay as described for *Uncoated tablets*, omitting the use of disks. Using a stainless steel wire mesh with an opening between 1.8mm to 2.2mm, adapted to the basket cover, as described in the *Apparatus* section. Observe the capsules after 45 minutes or as specified in the drug monograph. All capsules must be completely desintegrated, or only insoluble fragments with soft consistency should remain in the mesh.

Soft capsules

Carry out the assay as described for *Uncoated tablets*, omitting the use of disks. Observe the capsules after 30 minutes or as specified in the drug monograph. All capsules must be completely desintegrated, or only insoluble fragments with soft consistency should remain in the mesh. If the capsules do not disintegrate due to the adherence to the disks, repeat the assay with other six units, omitting the disks. All tablets or capsules should be completely disintegrated by the end of the assay.

5.1.4.2 DISINTEGRATION ASSAY FOR SUPPOSITORIES, OVULES AND VAGINAL TABLETS

This assay allows checking the highest or the lowest capacity of these dosage forms to soften or disintegrate in a liquid mean, within the prescribed time.

The complete desintegration takes place when the suppository or ovule features:

complete dissolution;

b) complete separation of the components, accumulating fat substances fused on the liquid surface, depositing insoluble powders in the bottom of the vessel and dissolving the soluble components of the sample; the components distribution occurs in one or more of the ways described above;

c) softening of the sample which may happen after the change of its shape, without occurying the complete separation of its components; the softening should happen in manner that the existence of the stiffer layer on the surface is not perceivable by pressing the soft sample with glass rod;

d) rupture of the gelatinous capsule of ovules, allowing the release of its components;

e) absence of the residue over the perforated disk, or if applicable, when there is the consistency of a soft mass that is not resistant to the pressure of the glass rod.

APPARATUS

The apparatus (**Image 1**) is composed by a glass or see-through plastic cylinder, with walls of a suitable thickness, where a metal device with two stainless steel perfored disks, provided with 4-mm 39 holes is attached by three metal hooks. The diameter of each disk allows the introduction into the transparent cylinder, the disks areapproximately spaced from one anotherin 30 cm. The determination is carried out using three apparatus, each one should contain a single sample. Each apparatus is introduced into the beaker with at least 4 liters of capacity containing water at a temperature of 36 ° C to 37 °C, unless otherwise indicated in the individual monograph. The beaker is provided with an stirrer operating at slow speed and a device for reversing the cylinder without removing it from the water.

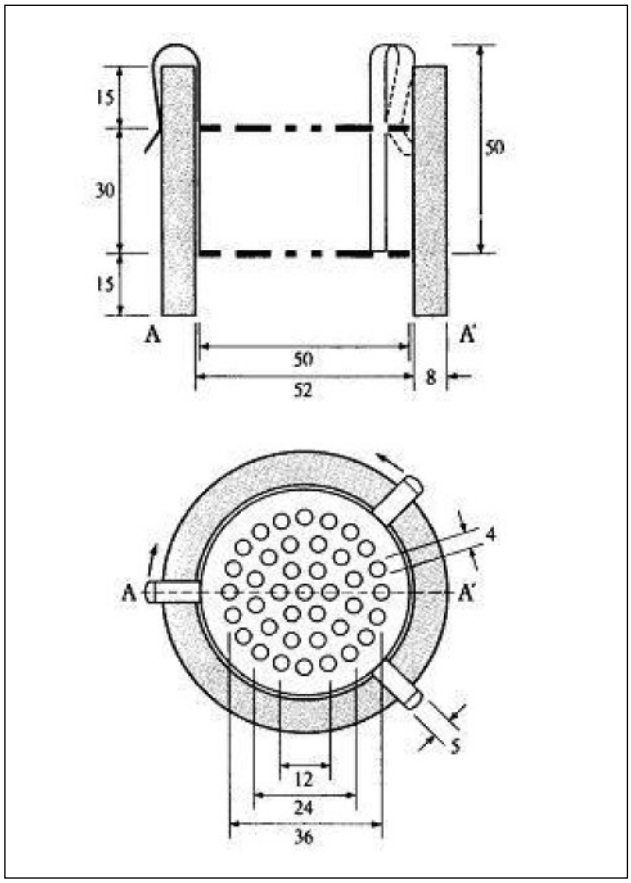

Image 1 – Device for the disintegration assay for suppositories, ovules and vaginal tablets (dimensions in mm).

PROCEDURE

Suppositories and ovules

Use three suppositories or ovules. Put each one over the lower disk of the device, introduce and attach the disk inside the cylinder. Invert the device every 10 minutes. Examine the samples after the time prescribed in the monograph. The assay is considered satisfactory if all samples are disintegrated. The time limit established as a general criterion for disintegrating suppositories, ovules and vaginal tablets with hydrophobic basis is 30 minutes, and 60 minutes for suppositories with hydrophilic basis, unless otherwise stated in the individual monograph.

Vaginal tablets

Use the apparatus described in *Disintegration of suppositories and ovules*, assembled as shown in **Image 2**. Insert the cylinder into the beaker witha suitable diameter containing water at 36 ° C -37 ° C which must uniformly cover the disk perforations. Use three devices, put one vaginal tablet over the disk above. Cover the device with a glass plate to ensure the adequate moisture. Examine the state of each sample after the time prescribed in the monograph. The assay is considered satisfactory if all samples are disintegrated.

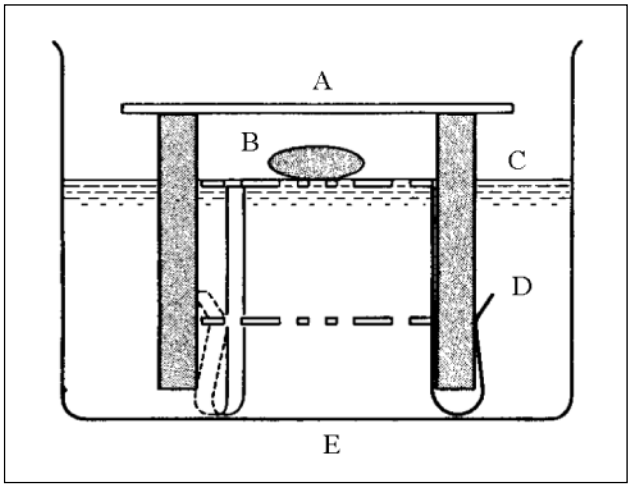

Image 2 – Device for the disintegration assay for suppositories, ovules and vaginal tablets

A, glass plate; **B**, vaginal tablet;**C**, water surface; **D**, water; **E**, vessel bottom.

5.1.5 ASSAY FOR DISSOLUTION

The dissolution assay allows determining the amount of active ingredient dissolved in the dissolution medium, when the product is subjected to the action of specific apparatus, under described experimental conditions. The result is expressed as a percentage of the amount indicated on the label. The assay is intended to demonstrate if the product meets the requirements stated in the monograph of the drug in tablets, capsules and other cases in which the assay is required.

APPARATUS FOR METHODS 1 AND 2

The dissolution apparatus consists of a three-component system described below.

(1) Open vessels with cylindrical shape and hemispherical bottom (tanks), made of borosilicate glass, plastic or other transparent inert material, which an inert cover can be adapted, with appropriate openings for the stirrer, sampling collection and thermometer insertion. The tanks may have the following dimensions and capacities:185 ± 25 mm height and 102 ± 4 mm internal diameter for a nominal capacity of one liter, 290 ± 10 mm height and 102 ± 4 mm internal diameter for a nominal capacity of two liters; 290 ± 10 mm height and 150 ± 5 mm internal diameter for a nominal capacity of four liters.

(2) Stainless steel rods to provide the stirring of the medium, which may occur in two forms: baskets (*Method 1)* or paddels (*Method 2*) (**Images 1 and 2**). The rod should be centered so that, when actuated, its rotational axis is not displaced more than 2 mm from the vertical axis of the vessel containing the dissolution medium.

(3) A motor that allows adjusting the rotation speed of the rod specified in the individual monograph, keeping it within the limits of ± 4%. The rotation should not produce undesirable effects in the system hydrodynamics.

The tanks are immersed into a thermostated water-bath with a transparent material and suitable size, in which the temperature is maintained at 37 ° C ± 0,5 ° C throughout the assay. The device must be free of any vibration source, including external vibration, which may affect the system hydrodynamics. Preferably, the device must enable viewing of samples and stirrers throughout the assay.

Metho1:Baskets

When specified in the monograph, a stainless steel rod is used as an stirrer, which its end fits into a basket of the same material (**Image 1**). The default mesh used in the basket manufacture has a diameter of 0.25 mm and a square mesh opening of 0.40 ±

0.04 mm (mesh 40), unless otherwise specified in the individual monograph. The sample should be placed in the dry basket before starting the assay. When placed inside the vessel, the distance between the inner bottom of the vessel and the basket with the dissolution medium is 25 ± 2mm.

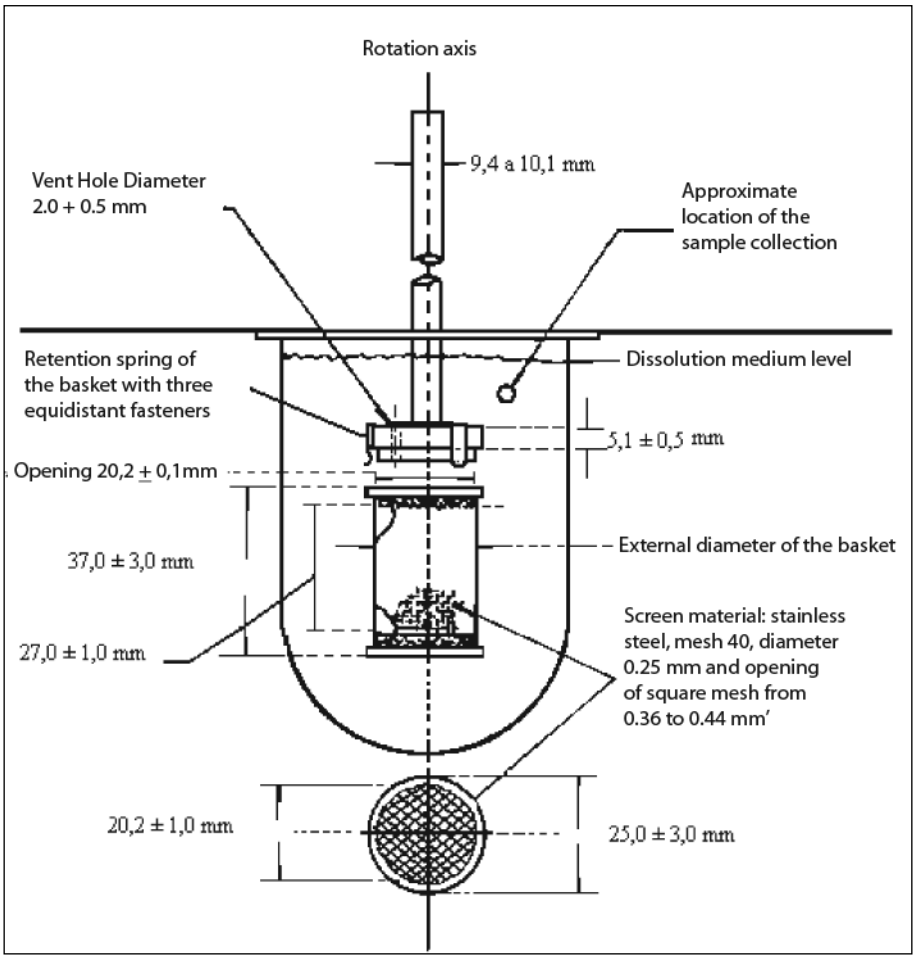

Image 1– Method 1 (Baskets).The basket and the tank do not have the same ratio.

Method 2: Paddels

When specified in the monograph, a stainless steel rod is used as an stirrer, coated or not with inert materials, which its end has the shape of a paddel (**Image 2**) able to smoothly rotate and without axis deviation during the time and speed specified in the corresponding monograph. The sample must be added whenever applicable before the starting the assay. When placed inside the vessel, the distance between the inner bottom of the paddles and the vessel with the dissolution medium is 25 ± 2mm.

It is important to prevent the samples from floating in the dissolution medium. A suitable device may be used, made of spiral wire with a suitable diameter to anchor the capsule or tablet, without deformation and preventing the reduction of the contact area with the medium.

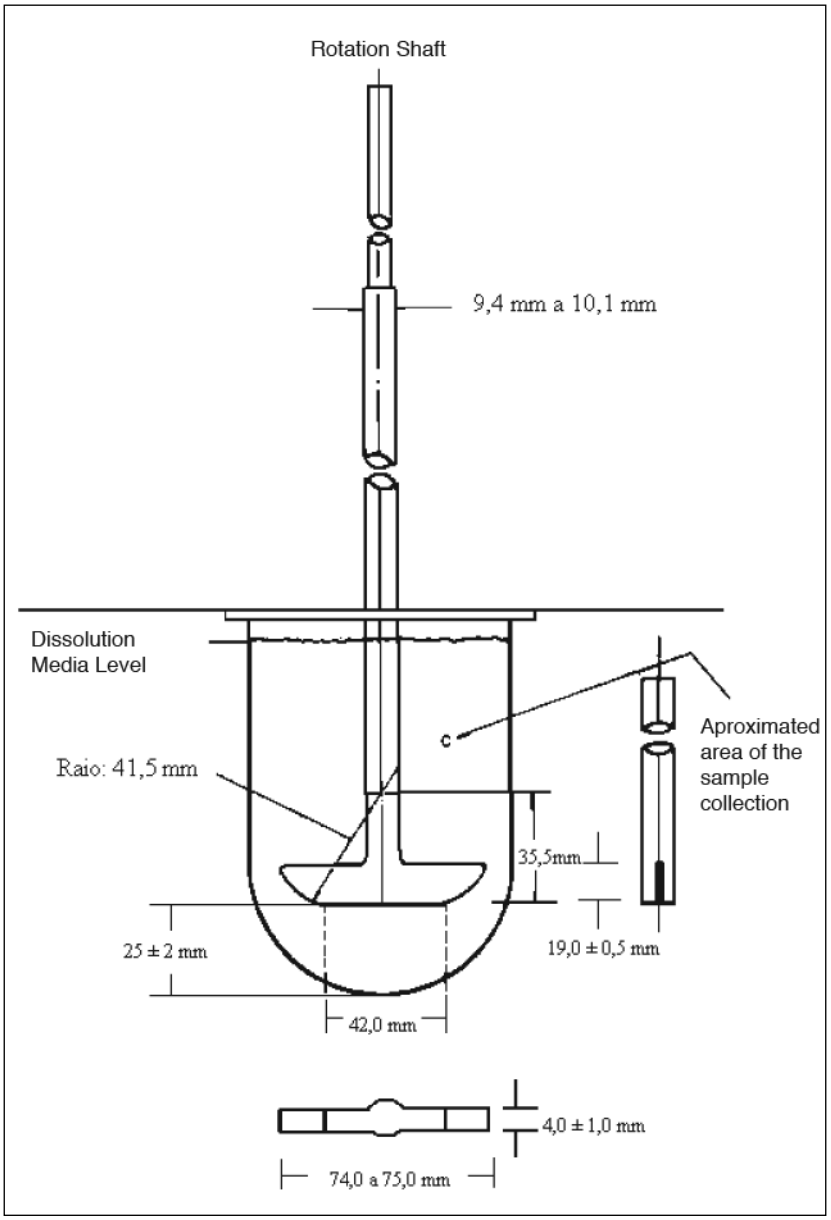

Image 2 – Method 2 (Paddles).The paddle and the tank do not have the same ratio.

APPARATUS FOR METHOD 3

Method 3: Alternative Cylinders

The dissolution apparatus for *Method 3* is composed by a series of cylindrical vials with a flat bottom; a series of glass cylinders with a closing system made of inert material (stainless steel or other suitable material) and meshes made of non-absorbent and non-reactive materials, intended to be attached in the:higher and lower parts of the cylinders. A motor and fitting device must enable the upward and downward vertical alternating movement of the cylinders in the vials and also facilitate the horizontal displacement of the cylinder to another flask arranged in a different row.

The vials remain partially immersed in a water-bath of appropriate size that enables the thermostatization at 37 ° C ± 0.5 ° C during the assay period. The device must be free of any internal or external vibration,

that may affect the smooth upward and downward movement of the cylinders. The device must have a device for adjusting the alternating movement speed, according to the recommendations in the individual monograph, with a maximum range of ± 5%.

Preferably, the device must enable the visualization of the cylinders and assay samples inside. The vials have proper cover, which must remain fixed during the assay. The components of the ensemble must feature the dimensions shown in **Image 3**, unless specified otherwise in the monograph.

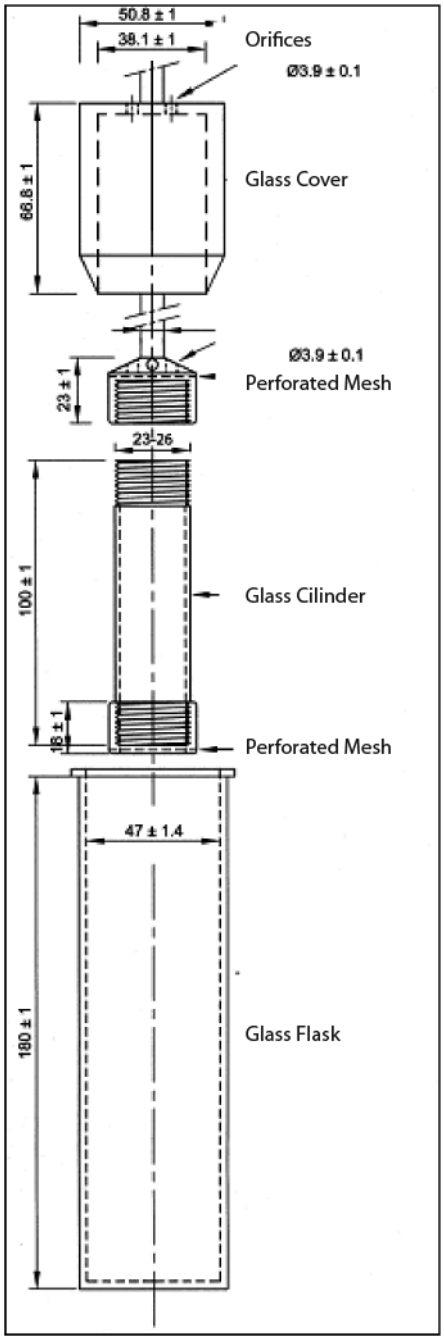

Image 3 – Method 3 (Alternating cylinders). The dimensions are indicated in milimeters.

DISSOLUTIUM MEDIUM

The dissolution medium specified in the monograph is used, which must be previously degassed by a convenient procedure when necessary to avoid formation of bubbles which could interfere with the dissolution rate to be measured. When the dissolution medium is a dissolution buffer, pH should be adjusted to ± 0.05 units of the pH value specified in the product monograph.

DISSOLUTION TIME

When a single time is specified in the product monograph, it represents the maximum time which the minimum amount of the active ingredient must be dissolved. When more than one time is specified in the monograph, aliquots appropriately measured must be taken at the end of each indicated time.

GENERAL PROCEDURE FOR METHODS 1 And 2

Assembly and check the apparatus according to the specifications mentioned above, in order to reduce to a minimum the factors that significantly change the system hydrodynamics (axis deviation, vibration, etc). Add the measured volume of the *Dissolution medium* specified in the monograph of the product suitably degassed, if necessary, to the dissolution vessel of the apparatus. Keep the medium temperature at 37 °C ± 0.5 °C, removing the thermometer before start stirring. In the case of Method 1, place the sample into the dry basket. In the case of *Method 2*, place the sample in the dissolution vessel, as previously described. In both cases, upon observing the formation of bubbles on the samples surface, when in contact with the dissolution medium, check the influence on the result. Immediately start stirring, according to a preset speed. In the interval (s) of time specified in the product monograph, remove the aliquot for analysis of the intermediate region between the surface of the dissolution medium and the top of the basket or paddles, not less than 1 cm from the inner wall of the vessel (**Images 1 and 2**). Keep stirring during the aliquot removal. If the filters coupled to the sampling system are not used, filter immediately the samples. The filters applied should be inert, should not adsorb the significant portion of the drug and have adequate porosity. According to that information specified in the product monograph, the volume of the sample removed may be replaced or not. If the replacement is required, the same dissolution medium warmed at 37 ° C should be used. In case the dissolution medium replacement is not performed, correct the volume of the calculations. After filtration and dilution (if necessary) of the aliquot, the drug quantification is performed by the method described in the product monograph. Repeat the assay with additional unit doses as necessary, considering the C*riteria of acceptance*.

*Capsules dissolution:*In case of insufficient result, repeat the assay as follows:when the dissolution media is water or buffer with a pH lower than 6.8, use the same dissolution media specified with the

addition of purified pepsin with activity up to 750,000 units/1000 mL. For dissolution medium with a pH equal to or higher than 6.8, add pancreatin up to 1,750 protease units/1,000 mL.

PROCEDURE FOR DELAYED-RELEASE DOSAGE FORMS

Apply *Method A* or *Method B* or the method indicated in individual monograph.

Method A

*Acid stage:*use 750 mL of HCl 0,1 *N* as a *Dissolution medium* in tanks when the Methods 1 and 2 area applied.

Mount the dissolution apparatus described in *Apparatus for Methods 1 and 2* and add a assay unit in each tank or basket, as appropriate. Proceed to the assay at a speed specified in the monograph for 2 hours. At the end of this time, remove an aliquot of the *Dissolution medium*, and immediately perform the *Buffer stage pH 6.8*. Determine the drug amount dissolved in the sampled aliquot using

the suitable analytical method.

Buffer stage pH 6.8: prepare the buffer stage

and the pH adjustment in 5 minutes. With the dissolution apparatus operating at the speed specified for the product, add to the Dissolution medium of the Acid stage 250 mL of tribasic sodium phosphate solution 0.20 M previously conditioned at 37 ° C ± 0.5 ° C. Adjust, if necessary, the pH to 6.8 ± 0.05 with HCl 2 *M* or NaOH 2 *M*. Keep operating the dissolution apparatus for 45 minutes or the time specified in the monograph. At the end of this time, remove the aliquote from the *Dissolution medium* of the *Buffer Stage* pH 6.8 and determine the amount of the dissolved drug, using appropriate analytical method.

Method B

*Acid stage:*use 1000 mL of 0.1 M HCl as a *Dissolution medium* in tanks and assemble the dissolution apparatus as described in *Apparatus for Methods 1 and 2*. Add a assay unit in each tank or basket, as appropriate. Proceed to the assay at a speed specified in the monograph for 2 hours. At the end of this time, remove the aliquote from the *Dissolution medium* and carry out the Buffer Stage pH 6.8. Determine the drug amount dissolved in the aliquot shown, using the appropriate analytical method.

Buffer stage pH 6.8: use phosphate buffer pH 6.8 previously conditioned at 37 ° C ± 0.5 ° C. Drain the dissolution medium of the *Tanks acid stage* and add 1000 mL of phosphate buffer dissolution medium pH 6.8. Alternatively, it is possible to remove each tank through the medium of the *Acid stage* of the dissolution apparatus and replace with another tank through the *Buffer stage* pH 6.8, carefully transferring the drug assay unit under assay. Keep operating the dissolution apparatus for 45 minutes or the time specified in the monograph. At the end of that time,

remove the aliquote from the dissolution medium of the *Buffer Stage pH 6.8* and determine the amount of the dissolved drug, using appropriate analytical method. The buffer pH 6.8 can be prepared by mixing 3 volumes of HCl 0.1 M and 1 volume of tribasic sodium phosphate solution 0.20 *M*, adjusting, if necessary, the pH to 6.8 ± 0.05 with HCl 2*M* or NaOH 2 *M*.

PROCEDURE FOR METHOD 3

*Immediate-release dosage forms:*by using the Method 3, add the volume of the *Dissolution mean* specified in the product monograph in each device vial, arrange the vials in the instrument bath at 37 °C ± 0.5 °C and remove the thermometers before starting the assay. Place one dosage unit of the sample in each of the six alternating cylinders, preventing the formation of air bubbles on the material surface, and immediately start the operation of the device as specified in the product individual monograph. During the upward and downward movement of the cylinders, the height amplitude should be between 9.9 and 10.1 cm. In the time range specified in the individual monograph, lift the cylinders and take an aliquot sample of the *Dissolution medium*of each vial, the intermediate region between the liquid surface and the bottom of the vial. After filtration and dilution (if necessary) of the aliquot, perform the quantitative analysis of the dissolved drug according to the information established in product individual monograph. If necessary, repeat the assay with the additional units of the drug. Replace the volume of the sampled medium with an equal volume of fresh *Dissolution medium* kept at 37 °C, or in situations where the medium replacement is not required, make the correction of volume changes during calculations. Keep the vials covered with their respective seals during the assay and periodically check the temperature of the medium. For the medium and the dissolution time, follow the general guidelines indicated in the *Dissolution medium*and *Dissolution time*.

*Extended-release dosage forms:*by using the Method 3, proceed as described in *Immediate-release dosage forms*and follow the general guidelines indicated in the *Dissolution medium and Dissolution time*. The times are expressed in hours and are normally indcated by at least three time intervals.

*Delayed-release dosage forms dosage forms:*by using the Method 3, proceed as described in Method B for Release dosage forms dosage forms, using a row of vials for the *Acid stage* and a successive row of vials for the buffer solution pH 6.8, adding the volume of the medium specified in the monograph (usually, 300 mL). The collection times are specified in the monograph or are indicated in the Method B for Delayed-release dosage forms dosage forms:

ACCEPTANCE CRITERIA FOR Immediate-release dosage forms:

The product is compliant with the assay if the results meet the requirements set out in **Table 1**, unless otherwise indicated in the individual monograph.

Table 1 – Acceptance criteria for immediate-release dosage forms.

Stages	***Nº. of samples tested***	***Acceptance criteria***
E_1	06	Each unit shows a result higher or equal to Q + 5%
E_2	06	Average of 12 units (*E1 + E2*) is equal to or higher than Q and no unit shows a result lower than Q – 15%
E_3	12	Average of 24 units ($E + E_2 + E$) is equal to or higher than Q, but not higher than two12 units show results lower than Q – 15% and if any unit shows a result lower than Q – 25%.

The word Q corresponds to the amount of drug dissolved, specified in the individual monograph, indicated as a percentage of the amount indicated. The values 5%, 15% and 25% also show the percentages of the amount indicated.

In specific circumstances, the maxium percentage for dissolution must be experimentally determined. In these cases, ensure a value of Q∞ (amount dissolved in an infinite time) by checking if two consecutive dosages are not different from each other in more than 2% after ten minutes.

Stage E_1

Six units are tested in the Stage E_1. If each unit individually showsa result equal to or higher than Q + 5%, the product is in compliance with the specified, thus, the Stage E_2 is not required.

Stage E_2

In case the criterion for Stage E_1 is not met, repeat the assay with other six units. If the average of the twelve units tests (*Stages* E_1 *and* E_2) is higher than ou equal to Q and if any of the units tested show a result lower than Q – 15%, the product is in compliance with the specified, and the Stage E is not required.

Stage E,

In case the criterion for Stage E_2 is not met, repeat the assay with other 12 units. If any of the 24 units tested (Stages E_1, E_2*e* E_3) show results equal to or higher than Q, up to two units show results lower than Q – 15% and if any unit show a result lower than Q – 25%, the product is in compliance with the specified. In case the criterion for *Stage* E_3 still is not met, the product is deemed as unsatisfying.

ACCEPTANCE CRITERIA FOR EXTENDED-RELEASE DOSAGE FORMS

The product is compliant with the assay if the results meet the requirements set out in Table 2, unless otherwise indicated in the individual monograph. The terms Q1 and Q2 correspond to the minimum and maxium amount of drug dissolved in each time interval specified in the monograph, shown as a percentage of the amount stated. In the last time, the specification may be shown only as a

Table 2 – Acceptance criteria for the dissolution assay (release) carried out for extended-release dosage forms.

Stages	*Nº of units tested*	*Acceptance criteria*
L_1	6	Each individual result falls within the interval established (Q1 and Q2) for each time established and any individual result is lower than the Q of the last time.
L_2	6	The average of 12 units (E + E) falls within the interval established (Q1 and Q2) for each time established and is not lower than Q of the last time. Any individual unit show any result overcoming the limits of Q1 and Q2 in 10% of the amount stated, for each time established, and any individual result provides a value lower than Q of the last time which overcomes 10% of the amount established.
L_3	12	The average of 24 units (E + E + E) falls within the interval established (Q1 and Q2) for each time established and is not lower than Q of the last time. Up to 2 units of the 24 units tested show the results overcoming the limits of Q1 and Q2 in 10% of the amount stated, for each time established, and up to 2 units of the 24 units assayprovide a value lower than Q of the last time which overcomes 10% of the amount established. Any individual unit show any result overcoming the limits of Q1 and Q2 in 20% of the amount stated, for each time established, and any individual result provides a value lower than Q of the last time which overcomes 20% of the amount established.

minimum Q value The terms L_1, $L_2$and L_3 refer to the three possible stages of the release evaluation (L).

ACCEPTANCE CRITERIA FOR DELAYED-RELEASE DOSAGE FORMS:

The product is compliant with the assay if the results meet the requirements shown in **Table 3**in the *Acid stage* (Methods A or B) and also the requirements shown in **Table 4** in the *Buffer stagepH 6.8* (Methods A or B), unless otherwise indicated in the individual monograph. Apply

Table 3 – Acceptance criteria for the Acid stage of the dissolution assay (Methods A or B) carried out for delayed-release dosage forms.

Stages	*Nº of units tested*	*Acceptance criteria*
A_1	06	Any individual unit shows the amount dissolved higher than 10% of the value stated.
A_2	06	The average of 12 units is not higher than 10% of the value stated and any individual unit shows the amount dissolved higher than 25% of the value stated.
A_3	12	The average of 24 units is not higher than 10% of the value stated and any individual unit shows the amount dissolved higher than 25% of the value stated.

Table 4 – Acceptance criteria for the *Buffer stage pH 6,8* of the dissolution assay (Methods A or B) carried out for delayed-release dosage forms.

Stages	*№ of units tested*	*Acceptance criteria*
B_1	06	Each unit shows a result higher or equal to Q + 5%
B_2	06	Average of 12 units (B_1 + B_2) is equal to or higher than Q and any unit show a result lower than Q – 15%.
B_3	12	Average of 24 units (B_1 + B + B) is equal to or higher than Q, but not higher than two units show results lower than Q – 15% e any unit shows a result lower than Q – 25%.

the Q value indicated in the product monograph, and when the value is not specified, apply 75% as a Q value in the *Buffer stage pH 6.8.* The terms A_1, A_2 and A_3 refer to A_3 the three possible stages of evaluation in the *Acid stage* (A) and the terms B_1, B_2 e B_3 stages of evaluation in the *Buffer stage* pH 6.8 (B).

5.1.6 UNIFORMITY OF DOSAGE UNITS

To ensure the administration of right dosages, each unit of the drug batch must have the amount of the active ingredient close to the amount stated. The assay for uniformity of dosage units allows evaluating the amount of the individual units of the batch and checking if this amount is uniform in the units tested. The specifications of this assay are applied

Table 1 – The application of the Uniformity of Content (UC) or Weigh range (VP) method according to the pharmaceutical form, dosage and proportion of the drug.

Pharmaceutical form	*Type*	*Subtype*	*Dosageand drug proportion*	
			≥ 25 mg and ≥ 25%	< 25 mg or < 25%
Tablets	uncoated		VP	UC
	coated	film	VP	UC
		others	UC	UC
Capsules	hard		VP	UC
	soft	suspensions, emulsionsorgels	UC	UC
		solutions	VP	VP
Solid stored in single-dose vessels	unique component		VP	VP
	several components	lyophilized solution in the final vessel	VP	VP
		others	UC	UC
Solutions stored in single-dose vessels			VP	VP
Others			UC	UC

to the dosage forms with one drug or provided with more than one active ingredient. Unless otherwise indicated in the individual monograph, the assay is individually applied to each active ingredient of the product.

The uniformity of the pharmaceutical unit dosage form may be evaluated by two methods: *Weight range and Uniformity of Content.* The application of each method considering the pharmaceutical form, dosage and proportion of the drug is shown in **Table 1.**

The *Uniformity of Content* method for single-dose preparations is based on the dosage of the individual content of the active ingredient of a number of single-doses to establish if the individual content is within the specified

limits. The *Uniformity of Content* method may be applied in all cases.

The *Weight Range* method may be applied to the following dosage forms:

1. solutions stored in single-dose vessels and in soft capsules;

2. solid (including powders, granules and sterile solids) stored in single-dose vessels which do not have other ingredients stored, whether they are active or inactive;

3. solids (including sterile solids) stored in single-dose vessels, containing or not active or inactive ingredients stored, which have been prepared from lyophilized homogeneous solutions in final vessels, labeled in order to indicate this method of preparation;

4. hard capsules, uncoated tablets or film-coated tablets, with 25g or more of the active ingredient comprising 25% or more, in weight, of the unit dosage, or in case of hard capsules, the capsule content, except that the uniformity of other active ingredients featured in smaller proportions may be stated by the *Uniform of Content* method.

The *Uniform of Content* method is required for all dosage forms which do not meet the conditions specified for the application of the method *Weight Range*.

UNIFORM OF CONTENT

In order to establish the uniformity of unit doses by the uniformity of content method, separate at least 30 units and proceed as described for the dosage forms indicated. When the amount of the active ingredient of a unit dosage is different from the value specified in the dosage, adjust the dilution of the solutions and/or the aliquot volume in order to obtain the concentration of the active component in the final solution, similar to the dosing. In case of dosing per titration, use the titrant with a different concentration, if required, for the suitable volume of the titrant consumption. Consider any change of the dilutions to perform the calculations.

When there is a special procedure for the uniformity of content assay in the individual monograph, make the necessary correction of the results as described below.

1. Weigh the amount of the product units enough to carry out the dosage and the special procedure for the uniformity of content assay presented in the individual monograph. Reduce the tablets to a fine powder (or mix the contents of the capsules, solutions, suspensions, emulsions, gels or solids in single-dose vessels) to obtain a homogeneous mixture. If it is not possible to obtain homogeneous mixture, use appropriate solvents or other procedures to obtain a solution containing the drug. Apply appropriate aliquots of this solution to the tests specified.

2. Separately analyze portions of the precisely measured sample, according to the procedure described for the dosage (*D*) and the special procedure indicated for the uniformity of content (*E*) described in the individual monograph.

3. Calculate the drug amount by the mean weight using the results obtained by the dosage procedure (*D*) and the special procedure (*E*).

4. Calculate the correction factor (F) according to the equation:

$$F = D/E$$

whereas

D= amount of the active ingredient by mean weight of the dosage form obtained by the dosage procedure;
E = amount of the active ingredient by mean weight of the dosage form obtained by the special procedure.
If $(100|D - E|)/D$ is higher than 10, the *F* use is not valid.

1. If *F* is between 0.970 and 1.030, there is no need for correction.

2. The correction is applied when the F value is between 0.900 and 0.970 and between 1.030 and 1.100 and should be carried out by calculating the drug amount in each unit, multiplying the quantities obtained in the special procedure by the correction factor *F*.

Solid dosage forms

Individually analyze 10 units as indicated in individual monograph for dosing, unless a special procedure for uniformity of content is described in the monograph. Calculate the *Value of Acceptance (VA)*.

Liquid dosage forms

Individually analyze 10 units as indicated in individual monograph for dosing, unless a special procedure for uniformity of content is described in the monograph. Individually conduct the assay, in equal amount of the material, which is removed from each vessel under normal use conditions. Show the result as the quantity dispensed per unit. Calculate the Value of Acceptance (*VA*).

Value of Acceptance for Content Uniformity.

Calculate the *Value of Acceptance (VA)* according to the equation:

$$VA = |M - \overline{X}| + ks$$

The terms are defined in **Table 2**.

WEIGHT RANGE

In order to establish the uniformity of single doses by the weight range method, separate at least 30 units and proceed as described for the pharmaceutical forms indicated. The drug amount per unit is estimated from the dosage result and individual weights, assuming the equal distribution of the active ingredient. The estimated individual amounts (x) are calculated according to the equation:

$$x_i = p_i \times A/P$$

whereas

p_1 = individual weights of the units or contents of the units tested;
A = amount of the active ingredient, expressed in percentage of the amount stated, determined in the dosing;
P = mean weight of the units used in the dosing.

Uncoated tablets or film-coated tablets

Individualand exactly weight 10 tablets. From the result of the dosing and the individual weight of each tablet, estimate the amount of the active ingredient of each unit and show the individual results in percentage of the amount stated. Calculate the Value of Acceptance (*VA*).

Hard capsules

Individual and exactly weight 10 capsules, maintaining the identity of each one. Carefully remove the content and weigh the empty capsules. Calculate the content weight of each capsule, and from the dosing result, estimate the quantity of the active ingredient in each capsule. Show the individual results as a percentage of the amount indicated on the label. Calculate the Value of Acceptance (*VA*).

Soft capsules

Individual and exactly weight 10 capsules, maintaining the identity of each one. Cut the capsules with a blade and remove the content, washing the shells with a suitable solvent. Leave the shells at room temperature for 30 minutes for the complete evaporation of the solvent, taking care to avoid the addition or loss of humidity. Weigh the empty capsules and calculate the content weight of each capsule. Estimate the active ingredient amount of each capsule from the dosing result and the content weight of each capsule. Calculate the Value of Acceptance (*VA*).

Solid dosage forms (except tablets and capsules)

Proceed as described in the *Hard capsules* paragraph. Calculate the
Acceptance Value.

Liquid dosage forms

Individual and exactly weigh the liquid amount which is removed from each one of the 10 vessels under normal use conditions. If required, calculate the volume equal to the content removed after determining the density. Estimate the active ingredient amount of each vessel from the dosing result and the content weight removed from the individual vessels. Calculate the *Value of Acceptance* (VA).

Value of Acceptance for Weight Range

Calculate the *Value of Acceptance* as described in *Value of Acceptance for Content Uniformity*, except the individual amounts of active ingredients in the units are replaced by the estimated individual amounts.

CRITERIA

Apply the following criteria, either for *Content Uniformity* and *Weight Range*, unless otherwise indicated in the individual monograph.

Solid and liquid dosage forms

The product complies with the assay for uniformity of dosage units if the *Value of Acceptance* calculated for the 10 first units tested is not higher than L1. If the *Value of Acceptance* is higher than L1, assay other 20 units and calculate the *Value of Acceptance*. The product complies with the assay for uniformity of dosage units if the *Value of Acceptance* calculated for the 30 first units tested is not higher than L1 andif the active ingredient amount of any individual unit is lower than $(1 - L2 \times 0.01)\ M$ or higher than $(1 + L2 \times 0.01)\ M$. Unless otherwise indicated in the individual monograph, $L1$ is 15.0 and $L2$ is 25.0.

Table 1 – Terms and expressions to calculate the Value of Acceptance (VA).

Variable	*Definition*	*Conditions*	*Values*
$\overline{X}$	Content average individuals (x_1, x_2,..., x_n), expressed as a percentage of the quantity stated.Individual contents of the units tested, shown as a percentage of the amount indicated.		
$x_1, x_2,..., x_n$	Conteúdos individuais das unidades testadas, expressos como porcentagem da quantidade declarada.		
n	Number of units tested		
k	Acceptability constant	If n = 10, so k =	2,4
		If n = 30, so k =	2,0
s	Sample standard deviation		$\left[\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n - 1}\right]^{1/2}$
M to be used when $T \leq 101,5$ (caso 1)	Reference value	If $98,5\% \leq \overline{X} \leq 101,5\%$, so	
		If $\overline{X} < 98,5\%$, so	$M = 98,5\%$ $(VA = 98,5 - \overline{X} + ks$
		If $\overline{X} > 101,5\%$, so	$M = 101,5\%$ $(VA = \overline{X} - 101,5 + ks$
M to be used when $T > 101,5$ (caso 2)	Reference value	If $98,5 \leq \overline{X} \leq T$, so	$M = \overline{X}$ (VA = ks)
		If $\overline{X} < 98,5\%$, so	$M = 98,5\%$ $(VA = 98,5 - \overline{X} + ks$
		If $\overline{X} > T$, so	$M = T$ $(VA = \overline{X} - T + ks$
VA	Acceptance value		General formula: $\lvert M - \overline{X} \rvert + ks$ Os cálculos são especificados acima para os diferentes casos
$L1$	Maximum value allowed for the acceptance value		$L1$ = 15,0 unless otherwise specified in the individual monograph
$L2$	Maximum deviation allowed for each unit test in relation to the M value, using the value of acceptance calculations.	Any individual result is lower than $(1 - L2 \times 0.01)M$ or higher than $(1 + L2 \times 0.01)M$	$L2$ = 25,0 unless otherwise specified in the individual monograph
T	Average of the limits specified in the individual monograph for the amount or capacity stated, shown as a percentage	T is equal to 100%, unless other value had been approved due to stability reasons; in these cases, T is higher than 100%.	

5.1.7 CONTAMINATION CAUSED BY PARTICLES

5.1.7.1 SUBVISIBLE PARTICLES

The contamination of injectables caused by particles is the presence of insoluble, mobile and foreign matters that are not air bubbles.

The specifications required for the pharmaceutical preparations are described in the specific monographs.

The contamination caused by particles, from preparations for parenteral use and perfusion is composed by non-soluble foreign and mobile particles, in addition to gas bubbles which are involuntarily found in these preparations. For the determination of contamination caused by particles, the 2 methods below are specified:*method 1* (assay for particle counting through a light block) and *method 2* (assay for counting the particles through optical microscopy). For determination of non-visible particles in injectable preparations and preparations for perfusion use *method 1*. In certain preparations, however, it may be required to carry out assays for particle counting by light blocking at first and then by optical microscopy in order to conclude as to the compliance of the results obtained.

The research of non-visible particles performed by applying one of these methods, or even two, it is not possible for all injections. When the *method 1* is not applicable, for example in the case of preparations that are not very clear or are highly viscous, the assay is carried out by *method 2* (in case of emulsions, colloidal solutions and preparations of liposomes). Similarly, an assay for particle counting by optical microscopy may also be required for products which form air or gas bubbles when passing through the detector. If the preparation viscosity is such that the examination of one of the methods is impossible, a quantitative dilution may be carried out with a suitable diluent to reduce the viscosity to the extent deemed sufficient to allow the assay.

The results obtained when a unit or group of units is examined can not be reliably extrapolated to other units that were not analyzed. Consequently, establish statistically valid sampling plans if it is desired to obtain valid conclusions from the data collected to determine the level of particulate contamination of a large group of units.

The water used in assays is free of particles. Water free from particles can be obtained by membrane filtration with a porosity of 0.22 μm.

METHOD 1 – PARTICLE COUNTING BY LIGHT BLOCKING

Equipment

Use a particle counter based on the operating principle of light blocking that allows the size determination of particles and their number, according to their dimensions.

Calibration

Calibrate the equipment with the aid of standard spherical particles of a size between 10 and 25 μm. These standard particles are dispersed in water free of particles. Prevent the aggregation of particles during dispersion.

Precautions

Carry out the assay under limited contamination conditions, preferably in a laminar flow cabinet. Wash the glassware and filtration equipment used, except the membrane filters, with a warm detergent solution and rinse with water until all detergent is removed. Immediately before the use, rinse the equipment from the top to the bottom, internally and externally, with water free from particles.

Be careful to avoind the introduction of air bubbles in the sample to be analyzed, especially when sample aliquots are transferred to the reading device.

To verify the suitability of the environment, water and glassware used to perform the particles counting in five samples of 5 mL of water free from particles, according to the method described in this chapter. If the number of particles greater than 10 μm is more than 25 for the total volume of 25 mL, the environment is not in good conditions to carry out the test.

Procedure

Homogenize the sample by 25 consecutive slow and smooth inversions of the container. Eliminate the bubbles leaving the sample to stand for 2 minutes. Transfer four portions not less than 5 mL, and determine the number of particles with size equal to or greater than 10 and 25 μm. Disregard the result obtained in the first aliquot, and calculate the average number of particles for the sample under test.

Evaluation

Employ the test A, B or C as well as the number of samples as indicated in specific monograph of the dosage form.

Test A – Solutions for injections in containers having declared volume greater than 100 mL. The sample meets the assay if the average particle size is equal to or greater than 10 μm present in the units tested does not exceed 25 particles per mL and the number of particles with size equal to or greater than 25 μm does not exceed 3 per mL.

Test B – Solutions for injections in containers having declared volume equal to or less than 100 mL. The sample meets the assay if the average particle number is equal to or greater than 10 µm, present in the units tested, does not exceed 6,000 particles per container and the number of particles with size equal to or greater than

25 µm does not exceed 600 particles per container.

Test C – Powders for injections in containers having declared volume equal to or less than 100 mL. The sample reconstituted with water or diluent free of particles meets the assat if the average particle number is equal to or greater than 10 µm, present in the units tested, does not exceed 10,000 particles per container and the number of particles with size equal to or greater than 25 µm does not exceed 1,000 particles per container.

METHOD 2 – PARTICLE COUNTING BY MICROSCOPY

Equipment

Use a suitable binocular microscope, a filtering device to retain the particulate contamination and a membrane filter. Microscope equipped with a calibrated ocular micrometer with a stage micrometer, one platinum of cross movements able to maintain and go through the filtering surface of the membrane filter, two appropriate illuminators that allow incident illumination and oblique illumination, set to expand 100 ± 10 times.

The ocular micrometer is a circular reticle and comprises a large circle divided into quadrants by crossed lines, transparent and black reference circles with a diameter of 10 µm and 25 µm with a rise of 100 and a linear graduated scale of 10 on 10 µm (**Image 1**).

The great circle is called the visual field of the reticle. The following items are required: two illuminators, an episcopic illuminator for light background, inside the microscope and an external auxiliary illuminator adjustable to enable an oblique illumination reflected off an angle of 10-20°. The filtration device to retain the particulate contamination comprises a glass filter support or other suitable material, a vacuum source and a suitable membrane filter. The membrane of appropriate dimensions is black or dark gray; is covered or not with a grate and the pore size is less than or equal to 1.0 µm.

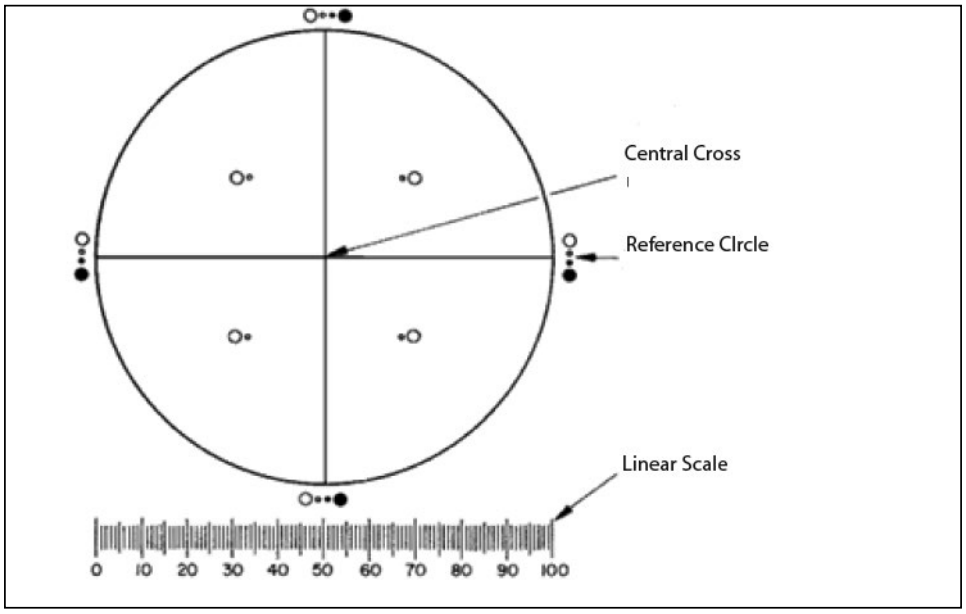

Image 1 – Circular reticle.

Calibration

It is calibrated with a stage micrometer certified by an international or national standards organization. A relative error of ± 2% for the reticle linear scale is acceptable.

General precauctions

Carry out the assay under limited contamination conditions, preferably in a laminar flow cabinet.

Wash the glassware and filtration equipment used, except the membrane filters, with a warm detergent solution and rinse with water until all detergent is removed. Immediately before the use, wash both sides of the membrange filter, rinse the equipment from the top to the bottom, internally and externally, with water free from particles.

To verify the suitability of the environment, water and glassware used, perform the particles counting in 50 mL of water free from particles, according to the method

described in this chapter. If the number of particles with 10 μm or more is higher than 20, or if more than 5 particles of 25 mL or higher are present, the environment is not in good conditions to carry out the test.

Procedure

Homogenize the sample by 25 consecutive slow and smooth inversions of the container. If necessary, carefully remove the closing device. Wash the external surfaces of the vial opening with a water jet free of particles and remove the clamp avoiding any contamination of the contents.

In case of large parenteral preparations, carry out the test in separate units. In the case of small or large parenteral preparations with volume equal to or greater than 25 mL, less than 10 packages may be sufficient for the test in accordance with an appropriate sampling plan. In regard to small parenteral preparations, which volume is less than 25 mL, gather the contents of 10 or more units in a clean container in order to obtain a minimum volume of 25 mL; in authorized justified cases, the problem solution can be prepared by mixing the contents of an appropriate number of vials and completing 25 mL with water free of R particle or an appropriate contamination free solvent, especially when the water free of R particles is not appropriate. The small parenteral preparations which volume is greater than or equal to 25 mL can be individually examined.

In the case of powders for parenteral use, retrace the preparation with water free of particles or an appropriate contamination-free solvent, especially when the water free of particles is not appropriate.

Moisten the inside part of the filter support provided with the membrane filter with some millilitres of water free of particles. Proceed to the filter the whole sample (mixture of the test portion or the unit under test) and apply vacuum. If necessary, slowly add portions of the solution until the total volume is filtered. After the last addition, start washing the internal walls of the filter support using a jet of water free of particles. Keep the vacuum until the surface of the membrane filter is free of liquid.

Place the filter in a Petri dish and dry it in the air leaving the board slightly open. When the filter is dry, place the Petri dish in the microscope platen, perform the sweep of the whole membrane filter on the reflected light from the illuminator and count the number of particles of size greater than or equal to 10 μm and the number of particles of size greater than or equal to 25 μm. It is also possible to perform the partial count and determine by calculating the total number of particles held in the filter. Calculate the average number of particles present in the sample. To determine the size of the particles with the aid of circular reticle, carry out the transformation of the image of each particle in a circle and then compare it with the reference circles of the reticle of 10 μm and 25 μm. Thus, the particles maintain its initial position within the visual field of the reticule and do not overlap with the reference circles for comparison purposes. The inside diameter of the reference circles of the reticle is used to determine the size of white or transparent particles while the size of dark particles is determined with the outer diameter of the black and opaque reference circles of the reticle. When performing a particles counting test in the microscope, try not to measure or enumerate amorphous, semiliquid or morphologically indistinct matters that resemble a stain or pallid zone of the membrane filter. These materials may present a weak brightness or no brightness and a gelatinous appearance or the semblance of a film. The evaluation construction may be facilitated by conducting a particles counting test by the light retention over a solution sample.

Evaluation

Apply the criteria below, in accordance with the volume of samples or as indicated in the specific monograph, in the pharmaceutical form.

In preparations stored in containers with a nominal content greater than 100 mL, preparation meets the test if the average number of particles present in the units tested is not more than 12 per millilitre for particles of size greater than or equal to 10 μm and does not exceed 2 particles per millilitre for particles of size greater than or equal to 25 μm.

In preparations stored in containers with a nominal content equal to or greater than 100 mL, preparation meets the test if the average number of particles present in the units tested is not more than 3000 per container for particles of size greater than or equal to 10 μm and does not exceed 300 particles per container for particles of size greater than or equal to 25 μm.

5.1.7.2 VISIBLE PARTICLES

The particle contamination of injectable preparations and injectable preparations for perfusion is constituted by foreign particles, which are not dissolved and are mobile, in addition to gas bubbles, which are involuntarily found in these solutions. The purpose of the test is to provide a simple method for the visual evaluation of the quality of the solutions in regard to visible particles. Other validated methods may be used.

Apparatus

The apparatus (Figure 1) is composed of an observation post, comprising: an opaque black panel, with appropriate dimensions, placed in a vertical position, an antireflective white panel with appropriate dimensions, placed in a vertical position beside the black panel, an adjustable lightning rail, with a protected white light source and an appropriate diffuser (a lighting system containing 2 fluorescent bulbs of 13W, with a wavelength of 525 nm each one, is appropriate).

The lighting intensity on the observation point is maintained between 2000 and 3750 lux; it is recommended a higher intensity for stained glass or plastic containers.

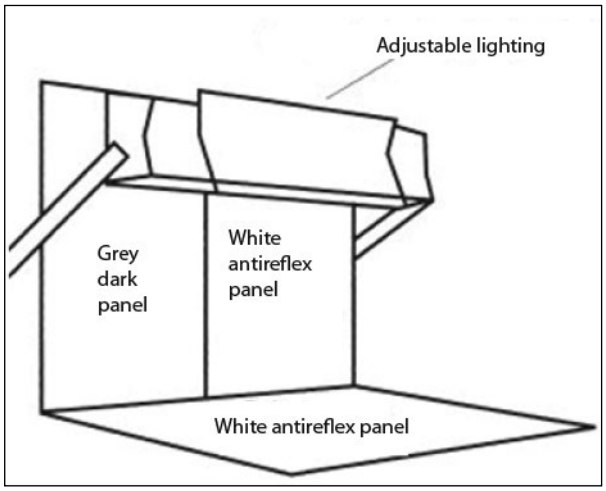

Figure 1 – Apparatus for visible particles.

Procedure

Remove any labels, wash and dry the outside of the container. Gently shake and invert each container carefully, avoiding the formation of air bubbles and observe it for about 5 seconds against the white panel. Repeat this procedure, observing the container against the black panel. Write down the presence of any particles.

5.1.8 DRIPPING TEST

The dripping test is intended to determine the ratio of the number of drops per milliliter and the drug amount per drop in liquid pharmaceutical forms stored in containers with integrated metering device. To carry out the test, it is required to know the number declared of drops per milliliter, or the declared drug ammount in mass per drop.

PROCEDURE

Determination of the number of drops per milliliter

The dripping should be carried out with the inverted vial in the vertical position or according to the dripping angle declared by the manufacturer, allowing the flow per gravity, at a constant rate, without any type of additional pressure. A slight pressure can be applied in polyethylene vials.

Separate 30 units. Proceed to the test using 10 units, in an environment with controlled temperature at 20 ± 2 ºC. For each unit, determine the mass relative to the number of drops corresponding to 1 ml, as declared by the manufacturer. If this ratio is not declared, use 20 drops for the test.

Calculate the number of drops per milliliter for each unit tested (*N*) according to the following equation:

$$N_t = \frac{(N_1 \times \rho)}{m}$$

whereas

N_1= the number of drops used in the test, which can be the number of drops declared per millilitre (*N*) or 20 drops;
ρ = product mass density, in g/mL, established at 20 ºC, as described in *Establishment of the mass density and relative density* (**5.2.5**).
m_1= mass, in g, corresponding to the number of drops used in the test.

Determination of the drug amount per drop

Calculate the drug amount, in mg/drop, for each unit tested (q), according to the equation:

$$q_1 = \frac{Q}{N_1}$$

whereas

Q = drug amount, in mg/mL, determined in the dosing;
N = number of drops per milliliter calculated for each unit tested.

Calculate the percentage in relation to the quantity declared, for each unit tested (*%Q* ou %q), applying one of equations below:

$$\%Q_t = \frac{q_t}{(Qd / N_d)}\ 100 \text{ ou } \%q_t = \frac{q_t}{q_d} \times 100$$

whereas

q = drug amount, in mg/drop, calculated for each unit tested;
Q= declared drug amount, in mg/mL;
N = declared number of drops per milliliter;
q = declared drug amount in mg/drop.

Calculate the average of the individual percentages obtained (%Q) and the relative standard deviation (*DPR*) according to the equations:

$$\overline{\%Q} = \frac{\sum \%Q_t}{n}$$

$$s = \sqrt{\frac{\sum (\%Q_t - \overline{\%Q})^2}{n - 1}}$$

$$DPR = \frac{100 \times s}{\%Q}$$

whereas

%*Q* = percentage in relation to the quantity declared calculated for each unit tested;
s = standard deviation;
n = number of units tested.

CRITERIA

The product complies with the test requirements if the individual percentages for each of the 10 units tested, are situated between 85.0% and 115.0% of the declared quantity and the relative standard deviation (*RSD*) is not greater than 6.0 %.

If a unit is out of range between 85.0% and 115.0% of the declared quantity, or if the RSD is greater than 6.0%, or if both conditions are met, test 20 more units.

The product complies with the test if one unit is out of range between 85.0% and 115.0% of the declared quantity, no unit is outside the range between 75.0% and 125.0% and the *RSD* of 30 units tested is not greater than 7.8%.

5.2 PHYSICAL AND PHYSICOCHEMICAL METHODS

5.2.1 MASS DETERMINATION

To perform the mass measurement, the scales should present capacity and sensitivity according to the degree of accuracy required and updated calibration certificate.

In regard to activities that require accurate weighings, the determination of masses equal to or greater than 50 mg, use analytical balance with a capacity of 100g to 200 g 0.1mg of sensitivity. For quantities lower than 50mg, use analytical balance with a capacity of 20.0g and 0.01 mg of sensitivity.

APPARATUS

The analytical balances to be used in this test should have a single pan, and must be preferably electronic.

The scales must have suitable device which enables the verification of the applied load, provided that they are periodically calibrated by means of measured reference masses.

The analytical balances should have the following characteristics:

- cabinet or protective housing, with apertures to allow operations in its interior and exclude air currents;

- to be installed on a compact and sturdy material

(marble, granite, metal or rubber, for example);

- level indicator (gravimetric or hydraulic) and device that enables its leveling;

- to be installed on shock absorber system (magnetic, pneumatic or hydraulic, for example) to reestablish the balance promptly;

- a system that enables the mass reading (through displays and/or scale optical projection, etc).

They must also bear their full load without suffering inadequate stresses that might compromise their sensitivity in successive weighings under these conditions.

The balance should not be overloaded.

Location of the analytical balance

The analytical balance must be placed levelled on a table or a firm and heavy shelf, protected by shock absorbers, such as cork mats or rubber blades, or even on a concrete bench, supported in pillars which are fixed on the ground or attached to elements of the building in order to prevent vibration. The balance must be placed in a secluded location that provides security and stability, in an environment with a relatively dry atmobead, protected from the attack of acid gases and vapors, away from heat sources (direct sunlight, furnaces, ovens, etc) and air currents.

Conservation and cleaning

The plate and other parts of the balance, including its protective housing, should remain clean, free of dust and substances that may accidentally fall on the weighing plate or on the box bottom. Such materials must be immediately removed.

The bodies to be weighed must not be placed directly on the plate. It is required to use papers or containers suitable to the mass, such as beakers, clock glasses, porcelain capsules, weighs filters with or without seals.

The moving parts of the balance and the weights should not be touched with the hands. For this purpose, appropriate clip must be used, which should be stored in the box of weights.

Desiccants agents, such as silica gel or calcium chloride, may be placed inside the protective housing, for maintenance of the relatively dry atmobead.

When the balance is not in use, its doors should remain closed and locked.

The sensitivity of the analytical balance must be periodically inspected and evaluated by a qualified technician.

Use of the analytical balance

The material to be weighed must be in thermal balance with the air from the inside of the protective housing of the balance in order to avoid errors due to the convection currents, as well as the moisture condensation on the cold bodies.

The balance must be leveled according to the occasion of its use. The balance position with or without load must be conferred several times with 10% of the total load and with the total load. The balance difference, found in two successive determinations, made with equal weights, should not exceed 0.1 mg for analytical balances (maximum 200 g) and 0.01 mg for analytical balances (maximum 20 g).

The weights and the material to be weighed must be deposited in the plate center. During the weighing operations, the doors of the protective housing must be closed.

5.2.2 DETERMINATION OF THE MELTING TEMPERATURE OR RANGE

Temperature or melting point of a substance is the temperature corrected in which the substance is completely melted.

The melting point of a substance is the one between the temperature corrected in which the substance begins to melt or to form droplets on the wall of the capillary tube and the temperature corrected in which is completely melted, which is evidenced by the disappearance of the solid phase.

There are four methods for determining the melting temperature or range.

METHOD I – CAPILLARY METHOD

Usually applied to substances easily transformed into dust.

Apparatus

Apparatus shown in **Figure 1**.

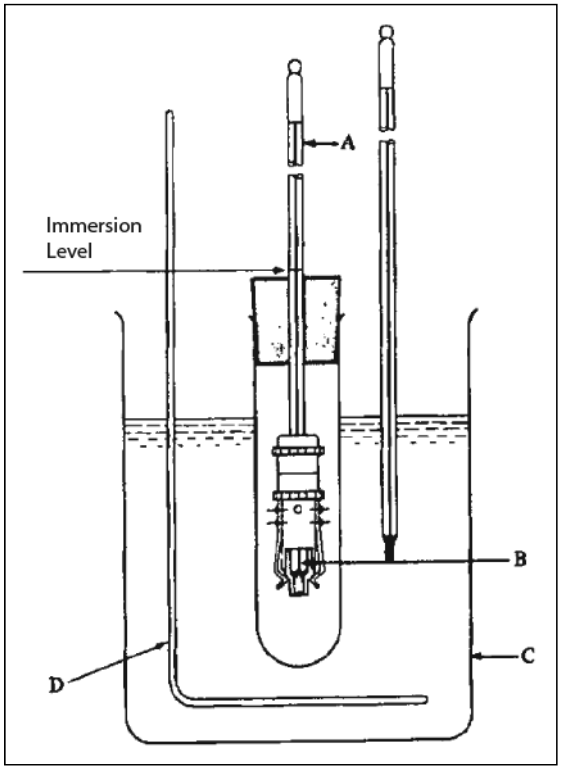

Figure 1 – apparatus for determination of the melting temperature or range by the capillary method.

The beaker should have capacity of 150 mL and contain liquid appropriate for the immersion bath according to the desired temperature. These liquids may be: liquid paraffin with high boiling point, fluid silicone with high boiling point, concentrated sulfuric acid, ethylene glycol or water.

The agitator must mix the liquid quickly, keeping the medium temperature homogeneous. The thermometer, calibrated until its immersion mark, must be in a range from -10 to 360 ºC,with divisions of 1 ºC and placed 2 cm from the bottom of the beaker.

The borosilicate glass capillary must be closed at one end and have approximately 8 to 9 cm in length, 0.8 to 1.2 mm of internal diameter and walls with thickness about 0.10 to 0.30 mm. A suitable magnifying glass should be used for observation of the capillary tube. As a heat source, use gas jet or electrical plate.

Procedure

Spray the substance under analysis and dry it in a vacuum desiccator over silica gel, phosphorus pentoxide or other suitable desiccant for 24 hours

Transfer a quantity of the dried powder to a dry capillary tube and pack the powder, by tapping the tube on a hard surface, so as to form a tightly packed column about 3-4 mm in height.

Heat the bath with constant stirring. When the temperature reaches 10 ºC below the assumed melting temperature,

regulate the temperature increase rate to 1-2 ºC per minute, depending on the stability of the substance under test.

When the bath is 10 ºC below the temperature range, introduce the capillary in the bath, so that its bottom is very near the middle of the thermometer bulb.

Before starting the determination of the melting range, an auxiliary thermometer is attached so that the bulb touches the standard thermometer at a point midway between the graduation for the expected melting point and the surface of the heating material.

The correction to be added to the temperature reading of the standard thermometer is calculated from the following formula:

$$0.00015\ N\ (T-t)$$

whereas

N = the number of degrees of the scale of the standard thermometer between the surface of the heating material and the level of the mercury,
T = the temperature reading of the standard thermometer;
t = is the temperature reading of the auxiliary thermometer.

The equipment must be calibrated through the application of melting temperature patterns among those internationally recognized or others.

METHOD II – OPEN CAPILLARY METHOD

Usually applied to substances easily transformed into powder.

Apparatus

A similar apparatus to that described under the *Capillary Method*, with the following modifications:

- water should be used in the heating vessel;

- an accurately standardized thermometer graduated in 0.2 ºC should cover the range -10 to +100°C; and

- a glass capillary tube should have the same dimensions as described under the *Capillary Method*, but must be open at both ends.

Procedure

Melt the substance as soon as the temperature do not exceed 10 ºC above the complete melting point. Stir and, if necessary, filter through a dry filter paper.

Insert the substance melted at the end of the capillary to form a column of 8 to 12 mm in height. Cool the capillary containing the sample at a temperature at 15 ºC, while maintaining it, at least, for 16 hours. Attach the capillary tube on the thermometer so that the substance column is located in the middle part of mercury bulb. Put the system in a water bath at 15 ºC, depth of 3 cm from the water surface. Warm up with constant stirring to increase the temperature in 2 ºC per minute.

The temperature at which the substance begins to ascend in the capillary is the melting point.

METHOD III – DROP METHOD

Usually applied for the determination of the melting point of greases substances with pasty consistency.

Apparatus

A similar apparatus to that described under *Method I* should be used with the following modifications:

- Thermometer with reading until 100 ºC graduated at 1 ºC
- capillary is not used
- the immersion liquid is water.

Procedure

Melt the sample, stirring until it reaches a temperature of 90 to 92 ºC and immediately leave the melted sample to cool down at a temperature of 8 to 10 ºC above the melting point expected. Cool the bulb of a thermometer until it is at 5 ºC, and while it is cold, submerge it into the sample melted until the height of the half of the bulb, approximately. Remove immediately and keep it in a vertical position until the surface of the sample deposited on the bulb is solidified, keeping it in the water bath for approximately 5 minutes at a temperature not higher than 16 ºC. Adapt the thermometer with the sample inside of a test tube by means of a rubber or cork stopper, so its lower end is about 15 mm above the bottom of the test tube. Suspend the test tube in a water bath at a temperature of 16 ºC and increase the bath temperature until 30 ºC at a speed of 2 ºC per minute and then at a speed of 1 ºC per minute, until the first drop pops out of the thermometer. The temperature at which this occurs is the melting point.

For each determination, employ a newly cast portion of the sample. If the variation of the three determinations is less than 1 ºC, calculate the average. If the variation is greater than 1 ºC, carry out more two determinations and determine the average of the five readings.

METHOD IV – METHOD OF HEATED METAL BLOCK

For widespread application in determining the point or melting range of substances.

Apparatus

Consists of metal block with high thermal conductivity, resistant to substances under analysis and flat and polished surface, such as brass, stainless steel and the like.

The block should contain an internal cylindrical cavity, parallel to its top surface and 3 mm away, with appropriate dimensions to accommodate the calibrated thermometer.

The block should be evenly heated by means of electrical resistance or micro-adjustable flame. The apparatus must be constantly calibrated with appropriate substances and its degree of purity must be proven.

Procedure

Heat the block quickly until temperature of 10 ºC below the melting point expected, and then adjust the heating to temperature increments at 1 ˚C per minute.

At regular intervals, put some particles of the sample, previously sprayed and drought, on the metal surface, in the region immediately above the thermometer bulb. Clean the surface after each test. Record the temperature (t) in which the substance melts immediately after the contact with the metal. Stop the heating. During the cooling, put again some particles of the sample, at regular intervals, at the same location of the block, cleaning the surface after each test. Record the temperature (t) in which the substance instantly solidifies upon the contact with the metal.

The instantenous melting point of the sample is calculated by the following expression:

$$\frac{t_1 + t_2}{2}$$

5.2.3 DETERMINATION OF THE BOILING TEMPERATURE AND THE DISTILLATION RANGE

Temperature or boiling point of a liquid is the corrected temperature in which the liquid boils under steam pressure of 101,3 kPa (760 mm Hg).

The distillation range is the temperature interval, corrected for a pressure of 101,3 kPa (760 mm Hg), within which a liquid, or a specified fraction of a liquid, entirely distils.

APPARATUS

Use the apparatus as recommended in **Figure 1** consisting of a distillation flask with a capacity of 100 mL connected to condenser B. On the bottom end, B is attached to adaptor C. A 50-mL beaker graduated at 0.2 mL is used as a collector. A thermometer is inserted in the neck of the flask so that the temperature sensor is in the center of the neck and 5 mm lower than the level of the lateral tube. The heating (gas, electric or through bath) must be selected according to the nature of the substance.

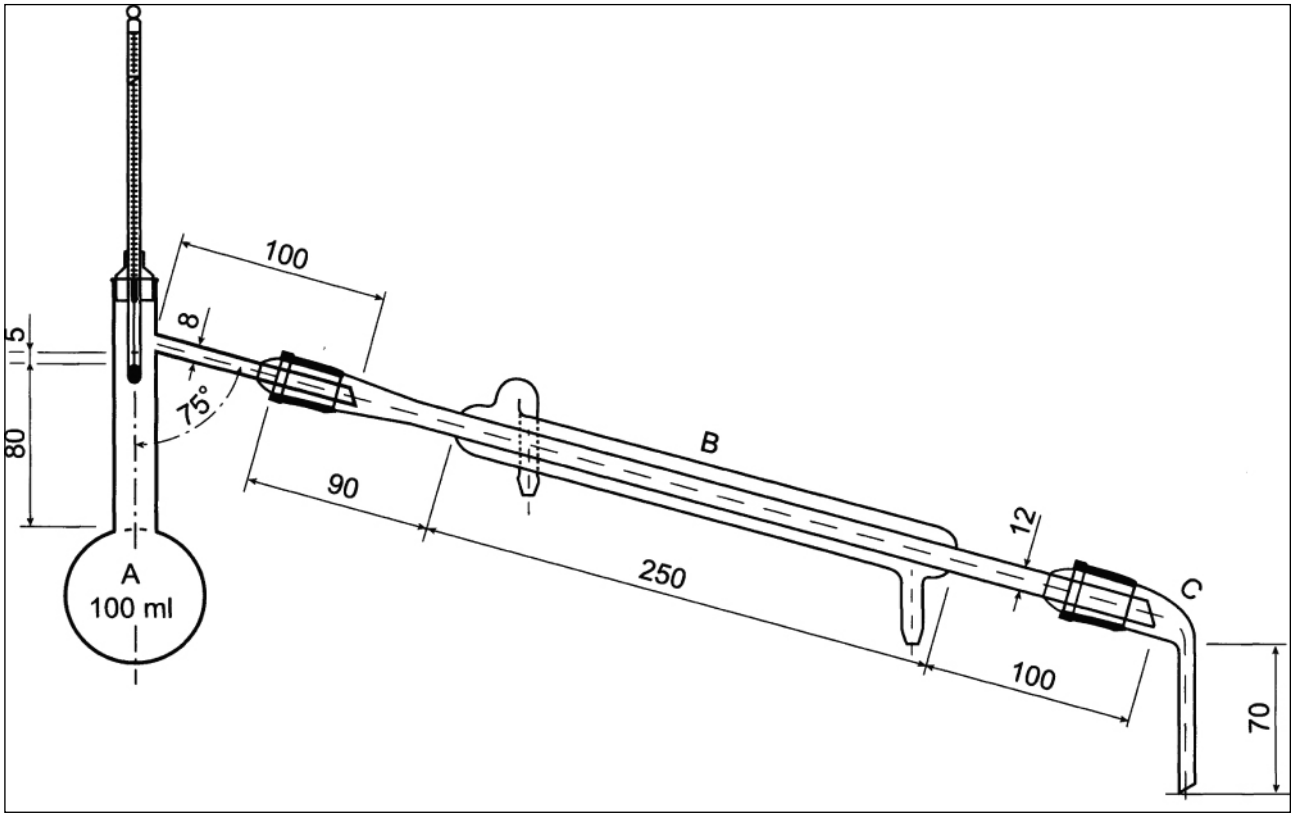

Figure 1 – Apparatus for the determination of distillation range (dimensions in mm). A, distillation flask; B, straight tube condenser; C, adaptor.

PROCEDURE

Place in the flask (A) 50.0 ml of the liquid to beexamined and avoid to not drain to the side tube.

Add glass beads or other suitable porous material. Adapt the thermometer to the flask and slowly heat, protecting the system against the current of air.

Record the temperature at which the first five drops of distillate were collected. Adjust the heating to obtain the distillate the flow rate of 3 to 4 mL per minute. Record the temperature at which the last drop evaporate from the distillation flask or when the specified fraction is collected. Keep the distillate at the same temperature at which the liquid was originally measured and record the distillate volume.

Compare the values obtained from the boiling point, distillation range and distillate volume with their monographs specifications.

Correct the observed temperature for barometric pressureby means of the formula:

$$t_1 = t_2 + k\,(101{,}3 - b)$$

Whereas:

t_1 = the corrected temperature;
= the observed temperature at barometricpressure b**;**
k = the correction factor **(Table 1),** unless this factor is not considered;
b = the barometric pressure, in kilopascals, at the time of the distillation.

Table 1 – Correction factors for different temperatures of distillation.

Distillation temperature	*Correction factor k*
Up to 100 ºC	0.30
Above 100 ºC and up to 140 ºC	0.34
Above 140 ºC and up to 190 ˚C	0.38
Above 190 ºC and up to 240 ºC	0.41
Above 240 ºC	0.45

Note 1: when the liquid is pure, most part distills at a constant temperature (in a range of 0.5 ºC). This temperature is the boiling point of the liquid.

Note 2: liquids that are distill below 80 ºC must be cooled to 10-15 °C prior to measure the volume and the beaker that receives the distillate must be immersed in an ice bath.

Note 3: when the boiling point is higher than 140-150 ºC, it is possible to replace the water condenser for air condenser.

5.2.4 DETERMINAÇÃO DA TEMPERATURA DE CONGELAMENTO

Temperatura ou *ponto de congelamento* de líquido ou de sólido fundido é a mais alta temperatura na qual ocorre solidificação.

Para substâncias puras que fundem sem decomposição, o ponto de congelamento do líquido é igual ao ponto de fusão.

APARELHAGEM

O aparelho (**Figura 1)** consiste em tubo de ensaio de aproximadamente 25 mm de diâmetro interno e 150 mm de comprimento suspenso por intermédio de rolha adequada dentro de um segundo tubo maior de 40 mm de diâmetro interno e 160 mm de comprimento formando uma camisa de ar que evita mudança brusca de temperatura. Esse sistema é fixo por garra no centro do béquer com capacidade de 1000 mL contendo água ou solução refrigerante.

The inside tube is sealed with a cork in order to hold the stirring rod and thermometer with 0.2 ºC divisions. The temperature sensor of the thermometer must be fixed approximately 15mm from the tube bottom. The stirrer is a glass rod adapted with a ring in its bottom end **(Figura 1).**

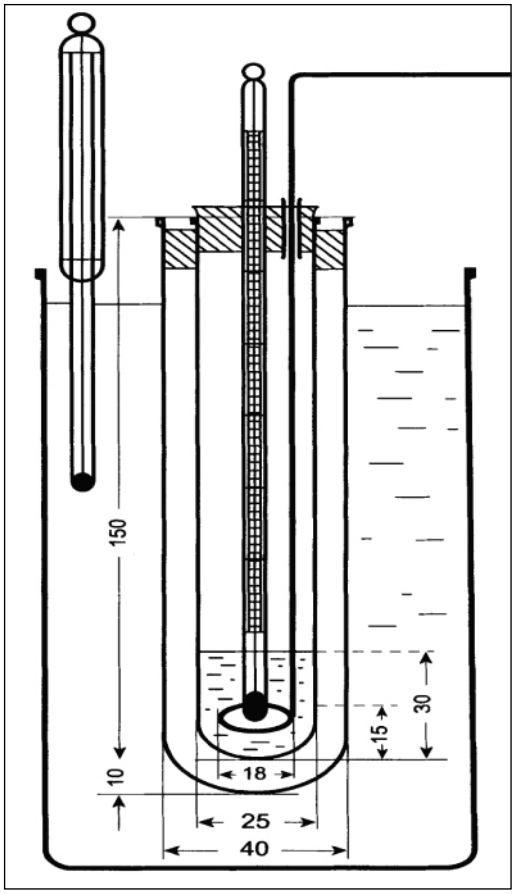

Figure 1 – Apparatus for determination of the defrosting point

PROCEDURE

Transfer the sample in sufficient quantity to reach 30 mm on the inner tube. Transfer to the beaker the coolant mixture suitable for 5 ºC below freezing point expected. When the sample is cooled down to about 5 ºC above the freezing point, move vertically the stirrer between the surface and the bottom, for approximately 20 cycles per minute and record the thermometer temperature every 30 seconds. Stop the stirring when the temperature remains constant or presents slight increase. Record the temperature every 30 seconds for at least 3 minutes after the temperature begins to decrease again.

Registrar o máximo na curva da temperatura-tempo que ocorre após a temperatura permanecer constante, ou apresentar leve aumento, e antes da temperatura começar a diminuir novamente. O ponto de congelamento é atribuído à média de não menos que três pontos máximos consecutivos que estejam dentro de uma faixa de 0,4 ºC.

Nota 1: se a substância é sólida a temperatura ambiente, fundir a substância e aquecer até no máximo 20 ºC acima da temperatura de congelamento esperada antes de transferir para o tubo interno.

Note 2: if the substance is liquid at ambient temperature use a bath at 15 ºC below the freezing temperature expected.

5.2.5 DETERMINATION OF MASS DENSITY AND RELATIVE DENSITY

Mass Density (ρ) of a substance is the ratio of its mass by its volume at 20 ºC. The mass density of the substance (ρ) at a given temperature (*t*) is calculated from its relative density (d_t^t) by

$$\rho_t = d_{(água)} \times d_t^t + 0{,}0012$$

expressed in g/mL or kg/L.

When the temperature, for example, is 20 ºC the formula is expressed as:

$$p_{20} = 0{,}99820 \; x \; d_{20}^{20} + 0{,}0012$$

Table 1– Water density from 0 to 40 °C.

Temp. (ºC)	*Density (g/mL)*	*Temp. (ºC)*	*Density (g/mL)*	*Temp. (ºC)*	*Density (g/mL)*	*Temp. (ºC)*	*Density (g/mL)*
0	0,99984	10	0,99970	20	0,99820	30	0,99565
1	0,99990	11	0,99961	21	0,99799	31	0,99534
2	0,99994	12	0,99950	22	0,99777	32	0,99503
3	0,99996	13	0,99938	23	0,99754	33	0,99470
4	0,99997	14	0,99924	24	0,99730	34	0,99437
5	0,99996	15	0,99910	25	0,99704	35	0,99403
6	0,99994	16	0,99894	26	0,99678	36	0,99368
7	0,99990	17	0,99877	27	0,99651	37	0,99333
8	0,99985	18	0,99860	28	0,99623	38	0,99297
9	0,99978	19	0,99841	29	0,99594	39	0,99259
10	0,99970	20	0,99820	30	0,99565	40	0,99222

Relative density of a substance is the ratio of its mass by the mass of the same volume of water, both at 20 ºC (d_{20}^{20}) or by mass of the same volume of water at 4°C (d_4^{20}):

$$d_4^{20} = 0{,}998234 \times d_{20}^{20}$$

PROCEDURE

The relative density of a substance may be determined through pycnometer, hydrostatic balance or hydrometer. The use of these last two is subject to the type of equipment available.

PYCNOMETER METHOD

Use clean and dry pycnometer with capacity of at least 5 mL, which has been previously calibrated. The calibration consists of the mass determination of the empty pycnometer and the mass of its contents with water, distilled and boiled at 20 °C.

Transfer the sample to the pycnometer. Adjust the temperature to 20 °C, remove the substance excess, if necessary, and weigh. Get the weight of the sample through the mass difference of the pycnometer filled and empty. Calculate the relative density (d_{20}^{20})determining the ratio between mass of the liquid sample and the mass of the water, both at 20 °C. Use the relative density to calculate the density of mass (p).

5.2.6 DETERMINATION OF THE REFRACTIVE INDEX

Refractive index (*n*) of a substance is the ratio of the speed of light in vacuum and its speed in substance. When a ray of monochromatic light passes from one transparent medium to another with a different optical density, this ray is reflected or refracted, except when focuses perpendicularly to the interface. The relationship between the sine of the angle of incidence (*sen i*) and the sine of the angle of refraction (*sen r*) is constant. This ratio is equivalent to the refractive index (*n*).

$$n = \frac{sen\ i}{sen\ r}$$

For practical purposes, the refraction is measured with reference to air and substance and not with reference to the vacuum and the substance, because the differences between the values obtained with both measures are not significant for pharmacopoeial purposes.

In isotropic substances, the refractive index is a constant characteristic in a given wavelength, temperature and pressure. For this reason, this index is useful not only to identify the substance, but, also, to detect the presence of impurities. It is applied to characterize mainly fats, oils, waxes, fatty sugars and organic solvents, as well as to identify certain drugs. It is also used to determine the purity of volatile oils.

The refractive index is usually determined in function of the sodium light at wavelength 589.3 nm (ray D) and at 20 ± 0.5 ºC. Then, express the refractive index value as n_D^{20}.

REFRACTOMETERS

The refractometers normally used in pharmacopoeia analysis use white light, but are calibrated so as to provide the refractive index in terms of wave length corresponding to the light of the sodium ray D.

The Abbé refractomer measures the range of refractive index for pharmaceutical substances. Other refractomeres of equal or greater accuracy may be applied.

Since the refractive index changes according to the temperature, during the reading you should adjust and maintain at 20 ºC.

The device calibration is performed with standard supplied by the manufacturer. For control of temperature and cleanliness of the equipment, the refraction index of the distilled water whose values are 1.3330 at 20 ºC and 1.3325 at 25 ºC should be determined.

5.2.7 VISCOSITY DETERMINATION

Viscosity is the expression of liquid resistance to flow, i. e. the displacement of part of its molecules on neighboring molecules. The viscosity of the liquid comes from internal friction, that is, the cohesion forces between molecules relatively together. With the increase of temperature, the average kinetic energy of molecules, decreases (on average) the time interval that the molecules passes together, the intermolecular forces become less effective and lower the viscosity.

The dynamic unit, CGS System, of viscosity is the *poise*. The CGS System of units is a physical measurement unit system, or dimensional system, with LMT typology, (length, mass time), whose basic units are the centimeter to length, the gram to mass and second to time.

The analogous dynamic unit in the International System of Units (SI) is the pascal second. The poise is often used with the prefix *centi*; a centipoise (cP) is a milipascal second (mPa·s) in SI units.

CGS System – *poise (P)*

$$1\ P = 1\ g \cdot cm^{-1} \cdot s^{-1}$$

By definition, *poise* is the strength, in dinas, required for the displacement of the flat layer of liquid, with an area of 1 cm^2, on another identical layer, parallel and distanced from the first at 1 cm, at a speed of 1 cm/s. The *poise* study is, however, too great for most applications, so is better to use *centipoise*, cP, corresponding to one hundredth of *poise*. Sometimes it is convenient to use the kinematic viscosity, which consists in the relation between the dynamic viscosity and density. In this case, the CGS system, the unit is the *stoke*. The example that occrus with absolute viscosity (measured in *poise*), it is more convenient to express kinematic viscosity in *centistokes* (100 *centistokes* = 1 *stoke*) to characterize the majority of liquids commonly used in Pharmacy and Chemistry.

International System of Units – *pascal second (Pa·s)*

$$1\ Pa{\cdot}s = 1\ kg \cdot m^{-1} \cdot s^{-1} = 10\ P$$

Pascal second equals 10 *poise*, but, normally, milipascal second (mPa·s) is used more often.

In **Table 1**, the viscosity of some liquids is registered.

Table 1 – Viscosity of some liquids.

Liquid	***Viscosity (P)[a] Units CGS***	***Viscosity Nsm Units SI***	***Viscosity cP = mPa.s***
Water	0,0101 (298 K)	0,00101	0,890
Aceton	0,00316	0,000316	0,306
Ethanol	0,01200	0,001200	1,074
Glycerin	14,9	1,49	934

[a] 1 poise (P) = 1 dina. s. cm-2 = 0,1 N s m-2. cP = centi-poise = mPa.s = mili Pascal times Sec.

Viscosity determination – test for which the temperature specification is essential due to its decisive influence on the outcome (in general, the viscosity is inversely proportional to temperature) – is performed based on various properties. The most frequent method is based on the time of disposal of liquids through capillaries (Ostwald viscometers,

Ubbelohde, Baumé and Engler) due to simplicity and affordable price of apparatus. Viscometers that has as a operation principle the time determination of the free fall of beads through tubes containing the liquid under test (Hoppler) or the rotation speed of metal axis immersed in liquid (Brookfield, among others) are equally applied.

Various methodologies that can be applied:

- liquid resistance to flow, flow time of a liquid through a capillary (Oswald viscometer, Ubbelohde, Baumé and Engler);
- time determination of the free fall of beads through tubes containing the liquid under test (Höppler);
- measuring the resistance to rotation movement of metal axis when immersed in liquid (Brookfield rheometer).

5

Although it is possible the determination of absolute viscosity, based on exact dimensions of the viscometer applied, prior calibration of apparatus with known liquid viscosity is the most frequent practice, allowing, by comparison, the relative evaluation on the viscosity of the liquid under test. Thus, employing the Ostwald viscometer or similar, the determination of flow time t_1 and t_2 of the volumes equal to reference liquids and sample, with density d_1 and d_2, respectively. Being h_2 the liquid viscosity of reference, absolute viscosity (cP) of the sample liquid, may be calculated by the equation:

$$\frac{\eta_1}{\eta_2} = \frac{t_1 d_1}{t_2 d_2}$$

or better

$$\eta_1 = \eta_2 \frac{t_1 d_1}{}$$

Quociente $\eta_2 / t_2 . d_2$ has a constant value, k, for each reference liquid, the same viscometer. Thus, this value (usually found in the apparatus manual), simplifies the equation:

$$\eta = k.t.d.$$

The k value can also be experimentally determined by measuring the flow time of the pure standard liquid, and by applying the following equation:

$$k = \frac{\eta}{t.d.}$$

Applying water as standard, usual for determination of low viscosity liquids, viscosity values recorded in **Table 2** are adopted, according to the test temperature:

Table 2 – Viscosity values, according to the test temperature.

Temperature (°C)	η*(cP)*
15	1,140
16	1,110
17	1,082
18	1,055
19	1,029
20	1,004
21	0,980
22	0,957
23	0,936
24	0,915
25	0,895

For very viscous liquids (glycerin and oils in general), it is possible to determine the relative viscosity by the method of the free fall speed of the f balls through the liquid, using the Höppler viscometer. This method is also appropriate to determine the absolute viscosity of liquids, by applying the following equation:

Whereas:

t = time of the ball fall (sec).
K = specific cte of the ball ($mPcm^3$), supplied by the manufacturer.
d_S = ball density (g/cm3).
d_L = liquid density (g/cm3).

The density of the liquid (d), for a certain temperature, can be obtained in reference books (such as handbooks), or be experimentally determined.

Viscosity concerning the Höppler method can be determined by applying the following equation:

$$\frac{\eta_1}{\eta_2} = \frac{(d_s - d_1)t_1}{(d_s - d_2)t_2}$$

where η, d and t are, respectively, the dynamic viscosity coefficient, the specific mass and the flow time with equal volume of liquids 1 and 2.

OSTWALD VISCOMETER

The operating principle of the Stokes viscometer is based on determining the speed of free fall of a ball through the fluid from which you want to obtain the viscosity.

The ostwald viscometer is the simplest and most popular among the available apparatus. It is composed by a bent U-tube (Figure 1), with one of the branches fitted with ampoule terminated in capillary. There are two dashes of reference, one immediately above the bulb and the other on the capillary. The other branch is sufficiently wide to allow the filling with the liquid under test up to the height of 5 mm below the lower reference trace. To allow a higher range of viscosities, likely to

be determined, viscometers collections are applied, with different sizes. The apparatus is suitable for a particular evaluation, which allows the sample flow in a period not less than 60 seconds.

For determining, transfer to the viscometer chosen, which should be washed and dried, sufficient quantity of fluid to reach the order level of 5 mm below the lower reference trace. Fix the apparatus in thermostat (20 ºC), after waiting that the liquid inside the apparatus obtain the controlled temperature, aspirate the liquid by capillary tube/ampoule (by means of a rubber hose attached to the end) until the liquid level slightly exceeds the upper reference trace. Release the pipe and, at the moment the meniscus reaches the upper reference mark, start the accuracy timer, blocking again when the meniscus pass by the lower reference trace. Record the elapsed time and repeat the test several times with intervals of a few minutes until the successive times do not differ in more than 0.5 seconds. Determine the density of the liquid under test (**5.2.5**), correcting the value for the relative density to water, at 20 ºC, and calculate the viscosity of the sample liquid by the formula indicated, employing the constant k supplied or determined by similar procedure.

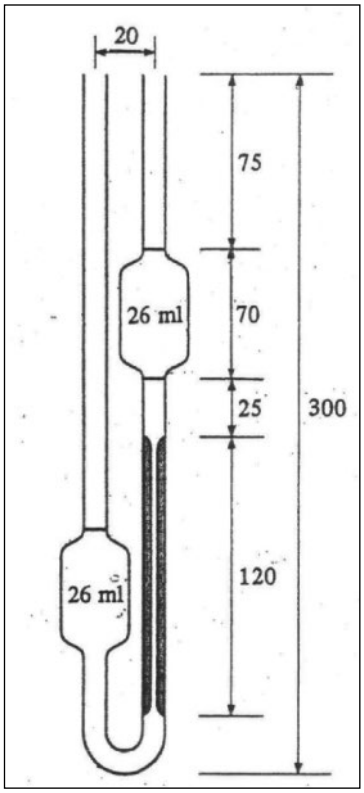

Figure 1 – Ostwald Viscometer (dimmensions em mm).

HÖPPLER VISCOMETER

The Höppler measurement system measures the time that a solid bead needs to travel a distance between two points of reference within a sloped tube with a sample. The results obtained are considered as dynamic viscosity in the standardized measurement on the International System (mPa. s). Precisely determines the viscosity of Newtonian liquids and gases (with a special ball for gases). Its applications include research, the control of processes and quality control, mainly used for low viscosity substances, between 0.6 and 10.000 mPa.s.

The Höppler viscometer is composed of a glass tube with two marks (A and B) spaced 10 mm from each other in the column, which define the measuring distance. A ball (glass, nickel and iron alloy or steel), with a diameter that is compatible with the size of the glass tube is installed at the top of the liquid contents. The tube is surrounded by a glass cylinder filled with water in circulation, under controlled temperature. The whole assembly is arranged in a slightly sloped position (10% in the vertical), and can be rotated

180º around an axis perpendicular to both tubes, to allow the repetition of determinations and the return of the ball to the starting position. The technique consists in clocking the time (fall) that a bead (with density and diameter variable with the respective structural constitution) needs to travel through the space between those two marks (A and B) existing in the ends of the glass tube. The higher the viscosity, the bigger time that the ball will pass through that space. The type of ebead to be used must be chosen depending on the presumed value of the viscosity of the liquid under observation. In the case of blood, glass beads are used. The results of the viscosity, newtonian liquids are expressed in international standards of absolute units (milipascal, seconds, mPa.s).

For determining, rinse the viscometer chosen, which should be washed and dried, with the liquid that will be used to determine the viscosity. Adjust the apparatus plummet. Choose the appropriate bead for each liquid (water = glass bead). Completely fill the inner tube of the viscometer with water. Record the fall time of the bead between the marks A and B in the viscometer. Make two more determinations to obtain the best average.

BROOKFIELD VISCOMETER

The viscosity of a pharmaceutical form can be determined by a Brookfield viscometer, which measures the viscosity by force required to rotate the spindle in the liquid under test.

To use this apparatus, it is required to proceed as follows:

- add the sample to be analyzed in container of the apparatus, until the desired mark;
- set the apparatus, by choosing a spindle number and a rotation to be tested, in accordance with specific methodology;
- immerse the spindle in the sample to be analyzed;
- start the apparatus and, after stabilizing the value, which will appear on the apparatus display, record this value which will be expressed in centipoise (cP);

if there is no value stabilization, test again, using another spindle number or another rotation.

EFFLUX VISCOMETER – FORD MODEL TYPE

Select the proper hole. The matrix for the hole selection should be obtaining an efflux time of the liquid under test for 60 seconds. The efflux time must be between 20 and 100 seconds, for the sample at 25 ºC.

The sample should be perfectly homogenized. At the time of the test, the viscometer and the material to be tested shall be at 25 ± 0.1 ºC. Close the hole with a flat glass blade and fill the beaker with the sample up to the highest level. Pour the sample slowly, avoiding the formation of bubbles. Level the sample in the beaker using a flat glass plate. Remove the blade from the hole. The sample will be retained in the beaker. Remove the flat glass plate and start the timer when the sample starts to flow through the hole. When the efflux flow interrupts, stop the timer and record the elapsed time in seconds. Carry out the test at least in triplicate. The viscosity will be the average of the values obtained, expressed in mm2/s or Centistokes, a maximum standard deviation of 3% is allowed.

The conversion of seconds to mm2/s or Centistokes is determined in accordance with the manual of the equipment used.

5.2.8 DETERMINATION OF ROTATING POWER AND THE SPECIFIC ROTATING POWER

Many pharmaceutical substances are optically active, they quick deflect the plane-polarized light so that the transmitted light is deflected at a given angle in relation to the incident.

Substances with the same structure containing one or more chiral centers, which are not superimposable mirror images of one another, are called enantiomers.

One of the enantiomers deflects plane-polarized light to the right (+) and is called dextrorotatory, or *d*; the antipodal deflects to the left (-) and is known as levorotatory or *l*. The angle of this deviation is equal in module for the enantiomers, but with opposite signs.

The physico-chemical properties of the enantiomers as density, refractive index, dipole-dipole interaction, boiling and melting points are identical since the chemical environment in which is inserted, each atom is equal to the enantiomers.

The polarimetry, that is, the measurement of the rotating power of a substance with polarimeter is one of the most practical methods to distinguish the enantiomers and, therefore, is an important criterion for the identification, characterization and determination of enantiomeric excess of drugs.

The rotating power varies with temperature, the wavelength of the incident light, the solvent used, the nature of the substance and its concentration. If the solution contains two optically active substances and these solutions do not react among themselves, the angle of deviation will be the algebraic sum of their angles of deviation.

Polarimeter

Historically, the polarimetric method was performed using an instrument in which the rotating power is estimated by viewing the intensity of splitting fields. For this reason, the sodium D-line lamp in the visible wavelength at 589 nm was the most frequently used. The specific rotating power determined on D-line is usually expressed by the symbol:

$$[\alpha]\frac{25}{D} \text{ or } [\alpha]\frac{20}{D}$$

The use of lower wavelengths as those available with the lines of mercury lamp isolated by means of filters with maximum transmittance at about 578,

546.436.405 and 365 nm in a photoelectric polarimeter, showed a greater sensitivity; consequently, there has been a reduction in the concentration of the substance in the test. In general, the rotating power observed at 436 nm is approximately twice and at 365 nm, is approximately three times higher than the power in 589 nm.

Rotating power and specific rotating power

The general equation used in polarimetry is:

$$[\alpha]\frac{t}{\lambda} = \frac{100a}{lc}$$

That [α] is the specific rotating power in the wavelength λ and at temperature *t*, *a* is the rotating power observed in degrees (°), *l* is the optical path in decimetres and *c* is the concentration of the substance in grams per 100 mL. Thus, [α] is 100 times α for a solution containing 1g in 100 mL in a cell with an optical path of 1.0 decimetre under defined conditions of the wavelength of the incident light and temperature.

Procedure

The specific rotating power of a drug is a reference value and must be calculated from the rotating power observed for the sample solution or fluid as specified in the monograph. The measures of the rotating power are carried out at 589 nm at 20 °C, except when specified. Whenever a photoelectric polarimeter is used, a single measurement corrected by white is performed. For a polarimeter visual, the average of at least five determinations corrected by white is used. In both cases, the solvent used for the preparation of the sample solution should be used as white or the empty tube in case of liquids. The experimental temperature should be maintained at ± 0.5 °C in relation to the specified value. Use the same tube of the polarimeter in the same angle orientation for the sample and white. Place the tube in a manner to allow the light to pass through it in the same direction each time.

The rotating power of solutions should be determined within 30 minutes after preparation. In cases where racemization or mutarotation occurs, all conditions must be standardized, from the preparation time of the solution to the polarimeter measurement.

The rotating power and the specific rotating power refer to dry, anhydrous or free of solvent substance, in all monographs which provide moisture values, loss on drying or solvent content.

5.2.9 DETERMINATION OF THE LOSS ON DRYING

This test is intended to determine the quantity of volatile substance of any nature eliminated under the conditions specified in the monograph. In the case of the water, the only volatile substance, it is enough to determine its contents by one of the methods described in *Water determination* (**5.2.20**).

For all other cases, the procedure adopted is described below, and the method to be adopted is specified in the monographs.

PROCEDURE

Gravimetric Method

Reduce the substance to a fine dust, if a form of large crystals is presented. Weigh, exactly, about 1 to 2g and transfer to a flat weighs-filter which has been previously dried during 30 minutes in the same conditions to be applied in the determination. After cooling in a desiccator, weigh the weighs-filter, which must be sealed, containing the sample. Stir the weighs-filter softly to distribute the sample as uniformly as possible, at an ideal height of 5mm. Put the weighs-filter in the oven, remove the seal, leaving it also in the oven. Dry the sample (usually at 105 ºC) and by a certain time (usually 2 hours) specified in the monograph. Cool to room temperature in a desiccator. Weigh. Repeat the operation until constant weight is obtained.

Note: In case the substance melt at a temperature lower than specified for the determination, keep the weigh-filter with its contents by to 1-2 hours at a temperature at 5-10 ºC below the melting point, before drying at the specified temperature. When the substance decomposes at a temperature of 105 ºC, it must be dried in a lower temperature. In both cases, it is possible to carry out the drying under a reduced pressure in a desiccator.

The percentage of loss on drying is given by the equation

$$\frac{Pu - Ps}{Pa} \times 100$$

whereas

Pa = weight of the sample,

Pu = weight of weighs-filter containing the sample before the dessication,
Ps = weight of weighs-filter containing the sample after thedessication.

Infrared balance or using halogen lamp

The procedure should be performed as follows.

- Remove the moisture from the equipment;
- weigh approximately 1g of the substance to be examined and distribute the material evenly in the aluminum collector inside the apparatus;
- set the drying time and temperature as described in the respective substance monograph. Most of the times, one (1) minute at 105 ºC is used.
- start the apparatus and record the moisture value, in percent, that will appear on the apparatus display.

Thermogravimetry

Proceed as described in *Thermal Analysis* (**5.2.27**).

5.2.10 DETERMINATION OF SULPHATED ASH (INCINERATION RESIDUE)

Sulphated ashes comprise the residues non-volatile to incineration in the presence of sulfuric acid, according to the technique specified. Usually, the test is intended to determine the contents of inorganic constituents or impurities contained in organic substances. It is also intended for the determination of inorganic components in mixtures and the amount of impurities contained in thermolabile inorganic substances.

PROCEDURE

Weigh exactly 1-2 g (or the amount specified in the monograph) of the sprayed substance, transfer to the crucible (as example: platinum, porcelain, silica, quartz) which has been previously calcined, cooled in a desiccator and weighed, and add about 1 mL of sulfuric acid. Gently heat until the carbonization at a temperature not higher than 600 ºC± 50 ºC. Cool and slowly add about 1 mL of sulfuric acid to moisten the residue, carbonize and incinerate with gradual warming up to 600 ºC± 50 ºC. Cool down, weigh again and incinerate for more

30 minutes. Repeat this procedure until the difference between two successive weighings is not greater than 0.5mg. A calibrated equipment, for example, muffle, should be used for the temperature control. Calculate the

percentage of the sulphated ash in relation to the substance under test, using the following calculation:

$$Cinzas\ sulfatadas\ (\%) = \frac{P_2 - P_1}{P_3} x100$$

whereas:

P_1= Weight of the crucible after calcination and cooling (tare do cadinho);
P_2= Weight of the crucible after calcination and cooling
P_3 = Weight of the original sample
100 = Percentage Factor.

5.2.11 DETERMINATION OF FINENESS OF SALES SOLUTION

The division degree or the powder granulometry is expressed by reference to the mesh nominal aperture of the sieve used. The sieves used are made of stainless steel, brass, the wire coating is not allowed.

In the description of the powders, the terms below are used:

Thick powder – all particles passes in the sieves with a mesh nominal aperture of 1.70 mm, and up to 40% in the sieve with a mesh nominal aperture of 355 cm.

Moderately thick powder – all particles passes in the sieves with a mesh nominal aperture of 710 cm and up to 40% in the sieve with a mesh nominal aperture of 250 cm.

Semi-fine powder – all particles passes in the sieves with a mesh nominal aperture of 355 cm and up to 40% in the sieve with a mesh nominal aperture of 180 cm.

Fine powder – all particles passes in the sieves with a mesh nominal aperture of 180 cm.

Ultra-fine Powder – all particles passes in the sieves with a mesh nominal aperture of 125 cm.

The determination of the powder granulometry is made by the process described below, with the aid of sieves, whose characteristics are standardized in the table attached.

PROCEDURE

The granulometry is determined with the aid of the sieves operated by a mechanical device. This type of device copies the horizontal and vertical movements of the manual operation, through the uniform mechanical action. To use this device, proceed as follows:

Separate, at least, 4 sieves which are described in **Table 1**, in accordance with the characteristics of the sample. Mount the assembly with the sieves with a greater aperture on the one with a smaller aperture. Place the assembly on the sieve receiver.

Weigh approximately 25 g of the sample (depending on the nature of the material, density of powder or granule and the diameter of sieves to be used). Transfer the sample to the upper sieve, evenly distributing the dust. Seal the assembly.

Start the apparatus, for about 15 minutes, with proper vibration. After the end of this time, using a suitable brush, remove the sample held in the upper surface of each mesh for a waterproof paper, and weigh the powder. Also weigh the powder held in the collector.

Calculate the percentage held in each sieve, using the following calculation:

$$\%\ Retida\ pelo\ tamis = \frac{P_1}{P_2} . 100$$

whereas:

P_1 = Weight of the sample held in each sieve (in grams);
P_2 = Sum of the weights held in each sieve and in the collector (in grams);
100 = Percentage factor.

Table 1 – Mesh aperture of the sieves.

Número do tamis (ABNT/ ASTM)	***Orifício do tamis***
2	9,5 mm
3,5	5,6 mm
4	4,75 mm
8	2,36 mm
10	2 mm
20	850 µm
30	600 µm
40	425 µm
50	300 µm
60	250 µm
70	212 µm
80	180 µm
100	150 µm
120	125 µm
200	75 µm
230	63 µm
270	53 µm
325	45 µm
400	38 µm
500	25 µm
635	20 µm

* The number of sieves corresponds to the Brazilian National Standards Organization— ABNT (1984), ISO 33101:2000.

5.2.12 LIQUID COLORS

The evaluation of liquid colors is carried out by comparing the solution under analysis – prepared according to the monograph instructions – and standard color solutions (SC). Such solutions are applied as reference for some drugs and in carbonization tests with sulfuric acid specified in several monographs.

The comparative process, unless otherwise specified, shall be carried out in glass test tubes with flat bottom, with a diameter of approximately 16 mm, of the type used in test limit of impurities. The tubes must be the most uniform possible.

For the evaluation, use 10 mL volumes for the preparation sample and for the standard preparation, ensuring approximate height of 50 mm for the liquids in tubes. Observe the tubes crosswise against white background, under diffuse light. It is important to compare the solutions under the same conditions, including temperature (25 ºC).

The sample preparation is prepared to show a coloring similar to the reference preparation specified.

BASIC STANDARDS

The color reference solutions (SC) are obtained from three basic solutions, to be prepared and stored in airtight bottles. Based on Table 1, containing volumes indication for the preparation of 20 color standard solutions (SC) designated with the alphabet letters, from A to T – prepare the solution or solutions specified for the comparison. Transfer the volumes indicated (the water should be the last) and homogenize directly in comparison tubes.

Cobalt chloride basic solution II

Prepare 25 mL solution of hydrochloric acid and 975 mL of water. Dissolve 65 g of sodium chloride of cobalt (II) in approximately 900 mL of this solution and complete the volume to 1000 mL with the same solvent. Transfer, using a pipette, 5 mL of this solution to the 250 mL iodine vial, add 5 mL of hydrogen peroxide SR and 15 mL of sodium hydroxide 5 M. Boil for ten minutes, cool down and add 2 g of potassium iodide and 20 mL of sulfuric acid 0.26 *M*. Titrate with sodium thiosulphate 0,1 *M* SV, adding 3 mL of starch SI as indicator. Correct the volume of titrant consumed by the blank determination. Each mL of sodium thiosulphate 0.1 *M* SV is equivalent to 23.79 mg of $C^oCl_2.6H_2O$. Adjust the solution volume of by adding sufficient quantity of hydrochloric acid solution and water to obtain a solution containing exactly MG $C^oCl_2.6H_2O$ per mL of solution.

Cupric sulphate basic solution

Prepare 25 mL solution of hydrochloric acid and 975 mL of water. Dissolve 65 g of cupric sulphate ($CuSO_4.5H_2O$) in 900 mL of this solution and fill up the volume to 1000 mL with the same solution. Transfer, using pipette, 10 mL of this solution to the 250 mL iodine flask, add 40 mL of water, 4 mL of glacial acetic acid, 3 g of potassium iodide and 5 mL of hydrochloric acid. Titrate the iodine released with sodium thiosulphate 0.1 *M* SV, mixing 3 mL of the starch SI as an indicator. Correct the volume of titrant consumed by the blank determination. Each mL of sodium thiosulphate 0.1 *M* SV is equivalent to 24.97 mg of $CuSO_4.5H_2O$. Adjust the solution volume of by adding sufficient quantity of hydrochloric acid solution and water to obtain a solution containing exactly 62.4 mg of $CuSO_4.5H_2O$ per mL of solution.

Ferric chloride basic solution

Prepare 25 mL solution of hydrochloric acid and 975 mL of water. Dissolve 55g of ferric chloride ($FeCl_3.6H_2O$) in approximately 900 mL of this solution and fill up the volume to 1000 mL with the same solution. Transfer, using pipette, 10 mL of this solution to the 250 mL iodine vial, add 15 mL of water, 3 g of potassium iodide and 5 mL of hydrochloric acid. Leave to rest for 15 minutes. Complete the solution volume to 100 mL with water and titrate the iodine released with the sodium thiosulphate 0.1 *M* SV, adding 3 mL of starch SI as an indicator. Correct the volume of titrant consumed by the blank determination. Each mL of sodium thiosulphate 0.1 *M* SV is equivalent to 27.03 mg of $FeCl_3\ 6H_2O$. Adjust the solution volume by adding sufficient quantity of hydrochloric acid solution and water to obtain solution containing exactly 45.0 mg of $FeCI_3\ 6H_2O$ per mL of solution.

Table 1 – Composition of the standard color solutions (SC).

SC	*Parts of*			
	Cobalt chloride basic solution II, in mL	***Base Solution of ferric chloride, in mL***	***Cupric sulphate basic solution, in mL***	***Water, to fill up to 10 mL.***
A	0,1	0,4	0,1	4,4
B	0,3	0,9	0,3	8,5
C	0,1	0,6	0,1	4,2
D	0,3	0,6	0,4	3,7
E	0,4	1,2	0,3	3,1
F	0,3	1,2	0,0	3,5
G	0,5	1,2	0,2	3,1
H	0,2	1,5	0,0	3,3
I	0,4	2,2	0,1	2,3
J	0,4	3,5	0,1	1,0
K	0,5	4,5	0,0	0,0
L	0,8	3,8	0,1	0,3
M	0,1	2,0	0,1	2,8
N	0,0	4,9	0,1	0,0
O	0,1	4,8	0,1	0,0
P	0,2	0,4	0,1	4,3
Q	0,2	0,3	0,1	4,4
R	0,3	0,4	0,2	4,1
S	0,2	0,1	0,0	4,7
T	0,5	0,5	0,4	3,6

5.2.13.1 ATOMIC ABSORPTION SPECTROMETRY

The atomic absorption spectrometry is used for the determination of various elements of the periodic table and basically consists of four techniques: flame atomic absorption, hydride generation, cold steam generation and graphite furnace. The techniques that use flame and graphite furnace as atomizers allow the determination of about 70 elements being metals the most part of these. The technique of hydride generation allows the determination of arsenic, antimony, selenium, bismuth, tellurium, lead, indium, tin, germanium and thallium; the generation of cold steam is basically used for the determination of mercury.

For the determination of the analyte concentration by atomic absorption, radiation from a source with a specific wavelength in accordance with the element tested occurs under the atomic steam containing atoms free from this element in the ground state. The radiation attenuation is proportional to the analyte concentration according to the Beer-Lambert law.

The instrumentation for atomic absorption basically consists of radiation source, atomizer, monochromator, detector and data processing system. As light sources, hollow cathode lamps and discharge lamps without electrode that emit intense radiation of the same wavelength of the absorbed by the element to be determined are used. The atomizer can be composed of a flame or graphite furnace. The monochromator is responsible for separating the wave length desired. The radiation focuses on monochromator by a narrow slot; then, it is separated by its different wavelengths in a diffraction grating and, after, directed to the detector. The detector is typically a photomultiplier, which transforms the light energy into electrical current, which is amplified and, then, interpreted by a reading system.

PROCEDURE

To operate the atomic absorption spectrometers, it is recommended to follow the manufacturer instructions. The determinations are made by comparison with reference solutions containing known concentrations of the analyte. The determinations may be made by the *Direct calibration method (Method I)* or by the *Standard addition method (Method II)*. The *Method I* is recommended, unless otherwise specified.

Direct calibration method (Method I): prepare at least four reference solutions of the element to be determined using the concentration range recommended by the equipment manufacturer for the analyte. All reagents used in the sample preparation should also be included, at the same concentrations, in preparation of the reference solutions. After equipment calibration with solvent, introduce in the atomizer three times each of the reference solutions and, after the reading, record the result. Wash the sample introduction system with water after each operation. Trace the analytical curve for the mean absorbances of three readings for each reference solution with the respective concentration. Prepare the sample as indicated in the monograph by adjusting its concentration; so the concentration falls within the concentration range of the reference solutions for the analyte. Introduce the sample in atomizer, record the reading and wash the sample introduction system with water. Repeat this sequence twice. Determine the element concentration by the analytical curve using the average of the three readings.

Standard addition method (Method II): at least, add four volumetric flasks with equal volumes of the substance solution to be determined prepared as indicated in the monograph. Into three volumetric flasks, add volumes determined from the reference solution specified in order to obtain a series of solutions containing increasing amounts of analyte. Fill up the volume of each flask with water. After calibrating the spectrometer with water, record three times the readings of each solution. Trace the analytical curve for the mean absorbances of three readings for each reference solution versus the respective quantity of analyte added to the solution. Record the analyte amount in module of the sample by extrapolation of the analytical curve on the x-axis.

5.2.13.1.1 Flame Atomic absorption spectrometry

The system consists of a pre-mix camera in which the fuel and oxidant are mixed and burner that receives the fuel-oxidant mixture. The solution is introduced through a pneumatic nebulizer, in which a fine aerosol is generated that is driven up to the flame. The energy amount that can be supplied by the flame for the dissociation and the sample atomization is proportional to the temperature. If a low temperature flame is used, the solution can not be converted into neutral atoms. On the other hand, if a flame with very high temperature is applied, the formation of a large amount of ions which do not absorb radiation from the source may occur. By modifying the ratio of oxidant and fuel used for each type of flame, it is possible to significantly change its temperature. The flames normally used are produced by air-acetylene (2100 – 2400 °C) and nitrous oxide-acetylene (2650 – 2850 ºC). The air-acetylene mixture is used for elements with lower atomization temperatures as Na, K, Mg, Cd, Zn, Cu, Mn, Co, etc. The flame generated by nitrous oxide-acetylene is applied to refractory elements as Al, V, Ti, Si, U, among others.

INTERFERENCES

Physical interferences: the use of sample preparation with physical properties such as viscosity and surface tension different from the standard preparation may result in differences in relation to aspiration and nebulization, leading to incorrect readings. Whenever possible, use preparations with the same physical properties and matrix constituents.

Iionization interference: usually occurs in alkaline and alkaline earth elements that are easily ionized. The higher the ionization degree, the lower is the absorbance. In order to reduce the ionization interferences, it is possible to use flames with lower temperatures or use "ionization suppressors" that are elements such as cesium, which ionize easier than the analyte, thus increasing the number of atoms in the ground state.

Chemical interferences: the formation of thermally stable compounds in the flame as the oxides of some elements (Ca, Ti, V, Cr, Al, etc) reduces the population of atoms in the ground state. This can be resolved by increasing the temperature of the flame which results in dissociation of these compounds. Another possibility is to use a "suppressor or liberating agent" that has greater affinity for oxygen in relation to the analyte, avoiding the oxides formation. The solution containing cesium chloride and lanthanum chloride, "Schinkel's Solution", is the most commonly used.

Spectruml interferences: they occur by means of absorption or scattering of radiation selected for the analyte. The spectruml interferences caused by atoms are not common and can be resolved by changing the spectruml line used. The interference caused by molecular species are more serious but are normally resolved through bottom correction.

5.2.13.1.2 Hydride generation atomic absorption spectrometry

The hydride generation atomic absorption spectrometry is a technique used for the determination of formative elements of volatile hydrides more usually to As, Se, Sb, Bi, Ge, Sn, Pb and Te. The process consists of three main steps: generation, transport and atomization of hydrides. The system can be built in batch or flow. The hydrides generation consists of the reaction of the analyte, usually in acid medium, with a reducer ($NaBH_4$). The hydrides transport from the reaction flask to the quartz cell is done through an inert gas, as argon or nitrogen. For elements that absorb at a wavelength lower than 200 nm, before the hydrides generation step, a purge for the removal of atmospheric gases should be done in order to avoid that these gases absorb the radiation from the source. The atomization is made in an electrically heated quartz cell or with a typical burner of flame atomizing systems; the internal temperature of the cell is 850º-1000ºC. Normally, the signal obtained is the transient type; approximately

20 seconds are required for the signal full integration for almost all elements.

INTERFERENCES

Oxidation State Influence: the analytes typically have more than one oxidation state. Arsenic and antimony, for example, have oxidation states III and V, and selenium and tellurium have oxidation states IV and VI, respectively. The superior oxidation states, in general, are inert for conversion to volatile hydrides; therefore, the pre-reduction before the determination is required in these cases.

Hydride Forming Elements: mutual interference can occur between the Hydride Forming Elements, for example, between arsenic and selenium. In these cases, the kinetics this process.

Transition Elements: some metal ions as $Cu^{2}+$ and $Ni^{2}+$, if present in high concentrations, are reduced, forming precipitates that may adsorb volatile hydrides.

5.2.13.1.3 Atomic absorption spectrometry with cold steam generation

The atomic absorption spectrometry with cold steam generation is used for the determination of mercury. The equipment and reagents are the same used in the hydride generation system, however, the quartz cell does not need to be heated, because mercury is reduced to metallic mercury, that is volatile at ambient temperature. Therefore, water steam can be transported by gas and interfere in the determination. To solve this problem, an infrared lamp is used to heat the quartz cell, preventing the water steam condensation. In this case, the purging is not required, because the wavelength used for the determination of Hg is 253.7 nm, which the radiation absorption by atmosphere gases is rare.

5.2.13.1.4 Atomic absorption spectrometry with graphite furnace

The atomic absorption spectrometry with graphite furnace is a comprehensive technique that has high sensitivity. The furnace consists of a graphite tube of 3 to 5 cm long and 3 to 8 mm in diameter coated with pyrolytic graphite. The sample amount injected into the furnace varies from 5uL to 50 uL and is generally introduced by an automated system. The furnace is electrically heated by the electrical current in a longitudinal or transverse manner. Flow of inert gases such as argon are internally and externally maintained to prevent the combustion of the furnace. Furthermore, the internal flow expels the atmosphere from the air furnace and also the vapors generated during the drying and pyrolysis stages.

A graphite furnace offers a durability of approximately 300 cycles depending on the model.

The analysis with the graphite furnace can be divided into the following steps: drying of the sample, pyrolysis, atomization and cleaning. The transition from one step to another is marked by the temperature increase, therefore, a special heating program must be planned. First, the drying of the sample is carried out; in this phase, the solvents and residual acids are evaporated. After drying, the temperature is incrased for the matrix removal (pyrolysis step). Then, the temperature increase leads to the analyte atomization

for subsequent quantification. Finally, the oven is cleaned at a high temperature (e. g. 2600 ºC) for few seconds. The temperature and the duration of each heating step can be controlled; this is essential for the development of analytical methodologies.

Curves of atomization and pyrolysis are used for the temperature optimization for such processes. The pyrolysis curve allows to determine the maximum temperature at which the analyte loss does not occur. The atomization curve allows to determine the atomization minimum temperature of the analyte with adequate sensitivity. It is recommended that the pyrolysis and atomization curves are made whenever an unknown sample is analyzed.

The atomization process in a graphite furnace is complex and depends on several factors such as the furnace and platform material, the atmosphere inside the tube, the heating speed, the temperature and the substances nature. For best results, it is recommended to use the L'Vov platform inside the tube and transverse heating. The signal obtained is the transient type; up to 12 seconds are required for the signal integration.

INTERFERENCES

Spectruml interferences: interferences caused by line overlap among atoms are very common. The attenuation of the radiation beam by species generated during the atomization process resulting from the matrix are more frequent. To solve this problem, the array must be eliminated efficiently. The use of a matrix modifier and a bottom corrector are essential to the reliability of the results.

Formation of volatile substances: in samples with high levels of halogens (especially Cl), there is the formation possibility of analyte volatile substances that may be lost in low temperatures causing an analysis error. In this case, the use of a chemical modifier able to form thermally stable complexes with the analyte reduces the formation of volatile substances. In addition, when the chemical modifier is combined with the L'vov platform, the matrix interference effects are considerably reduced. It is important to emphasize that a given chemical modifier can be very effective for some elements, but ineffective for others.

5.2.13.2 ATOMIC EMISSION SPECTROMETRY

Atomic emission spectrometry is the method that allows the determination of an element concentration in a sample by measuring the intensity of one of the element emission lines. The determination is made at the wavelength of this emission line. The emission sources in atomic emission spectrometry must have energy to generate neutral atoms and to excite the elements of interest.

5.2.13.2.1 Flame photometry

The flame photometry is a technique that presents good sensitivity, and is used mainly for the determination of alkali metals. The equipment consists of a flame normally produced by mixing oil liquefied air-gas, a monochromator and a detector. The solvent for the preparation of the sample solution and reference solutions should preferably be aqueous. The organic solvents may be used, provided that they do not interfere with the stability of the flame.

INTERFERENCES

The interferences that occur in flame photometry are very similar to those observed in *Atomic absorption spectrometry* **(5.2.13.1).** However, spectruml interferences may occur caused by the emission of molecular rotation-vibration bands, such as OH (310-330 nm), NH (around 340 nm), N2+ (around 390 nm), C2 (around 450 nm), etc.

SOLVENTS

The solvent must be selected with caution. If there is a significant difference of surface tension or viscosity between the sample and reference solution, variations will occur in aspiration and nebulization rates and, as a consequence, significant differences in signals produced. Thus, the solvent applied in the preparation of sample and references should be as similar as possible.

PROCEDURE

The equipment must be operated according to the manufacturer instructions and in the wave length specified. Adjust the zero with the solvent. Then, inject the concentrated reference solution and adjust the sensitivity desired. The determinations are made by comparison with reference solutions containing known analyte concentrations. The measurements can be carried out by the *Direct calibration method (Method I)* or by the *Standard addition method (Method II)* as described in *Atomic absorption spectrometry* (**5.2.13.1**).

5.2.13.2.2 Optical emission spectrometry with inductively coupled plasma

The optical emission spectrometry with inductively coupled plasma is a very comprehensive technique that has high sensitivity and with a multi-elementary characteristic. In a general way, in the spectrometry with inductively coupled plasma, the aerosol of the sample is introduced into a plasma source, where is evaporated and dissociated into atoms and free ions that are excited. The plasma is a partially ionized gas at a high temperature (6000 to 10.000 °C), electrically neutral and with good electrical conductivity. Due to the plasma high temperature, a polychromatic radiation is generated, arising from the issuance of multiple elements and ions present in the sample. Therefore, it is required to use a monochromator with high resolution capability for separation of wavelengths

of each element. The radiation detection generated by specific wave length can be applied to qualitative analysis and the intensities of these wavelengths can be used for quantitative analysis.

INSTRUMENTATION

The instruments used in spectrometry with inductively coupled plasma consist basically of the generator and the signal processor. The generator is formed by plasma source and sample introduction system (propelling pump and nebulizer). The signal processor is understood by optical and electronics systems and data acquisition unit.

Plasma sources: the most common is the inductively coupled plasma. The plasma is generated in a torch that consists of three concentric tubes usually made of quartz. Generally, argon gas streams are maintained in three compartments formed by concentric tubes. In the outer housing, the gas is used for the plasma formation. The intermediate compartment drives the auxiliary gas that is responsible for maintaining the plasma away from the inner housing and prevent deposition of carbon and salts from the sample in this slot. The internal argon stream drives the aerosol sample for the plasma center. When a given power (between 700 and 1500 W) is applied by radiofrequency generator in the induction coil, an alternating current is generated in the coil at a frequency of 27 or 40 MHz. This oscillation in the coil results in an intense electromagnetic field at the end of the torch. With the argon flowing by the torch, a high voltage electric discharge is applied to the gas generating electrons and argon ions. The electrons are accelerated by the magnetic field and collide with more argon atoms, generating more ions and electrons. The argon ionization continues in a chain reaction generating plasma which consists of argon atoms, electron and argon ions.

Detection system for Optical Emission spectrometry with Inductively Coupled Plasma: all elements present in plasma emit radiation at the same time, the use of a multi-elementar detection system is necessary. The spectrometers may be simultaneous or sequential. For optical emission spectrometry with inductively coupled plasma, both the sequential and the simultaneous spectrometers are widely used. The most common configuration for sequential spectrometers is the *Czerny-Turner*. The simultaneous spectrometers are basically found with the *Echelle* and *Paschen-Runge* configurations.

INTERFERENCES

The overlap of emission lines is one of the main interferences for optical emission spectrometry with inductively coupled plasma. This interference type can be eliminated with the use of high-resolution spectrometers and bottom correction procedures. Many spectruml interferences are observed in the range of 200 to

400 nm, in which more than 200.000 atomic emission lines and molecular bands are observed.

The physical interferences are similar to those in the *Atomic absorption spectrometry with flame* section **(5.2.13.1.1).**

SOLVENTS

The solvent optimal for optical emission spectrometry with inductively coupled plasma interferes the least possible in the emission processes. The solvent type should be selected with caution. If there is a significant difference of surface tension or viscosity between the sample and reference solution, variations will occur in aspiration and nebulization speeds and, as a consequence, significant differences in signals produced. Thus, the solvent applied in the sample preparation and references solutions should be as similar as possible.

PROCEDURE

The equipment must be operated according to the manufacturer's instructions and in the wavelength suitable for each element. The determinations are made by comparison with reference solutions containing known concentrations of analytes. The determinations may be made by the *Direct calibration method (Method I)* or by the *Standard addition method (Method II)* as described in *Atomic absorption spectrometry* **(5.2.13.1).**

5.2.13.3 MASS SPECTROMETRY WITH INDUCTIVELY COUPLED PLASMA

The mass spectrometry with inductively coupled plasma is used for the determination of various elements with high sensitivity, in the ppt range, and with multi-elementar capacity.

INSTRUMENTATION

As in the optical emission spectrometry with inductively coupled plasma **(5.2.13.2.2),** the mass spectrometry with inductively coupled plasma consists of two main units: the signal generator and the signal processor. The fundamental difference is that in mass spectrometry with inductively coupled plasma the signal processor is covered by an interface, a mass separator and a data acquisition unit. The interface is responsible for sampling and the efficient transport of the ions of the plasma at atmospheric pressure (760 Torr) until the mass separador (10^{-6} Torr) is made by the reduction of pressure through the vacuum application. The interface consists of two metal cones with too small holes (1 mm in diameter). After the generation of ions in the plasma, they pass by the first cone (cone of the sampling) and, soon after, the second cone (skimmer). After the passage of ions by the skimmer, due to the expansion, there is a need for them to be focused to ensure their arrival to the mass analyzer. Ions are focused by the action of an ionic lens or set of ionic lenses, which consists of a hollow metal cylinder (or a series of cylinders or perforated plates) subjected to a potential difference (typically in the range of 2-15 V direct current). Most part of the mass spectrometers

with inductively coupled plasma sold currently uses the quadrupole as a mass separator. The quadrupole consists of four metal cylindrical or hyperbolic bars of the same length and diameter. By the combined application of direct current (dc) and alternating current (ac) to the electrodes (quadrupole), only the ions with a specific mass/charge ratio (m/z) are conducted through the quadrupole. The other ions collide with the electrodes or are removed from inside the quadrupole. Thus, the ions are sequentially separated by the quadrupole. Several types of detectors can be used to collect the ions on the output of quadrupole and convert into an electrical signal, but the most popular are the discrete dynodes, Faraday cup and Chaneltron.

INTERFERENCES

As well as in other spectrometric techniques, mass spectrometry with inductively coupled plasma has spectruml interferences and not spectruml. The spectruml interferences are dependent on the species present and can be divided into four main types: polyatomic, isobaric, dual ions and refractory oxides ions. This type of interference can be corrected by the simulation of the matrix composition, by choosing another isotope (when possible) or by use of reaction and/or collision cell. In some cases, the spectruml interferences can be corrected with the use of an appropriate software.

The non-spectruml interferences can arise for several reasons: deposition on the interface cones, the presence of another easily ionisable element, load space effect, among others.

However, the majority of non-spectruml interferences can be corrected by use of an internal standard. In this case, the internal standard must have mass/charge ratio and ionization potential similar to the analyte. Scandium and Rhodium, for example, are widely used as internal standard for elements with low and high mass/charge ratio, respectively.

SOLVENTS

The solvent optimal for mass spectrometry with inductively coupled plasma should interfere the least possible in the ionization processes. The solvent type should be selected with caution. If there is a significant difference of surface tension or viscosity between the sample and reference solution, variations will occur in aspiration and nebulization speeds and, as a consequence, significant differences in signals produced. Thus, the solvent applied in the sample preparation and references solutions should be as similar as possible.

PROCEDURE

The equipment must be operated according to the manufacturer's instructions and with the isotope suitable for each element. Adjust the zero with the solvent. The determinations are made by comparison with reference solutions containing known analyte concentrations. The measurements can be carried out by the *Direct calibration method (Method I), Standard addition method (Method II)* or by the *Internal Standard Method (Method III)*.

Direct Calibration Method (Method I). Prepare at least four reference solutions of analytes, covering the range of concentrations recommended by the equipment manufacturer for the elements under analysis. All reagents used in the preparation of sample solution should also be included, at the same concentrations, to the reference solutions. After the equipment calibration with solvent, inject, three times, each of the reference solutions and, after the reading stabilization, record the result, washing the system with solvent after each injection. Trace the analytical curve, plotting the average of the readings of each group of three, with their concentration. Prepare the substance solution to be determined as indicated in the monograph, adjusting its concentration to be within the range of concentrations of the reference solutions. Insert the sample in the equipment, record the reading and wash the system with solvent. Repeat this sequence twice and, adopting the average of three measurements, determine the analyte concentration by the analytical curve.

Standard Addition Method (Method II). Add to each one of, at least, four similar volumetric flasks, equal volumes of solution of the substance to be determined, prepared as described in the monograph. Gather into all the balloons, with the exception of one, the measured volumes of the reference solution specified, so as to obtain a series of solutions containing increasing amounts of analytes. Dilute the volume of each flask with water. After calibrating the spectrometer with water, as indicated above, record three times the readings of each solution.

Internal Standard Method (Method III). Prepare at least four reference solutions of analytes, covering the range of concentrations recommended by the manufacturer of the equipment for the analytes. All reagents used in the preparation of sample solution should also be included, at the same concentrations, to the reference solutions. The internal standard should be added in all solutions (solvent, reference solutions and samples), with fixed concentration and in the same order of magnitude of the analytes. After the equipment calibration with solvent, inject, three times, each of the reference solutions and, after stabilization of the reading, record the result, washing the system with solvent after each injection. Trace the analytical curve, plotting a graph of the ratio between the average of the intensities of the readings for each group of three and the intensity of the internal standard, with its respective concentration. Prepare a solution of the substance to be determined as indicated in the monograph, adjusting its concentration to be within the range of concentrations of the reference solutions. Inject the sample into the equipment, record the reading and wash the system with solvent. Repeat this sequence twice and, adopting the average of three measurements, determine the analyte concentration by analytical curve.

5.2.14 ULTRAVIOLET, VISIBLE AND INFRARED SPECTROPHOTOMETRY

The spectrometric techniques are based on the absorption of electromagnetic energy by molecules that depends on the concentration and its structure. In accordance with the frequency range of electromagnetic energy applied, the absorption spectrophotometry can be divided into ultraviolet, visible and infrared, and can be used as a technique for the identification and quantification of substances.

ELECTROMAGNETIC RADIATION

The electromagnetic radiation is a form of energy that propagates as waves and generally can be subdivided into regions of a specific wavelength. Still, it may be also considered as a stream of particles called photons (or quanta). Each photon contains certain energy whose magnitude is proportional to the frequency and inversely proportional to the wavelength. The wave length (l) is generally specified in nanometers, nm (10^{-9} m), and in some cases in micrometers, μ (10^{-6} m). In the case of infrared, electromagnetic radiation can also be described in terms of the number of wave and expressed in cm^{-1}. The ranges of wavelength of electromagnetic energy of interest for spectrophotometry are described in Table 1.

Table 1 – Ranges of wavelength of interest for spectrophotometry.

Region	*Ranges of wavelength of wave*
Ultraviolet (UV)	190 – 380 nm
Visible (VIS)	380 – 780 nm
Near infrared (NIR)	780–2500nm (12800–4000cm^{-1})
Mid infrared (MIR)	4 – 25 μ (2500 – 400 cm^{-1})
Far infrared	25 – 300 μm (400 – 33 cm^{-1})

INTERACTIONS OF ENERGY AND MATTER

The total energy of the molecule involves the energy derived from vibration (vibrational energy, due to relative movement of atoms or groups of atoms constituents of the molecule); rotation (rotational energy, due to the rotation of the molecule around an axis) and, usually, the electronic power, generated by the configuration of electrons in the molecule.

When the molecules absorb energy, they suffer from a transition to a state with higher energy or excited state. The transition to the excited state is not of a continuous nature, it is generally performed in steps called transitions. In the ultraviolet and visible region, the transitions are electronic and occur in portions of the molecule called chromophores. These transitions include promotions of occupied molecular orbital electronsgenerally π* and s*, ligand or not, for the orbitals with energy immediately higher, π* and s* anti-ligand.

In the region of the mid infrared (MIR), only transitions with vibrational energy occur, because the radiation in this region is insufficiently energical to promote electronic transitions. The vibrations induced by infrared radiation comprise stretchings and inter-atomic bond stress and modifications of bond angles.

The spectrum in the near infrared (NIR) are characterized by the radiation absorption by overtones and combination of fundamental vibrational modes of bonds such as C-H, N-H, O-H and S-H. The bands of a NIR spectrum are generally weaker than the MIR spectrum bands. Chemical and physical information, with qualitative and quantitative specifications, can be obtained from the NIR spectrum. However, the direct comparison between the sample spectrum and the reference chemical substance is not recommended.

The NIR spectrophotometry is widely used for physical and chemical analyses, such as: quantification and identification of active ingredients and excipients, identification of crystalline and polymorphous forms, determination of the particle size, disintegration pattern and process control.

MODES OF SPECTRUMS ACQUISITION

The spectrums can be obtained using different modes of acquisition. In the case of UV/VIS spectrophotometry, the main mode is the transmission. In the case of

NIR and MIR spectrophotometry, the spectrums can be acquired using the transmission and reflection mode. This last mode is subdivided into diffuse reflection and attenuated total reflection. There is still the possibility of combination of the transmission and reflection modes, called transreflection.

Transmission: the measure of the intensity decrease of the radiation at certain wavelengths when the radiation passes through the sample. The sample is prepared in the optical beam between the source and detector. The transmission (*T*) can be calculated by the formula below:

$$T = \frac{I}{I_0}$$

I_0 = intensity of incident radiation
I = intensity of radiation transmitted.

The specters in transmission can be converted to the absorbance:

$$A = \log_{10}\left(\frac{I_0}{I}\right)$$

Diffuse reflection: the ratio measure of the intensity of light reflected by the sample and the light reflected by a reference reflective surface. The radiation not absorbed is reflected toward the detector.

Attenuated total reflection: the infrared radiation propagates within an internal reflection element (high refractive index) through reflections on the walls of this element. The sample is placed in contact with the wall of this reflection element where it interacts with the infrared radiation (evanescent wave).

Transreflection: this mode is the combination of the transmission modes and reflection. To measure the transreflection, a mirror or a reflective surface is used to reflect the radiation transmitted through the sample, focusing a second time in the sample, and then to fold the optical path. The non-absorbed radiation reflected toward the detector.

INSTRUMENTATION USED IN ULTRAVIOLET (UV) AND VISIBLE (VIS)

Spectrophotometers used in the ultraviolet and visible region are essentially provided with radiation source; wavelength selector; absorption cells (bucket), for insertion of samples solutions in the beam of monochromatic light; radiation detector and a reading unit and signal processing.

The lamps most used as the source of radiation in spectrophotometry in the region of the ultraviolet and visible are deuterium and tungsten, that provide radiation between 160 to 380 nm and 320 to 2500 nm, respectively. The instruments for the regions of UV/VIS are generally equipped with one or more devices to restrict the radiation that is being measured within a narrow band that is absorbed or emitted by the analyte. The most part of the equipment uses a monochromator or filter to isolate the band of wavelength of desired so that only the band of interest is detected and measured. The monochromators generally have a diffraction grid, while that filters may be of interference or absorption. The photometers or colorimeters are the simplest instruments that use a filter for selection of the wavelength and are used generally in the visible region. The spectrophotometers, by its turn, use monochromators for selection of the wavelength and are used in the regions of UV/VIS.

The compartments used to receive the sample are called buckets that should show windows which are transparent in the spectruml region of interest. For the UV region, quartz buckets are necessary, whereas for the region of the VIS, it is possible to apply the glass or acrylic concentration.

The main types of detectors are the phototubes, the photodiodes arrangements and the devices for load transfer. The phototubes detectors are the simplest and its response is based on photoelectric effect. The diode array detector allows that all wavelengths can be monitored simultaneously. The load transfer devices have been applied in increasing numbers in spectroscopic instruments.

The spectrophotometers can be found in the configuration of single beam, double beam and multichannel. The double beam instruments have the advantage to compensate for any fluctuation in the radiant power source, when compared with the simple beam instruments. The multichannel instruments are new, they use detectors of the diode arrangement type and load transfer devices and allow the obtainment of the full spectrum of a sample in less than a second. In these instruments, the dispersive system is a grid spectrograph placed after the sample cell.

Spectrophotometers can be provided with graphics registers that allow the obtainment of the absorption spectrum. This feature is important for characterization purposes of the substance from obtaining of wavelengths where the highest absorbance is obtained ($l_{maximum}$). Currently, most spectrophotometers features a connection to a microcomputer and appropriate software, which allow the obtainment of substances absorption spectrums in digital copy.

INSTRUMENTATION USED IN MID INFRARED (MIR) AND NEAR INFRARED (NIR)

The spectrophotometers used for acquiring spectrums in near and mid infrared consist of a light source, monochromator and interferometer and detector, and allow obtaining spectrums in the region from 750 to 2500 nm (1330.440 cm^{-1}).

Currently, the average infrared spectrophotometers (4000 to 400 cm^{-1}) using an interferometer instead of the monochromator and the polychromatic radiation focuses under the sample and the spectrums are obtained in the frequency domain using the Fourier transform.

Cells of transmission, accessories for diffuse reflection and total attenuated reflection are the most common accessories for the acquisition of spectrum.

The spectrophotometry in near infrared (NIR) is a technique that allows obtaining the spectrum in the region from 13300 to 4000 cm^{-1} (750 to 2500 nm). The spectrophotometers in NIR region are comprised of appropriate radiation source, monochromator or an interferometer and detector. Conventional buckets, optical fibers, Cells of transmission, accessories for diffuse reflection and total attenuated reflection are the most common accessories for the acquisition of spectrum.

IDENTIFICATION BY SPECTROPHOTOMETRY

The identification of various pharmaceutical substances can be made using the ultraviolet, visible, mid infrared and near infrared regions. In a general way, the spectrophotometry in UV/VIS regions require solutions with concentration in the order 10 μ mL^{-1} of substance; while for MIR and NIR concentrations in the order 100 mg mL^{-1} are required. Although the spectrums obtained in regions of UV/VIS are more sensitive, they feature lower specificity when compared with the spectrum in the region of the MIR. In the case of MIR, measurements using the modes of reflection (diffuse and total attenuated) provide spectrum information equivalent to the one obtained by mode of transmission. When possible, the comparison of

the spectrum obtained before the spectrum of the reference chemical substance must be done.

Ultraviolet (UV) and visible (VIS)

Several monographs include absorption spectrum in the ultraviolet as identification proof. In such cases, there is no specification of the extension of the sweep, solvent, concentration of the solution and thickness of the bucket. Some drugs require the use of reference standards. The standard readings and sample are simultaneously made and carried out under the same conditions as the wave length, bucket size, etc.

For characterization using spectrophotometry UV/ VIS, the drug is dissolved using appropriate solvent. Many solvents are appropriate including water, alcohols, ethers and acidic solutions and diluted alkaline. It should be noted that the solvent does not absorb in the spectruml region that is being used.

Mid Infrared (MIR)

The spectrophotometry in the MIR is an identification test by excellence being able to differentiate substances with structural differences. From three regions of the infrared (near, mid and far) the region between 4000 to 400 cm^{-1} (mid infrared) is the most commonly used for identification purposes.

The transmission specters of solid samples are obtained from the its dispersion in mineral oil or through the preparation of potassium halide and Sodium tablets. The samples dispersion is prepared by crushing about 5 mg of the substance in a drop of mineral oil in a spectroscopic grade. The cake obtained is spread between two potassium bromide or sodium chloride windows. For the preparation of tablets, about 1 mg of the sample is crushed with approximately 300 mg of potassium bromide spectroscopic grade.

For solid powder samples opaque to the transmission of infrared radiation, the spectrum can also be acquired through the use of accessory for diffuse reflection. In this accessory, the infrared radiation reflects directly on the powder sample. Part of the radiation is absorbed and then is reflected diffuse toward the detector. In this case the sample in the form of powder is mixed with potassium bromide, approximately 5% (p/p) and prepared in the diffuse reflection accessory.

Finally, the spectrum of powder and pasty solid samples can be obtained using accessory for total attenuated reflection. The powder sample is prepared under the crystal with high index of refraction where it comes into contact with the infrared radiation; the prior preparation of the sample is not required.

QUANTITATIVE USE OF CHROMATOG- RAPHY

Spectrophotometry in the UV/VIS

The quantitative spectrophotometric analysis by absorption has as a principle the direct relation between the amount of light absorbed and the concentration of the substance, also known as Beer's law.

When the concentration (c) is expressed in mol. L^{-1} and the optical path (b) in centimeters, the equation becomes:

$$A = \varepsilon\ b\ c$$

Whereas

A = absorbance, logarithm of the inverse of the transmittance (A = – log T)
ε = molar absorptivity.
T = transmittance

Since the transmittance is the quotient between the intensity of the radiation transmitted by the solution (I_0) and the intensity of the incident radiation (I), the equation is:

$$\log_{10} (I_0/I) = A = \varepsilon\ b\ c$$

The intensity of the ultraviolet light absorption by chromophores substances is, in general, expressed as molar absorptivity, in conditions of maximum absorption. If the molar mass of the substance is not known, it is possible to express the absorption intensity by the equation of specific absorptivity – A (1%, 1 cm):

$$A\ (1\%, 1\ cm) = A / b\ c$$

Whereas A (1 %, 1 cm) corresponds to the absorbance of the solution at 1% (w/v) of the substance when the optical path is 1 cm.

To avoid possible deviations in the Beer's law, try to work with diluted solutions (of the order 0.01 M), avoiding associations between the molecules, and with monochrome radiations.

Near Infrared Spectrophotometry

The quantification by NIR spectrophotometry can be performed using data obtained from a reference method or from a set of calibration with samples with known composition. The spectrum can be obtained using the transmission modes and the reflection can be obtained with the aid of appropriate accessories. At first glance, the spectrum data are treated by means of mathematical transformations with the aim at reducing unwanted variations sources before the calibration step. The calibration process consists of the construction of a mathematical model that relates the response of the spectrophotometer to a property of the sample. There is a series of chemometrical algorithms that can be used in calibration. Typically, these algorithms are available in software and made available with the spectrophotometer. The main calibration algorithms are: multiple linear

regression – MLR, partial least squares – PLS and principal component regression – PCR.

Validation of a methodology that uses the NIR spectrophotometry is similar to the one required for any analytical procedure and generally is established from the chemometry tools. The main parameters to be evaluated are: specificity, linearity, range, accuracy, precision and robustness.

The specificity extent is dependent on the procedure used. The demonstration of the specificity of the NIR methods can be made through the following manners: (*i*) the wavelengths used in calibration models should correspond to the bands of the analyte of interest; (*ii*) for calibration using PLS, coefficients should be plotted and the regions with greater coefficient must be compared with the analyte spectrum; (*iii*) changes in the sample matrix should not significantly affect the quantification of the analyte.

The NIR linearity validation method involves the demonstration of linear response of the technique for samples distributed by means of a defined range of calibration. The coefficient of correlation, r, is not a suitable tool for the linearity verification, but is a variation measure of the the data that is adequately modeled by the equation. The best way to demonstrate the NIR methods linearity is through the statistical evaluation of the slope values and intercept values obtained for the validation set.

The operational range of the reference values of the analyte from the validation set defines the NIR method operational range. Controls must be established to ensure that the results outside the operational range are not accepted. The validation of a RIN method must generate an anomalous value when a sample containing analyte outside the operational range is analyzed.

The accuracy of a NIR method is demonstrated by the correlation of NIR results with the data of the reference technique. In addition, the accuracy can be checked from the proximity of the prediction standard error (SEP) with the reference method error. The reference method error should be known based on historical values. Different statistical methods can be used to verify statistical differences between the results obtained by the NIR method and reference method.

The accuracy of a NIR method expresses the concordance between a series of measurements obtained under pre-determined conditions. There are two levels of accuracy that can be considered: the repeatability and intermediate precision. The accuracy of a NIR method is typically expressed as coefficient of variation.

The robustness of the NIR method can be checked by means of changes in the method parameters such as: environmental conditions, sample temperature, sample characteristics and instrumental changes.

5.2.15 FLUORESCENCE SPECTROPHOTOMETRY

Some substances can be analyzed with greater sensitivity and specificity by means of fluorometric methods instead of other spectrometric techniques. The fluorescence spectrophotometry, or spectrofluorimetry, comprises the measurement of fluorescence emitted when these fluorescent substances are exposed to ultraviolet radiation, visible or other electromagnetic nature. Such radiation promotes the excitation of molecule electrons to higher energy levels. After a short period in the excited state – approximately 10^{-8} to 10^{-4} seconds – the electrons return to the ground state by means of non-radioactive process, called deactivation by collision, with the radioactive process called luminescence (fluorescence or minimum background phosphorescence), opposite to what occurs with the majority of the substances in which the return to the less energic state does not include emission of light. In deactivation by collision, the energy is lost as a heat shock between the molecules. In the radiant process, the excess energy is reemitted with maximum intensity at longer wavelength (in approximately 20 to 30 nm) than the excitatory radiation absorbed, due to energy loss happened in the process. Because it is from a fluorescent nature, the radiation emitted by the substance ceases when the energy source is removed and this characteristic distinguishes it from the phosphorescence, which continues for some time after the end of the excitation.

The intensity of the light emitted by a fluorescent solution is, under certain conditions, proportional to the concentration of the solute and, in consequence, used for analytical purposes. The measurement of the fluorescence intensity can not be used directly for the determination of the analyte concentration. For this reason, the determination is made by comparing the fluorescence intensity obtained for a sample solution with standard solutions, whose concentrations are known. The spectrofluorescence fundamentals consists of exciting the substance with radiation at the wavelength of maximum absorption and comparetively measure the intensity of the fluorescent light emitted before a standard.

DEFINITIONS

Fluorescence intensity: Empirical Expression of the fluorescent activity in arbitrary units proportional to the response of the detector.

Fluorescence excitation spectrum: graphical representation of the activation spectrum, showing the intensity of the radiation emitted by a substance enabled (ordered) and the wavelength of the excitatory incident radiation (abscissa).

Fluorescence emission spectrum: Graphical representation of the spectrum distribution of the radiation emitted by an activated substance, showing the intensity of radiation emitted as ordered and the wavelength as the abscissa.

EQUIPMENT

The determination of the fluorescence intensity can be performed in simple filter fluorimeter (fluorimeter) in adapted absorption spectrophotometer or in fluorescence spectrophotometer (spectrofluorimeter).

The filter fluorimeter comprises light source, primary filter, sample chamber, secondary filter and detection system. In fluorimeters of this type, the detector is 90° in relation to the incident light. Such arrangement in a right angle allows the incident light to pass through the sample solution without interfering with the fluorescent signal caught by the detector. This mechanism does not prevent that part of the diffused light reaches the detector due to diffusing parts inehrent to solutions or due to the presence of suspended solid particles. This residual dispersion is controlled with the use of filters. The primary filter selects the wavelength radiation appropriate to the excitation of the sample while the secondary filter selects the fluorescent radiation of the greater wavelength, blocking the access of scattered radiation to the detector.

Most part of the filter fluorimeter detectors are fitted with photomultipliers valves, with differences between equipment types for the spectruml region with maximum sensitivity. The electrical current generated by the photomultiplier is increased, and the corresponding reading is present in the analog or digital instrument.

Fluorescence spectrophotometers, in their turn, are distinguished from fluorimeters because they do not have filters, but have prism monochromators or diffraction grating, providing greater selectivity of wave length and flexibility.

Fluorimeters and fluorescence spectrophotometers allow the use of various sources of light. Mercury or tungsten bulbs, although common, are replaced with the advantage of a xenon arc lamp at high pressure, because this provides, unlike the other, continuous spectrum from the ultraviolet to the infrared. Either way, the radiation is very intense and should never be seen with the naked eyes, under risk of permanent injury.

The monochromators have to adjust the slot width. Narrow slots provide greater spectrum resolution and less noise while wide slots ensure higher intensity of light. The slot width to be adopted is a difference function between the wavelengths of incident and emitted light, as well as the level of sensitivity required for analysis.

The sample chamber usually allows the use of round tubes and square buckets, similar to those applied in absorption spectrophotometry, except by the need that the four vertical walls are polished. Sample volumes of the order form 2 to 3 mL are adequate although some instruments may be provided with small buckets, with capacity for 0.1 to 0.3 mL or even capillary holders that require even smaller volumes.

Equipment calibration

Fluorimeters and spectrofluorometers must be calibrated with stable fluorophore substances to ensure reproducible results. The variations are, in general, due to changes in the intensity of the bulbs or the sensitivity of the photomultiplier tube. The fluorophore can be a pure sample of the substance to be examined or any other fluorescent substance which has an easy purification, whose absorption and fluorescence wavelengths are similar to those of the substance under examination. For example, quinine in 0.05 *M* sulfuric acid is a suitable standard for blue fluorescence. On the other hand, fluorescein sodium hydroxide 0.1 *M* is appropriate for green fluorescence and rhodamine is the fluorophore of choice in red fluorescence. The wavelengths scale of the fluorescence spectrophotometer also requires periodic calibration.

PREPARATION OF SOLUTIONS

The choice of solvent used in the preparation of fluorescent solutions requires precautions. Nature, purity and pH of the solvent are relevant parameters in the intensity and spectrum distribution of fluorescence. As a result, it is recommended to use the specified volume in established methods. Many substances exhibit fluorescence in organic solvents, but are virtually non-fluorescing when dissolved in water. Thus, it is required the experimentation in various solvents to determine the fluorescent property of a substance.

For quantitative purposes, it is crucial that the fluorescence intensity store linear relationship with the concentration of the sample within limits compatible with the technique. If the solution is too concentrated, a significant proportion of the incident light will be absorbed in the periphery of the bucket and the smaller is the amount of radiation to reach the central region. This means that the substance will act as “internal filter”. However, this phenomenon is rare, considering that the fluorescence spectrophotometry is a high sensitivity technique, allowing the use of solutions of concentrations of the order 10^{-5} to 10^{-7} *M*.

Because the concentration limits are usually narrow in which the fluorescence is proportional to the concentration of the substance, the compliance with the rule is required $(c-d) / (a-b) = 0.40$ to 2.50. In this case, *a* is the fluorescence intensity of the reference solution, *b* is the intensity of the corresponding white, *c* is the intensity of the solution-sample and *d* is the intensity of the corresponding white.

The determinations of fluorescence are sensitive to the presence of solid particles in solutions. Such impurities reduce the intensity of the incident beam, producing false high readings due to multiple reflections in a bucket. It is necessary to eliminate these solids by centrifugation or filtration before reading, taking into account, however, that some filter papers may contain fluorescent impurities.

The presence of oxygen dissolved in solvent exerts an attenuator effect on the fluorescence intensity and it

should remove, by using, for example, passage of current of nitrogen, helium or any inert gas in solution, prior to reading.

The temperature control is also important. In some substances, the emission of fluorescence can decrease from 1 to 2% for each increase in temperature at 1ºC. In view of this, when the maximum accuracy is required, it is recommended the use of thermostated concentration. However, for routine analysis, there is no need of this feature since that the determinations are made quickly enough to avoid heating due to solution exposure to intense light.

Some fluorescent substances are sensitive to light and, when exposed to bright intense radiation of the fluorescence spectrophotometer, can decompose into more or less fluorescent products. This effect can be detected by observing the detector response in relation to time and attenuated with the intensity reduction of the light incident through the use of filters.

5.2.16 TURBIDIMETRY AND NEPHELOMETRY

Turbidimetry and nephelometry – variants of spectrophotometry – are intended to quantitative assessment of substances in function of turbidity of their suspensions, proportional to its diffraction power on incident light (Tyndall effect).

In turbidimetry, also known as opacimetry, the intensity of the transmitted light in the same sense of direction of the incident light is measured. Although there are turbidimeters, specifically intended to measure conventional turbidity, colorimeters and spectrophotometers are satisfactory to the extent of the transmitted light since they are adjusted to appropriate wavelength.

Nephelometry comprises the measure of the light scattered intensity (reflected) by suspended particles, at a right angle of incident light beam. In addition to nephelometers, it is possible the use of colorimeters and spectrophotometers in nephelometric measurement. They should be modify in such a way to allow capturing perpendicular to the angle of the incident light, whether by transfer of the light source or by the modification of the detector position. Fluorimeters, the example of nephelometers, is intended for the measurement of light scattered (positioning of the detector at an angle of 90 ° with respect to the incident light) and is, therefore, compatible with nephelometry.

Turbidance

Turbidance (S) in analogy to the transmittance (*T*), defined in *Utlraviolet, visible and infrared absorption spectrophotometry* (**2.5.14**) is the official expression for light dispersion produced by suspended particles. It is determined by turbidimetry or nephelometry, corresponding to equation

$$s = \frac{P_0}{P} k \frac{bd^3}{d^4 + \lambda^4}$$

Whereas

P_0 = intensity of incident radiation;
P = intensity of transmitted radiation;
b = the thickness of the sample (bucket);
C = concentration of the sample;
d = average diameter of particles;
λ = wavelength;
k = proportionality constant, dependent on the nature of the suspension and the method of measurement.

A suspension evaluted in a given instrument, under monochromatic light, presents turbidance that corresponds to the product of the concentration C by a proportionality constant k, which combines the other parameters of the equation above. The expression S = *k*C is obtained, expression of the Beer-Lambert's law, allowing that turbidity and nephelometric procedures are similar to those adopted in spectrophotometry. However, it is important to note that the proportionality is only true for very dilute suspensions, because secondary reflections cause excessive deviation from linearity when the number of particles in suspension exceeds a given threshold.

Another source of error in turbidity and nephelometric measures is the sedimentation of particles in suspension. Such occurrence can be minimized with the increase of the viscosity, with the incorporation of protective colloid

- Gelatin, gum arabic or starch – the liquid medium of the suspension.

PROCEDURE

The basic procedure for the use of turbidity or nephelometric techniques complies with the principles of spectrometric techniques, including the reference solutions preparation with suspensions of known concentration. In practice, it is permissible to plot against values of transmittance instead of turbidance.

The procedure steps comprise, in summary: (1) adjust the instrument in wave length specified in the monograph (for colorimeters, in the absence of specification, use filter that provide light in the blue strip); (2) fill the bucket with the more concentrated suspension and adjust the reading of transmittance to 100% (transmittance provides more linearity than absorbance); (3) measure the transmittance of other standard-suspensions and trace the calibration straight line (using the method of smaller squares) and (4) measure the transmittance of the sample by determining its concentration by the calibration straight line.

Visual Comparison

Carry out turbidity comparison in tubes that are matched as closely as possible in internal diameter and in all other respects. Flat-bottomed comparison tubes of transparent glass of about 70 ml capacity and about 23 mm internal diameter are suitable. For turbidity comparison the tubes should be viewed horizontally, against a dark background, with the aid of a light source directed from the sides of the tubes.

5.2.17 CHROMATOGRAPHY

5.2.17.1 THIN-LAYER CHROMATOGRAPHY

A chromatographic system in which the separation of the mixture components occurs through differential migration on a stationary phase composed of a thin layer of adsorbent applied on a flat support, which can be composed of various materials such as glass, aluminum or polyester. The mobile phase in its turn is composed of various mixtures of solvents and remains within a container or cup made of transparent and inert material, usually glass, remaining sealed where the chromatoplate is deposited in vertical position under a saturated atmosphere of the mobile phase.

EQUIPMENT AND PROCEDURES:

The equipment used for thin layer chromatography consist of: plate, cup or elution chamber, stationary phase and mobile phase, developer system. The plates are usually made of glass, aluminum or plastic. The sizes vary as follows: 20 cm x 20 cm; 10 cm x 20 cm; 10 cm x 10 cm; 5 cm x 10 cm.

Stationary Phase (adsorbent)

Silica – is more widely used in CCD. It is an amorphous, porous adsorbent. It is also used in column chromatography, but the silica used in CCD is thinner. The silica is prepared by spontaneous polymerisation and dehydration of silicic acid. The substances are adsorbed by silica through the hydrogen bond and dipole-dipole interaction. A silica with a satisfactory condition has 11 to 12% of water by weight. A humidity level at 11 to 12% is reached when the silica is in balance with the air, a relative humidity of 50% and a temperature of 20 °C.

The commercial silica have pore sizes varying between 40 to 150 ångström. The particle sizes vary from 5 to 40 µm, with an average of 10 to 15 µm, depending on the manufacturer.

Reducing the size of the particles increases the efficiency of silica. Particles of size 5 to 6 µm are used to CCDAE (high efficiency thin-layer chromatography). The pore sizes affect the selectivity and, therefore, can be used for the rates of migration and components resolution of the samples.

The sizes of most commercially common silica pores are 40.60.80 and 100 ångström. The silica 60 ångström is the most versatile and widely used. The fused silica are used for separation of lipophilic compounds such as aldehydes, ketones, phenols, fatty acids, amino acids, alkaloids, terpenoids and steroids, using the mechanism of adsorption.

Alumina – After silica, is the most commonly used adsorbent. The physical properties of alumina are similar to silica in terms of particle size, average pore diameter and surface area. Acidic (pH 4.0 – 4.5), neutral (7.0 – 8.0) and basic (9.0 – 10.0) alumina are commercially available. As the silica, alumina separates the components of the samples by polarity, hydrogen bonds or dipole forces. The selectivity of alumina in adsorption CCD is similar to silica-gel, therefore, alumina is a better adsorbent than silica, for separation of lipophilic acidic substances. The alumina with acid character strongly attracts basic compounds, while the basic alumina strongly attracts acidic compounds. The alumina retains aromatic compounds stronger than the silica-gel. It has the advantage of promoting catalysis of some reactions of labile substances. It is used in the separation of liposoluble vitamins, alkaloids, certain antibiotics, polycyclic hydrocarbons.

Kieselguhr – It is the thermally treated diatomaceous earth with granulation at 5 to 40 µm. Its main constituent is SiO_2. A variety of other inorganic compounds are also present. The pore sizes are variable, its characteristics make it suitable for the separation of sugars, amino acids and other similar polar substances.

Cellulose – Cellulose is polysaccharide which is highly polymerized by monomers of celobiose. The presence of a large number of free hydroxyl groups allows the hydrogen bond with low molecular weight liquids such as water and alcohols. Cellulose is suitable for the separation of hydrophilic substances, such as carbohydrates and amino acids.

Polyamide – In contrast with cellulose, polyamide is a synthetic resin. Two types of polyamide are used: polyamide 6 and polyamide 11. The polyamide 6 comes from aminopolicaprolactama, while the polyamide is prepared from the poliaminoundecanoico acid. Polyamides are used for the separation of polar compounds that are capable of interacting with the amide group by hydrogen bonds due to their molecular structure. Among them, there are amino acids and derivatives, benzodiazepines, carboxylic acids, cyclodextrins, fatty acids, flavonoids, preservatives, pesticides.

Magnesium silicate – ideal for the separation of sugars, anthraquinones, flavones, glucosides, steroids, lipids, residues of pesticides, vitamins, carbazois, hydrocortisone acetate.

Revealing and detection methods

After the development of chromatography and the evaporation of solvents, there is the method of spots revelation. This can be physical or chemical. The physical

methods include: ultraviolet light (bulbs with emission of radiation between 254 to 366 nm), in the case of substances that become fluorescent when excited by UV light or visible. The chemical methods include use of reagents not requiring chromogens. There is an extensive list of revealing appropriate for each group of compounds.

Identification

The final position of each stain is designated by Rf. After the revelation of chromatoplate, the distance reached by each stain is measured from the origin. This distance is a fraction of the total distance travelled by the solvent in the stationary phase.

Rf = (distance reached by stain from the origin) / (distance travelled by the solvent from the origin)

5.2.17.2 PAPER CHROMATOGRAPHY

It is used for the separation and identification of substances or components of the mixture to the differential migration over the surface of a pecial quality filter paper (stationary phase). The mobile phase may be a pure solvent or a mixture of solvents.

On paper, the chromatographic adsorbent is a layer of paper of appropriate thickness and texture. The chromatographic separation proceeds through the action of the mobile phase liquid similar to the process of column chromatography adsorption. Due to the intrinsic water content of the paper, or selective inhibition component, hydrophilic of the liquid phase by paper fibers, which can be considered as stationary phase, a partition mechanism may significantly contribute to the separation.

The chromatogram is developed by the slow passage of the mobile phase on the layer. The development may be ascending, in the case of solvent transported upwards through capillary forces, or downwards, in the case in which the solvent flow is aided by the gravity force.

The simplest form of paper chromatography is the ascending chromatography that uses a paper strip with variable width and length, in function of the chromatographic basin to be used.

This method is very useful for separating very polar substances, such as sugars and amino acids. It has the disadvantage of being able to fit a small substance quantity of each time. It is required to work under the closest conditions, quality and quantity as possible, between standard and sample, using the same paper, mobile phase, temperature, etc.

EQUIPMENT AND PROCEDURES

It is composed by a camera or glass chromatographic basin, provided with edges and grinding seal with adequate size to contain the chromatographic paper, which can be adapted for ascending or descending chromatography. It is important to not miss the vapors of the mobile phase.

Use filter paper special for chromatography, cut in the fiber direction in strips with variable length and width not less than 2.5 cm. There are several types of chromatography paper with different purposes for separation of hydrophilic or hydrophobic substances, organic or inorganic, amphoteric or with many hydroxyls, among others.

For descending chromatography, use the basin with a seal provided with central hole, closed by glass stopper or other inert materials. In the upper part of the basin, there is a suspended bucket, which contains device to secure the paper (usually stem or glass rod). On each side of the bucket there are glass guides, which sustain the hole, so do not touch the walls of the chromatographic bucket. The width of the chromatographic paper cannot be greater than the bucket that was suspended and the height should be approximately equal to the height of the chromatographic chamber.

For ascending chromatography, on the top of the bucket, there is the device that allows to sustain the chromatographic paper and that may fall without opening the chromatographic camera. The paper must be handled carefully and by the edges, and strips are cut in sizes that can be contained in the basins. It is important to cut the paper along the axis of the fibers, because the pulp is in this direction, which will facilitate the passage of the mobile phase. The paper strip should not touch the basin walls.

To add the paper in the basin (it should not take long to place the paper so that there is no loss of saturation), taking care that the sample does not come into direct contact with the eluent, leaving it to ascend or descenda across the surface of the paper, only by capillarity.

When the technique used is the ascending chromatography, draw a thin line with pencil 3 cm from the bottom edge of the paper; if the chromatography is descending, draw line at a distance, that it rests a few centimeters below the dipstick that secures the paper in the eluent bucket. You should also mark the finish line of the mobile phase (or the solvent front), usually 10 cm from the initial point.

Apply the solutions in the form of circular spots (capillary tubes or micropipettes are used), containing 1 to 20µ of the sample, and each stain should produce a width between 6 to 10 mm on the line drawn with pencil. Depending on the width of the paper, it is possible to put only one aliquot of the standard or sample, centralizing-if this application on the initial line. In the case it is possible to put than an aliquot at the initial point, you should put 2 cm away from the side edges and a 3 cm interval between the application points. If each stain produced is greater than 6 to 10 mm, apply the sample into portions, leaving the solvent to evaporate before applying the next portion.

The mobile phase must be below the initial point of the substance, the basin must always be selased to ensure that the steam of this phase is lost. At the end of the run,

wait for the paper to dry and submit it to some process of revelation.

DESCENDING CHROMATOGRAPHY

In descending chromatography, the mobile phase has a flow pointing downwards and counts with the action of gravity.

Insert into the camera a layer of eluent specified in the monograph, seal and leave to rest for 24 hours. Apply the sample in the paper, placing it properly on the tabs so that the upper end remains inside the bucket suspended and attach it with the glass rod. Close the bucket and leave to rest for 1 hour and a half. Then, through the hole in the seal, insert the eluent in a bucket. Develop the chromatogram until the distance or time prescribed, protecting the paper from the incidence of direct light. Remove the paper, mark the route of the mobile phase, dry and show the way prescribed in the monograph.

ASCENDING CHROMATOGRAPHY

The ascending flow of the mobile phase on the chromatographic paper is allowed by the action of capillarity.

Put on the bottom of the chamber container containing the eluent, close the basin and keep it at rest for 24 hours. Apply the sample in the paper by inserting it into the bowl

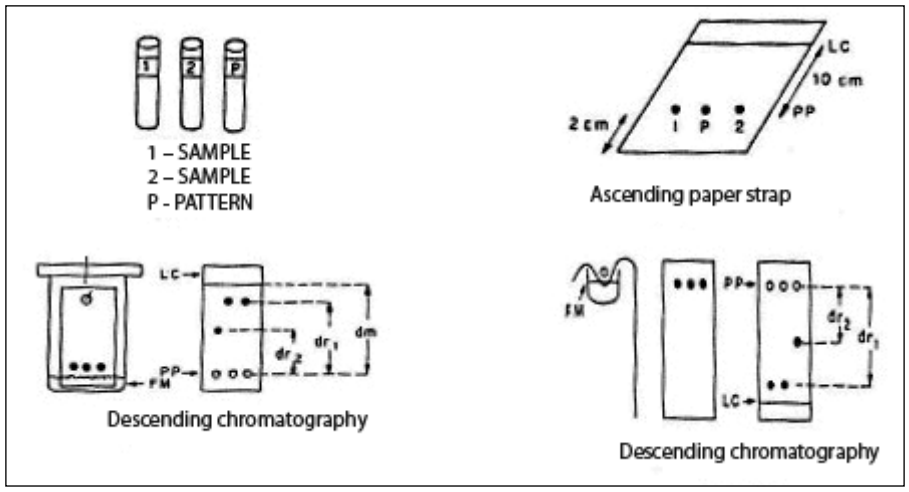

Figure 1 – Different types of paper chromatography in accordance with the development techniques

FM: Mobile Phase; PP: Initial Point; LC: Finish Line; dr1 and dr2: distances travelled by substances; dm: migration distance of the mobile phase

and leave to rest for 1 hour and a half. Without opening the camera, lower the paper to put its lower end in contact with the eluent and develop the chromatogram until the distance or time prescribed. Remove the paper, mark the route of the eluent, dry and show the way prescribed in the monograph.

5.2.17.3 COLUMN CHROMATOGRAPHY

In preparative chromatography column, there is a separation method that has an important role in the compounds purification of value in research, in the operation of a pilot plant and production of pharmaceutical products. It is a method that can be used to quickly, and is economic, for obtaining substances with high purity. In practice, standardized adsorbents are used, because they provide a high degree reliability of the method, the direct transfer of analysis scale and an optimized processing. The types of column chromatography may be: by adsorption (liquid-solid), per partition (liquid-liquid) or by ion exchange.

EQUIPMENT

The apparatus used for procedures in chromatography columns consist of a cylindrical tube, in vertical position, glass (or other inert and transparent material specified in individual monograph) length and diameters varying in which lower part there are bottlenecks (reduced route) and faucet for adjusting the flow rate of the various types of solvents or elution systems used. In some columns, the bottom shows on its base, a porous glass disc which purpose is to prevent the output of the stationary phase (silica-gel). The columns have variable sizes, however, in pharmaceutical analysis, most commonly rates used are from 10 to 30 mm in diameter along the tube and 3 to 6 mm in its lower part, where the faucet is attached. The length of the tube is usually from 150 to 400 mm. At the top of the column, there may be a spherical shape dilatation, intended to contain a larger volume of solvent followed by a cylindrical griding connection, tapped by a cylindrical stainless steel, aluminum, plastic or glass stopper, (or

other material specified in individual monograph) firmly attached to the vein. The rod vein is substantially smaller than the diameter of the column and has, at least, 5 cm more in relation to the effective length of the column. The stopper has a smaller diameter at approximately 1 mm in relation to the inner diameter of the column.

PROCEDURE

Column chromatography by adsorption

Start the preparation of the column, if necessary, sealing its bottom part, next to the faucet, with a piece of cotton or glass wool at the base of the tube in order to prevent the passage of the adsorbent material and air inlet (avoiding formation of bubbles). Evenly fill the pipe (as specified height) with this adsorbent material (such as activated alumina or silica gel, diatomaceous silica or calcined silica) previously suspended in the mobile phase (solvent system), performing the removal of the excess eluent. After sedimentation of the adsorbent material, apply the mixture of substances previously solubilized in a small amount of solvent in the top of the column until it can penetrate the adsorbent material. A certain amount of solvent can be added to the top to help on the adsorption of substances in adsorbent material, and then, sedimentar by the action of gravity or by the application of positive pressure of air leaving the mixture adsorbed in a narrow horizontal strip at the top of the column. The movement rate of a substance is determined or affected by several variables, including the low or high adsortivity of the adsorbent material, the particle size and the surface area (contact surface), the nature and polarity of the solvent, the applied pressure and the temperature of the chromatographic system.

A flow chromatogram is widely used and is obtained by a process in which solvents travel through the column, until the substance is separated in effluent solution, known as eluate. The eluate is controlled by collecting fractions as specified in the monograph and examining each fraction by suitable method. The substance may be determined in eluate by various methods: titration, colorimetry, spectrometry or be isolated (purified) upon the evaporation of the solvent. The efficiency of the separation can be gauged by thin layer chromatography (TLC) of each fraction collected along the chromatography run.

Column chromatography per partition

In partition chromatography, the substances to be separated are divided between two immiscible liquids, one of which, the stationary phase, is adsorbed on a solid support, thus presenting a surface area wide enough for the circulating solvent or mobile phase. The high number of successive contacts between liquid-liquid allows an effective separation, which does not occur through the usual liquid-liquid extraction.

The solid support is usually polar, when the adsorbent stationary phase, is more polar than the mobile phase. The most commonly used solid support consists of chromatographic silicon, whose particle size is satisfactory for the proper flow of the eluent. In reversed-phase partition chromatography, the adsorbed stationary phase is less polar than the mobile phase, and the adsorbent solid, becomes nonpolar by treatment with an silanising agent (e. g. diclorodimetilsilano; paraffin), to produce a silanized chromatographic sand.

The sample to be chromatographed is usually inserted into a chromatographic system in two ways: (a) a solution of the sample in a small volume of the mobile phase at the top of the column; or (b) a solution of the sample in a small volume of the stationary phase is mixed with the solid support and transferred to the column forming a transverse layer on the adsorbent material.

The development and elution are achieved through the "run" of the circulanting solvent. The (mobile phase) solvent is usually saturated with the (stationary phase) solvent before use.

In the case of conventional liquid-liquid partition chromatography, the degree of partition of a given compound between the two liquid phases is expressed through its partition or distribution coefficient. In the case of compounds that dissociate, it is possible to control the timing to change the pH, dielectric constant, ionic strength, and other properties of the two phases. The selective elution of the mixture components can be reached with the successful change of the mobile phase to the phase that provides a more favorable partition coefficient, or by changing the pH of the in situ stationary phase with a stationary phase composed of the acid solution or base in a suitable organic solvent.

Unless otherwise provided in the individual monograph, tests and trials using the partition chromatography column are carried out according with the conventional methods described below.

Solid Support – Use purified sand and silica. For reverse-phase partition chromatography, use chromatographic silica sand.

Stationary phase – Use the solvent or solution specified in the individual monograph. If a mixture of liquids in stationary phase is used, mix before entering the solid support.

Mobile phase – Use the solvent or solution specified in the individual monograph. Balance with water, if the stationary phase is an aqueous solution; if the stationary phase is an organic polar fluid, balance with this fluid.

Preparation of a Chromatographic Column – The chromatographic tube measures about 22 mm in internal diameter and 200 to 300 mm in length, without the porous glass disc, which is coupled with a distribution tube, without the faucet, with approximately 4 mm in internal diameter and approximately 50 mm in length. Introduce a thin glass wool buffer at the base of the tube. Add the specified amount of solid support in

a becher (beaker) of 100-250 mL and mix to produce a homogeneous paste. Transfer the mixture to the chromatographic tube, seal, lightly pressing them, until a uniform mass is obtained. If the quantity of solid support specified is more than 3 g, transfer the mixture to the column portions of approximately 2 g, by sealing each portion. If the test requires a threaded column, with a stationary phase different for each segment, seal after the addition of each segment, and add each segment directly to the previous one. If a solution of the analyte is incorporated into the stationary phase, complete the transfer quantitatively to the chromatographic tube by washing the becher used for preparation of the test mixture with a mixture of approximately 1 g of solid support and several drops of solvent used to prepare the test solution. Enter a thin glass wool buffer at the top of the column to fill completely. The mobile phase flows through a column properly filled as a moderate current or, if reversed-phase chromatographyis used, slowly, drop by drop, transfer the mobile phase for the space of the column on the filler column, and allow it to flow through the column under the action of gravity. Moisten the tip of the chromatographic column with approximately 1 mL of the mobile phase before each change of composition of the mobile phase and after completing the elution. If the analyte is introduced in the column as a solution of the mobile phase, let it pass completely through filler column, then add the mobile phase in several smaller portions, allowing each to be completely removed before adding the stocked mobile phase.

Column chromatography by ionic exchange

Use as stationary phase the ion exchange resin. The ion exchange consists of reversible ions exchange present in solution with ions of resinous polymer (modified cellulose or silica-gel support). The choice of resin, strong or weak, anion or cation, will depend in most part of the pH at which the ionic exchange should occur and the nature of ions (anions or cations) to be exchanged. The strong acidic resins and strong basic are convenient for the majority of analytical applications. The large excess (200 – 300 %) of resin are applied on the quantity of the sample stoichiometrically calculated; the capacity of resins varies from 2 to 5 mM/g (dry weight).

Resin treatment and column preparation – Suspend the ion exchange resin in the water and leave to rest for 24 hours. Insert it in appropriate column and, in the case of anion resin, convert it into basic passing through the column, sodium hydroxide solution SR, the speed of 3 mL/ min., until the eluate provide negative reaction for chloride. Then, pass the water free of carbon dioxide. In the case of cationic resin, conversion to the acid form occurs through passage of hydrochloric acid SR through the column, followed by washing with the water free of carbon dioxide until the eluate provides neutral reaction.

The ion exchange column is developed in a manner analogous to that described for adsorption chromatography. Upon the operation completion, the resin is regenerated by washing it with sodium hydroxide SR (anionic) columns or with hydrochloric acid SR (columns cationic) and, then, with water free of carbon dioxide until the neutral reaction is provided.

5.2.17.4 HIGH EFFICIENCY LIQUID CHROMATOGRAPHY

The high efficiency liquid chromatography (HPLC) is a separation technique based on the distribution of the components of a mixture between two immiscible phases, the mobile phase, liquid, and the solid stationary phase, contained in a cylindrical column. The separations are achieved by partition, adsorption, ion exchange, size exclusion or stereochemical interactions, depending on the type of stationary phase used. The CLAE has advantages over gas chromatography for the analysis of organic compounds. Non-volatile and thermolabile samples are preferably analyzed by HPLC. The majority of pharmaceutical analysis is based on the method of separation per partition and must occur in short time of analysis. Several chemical and physico-chemical factors influence on chromatographic separation, which depends on the chemical nature of the substances to be separated, the composition and flow rate of the mobile phase, the composition and surface area of the stationary phase.

APPARATUS

The equipment used is in a reservoir that contains the mobile phase, a pump with the purpose of spurring the mobile phase by chromatographic system, an injector for introducing the sample into the system, a chromatographic column, a detector and a data capture device, such as a software, integrator or recorder. In addition to receiving and sending information to the detector, software are used to control the entire chromatographic system, providing greater operational and logistics analysis.

The modern chromatographic systems consist of pumps to pressurize the mobile phase, controlled by software, which can be programd to vary the ratio of components of the mobile phase, as it is required for chromatography by gradient of solvent, or to mix, in isocratic manner, the mobile phase (mobile phases with fixed relationship of solvents). Operational pressures up to 5000 psi (approximately 345 bar) and flow rate of up to 10 mL per minute can be used. Higher pressures depends on the development of the instruments.

After dissolving the sample in the mobile phase or in another suitable solvent, the solution is injected in chromatographic system, in a manual manner, using suitable syringe, or by means of an injector or automatic sampler. This consists in a carousel or tray able to accommodate various vials containing the samples. Some automatic samplers can be programd to inject different volumes of sample, various quantities of injections,

control the interval between injections and other operational variables.

When working at high pressures, an injection valve is essential. This presents a calibrated system, with analysis volume, called injection ring or sampling handle, which will be completed with the solution to be analyzed and subsequently transferred to the column.

For the majority of pharmaceutical analysis, the separation is achieved by partitioning the components present in the solution to be analyzed, between mobile and stationary phases. Systems that consist of stationary phases, polar and apolar mobile phases are defined as normal phase chromatography, while the opposite, mobile phases polar and apolar stationary phases, are called reverse-phase chromatography. The affinity of a substance by stationary phase and, consequently its retention time in the column is controlled by the polarity of the mobile phase.

The stationary phases used in reversed-phase chromatography typically consist of an organic molecule chemically linked to silica particles or other media, such as porous graphite. The diameter of the particles is, normally, 3µ to 10 µ. The smaller the particle diameter and the film that covers the support, the faster and more efficient will be the transfer of substances between the stationary and mobile phases. The polarity of the column depends on the functional groups present, the most common are apolar groups, *octy, octadecyl, phenyl, cianopropil* and polar, *nitrile*. The proportion of silanol groups not linked to functional group significantly influence in the efficiency of the chromatographic separation and on the size of the eluate peak. Chromatography columns are commercially availablewith different qualities of stationary phases, including those with small proportion of groups free of silanols, called capeadas. Generally, silica columns in reverse phase feature useful life in a pH range of 2 to 8, however, columns containing graphite or porous polymeric materials, such as the styrene-divinylbenzene, are stable in a broader range of pH. In a less common way, liquids can be used and must be not connected, as coating of the silica support and, therefore, must be immiscible with the mobile phase. The columns normally used for analytical separations have internal diameters from 1 mm to 5 mm. These can be heated, providing more efficient separations, but are rarely used at temperatures above 60 °C, due to the potential for degradation of the stationary phase or the volatility of the mobile phase. Unless specified in the monograph of the substance to be analyzed, the columns are used at ambient temperature.

The detectors most frequently used in the liquid chromatography high efficiency are the spectrophotometric (UV/Vis). The spectrophotometric detectors are used to detect compounds with grouping chromophore. Such detectors consist of a flow cell located at the end of the chromatographic column. The ultraviolet radiation crosses, constantly, by flow cell and is received at the detector. With the system in operation, the substances are eluted from the column, are replaced by the cell of detector and absorb the radiation, resulting in measurable changes in the level of energy. These detectors can present fixed, variable or multiple wavelength. Fixed wavelength detectors operated in a single value, typically 254 nm, issued by a mercury lamp for low pressure. Those with variable wavelength contain a continuing source of emission, as a high pressure deuterium or xenon lamp, and a monochromator or an interference filter, in order to generate monochromatic radiation to a value selected by the operator, and can be programd to change the wavelength during the development of the analysis. The detectors of multiple wavelength measure, simultaneously, the absorbance at two or more wavelengths, called diode-array detector (DAD). In these, the ultraviolet radiation is transmitted through the flow cell, absorbed by the sample and then separated into its original components, that are detected, individually, by the detector of photodiodes, logging data of absorbance in the entire range of the spectrum from ultraviolet and visible and, additionally, the spectrum of each peak recorded in chromatogram.

The detectors of index of refraction measure the difference between the index of refraction of pure mobile phase and the mobile phase containing the substance to be analyzed. They are used to detect substances that do not absorb in the ultraviolet or visible, however, they are less sensitive than the spectrophotometric detectors. The detectors of index of refraction have the disadvantage of being sensitive to small changes in the composition of the mobile phase solvents, flow rate and temperature.

The fluorometric detectors are used to detect compounds with fluorophore grouping or that can be converted into fluorescent derivatives, by chemical transformation or adding fluorescent reagents specific to functional groups. If the chemical reaction is required, it is possible to do it at the time of preparation of the sample or, alternatively, the reagent can be introduced in the mobile phase, with the reaction occurring before the detection.

The potentiometric, voltammetric or electrochemical detectors are useful for quantification of substances that can be oxidized or reduced in an electrode. These detectors are highly selective, sensitive and safe, but require mobile phases free of oxygen and reducible metal ions. A continuous flow pump must be used, ensuring that the pH, ionic strength, and temperature of the mobile phase remain constant. Electrochemical detectors with carbon specific electrode can be used to quantify nanograms of easily oxidizable substances, such as phenols and catechols.

The detectors of mass spectrometry has the ability to measure the molar mass of a substance, combined with the liquid chromatography provide a high selectivity since the non-resolved peaks may be isolated by monitoring a selected mass value. These detectors can be simple denominated quadrupole (MS) or tandem (MS/MS), when associated, to exemplify some of the models used. The sources of ionization are the most common type of “ionization by eletrospray” and the “chemical ionization at atmospheric pressure”.

The detectors of conductivity has application in the ion-exchange chromatography and measure the conductivity of the mobile phase continuously which is modified with the presence of analytes in the cell.

Currently, data collection systems are available with modern functions to receive and store the signals coming from the detector and, subsequently, to provide the management of such information, generating the chromatograms with area data and peak height, identification of the sample and methods. The information may also be collected in simple systems of recording data, such as recorders, to ensure the integrity of the data generated.

PROCEDURE

The length and internal diameter of the column, the type and the particle size of the stationary phase, the operating temperature, the composition and the flow rate of the mobile phase and the type of detection are described in the individual monographs.

The composition of the mobile phase has significant influence on the performance and on chromatographic separation of substances present in the solution to be analyzed. For a precise quantitative analysis, high purity reagents or organic solvents of chromatographic purity should be used. The water, provided with adequate quality, must present low conductivity and absorption in the ultraviolet range. In partition chromatography, the partition coefficient and, consequently, the separation can be modified by the addition of another solvent to the mobile phase. In ion-exchange chromatography, the retention of substances is affected by pH, ionic strength and other changes in the composition of the mobile phase. The technique of changing continuously the composition of the mobile phase solvents during the chromatographic run is called gradient elution, and is applied to separate complex mixtures

for substances with different capacity factors. However, detectors that are sensitive to changes in the composition of the mobile phase, such as the refractometers, have limited use with the technique of gradient elution.

The detector must submit a wide range of activity and the substances to be analyzed must be separated from any interferer. The linear range for a substance is which the detector response is directly proportional to its concentration.

The CLAE systems are calibrated by comparing the responses of the peaks obtained with their concentrations of reference chemical substances (SQR). Reliable quantitative results are obtained by means of calibration with external standard, when injectors or automatic samplers are preferably used. This method involves the direct comparison of the responses obtained with the peaks – analyzed separately – of standard solutions and sample. In cases in which the external standardization is used, the calculations can be carried out according to the following equation:

$$Ca = Cp\ (Ra / Rp)$$

whereas,

Ca = concentration of the sample solution;
Cp = concentration of the standard solution;
Ra = response area (or height) of the peak of the sample solution;
Rp = response area (or height) of the peak of the standard solution.

If the injection is performed by means of syringe, better quantitative results are obtained by means of calibration with internal standard, adding a known quantity of a chemical substance not interfering reference standard solutions and sample. The ratio of the responses obtained with the substance to be analyzed and with the internal standard is used to express the quantitative result. In cases in which the internal standardization is used, the calculations can be carried out according to the following equation:

$$Ca = Cp\frac{(Ra / Rai)}{(Rp / Rpi)}$$

whereas,

Rai = response area (or height) of the internal standard peak in the sample solution;
Rpi = response area (or height) of the internal standard peak in standard solution.

Due to normal variations between equipment, solvents, reagents and techniques, it is necessary a test of adequacy of the system to ensure that the method described is applied in an unrestricted manner. The main parameters of the adequacy of the system are described in *Interpretation of chromatograms* and *Adequacy of the system*.

INTERPRETATION OF CHROMATOGRAMS

In **Figure 1**, there is a typical chromatographic separation of two substances, t_1 and t_2 are the respective retention times. The terms h, h/2 and $W_{h/2}$ correspond to the height, half height and the width at half height, respectively, and W represents the peak width at baseline, by triangulation method. The signal on the dead time, t_0, relates to a substance not retained on the chromatographic column.

Retention time (t), Retention factor (k) and Relative retention time

The retention time in chromatography is characteristic of the substance analyzed, however is not unique. The comparison between the retention times of the sample and the reference chemical substance can be used as indicative of the identity of the substance, but is insufficient to ensure the total sample characterization. The absolute retention time may vary between equipment and the use of different

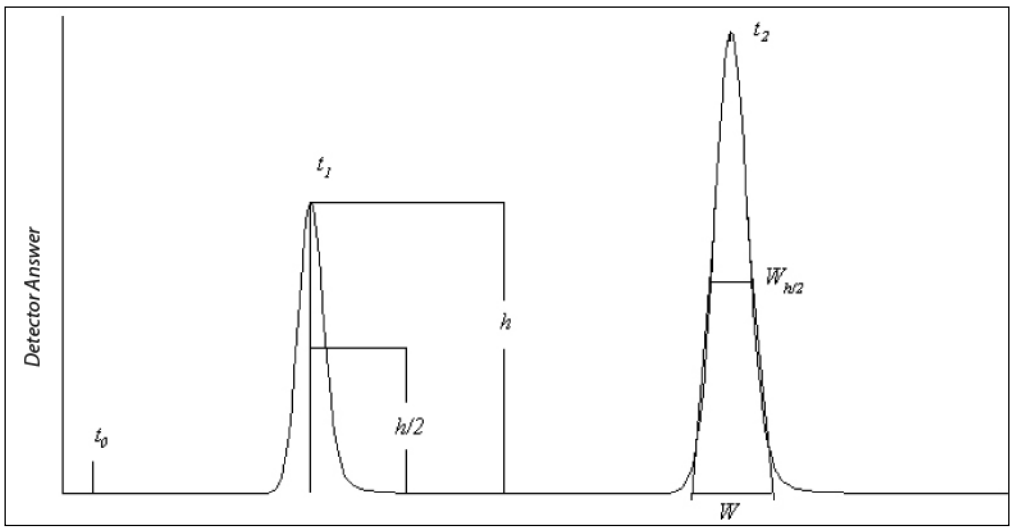

Figure 1 – Chromatographic separation of two substances.

solvents and reagents. In this respect, the comparisons are made in terms of retention factor, *k*, calculated according to the expression:

$$k = \frac{(t-t_0)}{t_0}$$

whereas,

T = the retention time of the analyte;
t_0 = dead time.

The retention factor, *k*, is the ratio between the quantity of substance with affinity for stationary phase and the quantity with affinity for mobile phase. The higher the affinity of substance by stationary phase the higher is the retention.

The concept of relative retention time can also be applied. A substance of a mixture must be defined as the main. This will be the relative retention time of 1. All other substances have their retention times related to the retention time of the main substance.

Number of theoretical plates (N)

The number of theoretical plates, *N*, is indicative of the efficiency of the column. It can be expressed in numbers of theoretical plates per column or the number of theoretical plates per meter. For peaks with gaussian format, the number of theoretical plates per column is calculated according to the expressions:

$$N = 16\text{x}\left(\frac{t}{W}\right)^2 ou\, N = 5{,}54\text{x}\left(\frac{t}{W_{h/2}}\right)$$

The N value depends on the substance to be examined and the conditions of the analysis, as mobile phase, temperature and stationary phase.

Resolution (R)

The resolution, *R*, is the chromatographic parameter that indicates the degree of separation between two substances in a mixture, and is calculated according to the expressions,

$$R = \frac{2(t_2 - t_1)}{W_1 + W_2} \; ou \; R = 1{,}18\frac{(t_2 - t_1)}{(W_{1,h/2} + W_{2,h/2})}$$

Whereas,

T_2 and t_1 = retention times of the two substances in the mixture;
W_1 and W_2 = respective widths of the peaks in the baseline, by triangulation method;
$W_{1,h/2}$ and $W_{2,h/2}$ = respective widths of peaks at half height.

The area or peak height are usually proportional to the quantity of the substance eluted. The area under the peak is more used, however it may be less accurate if there are other interfering peaks. For manual measurements, the graph should be obtained at a greater speed than usual, minimizing the errors in obtaining the width and width at half height of the peaks. For quantitative analysis, the substances must be completely separated from any interfering substances.

Tail factor (T)

The tail factor, *T*, which indicates the peak symmetry, presents value equal to 1 when the peak is perfectly symmetrical. This value increases as the peak asymmetry becomes more pronounced. In some cases, values lower than 1 can be observed. As the peak asymmetry increases, the integration and accuracy become less reliable. The tail factor is calculated according to the expression:

$$T = \frac{W_{0,05}}{2f}$$

Whereas,

$W_{0,05}$ = peak width at 5% of the time;
f = value of the previous portion of the peak, in relation to the width to 5% of the time, according to **Figure 2**.

SYSTEM SUITABILITY

The system suitability tests of the system an integral part of the methods of liquid chromatography. They are applied with the purpose of checking whether the resolution and reproducibility of the chromatographic system are adequate for the analyzes to be carried out. The main parameters required for checking the suitability of the system are described below.

The resolution, *R*, is a function of column efficiency, *N*, and is specified to ensure that substances eluted shortly show satisfactory separation without mutual interference.

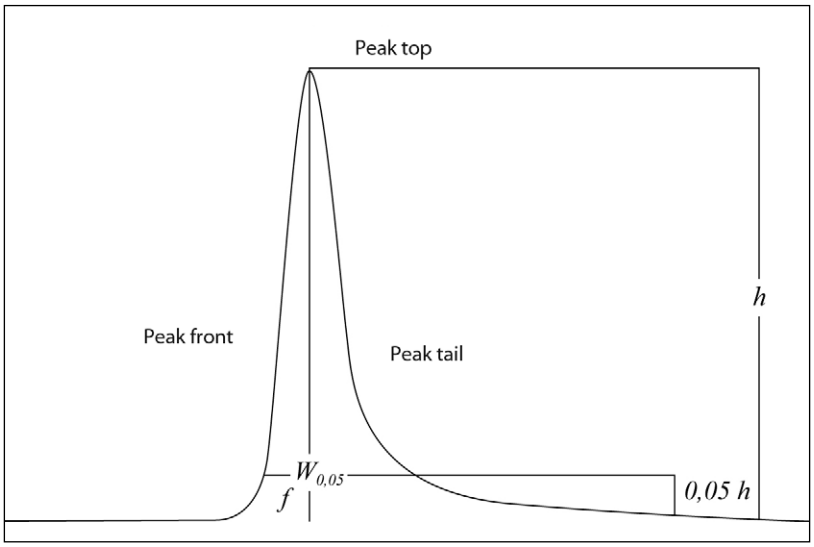

Figure 2 – Chromatogram representing the peak asymmetry.

Replications of standard solution injections statistically used to check whether the requirements for the accuracy of the analysis were achieved. Unless specified in individual monograph, the data of five replications of injections are used to calculate the relative standard deviation (RSD), if the specification is less than or equal to 2.0 %. If the relative standard deviation specified is greater than 2.0 %, the data of six replications should be used.

The tail factor, T, which indicates the peak symmetry, is equal to 1 for perfectly symmetrical peak and greater than 1 for peaks that feature asymmetry. In some cases, values smaller than 1 can be observed.

These tests are performed after collecting the results from replications of injections of standard solution or another solution specified in individual monograph. The specification of these chromatographic parameters, in a monograph, does not prevent the modification of the conditions of analysis. Adjustments in working conditions, so as to achieve the parameters of suitability of the system, may be required. Unless specified in individual monograph, the suitability parameters of the system are determined from data obtained with the peak of the substance of interest. The system accuracy, as demonstrated by means of replications of the standard solution must be achieved before the injections of solutions samples. The suitability of the system should be checked throughout the chromatographic analysis, by injection of standard solution at appropriate intervals. When there is significant change in the equipment or a reagent, the suitability tests of the system must be carried out before the sample injections. The analysis is not valid unless the requirements of the suitability test system are achieved.

5.2.17.4.1 Ion Chromatography

The ion chromatography refers to separation method and determination of ions using high efficiency liquid chromatography (HPLC). This technique is based on a separation process of the sample components between two phases: mobile phase and stationary phase. The separation process is resulting from specific interactions between the species present in the sample in both phases. The mechanism of interaction with the stationary phase is the ionic exchange, where the columns used are constituted by a functional group loaded, generally SO_3^-, COO^-, NH_3^+, NR_3^+ connected to a polymeric matrix, such as silica or copolymer of polystyrene-divinylbenzene type. The mobile phase also contains ionic species occurring, in this way, a competition between the distribution of the species present in the sample between the mobile phase and the stationary phase. For each ion, the exchange process is characterized by the balance of distribution between the mobile phase and the stationary phase.

The exchangers used can be classified as strong, medium and weak, depending on the functional group attached to the polymeric matrix. The strong ion exchangers are those that ionize completely in a wide range of pH, such as quaternary ammonium group and sulfonic acid. The degree of dissociation of ionic exchangers weak and medium is dependent on the pH and, in this way, the capacity of these exchangers varies according to the pH. It is possible to say as an example, the carboxylic acid and polyamine group.

This technique allows the electrical conductivity is used for the detection and quantitative determination of ions in solution, after the separation. Generally, the ion chromatography with anion exchange column and detector by conductivity may be used for the determination of ions (F^-, Cl^-, Br, SO_4^{2-}, PO_4^{3}-, I-, among others. Due to electrical conductivity is a property common to all the ionic species in solution, the detector by conductivity has the ability to monitor all the ionic species. The problem that occurs in the use of electrical conductivity to quantify the ionic species eluted can be caused by high conductivity of ions present in the mobile phase, mainly due to sodium ion, making it infeasible to quantification of other ions. This problem is overcome with the use of a suppressor eluent, positioned after the column separation, where occurs the conversion of the eluent ions in species that contribute to a low or zero conductance. The carbonic acid, resulting from the cation exchange, is weakly dissociated, possessing a low conductivity (conductivity sign of the base-line is less significant). This way, the sensitivity for the determination of anions can be significantly increased by a factor of 10 times or higher, when suppressors are used.

A product of ion chromatography consists, basically, in the same system used for CLAE. This system consists of a high propulsion pump, an injection valve handle with adequate sampling, column separation (for separation of anions an anion exchange column should be used), a post-column, if necessary, for conversion of the eluent ions in species with lower conductivity and a conductivity detector.

PROCEDURE

To operate the ion chromatograph, it is recommended to follow the instructions of the manufacturer. The determinations are made by comparison with reference solutions, containing known concentrations of the analyte.

Mobile phase: prepare the mobile phase in accordance with the specifications recommended by the manufacturer of the anion exchange column used. It is recommended that the use of mobile phase consisting of a mixture of carbonate and sodium bicarbonate (Na_2CO_3/$NaHCO_3$), in the range of concentration of 1.0 to 4 mmol/L, depending on the column used. Use the flow rate of the mobile phase recommended by the equipment manufacturer and in accordance with the ion exchange column used. During the analyzes using the detection by conductivity, regenerate the column of suppression chemistry, as the manufacturer's recommendation. It is recommended the use of H_2SO_4 0.005 mol/L and subsequent washing with purified water.

Calibration: prepare at least four reference solutions of the element to be determined, covering the range of concentration recommended by the manufacturer of the equipment for the analyte in analysis and inject separately, each reference solution in the product, using the adequate sampling handle. It is recommended the use of handle for sampling of 20 to 100µ. Record the chromatograms and integrate the signals in area or peak height. After calibration, plot the calibration curve. Prepare the sample solution as indicated in the monograph, adjusting its concentration for this tp be placed within the range of concentration of the reference solutions. Inject the sample into the gas chromatograph, record the reading and repeat this sequence three times, adopting the average of three readings. Determine the concentration of the element from the calibration curve. If the simultaneous determination of several anions is done, reference solutions can be made containing all analytes.

5.2.17.5 GAS CHROMATOGRAPHY

Gas Chromatography (GC) is a technique of chromatographic separation based on the difference of species distribution of a mixture between two non-miscible phases, in which the mobile phase is a gas that moves through the stationary phase contained in a column. CG is based on adsorption mechanism, mass distribution or exclusion by size. It is applied to substances and their derivatives which volatilize under the temperatures applied, and is used for identification, purity test and quantitative determination.

When a constituent vaporized is driven by gas inside the column, it is partitioned between the gaseous mobile phase and the stationary phase by a process of dynamic countercurrent distribution, showing a larger retention or less due to the phenomena of sorption and desorption on the stationary phase.

EQUIPMENT

The equipment consists of a source of gas and a flow controller, an injection camera, a chromatographic column contained in a furnace, a detector and a data acquisition system (or an integrator or recorder). The gas circulates through the column with controlled flow and pressure and follows directly to the detector. The injector, the column and the detector feature controlled temperature. The chromatography is carried out at a constant temperature or using a suitable temperature program. The compounds to be analyzed under both in solution such as gases, are injected, coming in contact with the gas injection on camera. Depending on the configuration of the equipment, the mixture to be analyzed should be injected directly in

column or must be vaporized on the injection camera and mixed in the gas before entering the column.

Once in the column, the constituents of the mixture are separated on the basis of their different linear retention indices, which are dependent on the steam pressure and the degree of interaction with the stationary phase. The retention rate, which sets the resolution, the retention time and the efficiency of the column in relation to the components of the mixture, also is temperature-dependent. The use of temperature programs for furnace where is the column has an advantage in efficiency of separation of compounds that behave differently in steam pressure.

The compounds are separated from the column, passing by a detector, which responds to the amount of each compound present. The type of detector to be used depends on the nature of the compounds to be analyzed and is specified in each monograph. The detectors are heated to prevent condensation of compounds eluted. The detector output is given as a function of retention time, generating a chromatogram, which consists of a series of peaks in the time axis. Each peak represents a composite of vaporized mixture, although some peaks can excape overlapped. The elution time is characteristic of an individual compound and the instrument response, measured as the peak area or peak height, is dependent on the quantity present.

Injectors

Direct injections of solutions is the usual way of injection, unless indicated otherwise in the monograph. The injection may be carried out directly at the head of the column using a syringe or an injection valve, or in a chamber of vaporization which can be equipped with a flow divider. The amount of sample that can be injected into a capillary column without saturate is lower when compared to the amount that can be injected into columns packaged. Capillary columns, therefore, are often used with injectors capable of dividing the sample into two fractions (split mode), a smaller that enters the column and another greater that is discarded. These injectors can be used without sample divider (splitless mode) for analyzes of components in smaller quantity or dashes.

The injections of the steam phase can be performed by injection system in static or dynamic headspace.

Injection System in confined space (headspace) static (purge and trap) includes a device for concentration, where the volatile substances of the solution are dragged up a column adsorbent, maintained at low temperature where are adsorbed. The substances are then retained desorbed in a mobile phase for rapid heating of the adsorbent column.

Fuel injection system in a dynamic headspace includes a heating thermostatically controlled chamber of the samples, in which closed vials are put where solid or liquid samples are placed in a given time period, to allow the volatile components of the samples to reach the balance between the gas phase and not the steam phase. Once the balance is established, a pre-determined quantity the confined space of the bottle is injected into the chromatograph.

Stationary Phases

The stationary phases are contained in columns that can be:

- A fused silica capillary column whose wall is coated with a stationary phase;
- A column packaged with inert particles impregnated with the stationary phase;
- A column packaged with the solid stationary phase. Capillary columns, usually made of fused silica, have an internal diameter (ø) of 0.10 to 0.53 mm and a length of 5 to 60 m. The liquid phase or stationary that can be chemically linked to the inner surface is a film with thickness of 0.1 to 5.0 m, although non-polar stationary phases can reach 5 µ of thickness.

The packaged, glass or metal columns feature length of 1to 3 m with an internal diameter (ø) of 4 mm. The stationary phases consist of polymers or porous solid support impregnated with the liquid phase at approximately 5% (p/p). Columns of high capacity, with the liquid phase at approximately 20% (p/p), are used for a wide range of compounds and for determination of compounds with low molecular weight as water. The ability required influences the choice of solid support.

The brackets for the analysis of polar compounds in columns packed with a stationary phase of low polarity and low capacity must be inert to prevent an excessive extension of the peaks. The reactivity of support materials can be reduced by silanization before filling with the liquid phase. Diatomaceous earth washed with acid and calcined is normally used. The materials are available in various sizes of particles, and the particles most commonly used have 150 to 180 µ (80 to 100 mesh) and 125 to 150 µ (100 to 120 mesh).

Mobile Phases

The gas supply can be obtained from a high pressure cylinder or by a gas generator with high purity. In both cases, the gas passes through a pressure reducing valve and the flow is measured, and then enter the chamber of injection and column. The retention time and the efficiency of the peak depend on the quality of the gas drag; the retention time is directly proportional to the length of the column and the resolution is proportional to the square root of the length of the column. For columns packaged, the average flow of the carrier gas is usually expressed in milliliters per minute, at atmospheric pressure and ambient temperature. The average flow is measured at the output of the detector, or with a calibrated mechanical device or with a “bubbling” tube, while the column is within the operating temperature. The linear velocity of the gas through the column packaged is inversely proportional to

the square root of the internal diameter of the column for a given volume flow. Flows of 60 mL/min in a column of 2 mm in internal diameter and 15 mL/min in a column of 2 mm internal diameter, provide identical linear speeds and similar retention times. Unless specified in the monograph, the average flow for columns packaged is approximately 30 to 60 mL/min. For capillary columns, the linear velocity of the flow is usually used instead of the mean flow. This is determined from the length of the column and the retention time of a sample of methane diluted, using a flame ionization detector. Operating at high temperatures, there is enough steam pressure to occur a gradual loss of the liquid phase, a process called bleeding.

Helium or nitrogen are generally applied as gases for columns packaged, while the gases used for capillary columns are nitrogen, helium and hydrogen.

Detectors

Flame ionization detectors are the most widely used but, depending on the purpose of analysis, other detectors can be applied, including: thermal conductivity, electron capture, nitrogen-phosphorus, mass spectrometry, infrared spectrometry with Fourier Transform, among others. For quantitative analysis, the detectors should present a wide linear dynamic range: the answer should be directly proportional to the quantity of compound present in detector in a wide range of concentrations. Flame ionization Detectors have a wide linear range and are sensitive to the majority of the compounds. The response of the detectors depends on the structure and concentration of the compound and the mean flow of combustion, the air and gas. Unless otherwise specified in the monograph, flame ionization detectors operate both with helium as with nitrogen as gas for packaged columns, and with helium or hydrogen for capillary columns.

The detectors by thermal conductivity use heated metal wire located in gas stream. When an analyte enters the detector with the gas, the difference in thermal conductivity of the gas stream (gas and components of the sample) concerning a reference flux of gas without analyte is measured. In general, thermal conductivity detectors respond uniformly to volatile compounds without considering its structure; however, they are considered less sensitive than the flame ionization detector.

Alkaline flame ionization detectors, also called NP or nitrogen-phosphorus detector, contains a thermionic source, with a salt-alkali metal or a glass element containing rubidium or another metal, which results in an efficient ionization of nitrogen and organic compounds containing phosphorus. It is a selective detector that presents low response to hydrocarbons.

Detectors per capture of electrons contains a radioactive source of ionizing radiation. They show an extremely high response to halogenated compounds and nitrocellulose group, but little responses to hydrocarbons. The sensitivity increases with the number and the atomic weight of halogen atoms.

Devices for data treatment

Data treatment stations connected at the output of the detectors must calculate the area and height of the peaks, and feature the complete chromatograms containing run parameters and the peaks data. The data of the chromatograms can be stored and reprocessed by integrating electronic or other type of calculation that is necessary. These stations for processing data are also used to program the runs dissolved.

PROCEDURE

Columns packaged and capillaries must be conditioned before use until the baseline is stable. This should be accomplished operating at a temperature above the specified or by repeated injections of compound or the mixture to be chromatographed. The manufacturer column generally provides instructions for the proper procedure for conditioning of the column. In the case of methyl and thermally stable substituted phenyl polysiloxanes, a special sequence increases the efficiency and downtime: keep the column at a temperature of 250 °C for 1 hour, with a flow of helium gas to remove oxygen and solvent. For the helium flow, heat up to 340 °C for 4 hours, and then reduce the heat until temperature of 250 °C, and prejudge with helium flow until the stability of the base line.

After the conditioning procedure, balance column, injector and detector temperatures and gases flow specified in the monograph until obtaining a stable baseline. Prepare the solution (s) sample and reference as described. The solutions must be free of solid particles.

Many drugs are polar molecules reactive. Whereas case, it may be necessary to convert these less polar and more volatile derivatives, for treatment of reactive groups with appropriate reagents.

The tests require quantitative comparison of a chromatogram with another. The largest source of error is the non-reproducibility of the injected sample amount, notably when injections manuals are carried out with the aid of a syringe. The effects of variability can be minimized by the addition of an internal standard, a compound does not interferer added at the same concentration in sample and standard solutions. The average of the responses of the analyte peak in relation to the internal standard is compared between the chromatograms of the sample and the standard. When the internal standard is chemically similar to the substance to be examined, there is also a compensation for minor variations in column and the characteristics of the detector. In some cases, the internal standard can be conducted through the preparation of the sample before the chromatographic analysis to control other quantitative aspects of the test. Automatic injectors increase the reproducibility of injections of samples and reduce the need for internal standards.

5.2.17.5.1 Gas Chromatography in headspace

The gas chromatography in headspace is a technique particularly suitable for the separation and determination of volatile compounds present in solid and liquid samples. This method is based on the analysis of a steam phase in balance with a solid or liquid phase.

EQUIPMENT

The equipment consists of a gas chromatograph to which adapts a device for the introduction of the sample, which can be connected to a programming module that automatically control the pressure and the temperature. If necessary, it is possible to attach a device for disposal of solvents. The sample to be analyzed is introduced into a flask fitted with a suitable shutter that closes and a valves system that allows entry of a gas. The flask is placed in a chamber thermostated to a certain temperature for the sample to be examined. The sample is left at this temperature for sufficient time to allow them to establish a balance between the solid phase and the gas phase. The gas drag is introduced in the flask, and after a certain time, a valve is opened to allow the gas to expand until the chromatographic column, dragging the volatile components.

Instead of using a gas chromatograph specially adapted to the introduction of samples, gastight syringes can also be used and a conventional chromatograph. In this case, the balance between the two phases is conducted in a separate chamber and the steam phase is transferred to the column, taking the necessary precautions to avoid any modification of the balance.

PROCEDURE

Adjust the working conditions of the equipment in order to obtain a satisfactory response, using the reference solutions.

Direct calibration

Enter separately, in identical bottles, the preparation to be examined and each of the reference solutions, according to the conditions described in the monograph and avoiding contact between the sample and the injection device. Stopper the flasks and insert them in the thermostated chamber temperature and pressure as described in the monograph. After achieving the balance, proceed to chromatographic analysis under the conditions described.

Adding standards

Add a series of identical bottles, equal volumes of solution to be examined. Add all the bottles, except for one of them, increasing quantities of a reference solution of known concentration of the substance to be examined. This way, a series of preparations containing increasing amounts of substance is obtained. Close the flasks and insert thems in thermostated chamber, under temperature and pressure conditions as described in the monograph. After achieving the balance, proceed to chromatographic analysis under the conditions described.

Calculate the equation of the straight line by linear regression, using the least squares method, and from it, obtain the substance concentration under examination in preparation of the sample, indicated by the equation intercept.

5.2.18 POLAROGRAPHY

The polarography, electrochemical analytical method, is based on the measurement of electrical current resulting from the electrolysis of electroactives substances (reducible or oxidizable) under certain potential of electrode and controlled conditions. In other words, the technique involves in the record of the current increase in polarizable electrode, during the electrolysis of dissolved substance in electrolytic, depending on the increase of the voltage applied to the system. The graph of this evolution of the chain in relation to voltage – the polarogram – provides information about quali and quantitative electro-reducible or electro-oxidable constituents of the the sample.

Among the variants of polarografic methodology, the most simple technique is in continuous current. It requires, by the example of potentiometry, the use of two electrodes, the reference electrode (usually saturated calomel electrode, ECS) and the microelectrode indicator (usually mercury electrode dripping, EMG). In some cases, it uses an auxiliary third electrode. The ECS – of a high surface area – provides constant potential during the test, while the EMG – mercury drops of dimensions reproducible flowing periodically from end of capillary connected to metal reservoir – assumes the potential conferred upon it by external source. The polarographic equipment comprises, in addition to the leads, the polarographic cell (electrolysis basin), variable power supply, provided with voltmeter and microammeter (galvanometer) and graphic or digital recorder.

In simplified form, the technique consists of dissolving the sample (the method has a sensitivity for concentrations of species resulting electroactive surface in the range of 10-2 to 10-4 M) in electrolyte support, responsible for the maintenance of small residual current, but that shows inert in the range of potential transformation of the sample (window of potential). Initially, without application of voltage at source, (accuracy pontentiostat), the voltage supplied to the microelectrode is null and there is no current indication at microammeter. The growing increase in voltage will cause small potential reach the electrodes. Under this voltage, further reduced, any impurities of electrolyte support and small concentrations of oxygen can suffer reduction in EMG (cathode, in this case), thus reducing and causing the indication of small passage of current. The progressive increase of the applied voltage will accentuate the process of reduction and the increase almost proportional of the chain. Finally, the potential required for

the analyte reduction in the sample solution is obtained, which is reflected in marked elevation of current read on microammeter (galvanometer) and recorded in polarogram. There are limits to the proportionality of the elevation voltage-current. While current rises (and the reduction is processed), progressive decrease of the concentration of the original species occurs, resulting electroactive surface near the electrode surface. At a given moment – the speed of electrolysis being constant – such concentration reaches low level to allow additional lift from the chain, and the latter becomes limited by diffusion with which the kind resulting electroactive surface can be spread in (inside) of the electrolyte solution to the surface of the EMG. The level observed in polarograma appears (**Figure 1**), and the current measured – called diffusion chain – a parameter proportional to the concentration of species resulting electroactive surface in the sample (quantitative aspect of derivative pulse polarography). After certain level of voltage is reached, the current continue to rise. This increase is caused by the reaction of electrolyte support. Their presence, in high concentrations, prevents the electroactive molecules sample reach the microelectrode by electrical migration and ensures, therefore, that the current limit is effectively governed only by diffusion.

When a microelectrode of dripping mercury is used, the electrode surface is constantly renewed (a new drop is made every 3-5 seconds), occurring variation in current measured within a given time interval; the current is lowest when the drop if so, reaching the maximum at the moment of the fall. The phenomenon explains the form "saw tooth" of the polarographic wave.

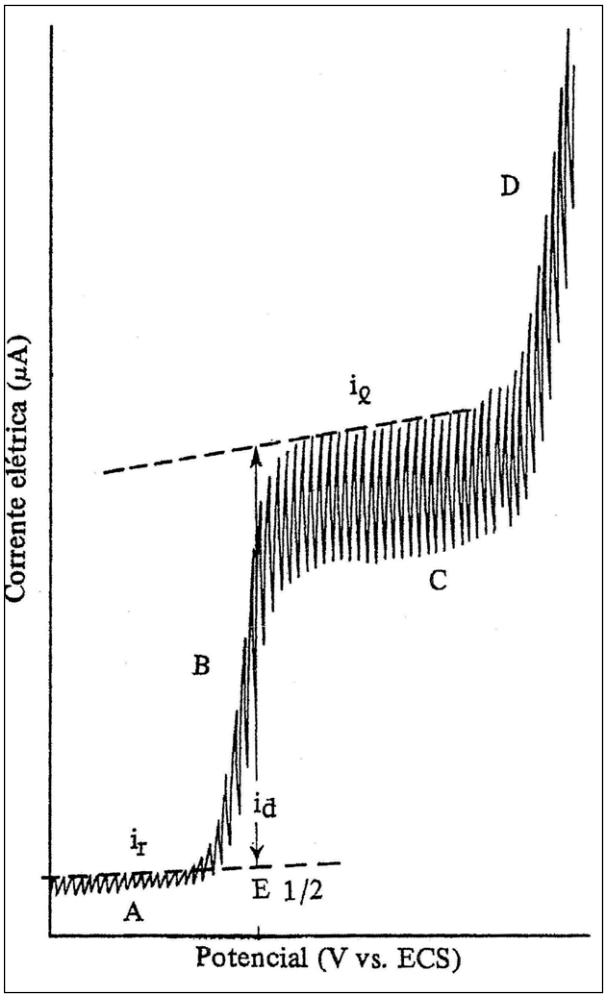

Figure 1– Polarogram of the electro-reducible type.

POLAROGRAM

In **Figure 1**, there is a typical polarogram (EMG), characterized by 4 distinct phases. The segment is due to the capacitive current, i_c, incorporated into the current normalized faradaic charge, i_f resulting from the oxidation or reduction of impurities of electrolyte support, or sample and small concentrations of oxygen, when this is not removed by the complete solution. The assembly of these currents is called residual current, ir ($i_l = i^l + i_r$). In the B segment of polarogram, the current normalized faradaic charge i_f occurs due to the conversion of the substance under test. The electrode composition leads to shortage of this substance along the microelectrode, observing that the level (segment C) where the limit current, i_l appears. This comprises the sum of residual currents and diffusion ($i_d = i_r + i_d$) in the distribution chain – proportional to the concentration of the species resulting electroactive surface in the sample – has its value determined by:

$$i_d = i_l + i_r$$

Two other chains – the undesirable migration and convection – may incorporate the current limit. The first is deleted by employment of electrolyte inert support in the range of potential applied in concentrations, at a minimum,

100 times larger than those of the species resulting electroactive surface.

The convection current, in its turn, is not eliminated by stirring of the solution.

Finally, the segment D of polarogram, in which the reversion of proportionality voltage-current, corresponds to the reduction of other eletroactive species, when present, or, more frequently, the electrolysis of support.

Ilkovic Equation

The Ilkovic equation establishes relations between variables included in polarographic measure and dissemination chain in EMG:

$$i_d = 708nD^{\frac{1}{2}}Cm^{\frac{2}{3}}t^{\frac{1}{6}}$$

Whereas

I_d = current broadcast, in which μA
708 = Constant dependent on several parameters, including the unit adopted for the variables, the size of the mercury drop and instant of measurement of i_d,
n = number of electrons needed to reduction or oxidation of a molecule or ion of substance resulting electroactive surface,
D = coefficient of diffusion, in cm^2/s,
C = concentration of the test substance resulting electroactive surface, in millimoles/L,
m = mass of the flow of mercury, in mg/s,
t = time of the drop life, in S.

The constant 708 – encompassing natural constant and the faraday value – is established for operation at 25 ºC and is applicable to sampled continuous current polarography, in which, instead of the continuous recording of the current, only the current reading is performed at the end of the life of the mercury drop, allowing obtaining the linear polarogram. However, by using instruments used with "saw tooth" shock absorber on the recorder, the average current of the pulses is recorded. The diffusion chain obtained according to the Ilkovic equation becomes the average for the whole life of the mercury drop. In this case the constant acquires the value 607.

The variables included in the Ilkovic equation should be controlled so that the distribution chain is effectively proportional to the concentration of electroactive species in the sample analyzed. Some ions and organic molecules in aqueous solution modify its diffusion coefficient to the ratio of 1 to 2% for each increased centigrade degree, it is required that the polarographic cell has its temperature controlled with tolerance at ± 0.5 ºC. The parameters *m* and *t*, related with size and renovation speed of the mercury drop, depend on the capillary geometry, the distribution chain must be proportional to the square root of the height of the mercury column. Appropriate Heights – the measurement from the end of the capillary up to the mercury level in the reservoir is between 40 and 80 cm. The internal diameter of the capillary is 0.04 mm for lengths between 6 and 15 cm. The exact height of the capillary is adjusted to allow the formation of a drop every 3-5 seconds, with open circuit and capillary immersed in electrolyte under test.

Thus, if during a particular test all parameters – except the concentration of species resulting electroactive surface – are kept constant, the Ilkovic equation can be written as

$$I_d = KC$$

Whereas K represents the variables set kept constant.

This direct relationship between current diffusion and concentration is usually adopted by the prior determination of the current dissemination of reference standard solution with known concentration. Then, under identical conditions, the distribution chain of the sample is determined and, finally, their concentration is also determined:

$$\frac{(i_d)_P}{(i_d)_A} = \frac{C_p}{C_A}$$

Whereas P and A respectively correspond to standard and sample.

Since most of the polarographers are provided with automatic recorders, it is easier to graphically determine dissemination currents by measuring the polarographic wave height (see **Figure 1**). The values recorded in cm can be directly applied to the formula, without the need for the conversion to electrical current units:

$$\frac{A_P}{A_A} = \frac{C_P}{C_A}$$

Whereas A_P and A_A match the heights of polarographic waves of the standard and the sample, respectively.

Half-wave potential

To measure the polarographic wave height for the purposes of quantitative analysis, straight lines must be drawn next to the peaks of the oscillations of the residual current and current limit and uniting, by means of third straight line parallel to the abscissa axis, with the extensions of the first two lines. The vertical straight line is drawn through the inflection point of the polarographic wave, corresponding to half the distance between the current and the residual current limit ($I = l / 2i_d$). The projection of this line on the ordinates axis provides the so-called half-wave potential, parameter used to characterize electroactive substances (qualitative aspect of derivative pulse polarography). The half-wave potential, $E_{1/2}$, is given in volts versus ECS (reference electrode), except when there is different specification, and its value as an identification parameter arises from its independence from concentration and EMG characteristics. However, this parameter varies as a function of composition, pH and temperature of the electrolytic environment. It is worth noting that, for the modern equipment, the measurement of the the polarographic wave

height can be done automatically by employing specific softwares for data acquisition and processing.

Oxygen removal

The oxygen is reduced in EMG in two steps, by initially converting into hydrogen peroxide, and then, in water. The fact of such reactions occur at potentials more negative than zero volts, *versus* ECS, it may interfere with the sample polarographic wave, it is necessary to eliminate the gas dissolved in the solution prior to the determination. The best way consists in bubbling oxygen-free nitrogen through the solution for a period of 10 to 15 minutes immediately before the test, taking the precaution of previously saturate the nitrogen (to avoid changes in electrolyte solution due to evaporation) bubbling it through small volume of electrolyte solution in separate container.

It is important to keep the electrolytic basin stopped and without vibrations during the polarographic recording to avoid the formation of convection currents. As a consequence, it is necessary to remove the solution nitrogen tube during the registration, and leave the tube over the solution surface to fill the upper part of the polarographic cell with nitrogen ($N_{2\,(g)}$) thus preventing the air inlet into the polarographic cell. Alkaline solutions may be deoxygenated by adding sodium bisulphite, provided that this does not interact with members of the electrolyte solution.

Maximum polarographic

Upon the reduction of electroactive species (cathodized EMG), many times the polarographic wave rises sharply before falling, equally sharp, up to the value of the current limit. The phenomenon is called maximum polarographic and the corresponding current receives the name of current of adsorption (i_a). The inconvenience is the difficult to measure polarographic wave (diffusion current) and its causes – still unclear – comprises the electrolyte adsorption to the surface of the mercury drop. The elimination of the maximum polarographic is, however, easily performed by addition of small quantities of certain surfactants (maximum suppressors) to the electrolytic environment. For such purpose, the use of a gelatine solution of 0.005% (p/v) and methyl red solution of 0.01% (p/v), among others, is required.

Warning

Mercury steams are toxic. When handling the metal, work in ventilated area and prevent spills; if they occur, they should be immediate and carefully collected.

PULSE POLAROGRAPHY

Pulse polarography consists in a superior technique variant by the precision and sensitivity, to the dosing continuous current and identification of high number of substances at low concentrations, including trace elements, metabolites and, of course, drugs. Its sensitivity, about 10 times higher than the derivative DC polarography, allows determinations in the order of 10^{-6} *M*.

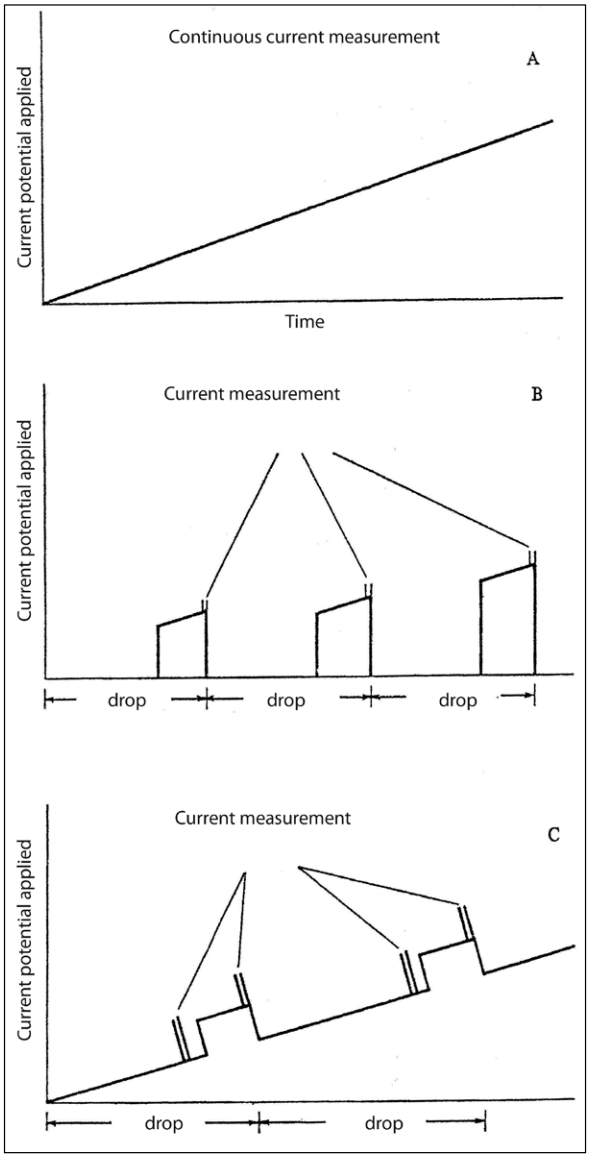

Figure 2 – Current measurement in relation to time in continuous current polarography (A); in pulse polarography (B); and in the differential pulse polarography (C).

Instead of the potential linearly progressive application and continuous measurement of the developed current, the polarography pulse comprises the application of increasing potential pulses to EMG, coinciding with the final period of the life of mercury drops, each pulse showing potential slightly higher than the previous one. The chain, in its turn, is sampled in the final moment of the potential pulse duration, period in which the capacitive current acquires value virtually zero and the residual current is composed almost exclusively of the distribution chain.

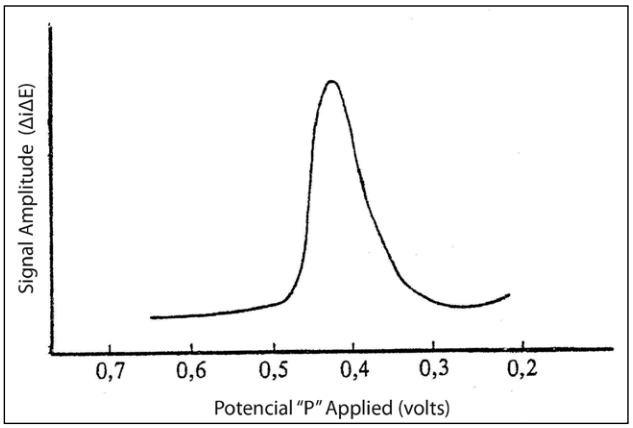

Figure 3 – Polarogram obtained in differential pulse polarography.

On the other hand, the pulses technique does not cause accelerated decrease of the diffusion layer (concentration of electroactive species along the electrode), providing higher diffusion chains for equivalent concentrations. Hence the increase of sensitivity inherent to the technique. Another favorable aspect of pulse polarography is the greater ease of the current limit measurement, free of oscillations, unlike what occurs in continuous current polarography.

In derivative differential pulse polarography, constant pulses, with small amplitude, are overlapped to a linearly increasing tension potention. The current measurement is performed twice to each pulse – immediately before the application of pulse and, again, in its final moment – recording only the difference between the two measured values (**Figure 2**). The graphic recording of this measurement system provides differential curve similar to the derivative of the polarographic wave, showing a characteristic peak (**Figure 3**). The potential of polarographic peak corresponds to $E_{1/2} - \Delta E/2$ in which ΔE represents the height of the peak. Due to the nature of the polarogram, which presents peaks instead of the traditional polarographic waves, the differential pulse polarography provides higher resolution,allowing simultaneous determinations of electroactive species with half-wave potential next from each other, at concentrations of the order of 10^{-7} M.

5.2.19 PH DETERMINATION

POTENTIOMETRIC pH DETERMINATION

The pH value is defined as the measure of the activity of the hydrogen ion in a solution. Conventionally, a scale of the hydrogen-ion concentration of the solution is used. The water is an extremely weak electrolyte, whose self-ionization produces hydronium ion (hydrated hydrogen) and hydroxide ion:

$$H_2O + H_2O <—> H_3O+ + OH^-$$

The concentrations of the hydronium ion in aqueous solutions may vary between wide limits, which experimentally are from 1 to 10^{-14} *M*, which is defined by the simplified relationship:

$$pH = – \log [H3O+] = \log 1/[H_3O+],$$

In this way, the pH scale is a scale inverted in relation to the concentrations of hydronium ion, i. e., the lower the concentration of hydronium ion, higher is the pH value.

The potentiometric determination of pH is made by the measreument of the potential difference between two electrodes immersed in the solution, suitable for examination. One of these electrodes is sensitive to hydrogen ions and the other is the reference electrode with constant potential.

The equation that expresses the potentiometric measurement of a cell is:

$$pH = pH_t = (E - E_t)/ K,$$

Whereas

E = potential measured when the cell contains the sample solution,
Et = potential measured when the cell contains the buffer solution,
pH = pH value in the sample solution
pHt = ph value in the buffer solution, and
K = variation of potential per unit of variation of pH – theoretically equals to 0.0591631 + 0.000198 (t-25), in which t is the temperature expected.

The pH value is expressed by the equation in relation to the pH of the standard solution (pHp) and determined in pH meter using glass electrode.

The apparatus commercially used for the determination of pH are potentiometric instruments, provided with electronic amplifiers with a chain with a calomel glass cell, which are capable to show values corresponding to 0.02 units of pH. The pH scale is calibrated not only in millivolts, but also in corresponding units of pH. This way, there is no need to apply the above equation, which expresses the pH eletrometric measurement. Since the measures of hydrogenic activity are sensitive to temperature variations, all pH meters are equipped with electronic temperature adjustment.

Buffer solutions for the pH meter calibration

They are applied in order to calibrate the device, allowing linearity in responses in relation to the potential changes observed. The most important are: tetraoxalate potassium phosphate, 0.05 M equimolar 0.05 M, 0.01 M sodium tetraborate, sodium carbonate and calcium hydroxide saturated at 25 °C.

The buffer solutions are prepared as follows:

Tetraoxalate potassium, 0.05*M* – Reduce the tetraoxalato potassiumto fine dust and dissicate in desiccator with silica. Dissolve exactly 12.71g of KH_3 (C_2O_4) $-2H_2O$ in water to 1000 ml.

Potassium biphtalate, 0.05*M* – Reduce the potassium biphtalate to fine dust and dissicate at 110 ºC until constant weight.

Dissolve exactly 10.21g of $KHC_8H_4O_4$, previously dried at 100 °C for 1 hour, in water, to dilute to 1000 ml.

Equimolar Phosphate, 0.05*M* – Reduce the KH_2PO_4 to fine dust and dissicate at 110 ºC until constant weight.

Dissolve 3.55 g of Na_2HPO_4 and 3.40 g of KH_2PO_4, in water, for 1000 ml.

Sodium tetraborate, 0.01M – Desiccate the sodium tetraborate in desiccator containing aqueous solution of sodium bromide until constant weight. Dissolve 3.81g of NaμmAμmWμm, in water, to dilute to 1000 ml. Prevent absorption of carbon dioxide.

Calcium hydroxide, saturated at 25°C – Reduce the calcium hydroxide to fine dust and dissicate with silica in desiccator until constant weight. Transfer 5 g to the volumetric flask and add water up to 1000 ml. Stir well and keep at a temperature of 25 ºC ± 2 ºC, for adequate saturation (Approximately 0.02 *M*). Decant at 2°C before use. Protect to avoid absorption of carbon dioxide.

Sodium carbonate – Dissicate the sodium carbonate in desiccator with silica gel until constant weight. Weigh exactly 2.10 g. Dissicate in oven 300 to 500 °C until constant weight. Weigh 2.65 g. Dissolve both samples in water up to 1000 ml.

Such solutions must be prepared with water free of carbon dioxide and applied for three months, taking care to prevent the bacteria and fungi growth. The use of preservatives is allowed since that does not interfere with the potentiometric measurement of pH.

The water used in the preparation of solutions should be freshly distilled, heated to boiling for at least 15 minutes, cooled and kept in airtight container to carbon dioxide. Prepare, individually, the six standard solutions and store them in glass vials or polyethylene suitable. Observe the expiration date of solutions, since the pH changes throughout time.

5

Table 1 – Relationship between the temperatures and the pH values of buffer solutions for the pH meter calibration

Temperature (ºC)	*Tetraoxalate potassium 0.05M*	*Potassium Biphtalate 0.05M*	*Equimolar phosphate*	*Sodium tetraborate 0.01M*	*Calcium hydroxide saturated at 25°C*	*Sodium carbonate*
10	1,67	4,00	6,92	9,33	13,00	10,18
15	1,67	4,00	6,90	9,27	12,81	10,12
20	1,68	4,00	6,88	9,22	12,63	10,07
25	1,68	4,01	6,86	9,18	12,45	10,02
30	1,68	4,01	6,85	9,14	12,30	9,97
35	1,69	4,02	6,84	9,10	12,14	9,93
40	1,70	4,03	6,84	9,07	11,99	-

PROCEDURE

PH meter measurement

Remove the becher containing KCl solution in which the electrode is immersed when the meter is not in use;

Rinse the electrode with distilled water jets and wipe with filter paper;

Immerse the electrode in buffer solution of reference, by checking the temperature at which it will operate;

Adjust the pH value until the value established, upon the calibration value;

Rinse the electrode with several portions of the second reference buffer solution, immerse the electrode and check the pH value recorded. The pH value should not show variations that exceed 0.07 of the value established for the second standard solution. There are apparatus that have bottles coupled with anionic synthetic detergents, used as wash solutions between each of the operations for measurement of pH values. The water also has this function;

If there is no accuracy in measurements, check possible damage in the electrodes and change them.

Determination of pH in the sample solution

After the convenient measurement, rinse the electrode with water (or proprietary solutions) and with several portions of the sample solution. For dilution of samples, use distilled water free of carbon dioxide;

The first determination provides variable value, there is need for further readings. The values subsequently

found should not vary more than 0.05 of the unit in three successive readings;

For determinations that require high accuracy, the temperatures of buffer solutions and sample, the electrodes and the washing water should not be above 2 °C. Thus, to reduce the electrical or thermal hysteresis effects of the electrodes, the solutions must be the same temperature for at least 30 minutes before the start of the operation;

It is important that, after the use of the apparatus, to retain the electrode in appropriate solution, normally, KCl.

Contamination of the solutions-stock should be avoided by the adoption of systematic procedures, such as the immediate closure of the flasks containing the solutions, in order to prevent accidental introductions of pipettes or sticks, the use of individual pipettes for each solution.

Ph COLORIMETRIC DETERMINATION

It is based on the use of indicator solutions or papers, which have the property of changing color as the pH variation. In this case, it is of approximate measure, indicating only a range of values, more or less wide, depending on the indicator used. The determination is carried out by adding drops of indicator solution to the solution under test or wetting the indicator papers with the solution under test and observing the color change. The colors developed by indicators in various pH ranges are related in *Indicators* **(14.1)**

ACIDITY AND ALKALINITY: QUICK TESTS

A solution is considered to be neutral when it does not alter the color of the blue and red papers due to litmus test, or when the universal indicator paper acquires the neutral colors, or when 1 ml of the same solution is green with a bromothymol SI blue drop (pH 7.0).

It is considered *acidic* when the blue litmus paper becomes red or 1 ml becomes yellow due to a of red SI drop (pH 1.0 to 6.6).

It is considered *weakly acidic* when cora slightly red litmus paper blue or 1 ml if cora of orange by a drop of methyl red SI (pH 4.0 to 6.6).

It is considered *highly acid* when the blue paper becomes red to congo or 1 ml becomes red red by adding a drop of methyl orange SI (pH 1.0 to 4.0).

It is considered *alkaline* when the red litmus paper becomes blue or 1 ml becomes blue due to a bromothymol blue SI drop (pH 7.6 to 13.0).

It is considered *weakly alkaline* when the red litmus paper becomes blue or 1 ml becomes blue by a cresol red SI drop (PH 7.6 to 8.8).

It is considered *strongly alkaline* when becomes blue by a drop of thymolphthalein SI (pH 9.3 to 10.5) or red by a drop of phenolphthalein SI (pH 10.0 to 13.0).

5.2.20 WATER DETERMINATION

Many pharmacopoeial substances are found in hydrate form or contain absorbed water, making relevant its determination by specific methods such as the volumetric method (5.2.20.1); azeotropic distillation method (5.2.20.2), or semimicro method (5.2.20.3), indicated in the monograph specific for each substance.

5.2.20.1 VOLUMETRIC METHOD

Determine the water content, by the direct method, except when otherwise specified in the monograph of the substance under analysis.

DIRECT METHOD

It is based on quantitative reaction of water with solution of anhydrous sulfur dioxide and iodine in the presence of a buffer solution that reacts with hydrogen ions.

In the original volumetric solution, known as Karl Fischer reagent, the sulfur dioxide and iodine are dissolved in pyridine and methanol. The sample can both be directly titrated (direct method) or back (indirect method).

Apparatus

It is allowed the useof any equipment which permits the exclusion of appropriate atmospheric humidity and the determination of the end point of the titration. For colorless substances, it is possible to detect the point of equivalence by the color change of the reagent, canary yellow to amber. The opposite is observed when adopting the back titration. However, it is more frequent and accurate to determine the end of the titration electrometrically. The use of electrical device capable of generating potential difference of 200 mV between two platinum electrodes immersed in the solution to the titrate. To reach the point of equivalence, the slight excess of reagent causes sudden elevation of current flow between 50 to 150 A, from 30 seconds to 30 minutes, depending on the nature of the sample (the period is smaller when the substance is soluble in the reagent).

Some automatic titrators have mechanism for immediate closure of the valve that controls the titrant inlet, so that detects the change in potential. The apparatus commercially available have a closed system that consists of one or two burets, automatic titration beaker, magnetic stirrer and specific electrode. The air in the system is kept dry, with the use of suitable desiccants.

Karl Fischer reagent

Add 125 g of iodine to a solution containing 670 mL of methanol and 170 mL of pyridine. Let it cool down. Transfer 100 mL of pyridine in 250 mL beaker, kept cold in an ice bath, move the sulfur dioxide chain through the solvent until its volume reaches 200 mL. Slowly, and under stirring, add this solution to the cold iodine mixture which had been

previously prepared. Stir until complete iodine dissolution and wait 24 hours before standardizing. When freshly prepared, the reagent solution neutralizes approximately 5 mg/mL of water, but it deteriorates quickly, therefore, it is recommended the standardization immediately before use, or daily, when in continuous use. Protect from light when in use. Store the reagent under refrigeration in amber bottle, fitted with a grinded airtight seal. As an option to the preparation of the reagent, commercial reagents solutions may be used. Also, commercial reagents containing other solventscan be used, different basis of pyridine or other alcohol other than methanol. These reagents may be individual solutions or obtained by mixing two reagents from different solutions. When the monograph specify that the reagent must be diluted, follow the instructions of the manufacturer of the product. As diluent, it is possible to use methanol or other suitable solvent, such as ethylene glycol monoethyl ether.

Standardization of reagent

Put sufficient quantity of methanol or another suitable solvent in titration to cover the electrodes and add sufficient quantity of reagent to provide the characteristic color of the turning point or the indication of (100 ± 50) micro amps of continuous current upon the application of 200 mV between the electrodes.

For the determination of water traces (less than 1%), it is preferable to use reagents with an equivalence factor of water less than 2.0, such as the sodium tartrate dihydrate ($C_4H4Na2O_6.2H_2O$), previously dried at 150 °C for 3 hours. Weigh, rapidly, from 150 to 350 mg of salt, exactly heavy, by difference, in titration and titrate until the equivalence point. The title of the reagent, in mg/mL of reagent water, is supplied by the following equation:

$$2x\left(\frac{18,02}{230,08}\right)\left(\frac{p}{v}\right)$$

Whereas

18.02 = Molecular weight of water
230.08 = Molecular weight of dihydrate sodium tartrate
p = mass, in milligrams, of the test portion of salt, v = volume, in mL, of reagent consumed in titration.

For the accurate determination of significant amount of water (more than 1%), using water as a reference. Transfer 25 to 250 mg of water, exactly weighed by difference, for the titration flask and titrate until the equivalence point. Calculate the title of reagent, T, in mg/mL of reagent water, by the equation

$$T = \frac{p}{v}$$

Whereas

p = mass, in mg, of the water,
V' = volume, in mL, of reagent consumed,

Procedure

Unless otherwise specified in the monograph, transfer 35 to 40 mL of methanol, or other appropriate solvent, to the titration vessel and titrate with the standardized reagent until visual or electrometric turning, with the aim of eliminating all the moisture that may be present (disregard the volume consumed, because it is not present in the calculations). Quickly add the titration vessel quantity, exactly weighed sample, containing 10 to 250 mg of water, mix and titrate until visual or electrometric turning. Calculate the water content of the sample, in mg, by the equation

Water Content (mg) = v x T

Whereas

V = volume in mL of reagent consumed;
T = title of the reagent.

INDIRECT METHOD

This method has the same principle of the direct method, however, the excess of reagent is applied to the sample and, after waiting for the reaction time necessary to quantitative reaction, titrate the reagent excess with standard solution of water in methanol. This technique, for unrestricted use, is especially recommended for substances that slowly release its water content.

Apparatus and reagent

Use the same described in direct method.

Standardization of the standard solution of water (indirect method)

Prepare standard solution of water by diluting 2 mL of water in sufficient quantity of methanol, or other suitable solvent, to make up to 1000 mL. Standardize this solution as the previous procedure, using aliquots of 25 mL. Calculate the content of water, in mg/mL of solution, by the equation

$$\frac{v' \text{ x } T}{25}$$

Whereas

v' = volume, in mL, of consumed reagent
T = title of the reagent, in mg/mL.

PROCEDURE

When it is specified in the monograph, transfer 30 to 40 mL of methanol, or any other proper solvent, for the titraton vessel and titrate until visual or elastomeric turn, in order to eliminate the entire unit, which may be present (disregard the consumed volume, because it does not enter the calculation). Quickly, add to the titration vessel, na exactly weighed quantity of the sample, containing 10 to 250 mg of water and one exceeding volume, exactly weighed, of the reagent. Let it rest as much as required so that the reaction is completed and titrate the non-consumed

reagent with standard solution of water, until visual or elastomeric turn. Calculate the content, in mg, of water in the test by the equation:

$$Water\ content = T\ [v' - (v \times v_r)]$$

COULOMETRIC METHOD

For coulometric water determination, the reagent of Karl Fischer is used. The iodine, however, is not used as volumetric solution, but obtained by anodic oxidation of a solution containing iodide. The cell reaction is composed of a broad anodic compartment and a restricted cathodic compartment; separated, among themselves, by a diaphragm. Also, other suitable types of cells of reaction can be used, as for example, without the diaphragm. Each compartment has a platinum electrode that leads to the current through the cell. The iodine, which is produced in an anodic electrode, reacts immediately, with the water that is in the slot. When all the water is consumed, it produces an excess of iodine that is normally electrometrically detected, indicating the end point. It is not necessary to change the Karl Fischer reagent after each determination. A necessary requirement for this method is that each component of the sample is compatible with the other components and do not produce secondary reactions. Usually the samples are transferred to the titration vessel, in the form of solutions, by injection through a septum. The gases may be introduced into the cell using asuitable inlet tube. The accuracy of the method fundamentally depends on the moisture elimination in the system. The system control can be monitored by base line. This method is especially suitable for chemical substances as inert hydrocarbons, alcohols and ethers. In comparison with the volumetric method of Karl Fischer, the coulometry is a microtiter method.

Apparatus

It is allowed the use of any commercially available equipment that has a fully gasketed system, equipped with specific electrodes and magnetic stirrer. The microprocessor equipment controls the analytical procedure and allows the visualization of results. It is not necessary to carry out a prior equipment calibration, since the current consumed can be measured in absolute terms.

Reagent

Use the same described in direct method.

Sample Preparation

If the sample is soluble, dissolve adequate amount, exactly in heavy, in anhydrous methanol or another suitable solvent. If the sample is insoluble, it is possible to remove the water using an anhydrous solvent that can be injected, in adequate quantity, exactly heavy, in the solution of the analyte. Alternatively, the evaporation technique may be used, in which the liberated water is collected in an air stream tube with inert and anhydrous gas.

Procedure

With a dry syringe, inject quickly the sample previously prepared as described in *Preparation of the sample*, measured with accuracy and with an estimated water content of 0.5 to 5 mg, or according to the instructions of the manufacturer of the equipment. Mix and quantify by coulometry, electrometrically determining the end point. Determine the water content in the sample directly in the equipment spreadsheet and calculate the percentage of this substance. Perform a blank determination and make the necessary corrections.

5.2.20.2 METHOD OF AZEOTROPIC DISTILLATION

As the volumetric method, the azeotropic enables the determination of water contained in samples of multiple nature. The present water is distilled with toluene, a solvent which is practically immiscible, and separated into appropriate receiver tube after cooling. It is required to use toluene previously saturated with water to avoid low results due to dissolution of residual water in the anhydrous solvent.

APPARATUS

Use round-bottom flask (A) with capacity for 500 mL, connected by a cylindrical tube (D) to receiver tube (B) (**Figure 1**). The critical dimensions of the parts of the device are: cylindrical tube of 9 to 11 mm in internal diameter; tube receiver with capacity of 5 mL, and cylindrical portion, with 146 – 156 mm in length, graduated in subdivisions of 0.1 mL, so that the reading error is not greater than 0.05 mL for any indicated volume, and can optionally be equipped with a tap. The upper part of the cylindrical tube connects, always, by means of joints with grinded material, the vertical reflux condenser (C) approximately 400 mm in length by at least 8 mm in internal diameter.

Parts of the flask and the cylindrical tube can be provided with asbestos cloth for greater thermal insulation. The heat for the distillation should be, preferably, supplied by electric heater control provided by dimmer or oil bath.

Clean the condenser and receiver tube with chromo, rinse with water and dry in an oven. Introduce in dry flask 200 mL of toluene and approximately 2 mL of water and then destilate for 2 hours. Let it cool down, and after about half an hour, measure the volume of water accumulated in graduated tube.

PROCEDURE

Add to the flask the sample quantity, exactly heavy, which contains 2 to 4 mL of water. If the substance is pasty, wrap it in aluminum foil so that the size of the package is sufficient only for its passage through the vial neck. If the substance induces the boiling, add washed and drought sand in quantity sufficient to cover the bottom of

the flask or glass capillary tubes of the type used for the determination of melting point, sealed at one of the ends. Add 200 mL of toluene, connect the equipment and heat for 15 minutes. When the toluene starts to boil, adjust the heat to which distils at 2 drops per second. When the water is virtually distilled, accelerate the speed of distillation for 4 drops per second. Complete distillation of water, pour about 10 to 15 mL of toluene by the entrance of the reflux condenser and continue distillation for other 5 minutes. Remove the heat source, wait for the cooling of the tube receiver at ambient temperature and move any droplets of water trapped in the tube receiver wall with the aid of copper wire with end wrapped in rubber (latex). Once completed the separation of the phases, read the volume of water deposited on receiver (discounting the initial volume) and calculate the percentage of water in the test.

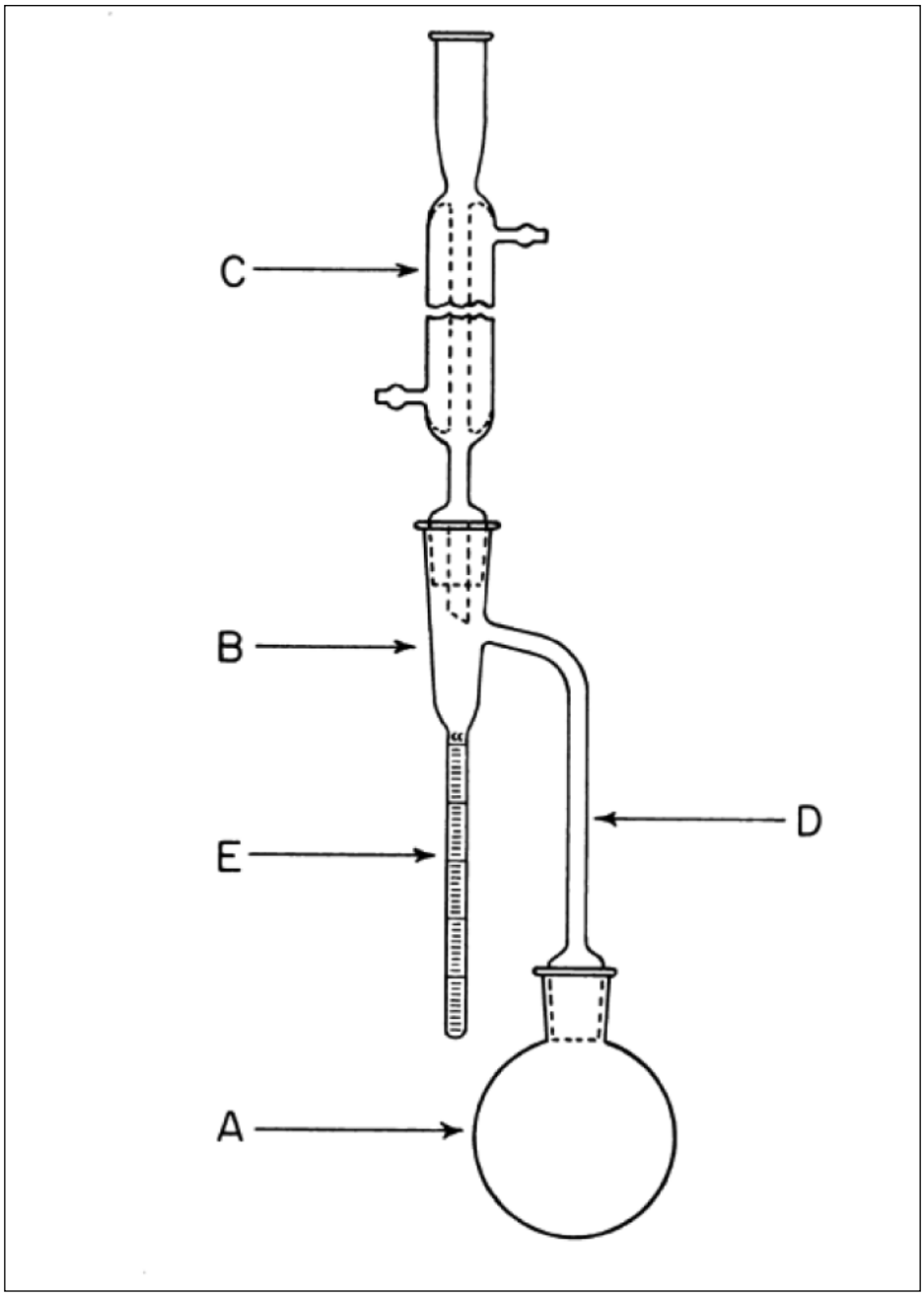

Figure 1 – Apparatus for water determination by azeotropic method.

5.2.20.3 WATER DETERMINATION BY SEMIMICRO METHOD

The determination of the water by the method is performed in a semi-micro titration apparatus with capacity of 60 mL, fitted with 2 platinum electrodes, a inlet tube for the nitrogen, a stopper adapted to the end of a buret and an air inlet tube protected by a drying agent. The test socket is introduced by a side tube fitted with a ground glass stopper. During the titration, stirring must be assured by the aid of a mechanical stirrer or through the bubbling of dry nitrogen.

The end of the reaction is determined by the intensity of the amperage. An appropriate circuit, comprising a potentiometer of approximately 2000 Q, connected to a 1.5 V battery, allows you to apply a variable potential difference. This is adjusted so as to conduct an initial weak current through platinum electrodes connected in series to a microammeter. The needle of the microammeter pulls after each addition of reagent, returning immediately to its original position. The order of the reaction is indicated by a detour that persists for at least 30 seconds.

Use the sulfur-iodine SR after determining its equivalent in water. The solutions and reagents used must be kept in anhydrous condition and preserved from atmospheric moisture during the determination or any manipulation.

The sulfur-iodine SR must be kept under the light, preferably in a flask fitted with an automatic buret.

The solutions of sulfur-iodine SR, commercially available have (or make) a composition that differs from the solution of sulfur-iodine SR by replacement of pyridine by several basic compounds. The use of these solutions reagents must be preceded by assessment that will allow, in each case, checking stoichiometry and absence of incompatibility between the substance to be tested and the reagent. Unless otherwise indicated, the Method should be used.

Method A. Introduce in titration approximately 20 mL of anhydrous methanol or the solvent prescribed in the monograph. Add the reagent sulfur-iodine SR solution until the amperometric turning. Introduce quickly the test, stir for 1 minute and titrate with the sulfur-iodine SR until new turning.

Method B. Enter in the flask of titration about 10 Ml of anhydrous methanol solvent or as prescribed in the monograph. Add sulfur-iodineSR until amperometric turning. Introduce, quickly, the test substance in a state of division, and then a volume of sulfur-iodine SR, enough to get an excess of approximately 1 mL. In this case, also, the prescribed volume can be used as defined in the monograph. Leave at rest in closed bottle and under the light during 1 minute or during the time prescribed in the monograph, stirring occasionally. Titrate the excess of sulfur-iodine SR with anhydrous methanol or with another solvent prescribed in the monograph, added to a quantity of water known and close to 2.5 g/L, until you return to the low initial current.

5.2.21 ANALYSIS OF SOLUBILITYBY STAGES

The solubility of pure substance in a given solvent, at a constant temperature, is a characteristic parameter of the substance, and may, therefore, be used for purposes of identification and assessment of degree of purity. In this principle, it is based upon the analysis of solubility by stages. The procedure consists in adding increasing portions of sample volumes contained in solvent in which the substance analyzed shows only slight solubility, aiming at obtaining saturated solution of this substance. Once the balance of the system is promoted – by prolonged stirring, under constant temperature – determine the total content of solute in supernatant solution (usually by gravimetric technique) and the solubility diagram by stages is drawn, plotting the composition of the solution, in mg of solute per g of solvent (ordered), by system composition, in mg of sample added per g of solvent (abscissa axis). The Figure 1 illustrates this diagram type. Along the segment AB, the solid totality dissolves and is found in the solution (slope corresponds to the unit). In section B the sample saturates the solution and subsequent additions do not entail an increase in its concentration. The slope of the line segment BC is, therefore, void and the intersection of the extension of this straight line with the Y-axis gives the value of the solubility of the substance.

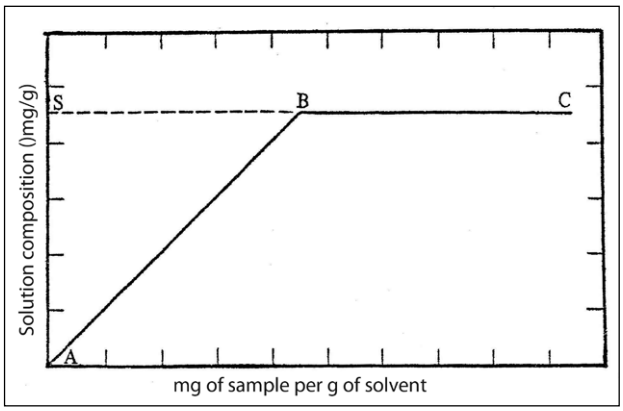

Figure 1 – Diagram of solubility by stages of a sample consisting of a single substance.

If the sample consists of two substances (one impurity of another, for example), the diagram takes the form illustrated in Figure 2. The AB segment presents unitary slope; point B indicates saturation of the solution with respect to one of the components of the sample (usually the one that is present in greater proportion); the segment BC indicates the solubilization of the second component and the segment CD to the saturation of the solution with the latter (tilt zero).

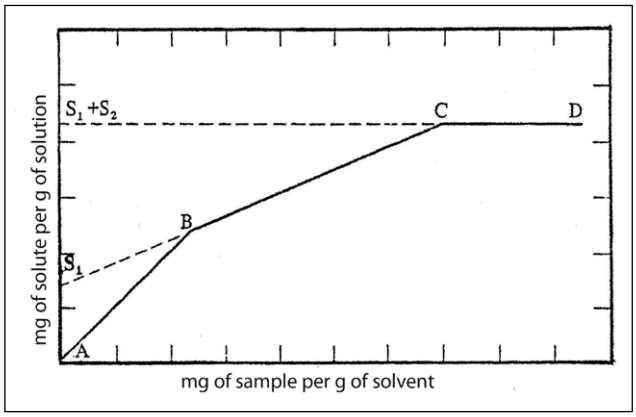

Figure 2 – Diagram of solubility by stages of sample containing two substances.

The slope value of the segment BC – phase in which only the second component is solubilized – corresponds to the proportion of this component in the sample. The subtraction of this value of the unit provides the contents of the first component in the sample, allowing the use of the formula (1-1). 100 to obtain the content. The slope, i, It is obtained by the formula $(Y_2-Y_1) / (X_2-X_1)$, in which, Y_2 and X_1, X_2 correspond, respectively, to projections of points of the line segment BC on the ordinate (composition of the solution) and the abscissa (composition of the system). The extrapolation of the segment BC provides the solubility limit, S_1, in mg of solute per g of solvent, the first component, while the extension of the straight line segment CD until the Y axis takes to the sum of solubilities of the two components, $S_1 + S_2$.

The occurrence of deviations pronounced in points which constitute the straight line segments of the diagram indicates a lack of balance in the system, although they also can be attributed to the existence of solid solution or deviations from theoretical behavior. If necessary, the tilt can be calculated by approximation or graphics from the statistical method of least squares.

A peculiarity of the analysis of solubility by stages is not be technique applicable to mixtures whose components are present in the sample in proportion to their solubilities. In this particular case, both components promote saturation at the same point, providing, as a result, phase diagram equivalent to the pure substance.

CHOICE OF SOLVENT

The choice of solvent for analysis of solubility by stages is based on the solubility of the component present in a greater proportion in the sample and the method of determination adopted for determining the concentration of the solution formed. The gravimetric technique is more common, the solvent must present volatility enough to allow the vacuum evaporation, but insufficient to hinder transfer and weighing operations. Solvents with boiling point between 60 ºC and 150 ºC are recommended. In terms of solubility, it is desirable that the solvent present capacity of sample solubilization in proportion not less than 4 mg/g and not more than 50 mg/g. The great solubility comprises the range 10 to 20 mg.

Additional recommendations include the inertia of the solvent before the sample components (it is expected, including the possibility of the formation of solvates or salts) and the use of solvent purity and known concentration (traces of impurities intensely affect the solubility), allowing the use of mixtures.

APPARATUS

Includes thermostatically controlled water bath, vials and appropriate ampoules and analytical balance, with an accuracy of ± 10 µmg.

The water bath is provided with thermostat with tolerance of temperature control not exceeding 0.1 ºC, especially in the range of 25-30 ºC, usual for the tests. The bath is equipped with rotary horizontal rod (25 rpm) provided with fastner claws for the ampoules. As an alternative, vibrator can be used (100,000 to 120 vibrations per second) also provided with claws fastner of ampoules.

The ampoule – with capacity for 15 mL – beside the solubility flask also applied in trials, is illustrated in Figure 3. Containers of different specification are admissible provided that they are airtight and compliant to the technique described.

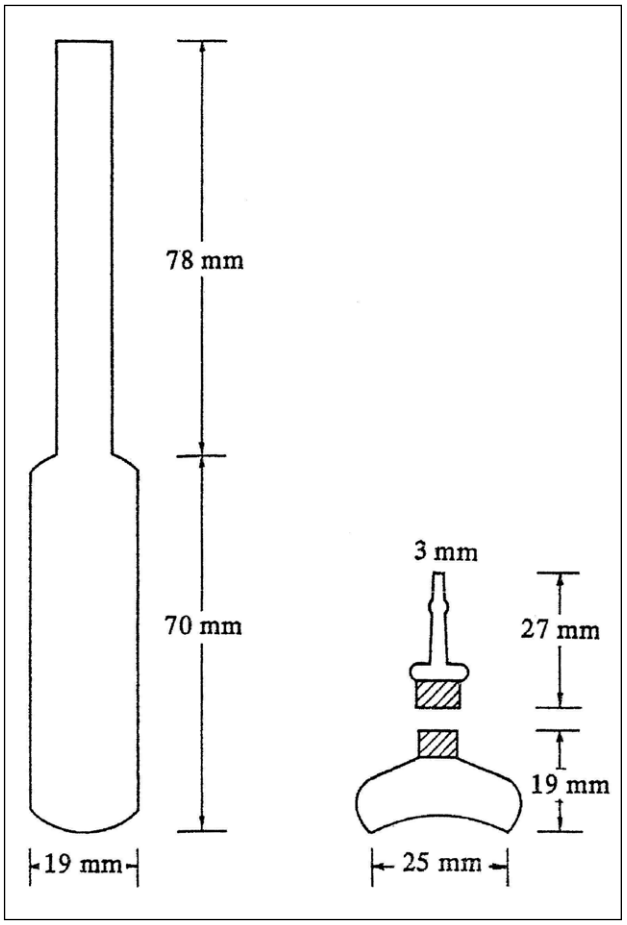

Figure 3 – Ampoule used in analysis of solubility by stages.

PROCEDURE

System Composition

Weigh accurately a minimum of 7 scrupulously clean ampoules of 15 mL. Transfer increasing quantities exactly weighed of sample for each vial, so that the first contains quantity only slightly smaller than the solubilizable in 5 mL of solvent and the last contains slight excess of sample. After transfer 5.0 mL of solvent for each ampoule, cool them down in a mixture of dry ice and acetone and seal them with torch air/gas, taking care to save glass fragments resulting from the process.

Allow the ampoules to reach the ambient temperature and weigh them, together with their respective glass fragments. Calculate the composition of the system, in mg/g for each ampoule, by the formula: $1000 (M_2-M_1) / (M_3-M_2)$, in which M_2 is the mass of the ampoule containing sample; M_1 is the mass of the empty vial and M_3 is the mass of the ampoule containing sample, solvent and any glass fragments.

Balance

The period necessary for the establishment of balance in systems contained in the vial is variable according to the nature of the sample, method of stirring (rotation or vibration) and temperature. The experience indicates average term of 1 to 7 days for stirring by vibration and 7 to 14 days for the rotational process. To confirm the promotion of balance, heat the penultimate ampulla of the series at 40 ºC with the aim of obtaining supersaturation. The result is positive if the point corresponding to this ampoule is consistent with the others in phase diagram. However, different result does not necessarily mean not having achieved balance. There are substances with a tendency to stay in supersaturated solution and, this being the case, it is up to the implementation of analyzes series, varying the waiting period in order to ensure the points consistency of the solubility curve.

Composition of the solution

When the balance is reached, put the ampoules in appropriate support to remain in a vertical position, with the bottlenecks above the water level of the thermostatted bath. Wait for the settling of solids in ampoules, open them and collect 2.0 mL of each one by means of pipette provided with cotton swab or other material capable of acting as a filter. Remove the filter material of the pipette and transfer the liquid crystal bottle of solubility **(Figure 3)** tared and duly identified, weighing each vial after the operation. Cool down the bottles in a bath of dry ice and acetone, and then evaporate the solvent under reduced pressure. Gradually increase the evaporation temperature, taking the precaution of not exceeding the limit compatible with the stability of the sample and dissicate the residue until constant weight. Calculate the composition of the solution in each flask, in mg/g, by Formula $1000 (P_3 – P_1) / (P_2 -P_3)$, in which P_3 corresponds to the mass of the flask containing the evaporation residue; P_1 is the mass of the empty bottle of solubility (tara) and P_2 is the mass of the flask containing the solution.

Trace phase diagram based on the values obtained and determine the purity percentage of sample on the basis of the slope of the line segment.

APPLICATION OF THE ANALYSIS OF SOLUBILITY BY STAGES IN PURIFICATION OF SUBSTANCES

While the solutions obtained in the analysis described contains essentially all the impurities present in the sample in proportion increased in relation to the original sample, after evaporation of solvent – the qualitative determination of impurities, the stage is adequate for high purity, preparation of reference standards for other analytical tests.

Procedure

Weigh appropriate sample quantity and suspending it in suitable solvent to – when the balance is reached – dissolve only 10% of the material. Close the bottle and wait for establishment of balance at ambient temperature (in general, 24 hours are sufficient). Then, collect the supernatant solution clear and evaporate at ambient temperature or close it, until dryness. The fact that the solution contain impurities of the original sample is obtained by this procedure, material in which the proportion of impurities is increased, being the ratio of enrichment approximately equal to the ratio of the mass of the sample by weight of dissolved solids in the amount of solvent applied. Purify the residue not dissolved by washing and drying (reference standard).

5.2.22 ELECTROPHORESIS

GENERAL PRINCIPLES

By the action of an electric field, the charged particles dissolved or dispersed in an electrolyte solution migrate toward the electrode of opposite polarity. In gel electrophoresis, the displacement of the particles is slowed by interactions with the gel matrix that constitutes the means of migration and it behaves as a molecular sieve.

The opposition interactions of the electric force and the molecular grading result in differential rate of migration according to the size, shape and particle load. Due to its physical-chemical properties, the various different molecules contained in a mixture will migrate at different speeds during the electrophoresis, thereby separated into fractions defined. The eletrophoretic separations can be conducted on systems without support phase (for example, separation in solution free in capillary electrophoresis), and or stabilized means as thin layer plates, films or gels.

FRONTIER, OR DIVISION, OR LIMIT FREE, OR IN MOTION ELECTROPHORESIS

This method is mainly used in the determination of mobilities, being the experimental features directly measurable and reproducible. The substances of relatively high molecular mass, little diffusible, are applied. The divisions are, initially, demarcated by a physical process as the refractometry or condutimetry. After the application of a defined electric field, during a certain period, it is possible to get new divisions and their respective positions are observed. The operational conditions allow the determination of divisions and constituents.

SUPPORT ELECTROPHORESIS OR ZONE ELECTROPHORESIS

This method is used only for reduced samples. The nature of the medium, such as paper, agarose gel, cellulose acetate, starch, agarose gel, methacrylamide or mixed, introduces a number of additional factors that modify the mobility:

a) Due to the sinuosity of the channelling of the support, the distance apparently travelled that is less than the actual distance;

b) Certain brackets are not electrically neutral, and as the environment constitutes a stationary phase, sometimes can result in a considerable important endosmotic current;

c) The heating due to the Joule effect can produce some liquid evaporation of support, which leads, by capillarity, a displacement of the solution of the ends to the center; thus, the ionic strength tends to increase gradually.

The speed of migration depends on four main factors: mobility of the particles, endosmotic current, chevaporation ain and intensity of the field. For these reasons it is necessary to proceed in well determined experimental conditions and use, if possible, reference standards.

Apparatus

An electrophoresis apparatus consists of:

– a continuous current generator of controllable and preferably stabilized voltage;

– a electrophoresis basin. Generally rectangular, glass or rigid plastic material, with two separate compartments, anode and cathode, which contains the conductive buffer solution. In each compartment there is an electrode, platinum or graphite, these are connected by means of a circuit properly isolated from the power supply to the corresponding terminal to form, respectively, the anode and cathode, connected by a circuit conveniently isolated the corresponding terminal of the generator. The fluid level in the two compartments is equal to avoid the effect of siphoning. The electrophoresis basin must be fitted with an airtight seal, allowing to keep inside a saturated atmosphere of unity and reduce the evaporation of the solvent during the migration. A safety device must be used to shut off the current, when the cover is removed from basin. If the measure of electrical current exceeds the 10 W it is preferable to cool down the support;

– a support device:

Electrophoresis in strips. In electrophoresis strips on bracket are previously impregnated with the same solution and each end immersed in the electrode. The strips are well extended, fixed on a suitable support to prevent the dissemination of conductive solution such as, for example, a horizontal frame, a support in inverted V, or a uniform surface, with points of contact at suitable intervals.

Gel electrophoresis. The device consists of a glass plate, such as, for example, a simple microscope slide, on which is deposited a layer of adhesive gel and of uniform thickness across the surface of the blade. The contact between the gel and the conductive solution varies depending on the type of apparatus used. It avoids any condensation of moisture or drying of solid coating;

– a measuring device or means of detection.

Procedure. Put the electrolyte solution in the compartments of the electrodes. Place the stand, conveniently soaked with the electrolyte solution in basin, according to the type of equipment used. Plot the line of departure and apply the test sample. Allow current to pass through during the time specified; then turn off the power, remove the support of basin, dry and reveal.

POLYACRYLAMIDE GEL ELECTROPHORESIS IN CYLINDRICAL TUBE

In polyacrylamide gel electrophoresis, cylindrical (tube) in the stationary phase consists of a gel prepared from acrylamide and N,N' -methylenebisacrylamide. The gels are prepared in tubes, usually with 7.5 cm in length and 0.5 cm internal diameter (cylindrical gel); a single sample is applied in each tube.

Apparatus. The apparatus consists of two shells for the receipt of buffer solutions and constructed with a suitable material, such as polymethyl methacrylate methyl. They are arranged vertically, one above the other, and are fitted with, each one, of a platinum electrode. These two electrodes are connected to a current source enabling to operate with intensity and constant tension. For cylindrical gels, the apparatus is based on top of the reservoir a number of elastomer seals located at an equal distance from the electrode.

Procedure. In a general way, it is recommended to degas solutions before the polymerization and use the gel immediately after its preparation. Prepare the gel according to the indications of the monograph. Put the mixture of gel in glass tubes, appropriate closed at the lower end with a stopper, up to a height equal to all of them, a distance of approximately 1 cm from the upper edge of the tube. Prevent the introduction of air bubbles in the pipes. Cover the mixture with a layer of water in order to prevent contact with the air and leave to rest. The gel formation requires

generally approximately 30 minutes and is complete when it appears a clear demarcation between the gel and the aqueous layer. Remove the aqueous layer. Fill the lower reservoir with the buffer solution, prescribed and remove the stoppers of the pipes. Clip the pipes in the joints of the upper reservoir so that its bottom immerse in the buffer solution from the lower reservoir and adjust so that the bottom of the tubes is immersed in buffer solution from the lower reservoir. Gently fill the pipes in the solution of the lower reservoir. Prepare the solutions problem and standard containing the dye indicated prescribed. Fill, carefully, the pipes with the buffer solution indicated. Apply the solutions, whose density was increased by the addition of sucrose, for example, the gel surface, using a different tube for each solution. Put the same buffer solution in the tank top. Connect the electrodes to the current source and proceed to electrophoresis, using the current or constant voltage and temperature prescribed in the monograph. Stop the current when the colored indicator reaches the lower reservoir. Remove immediately the pipes and extrude the gel. Locate the position of the bands in electropherograms according the procedure indicated.

POLYACRYLAMIDE GEL ELECTROPHORESIS WITH SODIUM DODECYLSULPHATE (DSS-EGPA)

Field of application. Electrophoresis in polyacrylamide gel is used for the qualitative characterization of the proteins contained in preparations for biological controls of purity and quantitative determinations.

Purpose. The analysis by gel electrophoresis is a process adapted to the identification and control of homogeneity of the proteins contained in pharmaceutical preparations. It is routinely used to assess the molecular mass of protein subunits and determine subunits that compose the purified proteins. On the market there is a great variety of gels and reagents are ready for use in time of which are described below, provided that the results obtained are equivalent and which could be met the conditions of validity described in Validation of the assay.

Characteristics of polyacrylamide gels

The properties of polyacrylamide gels sieves are related to your particular structure that is a three-dimensional grid of fibers and pores resulting from the formation of cross-links between the bifunctional bisacrylamide and adjacent polyacrylamide chains. The polymerization is catalyzed by a generator free of radicals composed of persulphate of ammonia (PSA) and N,N,N',N' – tetramethylethylenediamine (Temed). The actual size of the pores of a gel is lower as the higher concentration of acrylamide. As the concentration of acrylamide gel increases, their effective porosity decreases.

The real porosity of a gel is defined in an operational mode by their molecular properties of the molecular sieve, that is, the resistance that he opposes the migration of macromolecules. There are limits to the concentrations of acrylamide that can be used. At very high concentrations the gels run down more easily and are difficult to manipulate. When the size of the pores of a gel decreases, the speed of migration of a protein in this gel decreases too. ** Adjusting the porosity of a gel, changing the concentration in acrylamide, it is possible to optimize the resolution of the method for a given protein product. This way, the physical characteristics of a gel depend, therefore, of their content of acrylamide and bisacrylamide. In addition to the composition of the gel, the condition of protein constitutes another important factor for its electrophoretic mobility.

In the case of proteins, the electrophoretic mobility depends on the pK of groups with electrical load and the size of the molecule. It is also affected by the nature, concentration and buffer pH, temperature, intensity of the electrical field and the nature of the support.

POLYACRYLAMIDE GEL ELECTROPHORESIS UNDER DENATURING CONDITIONS

The method described for example is applicable to the analysis of polypeptides monomers of molecular mass between 1.000 and 10.000 daltons. It is possible to expand this range by different techniques (for example, by works with gradient gels or special buffer systems), but such techniques are not part of this text. The polyacrylamide gel electrophoresis under denaturing conditions using the sodium dodecylsulphate was also sodium (DSS-EGPA) is the technique of electrophoresis more used to evaluate the quality of pharmaceutical products and protein is mainly the focus of this text. In general, the analytical electrophoresis of proteins is performed in polyacrylamide gel under conditions that favor the dissociation of proteins in their polypeptide subunits and that limit the phenomenon of aggregation. This effect of sodium dodecylsulphate (DSS) is frequently used, a strongly anionic detergent, to decouple the proteins before their application in gel, in combination with the heat. The denatured polypeptides bind to DSS, acquire negative loads and is characterized by a constant load/mass ratio no matter the type of protein considered. Being the quantity of DSS connected almost always proportional to the molecular mass of the polypeptide and independent of its sequence, the DSS-polypeptide complexes migrate in polyacrylamide gels with mobilities which are a function of the size of the polypeptide.

The electrophoretic mobility of resulting complex detergent-polypeptides always presents the same functional relationship with the molecular mass. The migration of complex DSS is, as one would predict, in the direction of the anode to higher speed for the complexes of low molecular mass than for the high. It is, therefore, possible to determine the molecular mass of a protein from its relative mobility, after comparison with standard solutions of value of known molecular weight and the observation of a single band constitutes a criterion of purity. However, any changes in the constitution of the polypeptide, for example, a N- or O-glycosylation, has a significant impact on the non-negligible apparent molecular mass of a protein, not binds to a molecule of carbohydrates in a similar way to a polypeptide. In fact, the DSS is not switched on in the same

way to glucidic groupings or to peptidic groupings, so that the constancy of the ratio load/mass ceases to be checked. The apparent molecular mass of the proteins that have undergone post-translational changes not really reflects the mass of the polypeptide chain.

Reducing Conditions

The association of polypeptide subunits and the three-dimensional structure of proteins are based, many times, on the existence of disulfide bridges. One of the goals to be achieved in the analysis DSS-EGPA in reducing conditions is to break this structure by reduction of disulfide bridges. The denaturing and the complete dissociation of proteins by treatment with 2-mercaptoethanol or dithiothreitol (DTT) cause a breakdown of polypeptide chain followed a complexation with the DSS. Under these conditions, the molecular mass of polypeptide subunits can be calculated by linear regression with the help of molecular mass standards.

Non-reductive Conditions

For certain analyzes, the complete dissociation of the protein subunits in peptide is not desirable. In the absence of treatment by reducing agents, such as 2-mercaptoethanol or DTT, the disulfide bridges covalent bonds remain intact and the oligomerica conformation of protein is preserved. The complex DSS- oligomer migrates more slowly than the DSS-peptide subunits. In addition, the proteins not reduced may not be fully saturated in DSS and, consequently, it is not connecting to the detergent in a relationship of constant mass. This circumstance makes the determination of the molecular weight of these molecules by DSS- EGPA more difficult than the analysis of polypeptides completely denatured, because, for which a comparison may be possible it is necessary that the standards and the unknown proteins have similar configurations. However, to obtain the gel of a single band stained remains as criterion of purity.

CHARACTERISTICS OF THE ELECTROPHORESIS GEL IN NONCONTINUOUS BUFFER SYSTEM

The electrophoretic method most widely known for the characterization of complex mixtures of proteins is based on the use of a discontinuous buffer system that includes two continuous gels, but distinct: a gel (bottom) of separation or resolution and a gel (top) concentration. These two gels are porosity, different pH and ionic strength. In addition, the different mobile ions are used in gels and buffers of the electrode. The discontinuity of the buffer system leads to a concentration of large volume of samples in gel concentration and, therefore, an improvement in the resolution. When the electric field is applied, a bad voltage gradient establishes through the sample solution and drags the proteins from the gel concentration for the stacking gel. The glycinate ion contained in the buffer electrode follow the proteins in stacking gel. So, quickly, a zone of division mobile whose front is constituted by the chloride ions of high mobility and the back by glycinate ion more slow. A gradient of high voltage located establishes between the fronts of ionic head and tail and takes the complex DSS-protein to focus on a very narrow band that migrates between the fractions and chloride glycinate.

On a large scale, regardless of the volume of the sample used, the assembly of complex DSS-protein undergoes a effect of condensation and penetrates the gel separation in the form of a narrow band, well-defined, high-density protein. The stacking gel, wide-pore, not retardsgenerally the migration of proteins, but plays, mainly, the role of non-convective mean. At the interface of gels of stacking and separation, the proteins are faced with a sudden increase of the effect of delay due to the small diameter of the pores of the gel separation. When they penetrate the gel separation, this delay is continuing due to the effect of molecular sieve exercised by the matrix. The glycinate ions beyond the proteins whose migration continues, then, a means of uniform pH composed by buffer solution of ketorolac tromethamine (TRIS) and glycine. The effect of molecular Tamil leads to a separation of complex DSS-polypeptide based on its molecular weight.

PREPARATION OF DSS VERTICAL POLYACRYLAMIDE GELS OF DISCONTINUOUS BUFFER

Mold assembly

With a mild detergent, clean the two glass plates (for example size 10 cm x 8 cm), the comb of polytetrafluoroethylene, the two spacers and the tube of silicone rubber (for example, diameter 0.6 mm x 350 mm), and rinse, abundantly, with water. Dry all elements with a paper towel or tissue. Lubricate the spacers and the pipe with a lubricant other than a silicone base. Place the spacers 2 mm from the edge along the two short sides and one of the long sides of the glass plate. This latter will correspond to the bottom of the gel. Start installing the tube over the glass plate using a spacer as a guide. Reached the end of the spacer, bend the pipe with precaution to do so following the long side of the glass plate. Hold the tube in place with one finger, fold it again to do so following the second short side of plate, use the spacer as a guide. Put the second card in place, aligning it, perfectly, on the first, and keep the set by manual pressure. Put two clamps on each one of the short sides of the mold, and then, with precaution, four other tweezers on long side that will constitute the mold base. Check that the tube follows the edge of the plates and not moved after the placement of the clamps. The mold is ready and the gel can be placed in it.

Preparation of the gels

For gels of discontinuous buffer system, it is recommended to set the first gel separation and let it cure before putting gel concentration, because the content in acrylamide-Bisacrylamide in two gels, on the plug and the pH is different.

Gel Preparation of separation. a flask prepare the appropriate volume of a solution of acrylamide of desired concentration, using the values given in Table 1. Mix the components in the order indicated. Before you add the solution of ammonia persulphate and tetramethylethylenediamine (Temed), filter, if necessary, by suction using a cellulose acetate membrane (pore diameter; 0.45 µm); keep under stirring the suction filtration unit until no more form bubbles in the solution. Add the appropriate quantities of solution of PSA and Temed **(Table 1),** shake and enter, immediately, in the space that separates the two glass plates of the mold. Leave a free height sufficient for the gel concentration (height of a tooth of the comb over 1 cm). Using a glass pipette tapered, coat, with caution, the solution with iso-Butyl alcohol saturated OF water. Leave to polymerize the gel in vertical position, the ambient temperature.

Gel Preparation of stacking. When the polymerisation finish (about 30 minutes), drain the iso-Butyl alcohol and rinse several times the gel surface with water to eliminate completely the iso-Butyl alcohol and, if necessary, the acrylamide does not polymerized. Leave the minimum of liquid on the surface of the gel and, possibly, absorb the residual water with the tip of a paper towel. A erlenmyer, prepare an appropriate volume of a solution of acrylamide of desired concentration using the values recorded in Table 2. Mix the components in the order indicated.

Prior to joining the solution of PSA and Temed, filter if necessary, by suction by a cellulose acetate membrane (pore diameter: 0.45 µm); keep under stirring the suction filtration unit until no more form bubbles in the solution. Add the appropriate quantities of ammonia solutions of ammonium persulfate and Temed (Table 2), stir and add, immediately, on the gel separation. Place, immediately, on a comb of polytetrafluoroethylene clean in the solution of the gel concentration, taking the precaution of avoiding the formation of air bubbles. Add solution to the gel concentration so as to completely fill the interstices of the comb. Leave to polymerize the gel in vertical position, the ambient temperature.

Table 1 – Preparation of the Resolution Gel.

Solution Components	*Volume of the components in mL per volume of gel mold of:*							
	5 mL	*10 mL*	*15 mL*	*20 mL*	*25 mL*	*30 mL*	*40 mL*	*50 mL*
6% Acrylamide								
Water	2,6	5,3	7,9	10,6	13,2	15,9	21,2	16,5
Acrylamide Solution (1)	1,0	2,0	3,0	4,0	5,0	6,0	8,0	10,0
1.5 M Tris pH 8.8 (2)	1,3	2,5	3,8	5,0	6,3	7,5	10,0	12,5
(DSS) 100 g/L of Sodium Dodecyl sulphate (3)	0,05	0,1	0,15	0,2	0,25	0,3	0,4	0,5
(PSA) 100 g/L of Ammonium Persulphate (4)	0,05	0,1	0,15	0,2	0,25	0,3	0,4	0,5
(TEMED) Tetramethylethylenediamine (5)	0,004	0,008	0,012	0,016	0,02	0,024	0,032	0,04
8% Acrylamide								
Water	2,3	4,6	6,9	9,3	11,5	13,9	18,5	23,2
Acrylamide Solution (1)	1,3	2,7	4,0	5,3	6,7	8,0	10,7	13,3
1.5 M Tris pH 8.8 (2)	1,3	2,5	3,8	5,0	6,3	7,5	10,0	12,5
(DSS) 100 g/L of Sodium Dodecyl sulphate (3)	0,05	0,1	0,15	0,2	0,25	0,3	0,4	0,5
(PSA) 100 g/L of Ammonium Persulphate (4)	0,05	0,1	0,15	0,2	0,25	0,3	0,4	0,5
(TEMED) Tetramethylethylenediamine (5)	0,003	0,006	0,009	0,002	0,005	0,008	0,024	0,03
10% OF Acrylamide								
Water	1,9	4,0	5,9	7,9	9,9	11,9	15,9	19,8
Acrylamide Solution (1)	1,7	3,3	5,0	6,7	8,3	10,0	13,3	16,7
1.5 M Tris pH 8.8 (2)	1,3	2,5	3,8	5,0	6,3	7,5	10,0	12,5
(DSS) 100 g/L of Sodium Dodecyl sulphate (3)	0,05	0,1	0,15	0,2	0,25	0,3	0,4	0,5
(PSA) 100 g/L of Ammonium Persulphate (4)	0,05	0,1	0,15	0,2	0,25	0,3	0,4	0,5
(TEMED) Tetramethylethylenediamine (5)	0,002	0,004	0,006	0,008	0,01	0,002	0,016	0,02
12% OF Acrylamide								
Water	1,6	3,3	4,9	6,6	8,2	9,9	13,2	16,5
Acrylamide Solution (1)	2,0	4,0	6,0	8,0	10,0	12,0	16,0	20,0
1.5 M Tris pH 8.8 (2)	1,3	2,5	3,8	5,0	6,3	7,5	10,0	12,5
(DSS) 100 g/L of Sodium Dodecyl sulphate (3)	0,05	0,1	0,15	0,2	0,25	0,3	0,4	0,5
(PSA) 100 g/L of Ammonium Persulphate (4)	0,05	0,1	0,15	0,2	0,25	0,3	0,4	0,5
(TEMED) Tetramethylethylenediamine (5)	0,002	0,004	0,006	0,008	0,01	0,012	0,016	0,02
14% OF Acrylamide								
Water	1,4	2,7	3,9	5,3	6,6	8,0	10,6	13,8
Acrylamide Solution (1)	2,3	4,6	7,0	9,3	11,6	13,9	18,6	23,2
1.5 M Tris pH 8.8 (2)	1,2	2,5	3,6	5,0	6,3	7,5	10,0	12,5
(DSS) 100 g/L of Sodium Dodecyl sulphate (3)	0,05	0,1	0,15	0,2	0,25	0,3	0,4	0,5
(PSA) 100 g/L of Ammonium Persulphate (4)	0,05	0,1	0,15	0,2	0,25	0,3	0,4	0,5
(TEMED) Tetramethylethylenediamine (5)	0,002	0,004	0,006	0,008	0,01	0,012	0,016	0,02
15% OF Acrylamide								
Water	1,1	2,3	3,4	4,6	5,7	6,9	9,2	11,5
Acrylamide Solution (1)	2,5	5,0	7,5	10,0	12,5	15,0	20,0	25,0
1.5 M Tris pH 8.8 (2)	1,3	2,5	3,8	5,0	6,3	7,5	10,0	12,5
(DSS) 100 g/L of Sodium Dodecyl sulphate (3)	0,05	0,1	0,15	0,2	0,25	0,3	0,4	0,5
(PSA) 100 g/L of Ammonium Persulphate (4)	0,05	0,1	0,15	0,2	0,25	0,3	0,4	0,5
(TEMED) Tetramethylethylenediamine (5)	0,002	0,004	0,006	0,008	0,01	0,012	0,016	0,02

(1) Acrylamide Solution: acrylamide/Bisacrylamide (29:1) to 30% (p/v) Mr.

(2.1.5 M Tris buffer pH 8.8: hydrochloride 1.5 M pH 8.8.

(3) DSS: 100 g/L solution of sodium sodium dodecylsulphate was also 10% (p/v).

(4) PSA 100 g/L: solution of ammonium persulphate to 10% (p/v). The ammonium persulphate provides free radicals that induce the polymerization of acrylamide and Bisacrylamide. The solution of ammonium persulfate ammonia slowly decomposes and is renewed every week.

(5) Temed: N,N,N',N' -tetramethylethylenediamine.

Table 2 – Stacking gel preparation..

Solution Components	*Volume of the components in mL per volume of gel mold of:*							
	1 mL	*2 mL*	*3 mL*	*4 mL*	*5 mL*	*6 mL*	*8 mL*	*10 mL*
Água	0,68	1,4	2,1	2,7	3,4	4,1	5,5	6,8
Solução de acrilamida(1)	0,17	0,33	0,5	0,67	0,83	1,0	1,3	1,7
Tris *M* pH 6,8 (2)	0,13	0,25	0,38	0,5	0,63	0,75	1,0	1,25
(DSS) 100 g/L de Dodecil Sulfato de Sódio(3)	0,01	0,02	0,03	0,04	0,05	0,06	0,08	0,1
(PSA) 100 g/L de Persulfato de Amônia(4)	0,01	0,02	0,03	0,04	0,05	0,06	0,08	0,1
(TEMED) Tetrametiletilenodiamina(5)	0,001	0,002	0,003	0,004	0,005	0,006	0,008	0,01

(1) Acrylamide Solution: acrylamide/Bisacrylamide (29:1) to 30% (p/v) Mr.

(2) M Tris buffer pH 6.8: tris-hydrochloride M pH 6.8.

(3) DSS: 100 g/L solution of sodium dodecylsulphate was also 10% (p/v).

(4) PSA 100 g/L: solution of ammonium persulphate to 10% (p/v). The ammonium persulphate provides the free radicals Acrylamide and Bisacrylamide. The solution of ammonium persulfate ammonia Slowly decomposes and is renewed every week.

(5) Temed: N,N,N',N' -tetramethylethylenediamine.

Mount the gel in electrophoresis apparatus and electrophoretic separation

When the polymerization finish (about 30 minutes), remove the comb with caution. Wash the wells immediately with water or buffer for electrophoresis DSS- EGPA to eliminate acrylamide possibly not polymerized. If necessary, straighten the teeth of stacking gel, with a hypodermic needle, tip departure, attached to a syringe, one of short sides of the plate, remove with caution the tube and replace the clamps. Proceed in the same way the other short side and then at the base of the mold.

Enter the gel electrophoresis apparatus. Enter the earplugs of electrophoresis in upper and lower reservoirs. Eliminate the bubbles, eventually imprisoned, on the basis of gel between the glass plates. It is recommended that you employ for this purpose a hypodermic needle folded fixed in a syringe. Never establish electrical voltage gel without the samples because it can destroy the discontinuity of the buffer system. Before depositing the sample, wash or fill in the pits with caution with buffer for electrophoresis DSS-EGPA. Prepare the solutions problem and pattern using the recommended sample buffer and treated as if specifies in the monograph of the substance to be analyzed. Fit in the wells of the gel concentration of the appropriate volume of the different solutions. Proceed to electrophoresis under conditions recommended by the manufacturer of the device. Certain manufacturers of apparatuss to DSS-EGPA provide gels of various surfaces and thicknesses. To obtain an optimal separation, it may be necessary to vary the duration of electrophoresis and electrical parameters as indicated by the manufacturer. Check that the front of coloring moves in gel separation, she reaches the base of the gel, stop the electrophoresis. Remove the mold from the apparatus and separate the two glass plates. Remove the spacers, separate and reject the stacking gel and, immediately, the coloring.

DETECTION OF PROTEINS IN GELS

Staining with Coomassie blue is the method most commonly used for the proteins, with a level of detection of the order of 1 µg to 10 µg of protein per band. The staining with silver nitrate is the most sensitive method for the visualization of proteins in gels; enables the detection of bands with 10 ng to 100 ng of protein. All steps of the staining of the gels are carried out at ambient temperature; with moderate stirring and with orbital motion in a suitable equipment. It is necessary the use of gloves to avoid depositing the gel fingerprints which would also stained.

Staining with Coomassie blue. Immerse the gel during at least one hour in a large excess of Coomassie blue SR. Eliminate the staining solution. Soak the gel in a large excess of destaining solution (consists in a mixture of 1 volume of glacial acetic acid and 4 volumes of methanol and 5 volumes of water). Renew several times the destaining solution until the protein bands appear clearly on clear background. The stronger is the discoloration of the gel, both smaller quantities of proteins are detectable by this method. It is possible to accelerate the discoloration incorporating in destaining solution some grams of ion exchange resin or a sponge.

Note: the solutions acid-alcohol used in this method is not secure fully the proteins from the gel. You can, therefore, be no loss of certain proteins of low molecular weight during the operations of staining and destaining gels of fine. Can be achieved a permanent fix by placing the gel for 1 hour in a mixture of 1 volume of trichloroacetic acid, 4 volumes of methanol and 5 volumes of water, before you immerse yourself in Coomassie blue SR.

Silver nitrate staining. Immerse the gel for 1 hour in a large volume of fixation solution (consists in adding 0.27 mL of formaldehyde in 250 mL of methanol and dilute to 500 mL with water) Eliminate and renew the fixation solution and

let incubate for at least 1 hour, or during the whole night, if that is more convenient. Eliminate the fixation solution and place the gel in a volume in excess of water during 1 hour, then dive for 15 minutes in a solution of glutaraldehyde at 1% (v/v). Wash the gel by placing twice a excessive volume of water for 15 minutes and then immerse it for 15 minutes, under the light, silver nitrate SR1 recently prepared. Wash the gel by placing it by three times on an excessive volume of water for 15 minutes, and then immerse it for about 1 minute in a solution of development (consists in dilute 2,5 Ml of citric acid monohydrate to 2% (p/v) and 0.27 mL of formaldehyde in water 500 mL) until obtaining satisfactory staining. Suspend the development by immersion for 15 minutes in a solution of acetic acid in 10% (v/v). Wash with water.

Drying of polyacrylamide gels stained DSS

The treatment of gels is slightly different depending on the method of coloring used. In the case of staining with Coomassie, the step of discoloration is followed by an immersion gel in glycerol solution at 10% (p/v) during at least 2 hours (or overnight). In the case of staining with silver, the final flushing is followed by an immersion in glycerol solution at 2% (p/v) during 5 Minutes. Dive two sheets of porous cellulose in water for 5 to 10 minutes. Put one of the sheets in a frame for drying. Raise, delicately, the gel and deposit it on the sheet of cellulose. Eliminate bubbles that, possibly, they have been imprisoned and then add a few milliliters of water along the edges of the gel. Cover with a second sheet and eliminate any air bubbles trapped. Finish the whole framework of drying. Put in the oven or leave to dry at ambient temperature.

DETERMINATION OF MOLECULAR MASS

The molecular mass of the protein is determined by comparison of their mobility with the mobility of various protein markers of known molecular weight. There are, for the standardization of the gels, mixtures of proteins of molecular mass exactly known which allow obtaining a uniform staining. Such blends are available for different ranges of molecular mass. The mother concentrated solutions of proteins of known molecular weight are diluted in buffer for appropriate sampling and deposited in the same gel that the protein sample to examine. Immediately after electrophoresis, determine the exact position of the dye-marking (bromophenol blue) to identify the front of migration of ions. For this purpose, you can cut a small portion of the edge of the gel, or dive inside the gel, the level of forward migration of the dye, a needle wet in China ink. After staining the gel, determine the distance of migration of each protein band (markers and unknown bands) from the top edge of the gel separation and divide each of these migration distances by the distance travelled by the dye-marking. The distances of migration, thus, obtained are called mobility relating of proteins (in reference to front of coloring) and, conventionally, represented by Rf. Construct a graph using the logarithms of the relative molecular mass (Mr) of protein patterns in function of Rf corresponding. The graphs obtained are slightly sigmoids. The calculation of unknown molecular weights can be calculated by linear regression, or by interpolation from the calibration curve of variation of log (Mr) as a function of Rf provided that the values obtained for the unknown samples are situated in linear portion of the graph.

Validation of the assay

The test is only valid if the proteins used as markers of molecular mass distribute it in 80% of the length of the gel and if, in the interval of separation desired (for example, the interval that covers the product and its dimer, or the product and its impurities crossable) exist for the protein bands in question a linear relationship between the logarithm of the molecular mass and the value of the Rf. Additional validation Requirements concerns the preparation of the sample can be specified in the monographs in particular.

QUANTITATIVE DETERMINATION OF IMPURITIES

When it is specified in a monograph in particular an impurity content is convenient prepare a standard solution corresponding to this content by diluting the solution problem. If, for example, this limit is 5 %, the standard solution is a dilution 1:20 solution of the problem. The eletroforetograma obtained with the solution problem shows no band due to impurities (in addition to the main band) that is more intense than the main band of eletroforetograma obtained with the standard solution. Since that operate in conditions validated, it is possible to quantify the impurities by standardisation in relation to the main band, using a densitometer integrator. In this case, it is verified the linearity of response.

5.2.22.1 CAPILLARY ELECTROPHORESIS

Capillary Electrophoresis (CE) is a physical method of analysis based on migration, inside a capillary, solute loaded, dissolved in an electrolyte solution, under the influence of an electric current. Currently, the JV comprises a family of separation techniques eletrocineticas that separates compounds based, mainly, on the difference in electrophoretic mobility, partitioning between phases, isoelectric point, molecular size, or even, in combination of one or more of these properties.

GENERAL PRINCIPLES

In EC, the separation is governed by two factors. The first corresponds to movement of solutes in capillary due to electric field (E), also called electrophoretic velocity. The second occurs in function of the flow of the electrolyte due to the charged surface in wall of capillary flow, being called electroosmotic. The electrophoretic mobility of a solute (μ_{ep}) is related to the specific characteristics such as molecular size, shape and electrical load as well as properties inherent to the electrolyte in which the migration occurs (ionic strength of the electrolyte, pH, viscosity and presence of additives). Under the influence of tension,

the solutes loaded migrate through the electrolyte with a certain speed, V, given in cm/s, and calculated by the following equation:

$$V_{ep} = \mu_{ep} . E = \left(\frac{q}{6\pi\eta r}\right)\left(\frac{V}{L}\right)$$

Whereas:
μ_{ep} = electrophoretic mobility;
E = voltage applied;
q = solute effective load;
h = viscosity of electrolyte;
r = Stoke's radius;
V = Voltage applied to the system.
L = total length of the capillary.

When an electric field is applied along the capillary, a flow of electrolyte is generated inside the same. The migration of different solutes along the capillary toward the detector, independent of the presence of ionic charge, indicates that besides the electrophoretic mobility, this involved an additional strength. If there is additional strength, compounds with positive charge would migrate downstream into by capillary while anions would remain the distance of the detector and the solutes neutrals not simply would migrate downstream into. The additional force that directs all solutes through the capillary flow is called eletrosmotico (FEO) and has a major role in various types of EC.

The FEO has its origin from the ionization of groups silanois on inner wall of the capillary, which are transformed into groups silanoato (Si-O^-), at pH above 3. These groups with negative charge attract cations of electrolyte, forming an inner layer in the wall of the capillary. The double layer formed near the surface of the capillary is essentially static. The more diffuse layer, next to the double layer is mobile and, under the action of an electrical voltage, migrate toward the cathode directing along the water of hydration. Between the two layers there is a plan of friction and the electrical imbalance generated corresponds to the potential difference across the two layers, called zeta potential (ζ).

The flow rate is dependent on the electroosmotic mobility (μ_{eo}) which, in its turn, is directly related to the charge density of the inner wall of the capillary and the characteristics of the electrolyte. The electroosmotic flow velocity (V_{eo}) can be calculated by the following equation:

$$V_{eo} = \mu_{eo} . E = \left(\frac{\varepsilon . \zeta}{\eta}\right) \cdot \left(\frac{V}{L}\right)$$

Whereas:

ε = dielectric constant of electrolyte;
ζ = zeta potential of the surface of the capillary;
η = viscosity of electrolyte;
V = voltage applied to the system.
L = total length of the capillary.

The mobility and electrophoretic eletrosmotica a solute can act in the same direction or in opposite directions, depending on the load (positive or negative) of the solute and the speed of the solute (v), according to the equation below:

$$V = V_{ep} \pm V_{eo}$$

The sum or difference between the two speeds is used in dependence of mobility act in the same direction or in opposite directions. In capillary electrophoresis in its most usual form, superoxide anions migrate in the opposite direction to the electroosmotic flow and their speeds will be lower than the speed of the airelectroosmotic flow. Cations migrate in the same direction of electroosmotic flow and their speeds will be higher than the flow velocity eletrosmotico. In this condition, in which there is a rapid rate of electroosmotic flow in relation the electrophoretic velocity of the solutes, cations and anions can be separated in the same electrophoretic run.

Time (t) required for the solute migrate a distance (l) from the terminal of the capillary injection until the detection window of the capillary (effective length of capillary) is defined by the following equation:

$$t = \frac{l}{V_{ep} \pm V_{eo}} = \frac{l(\mathrm{L})}{V(\mu_{ep} \pm \mu_{eo})}$$

Whereas:

1 = Distance from terminal tip of the capillary to the capillary detection window (effective length of capillary);
V_{ep} = electrophoretic speed;
V_{eo} = electroosmotic flow velocity.

The reproducibility in the speed of migration of solutes is directly related to the maintenance of a constant value of electroosmotic flow eletrophoretic between different races. For some specific applications, it may be necessary to reduce or even eliminate the electroosmotic flow through changes in the wall of the capillary or in concentration, composition and/or pH of the electrolyte solution.

After the introduction of the sample in the capillary, each solute sample migrates along the electrolyte as an independent band, according to their intrinsic mobility. Under ideal conditions the single factor that can contribute to the enlargement of the band is derived from the molecular diffusion of the solute along the capillary diffusion (longitudinal). In this case, the efficiency of the band is expressed as the number of theoretical plates (N) according to the equation:

$$N = \frac{(\mu_{ep} \pm \mu_{eo}).(VL)}{2DL}$$

Whereas:

D = coefficient of molecular diffusion of the solute in the electrolyte;

The remaining terms were discussed previously.

The separation between two bands can be achieved by modification of the electrophoretic mobility of solutes, by eletrosmotico flow and by increasing the efficiency of the bands of each solute in analysis. The resolution can be calculated using the equation:

$$Rs = \frac{\sqrt{N}(\mu_{epb} - \mu_{epa})}{4(\mu_{ep} + \mu_{eo})}$$

Whereas:

μ_{epa} and μ_{epb} = eletrophoretic mobility of two solutes to be separated;
μ_{eo} = electroosmotic flow mobility
μ_{ep} = Average electrophoretic mobility of solutes $(\mu_{epb}+\mu_{epa})/2$

EQUIPMENT

A capillary electrophoresis product is composed of:

- a high voltage source.
- Two reservoirs of electrolytes, maintained at the same level, containing anode and cathode solutions;
- Two electrodes (anode and cathode), immersed in the reservoirs of the electrolyte and connected to the high voltage source;
- A fused-silica capillary provided with detection window for alignment to certain types of detectors. The terminals of the capillary are immersed in tanks containing the electrolyte solutions. The capillary should be filled with the electrolyte solution prescribed in monograph;
- The sample injection system of solute (s) per share hydrodynamics or eletrocinetica. The choice of the injection process and its automation are indispensable in quantitative analysis by capillary electrophoresis. The introduction of the sample by eletrocinetico mode should take into account the intrinsic electrophoretic mobility of each solute, allowing adequate discrimination of different components of the sample;
- Detector capable of monitoring the amount of solutes that pass through the segment of detection of capillary at specific interval of time. The most commonly used detectors are based on absorption spectrophotometry (UV and UV-VIS) or fluorimetry. Analysis can also be performed using electrochemical detectors or by mass spectrometry;
- Temperature control system capable of keeping it constant inside the capillary. Temperature Changes imply lack of reproducibility in separation of solutes;
- Computerized system for registration and integration of Electropherograms.

The monograph of each substance should detail the type of capillary, the electrolytic solutions, the method of pre- conditioning, the conditions of the sample and the electrophoretic migration.

The electrolyte solution should be filtered (filter 0.45 µm) to remove particles and deaerated to avoid the formation of bubbles that can interfere with the detection system or interrupt the electrical contact on capillary during the electrophoretic migration. The electrophoretic methods should establish a detailed procedure for flushing the capillary between each race in order to allow time for reproducible migration of solutes in analysis.

5.2.22.1.1 Capillary electrophoresis in free solution

PRINCIPLE

In this technique the solutes are separated in a capillary containing only electrolyte without any means anticonvectivo. The separation mechanism is based on differences presented by reason load / mass of analyzed species that migrate as bands at speeds differentiated. The solutes are separated by the combination between the electrophoretic mobility and the magnitude of the intrinsic eletrosmotico in capillary flow. Capillaries coated internally, with reduced electroosmotic flow, can be used to increase the capacity of separation of solutes that adsorb on the surface of the capillary.

The technique in free solution is suitable for analysis of solutes of small molecular mass (PM < 2000) and high molecular weight (2000 < PM < 10.000). Due to the high efficiency of the system, molecules with minimal differences in his reason earth / load can be broken down. The technique also allows separation of chiral solutes through the addition of chiral selectors in the electrolyte of separation. The optimization of separation requires the evaluation of different parameters related to instrumental and electrolyte solution.

INSTRUMENTAL PARAMETERS

Voltage – the separation time is proportional to the voltage applied. However, an increase in voltage used can cause production of excessive heat (*Joule effect*), determining elevation of temperature and gradients of viscosity in the electrolyte inside the capillary, which are responsible for enlargement of the band and reduction in the resolution of the solutes in analysis;

Polarity – the polarity of the electrode can be normal (anode and cathode inlet outlet). In this case the electroosmotic flow moves toward the cathode. If the polarity of the electrode is reversed, the direction of the electroosmotic flow is contrary to the exit and only solutes loaded with electrophoretic mobility higher than the electroosmotic flow migrate toward the exit;

Temperature – the main effect of temperature is observed in viscosity and electrical conductivity of the electrolyte. Changes in these two properties of electrolyte determine differences in speed of migration;

Capillary – the length and internal diameter influence analytical parameters such as time of total migration of solutes, efficiency of separations and load capacity. Under constant voltage, the increase of the total length and the

effective capillary can decrease the electrical current that, by its time, determines the increase in migration of analytes. Capillaries with smaller internal diameter have better ability to dissipation of heat generated by electrical current (Joule effect), allowing you to raise the applied voltage and reduction in analysis time. The detection limit of the method can also be influenced by internal diameter, depending on the volume of sample injected and the detection system used. The efficiency of separations can also be increased by reducing the inner diameter of the capillary.

The adsorption of the sample components on the inside wall of the capillary can limit the efficiency. For this reason, strategies to avoid these interactions must be considered in the development of a method of separation by capillary electrophoresis. This is a critical factor, for example, in samples containing proteins. One of these strategies (use of extremes pH (s) and adsorption of electrolytes loaded with positive charge) requires modification of the composition of the electrolyte to prevent the adsorption of proteins. Alternatively, it is possible encircle the inner wall of the capillary with a polymer through covalent bonds, by preventing the interaction of proteins with the surface of the silica loaded negatively. For this proposal, capillaries with inner wall previously coated with polymers of nature neutral-hydrophilic, cation and anion are commercially available.

PARAMETERS OF ELECTROLYTIC SOLUTION

Nature of the buffer and concentration – The electrolytes for capillary electrophoresis must submit adequate buffering capacity in the range of pH chosen and low mobility in order to minimize the generation of electrical current. To reduce the distortion of the electrophoretic peak it is important to combine the mobility of the ion of electrolyte the mobility of the solute. The choice of solvent of the sample is important to achieve a uniformity of the solute which allows the increase of the separation efficiency and improves the detection. In addition, an increase in the concentration of the electrolyte in a specific pH determines the decrease of electroosmotic flow and the speed of the solute.

PH of electrolyte – The pH of the electrolyte can affect the separation through the modification of the solute load or other additives, as well as the alteration of the electroosmotic flow. The change in the pH value of the electrolyte above or below the isoelectric point of proteins and peptides influence the separation of these solutes, through the modification of the liquid cargo from negative to positive. In general, an increase in pH of electrolyte causes increase of electroosmotic flow.

Organic Solvents – organic Solvents such as methanol, acetonitrile among others can be added to aqueous electrolyte to increase the solubility of the solute and/or other additives present in the electrolyte, or even influence the degree of ionization of the solutes in the sample. The addition of these solvents in the electrolyte usually causes the reduction of electroosmotic flow.

Additives for chiral separations – The enantiomeric separations should be carried out by adding chiral selectors to electrolyte of race. The chiral selectors most used are the cyclodextrins. However, crown ethers, polysaccharides and proteins can also be employed for this purpose. The enantiomeric discrimination is governed by different interactions between the chiral selector and each one of the enantiomers of solute in analysis. Thus, the correct choice of selector directly influences the resolution obtained for enantiomeric chiral solutes. During the development of a method for separating enantiomeric excess, it is recommended that you test cyclodextrins of different sizes of cavity (a, p, g), cyclodextrins modified with aromaticity neutral (methyl, ethyl, hidroxialquil, etc.), or with aromaticity ionizable (aminometil, carboxymethyl, sulfobutileter, etc.). The resolution of chiral separations is also controlled by the concentration of the chiral selector, the composition and pH of electrolyte and temperature of analysis. Organic Additives such as methanol and Urea can be employed to modify the resolution obtained.

5.2.22.1.2 Micellar Electrokinetic Chromatography (CEM)

PRINCIPLE

In Micellar Electrokinetic Chromatography, separation occurs in an electrolyte solution that contains a tenside a concentration above the critical micellar concentration (*cmc*). The molecules of the solute are distributed between the electrolyte and the pseudo-stationary phase consists of micelles, in accordance with the partition coefficient of the solute. It is a technique that can be used for separation of solutes neutral and/or ionized, while maintaining the efficiency, speed and suitability instrumental of capillary electrophoresis. The anionic tensid sodium dodecyl sulphate (DSS) is one of most surfactants used in CEM, although others are also used, such as, for example, cationic surfactants (salts of cetiltrimetilamonio).

At neutral pH or alkaline, a strong electro-osmotic flow is generated by moving the ions of the electrolyte of separation in the direction of the cathode. If DSS is used as tenside, the electrophoretic migration of the resultant miscella was maintained anion will be in the opposite direction, toward the anode. As a result, the speed of migration micellar total is reduced, in comparison to the flow of the electrolyte solution. In the case of solute neutral, since the IRT can be distributed between the resultant miscella was maintained and the electrolyte, and there is no mobility, the electrophoretic migration speed of analyte depend only of the partition coefficient between the resultant miscella was maintained and the electrolyte. In eletroferograma, the peaks corresponding to each solute neutral position are always located between the marker eletrosmotico flow and the resultant miscella was maintained (the time elapsed between these two peaks is called window of separation). For ionized solutes, the speed of migration depends on the partition coefficient of the solute between

the resultant miscella was maintained and electrolyte and the electrophoretic mobility of the solute in the absence of the resultant miscella was maintained.

In CEM, the mechanism of solute neutral and weakly ionised is essentially dissolved. Thus, the migration of the solute and the resolution can be represented in terms of retention factor of the solute (*k*), also called for reason of distribution of mass (D_m), which is the relationship between the number of moles of solute inside the And resultant miscella was maintained in the mobile phase. For a substance, neutral k can be calculated through the following equation:

$$k = \frac{t_R - t_0}{t_0 \times \left(1 - \frac{t_R}{t_{mc}}\right)} = K \times \frac{V_S}{V_M}$$

Whereas:

t_R = migration time of the solute;

t_0 =migration time of a solute does not retained (determined BY the injection of a marker of electroosmotic flow that do not connect to the resultant miscella was maintained, for example, methanol);
t_{mc} = time of migration of the resultant miscella was maintained (determined by injection of a marker of resultant miscella was maintained, as Sudan III, which migrates continuously associated with the resultant miscella was maintained along the Electrophoretic migration);
K = partition coefficient of the solute;
V_S = volume of micellar phase;
V_M, = volume of the mobile phase;

Also, the resolution between 2 adjacent peaks (R_s) is Given by:

$$R_s = \frac{\sqrt{N}}{4} \times \frac{\alpha - 1}{\alpha} \times \frac{kb}{kb + 1} \times \frac{1 - \left(\frac{t_0}{t_{mc}}\right)}{1 + ka \times \left(\frac{t_0}{t_{mc}}\right)}$$

Whereas:

N = the number of theoretical plates of each solute;
α = selectivity;
K_a and k_b = retention factors for both solutes respectively ($k_b > k_a$).

In a similar way, though not identical, the equations provide values of k and R_s for solutes with load.

OPTIMIZING

The development of methods for ONE HUNDRED involves instrumental parameters and the electrolyte solution:

Instrumental Parameters

Voltage – The separation time is inversely proportional to the voltage applied. However, an increase in voltage can cause excessive heat production, elevating the temperature gradients and viscosity of electrolyte in cross-section of the capillary. This effect may submit relevant impact in electrolytes that present greater conductivity as those that contain micelares systems. The systems that are less capable of dissipating heat determine enlargement of bands and lower resolution between the peaks.

Temperature – temperature Changes in capillary affect the partition coefficient of the solute between the electrolyte and the micelles, the critical micellar concentration and viscosity of electrolyte. These parameters directly influence at the time of migration of solutes during the electrophoretic separation. The use of a suitable cooling system increases the reproducibility of migration time of solutes.

- The dimensions of the capillary (length and internal diameter) contribute at the time of analysis and in the efficiency of separations. An increase of the total length and the effective capillary can decrease the electrical current (under constant voltage), increases the migration time and improves the efficiency of separation. The OD is too small for the internal capillary controls heat dissipation (in a given electrolyte and electrical current) and consequently the enlargement of the bands of the solutes.

Parameters of electrolyte solution

Nature of tenside and concentration – The nature of tenside, in a way similar to stationary phase in chromatography, affects the resolution, because it modifies the selectivity of separation. The log k of a neutral substance increases linearly with the concentration of tenside in mobile phase. Given that the resolution in ONE HUNDRED reaches a maximum when k presents value next to

$$\sqrt{t_{mc}/t_0}$$

changes in the concentration of tenside present in the mobile phase determine changes in resolution of the bands.

PH of electrolyte – the pH does not change the partition coefficient of non-ionised solutes, but can determine changes in electroosmotic flow in capillaries not coated. A decrease in pH of electrolyte reduces the electroosmotic flow, providing an increase in resolution of solutes neutral and at the time of analysis.

Organic Solvents – organic solvents (methanol, propanol, acetonitrile) can be added to the electrolyte solution to improve the separation of hydrophobic solutes. In general, the addition of these modifiers reduces the time of migration and the selectivity of the separation. The percentage of organic solvent added must take into consideration the critical micellar concentration of tenside, having in view that excessive values can affect, or even inhibit the process of formation of micelles and, therefore, the absence of the phenomenon of partition. The decoupling of micelles in the presence of high percentages of modifier does not

necessarily mean better results in separation. In certain situations, the hydrophobic interaction between the monomer of tenside and solute neutrals form complex solvofobicos which can be separated by electrophoresis.

Modifiers for chiral separations – the separation of enantiomers in CEM can be obtained through the inclusion of chiral selectors to micellar system, covalently bound to tenside or added to the electrolyte of separation. Micelles that have connections with properties of chiral discrimination include salts of N-dodecanoil- L – amino acids, bile salts, among others. The chiral resolution can also be obtained by means of chiral selectors, such as the cyclodextrins, added directly to electrolyte solutions containing surfactants not chiral.

Other additives – The selectivity can be modified through various strategies, by adding chemicals to the electrolyte. The addition of various types of cyclodextrins to electrolyte can also be used to reduce interaction of hydrophobic solutes with the resultant miscella was maintained, thus increasing the selectivity for this type of solute.

The addition of substances capable of modifying the interactions solute-resultant miscella was maintained by adsorption on the latter has been used to increase the selectivity of separations in a HUNDRED. These additives may be a second tenside (ionic or non-ionic surfactant) that causes mixing of micelles or metal cations which dissolve the resultant miscella was maintained forming complexes of coordination with the solute.

QUANTIFICATION

The areas of the peaks should be divided by the time of migration corresponding to provide the correct area with the aim of:

- compensate for the displacement in migration time between races, thus reducing the variation of the response;

- compensate for the different responses of the sample components with different times of migration.

When an internal standard is used, you should check if no peak of solute to be analyzed is superimposed to the internal standard peak.

CALCULATIONS

The content of the component (or components) in analysis should be calculated from the values obtained. When prescribed, the percentage content of one or more components of the sample to be analyzed is calculated by determining the area corrected (s) of peak (s) as a percentage of the total of the areas of all peaks corrected, excluding those resulting from solvents or reagents added (standardisation process). It is recommended to use a system of automatic integration (integrator or acquisition system and data processing).

SUITABILITY OF THE SYSTEM

The parameters of suitability of the system are employed to verify the behavior of the method by capillary electrophoresis. The choice of these parameters depends on the type of Capillary Electrophoresis used. The factors are: retention factor (k) (only for chromatography eletrocinetica micellar), apparent number of theoretical plates (N), a factor of symmetry (The) and resolution (Rs). The equations that allow to calculate the values of N and Rs through the Electropherograms are provided below.

Apparent Number of theoretical plates

The apparent number of theoretical plates (N) can be calculated using the expression:

$$N = 5{,}54 \text{ x} \left(\frac{t_R}{w_h}\right)^2$$

Where:

t_R = migration time, distance from base line from the next injection point of injection until the line perpendicular to the maximum point of the peak corresponding to the component; w_h = peak width at half height

Resolution

The resolution (R) between peaks of similar heights of 2 components can be calculated using the expression:

$$R_s = \frac{1{,}18 \text{ x } (t_{R2} - t_{R1})}{W_{h1} + W_{h2}}$$

$$t_{R2} > t_{R1}$$

Whereas:

t_{r1} and t_{R2} = migration times or distances from the base line from the point of injection until the perpendicular line of the maximum point of two adjacent peaks
w_{h1} and w_{h2} = peak widths at half height

When appropriate, the resolution can be calculated by measuring the height of the valley (H_v) between 2 peaks partially solved in a preparation standard and the height of the minor peak (H_p), calculating the ratio peak/valley (p/v):

$$\frac{p}{v} = \frac{H_p}{H_v}$$

Factor of symmetry

The factor of symmetry (A_s) of a peak can be calculated using the expression:

$$A_s = \frac{w_{0,05}}{2d}$$

Whereas:

$W_{0,05}$ = peak width determined to 5% of the value of the height;

d = distance between the perpendicular line of the maximum peak and the tangent of the peak to 5% of the height of the peak.

Tests for repeatability of area (standard deviation or areas of reason area / migration time) and for repeatability of migration time (standard deviation of the time of migration) are entered as parameters of suitability. The repeatability of the migration time provides a test for suitability of flushing procedures of the capillary. An alternative practice to avoid the lack of repeatability of the migration time is to use the time of migration on an internal standard.

A test to check the signal/noise ratio of a standard preparation (or the determination of the limit of quantification) can also be useful for determination of related substances.

Signal ratio: noise

The limits of detection and quantification correspond to signal: noise ratio of 3 and 10, respectively.

The signal: noise ratio (S/N) is calculated using the expression:

$$\frac{S}{N} = \frac{2H}{h}$$

Where:

H = height of the peak corresponding to the specific component, in eletroferograma obtained with the reference solution, measured from the maximum of the peak until the base line extrapolated to the signal observed over a distance equal to 20 times the width at half height of the peak;
h = interval from baseline on a eletroferograma obtained after injection of white, observed at a distance equal to 20 times the width at half height of the peak in eletroferograma obtained with the reference solution, and if possible, located near the retention time where this peak would be found.

5.2.22 ENANTIOMERIC ANALYSIS

CHIRAL DRUGS

The enantiomers generally exhibit different pharmacological and toxicological properties due to main molecular targets such as proteins, nucleic acids and polysaccharides are chiral. For example, the enantiomers of methyl ether levorfanol the dextrometorfane and the levometorfano, are used differently in the therapy. While the dextrometorfane is indicated as spasmodic, levometorfano is indicated as analgesic.

Due to the recognition of the importance of the clinical use of enantiomerically pure drugs in the treatment of various diseases, the pharmaceutical industries are encouraged constantly to make drugs settled in industrial quantities.

To ensure the safety and efficiency of available drugs and in development, it is necessary to resolve the enantiomers and examine each one regarding the activities pharmacological and toxicological characteristics. After the identification of the more active enantiomer must evaluate the enantiomeric excess of eutomero since the synthesis until the consumption to ensure the quality of the medicinal product.

DRUG ENANTIOMERIC SEPARATION AND DETERMINATION

The separation, or resolution of enantiomers by liquid chromatography of high efficiency (CLAE) began to be applied since the 1960s. In the 1970s, with the appearance of the columns of small particles for liquid chromatography, it started the development of chiral stationary phases for resolution of drugs racemicos.

The CLAE is considered one of the most efficient techniques for separation, detection and quantification of drugs. The use of chiral stationary phase (FEQ) adequate becomes a powerful method for the separation of the enantiomers.

The chromatographic resolution of the enantiomers can be achieved by various methods, however, is always required the use of some type of discriminator or chiral selector. The indirect method and the direct are the two paths for the separation of the enantiomers using liquid chromatography.

The indirect method, the enantiomers are converted into diastereoisomeros by reaction with a chiral substance. The diastereoisomeros are substances that have different physico-chemical properties and, therefore, may be separated using chiral stationary phase does not.

The indirect method was widely used in the past. However presents limitations as a necessity of the insulation of a substance of interest and their derivatization. These facts hinder the development of the automated process for large number of samples. In addition, the enantiomeric purity of agents derivatizing reagents is important to avoid false results. Another limitation are the different speeds and/or constants of reaction to the enantiomers already that the reactional states of transition are diastereoisomericos which can result in different proportions of initial enantiomeric composition.

In the direct method, a mixture of enantiomers to be resolved is injected directly into the gas chromatograph. For the separation of the enantiomers you can use a FEQ, or a solvent, or a chiral mobile phase with chiral additive. The resolution occurs due to the formation of complexes between diastereoisomericos enantiomeric mixture and the chiral selector used for resolution. The use of FEQ is today the most frequently employed method for resolution by HPLC.

In the following tables (**Tables 1.2.3.4 and 5**) are presented the main classes of stationary phases used for the resolution of racemic mixtures and some examples of chiral selectors in each class. Consult the manufacturer for the indication of use of each selector.

Table 1 – type of chiral stationary Phases Pirkle.

*Chiral discriminator**
(R) -DNB-phenylglycine
(S) -DNB-phenylglycine
(R) -DNB-leucine
(S) -DNB-leucine
Calcium phosphonate trifluorated of DNB-to-amino-2,2-dimethyl-4- penthenyl
DNB-tetrahydrofenantren
Naftylethylamide

* The majority of the Pirkle type columns are available in two enantiomeric forms.

Table 2– chiral stationary Phases type protein.

Chiral Discriminator
α-acid Glycoprotein
Bovine serum albumin
Human serum albumin
Celobioidrolase I
Pepsin
"Ovomucoid"

Table 3 – type of chiral stationary Phases cavity or inclusion.

Chiral Discriminator
α-Cyclodextrin
β-Cyclodextrin
γ-Cyclodextrin
O- (S) -2-Hydroxypropyl-β-cyclodextrin
O- (R/S) 2-Hydroxypropyl-β-cyclodextrin
O- (S) -Nafthylethylcarbamoyl-β-cyclodextrin

Table 4 – type of chiral stationary Phases carbohydrates.

Chiral Discriminator
Tris (dimethylphenylcarbamoyl) cellulose
Tris (4-ina methylbenzoate) cellulose Tris (phenylcarbamoyl) cellulose
Cellulose triacetate
Cellulose tribenzoate
Tribenzylic celulose ether
Cellulose Tricinamato

Table 5 – type of chiral stationary Phases macrociclicos antibiotics.

Chiral Discriminator
Vancomycin
Teicoplanin
Ristocetin

5.2.23 CONDUCTIVITY OF WATER

The electrical conductivity of water is a measure of the flow of electrons which is facilitated by the presence of ions. Water molecules dissociate into ions as a function of pH and temperature resulting in a specific conductivity. Some gases, particularly carbon dioxide, are dissolved in water and interact to form ions that affect the conductivity and pH of the water. These ions and its conductivity resulting may be regarded as intrinsic to the water. The exposure of the sample to the atmosphere can change the conductivity/ resistivity, due to loss or gain of dissolved gases.

The Chloride ion and the ammonium ion are some of the main impurities found in water, and also have an influence on its conductivity. These external ions can have significant impact on chemical purity of water and compromising its use in pharmaceutical applications.

The hydraulic conductivities combined of ions intrinsic dried and of external ions vary as a function of pH and are the basis for the specifications of the conductivity as described in table 3 and employed when performed at step 3 of the test. Two preliminary steps are included in this test. If the conditions of the test and the limits of conductivity are met in any one of these preliminary steps (Steps 1 and 2), the water meets the requirements of this test and it is not necessary to the implementation of Step 3. Only in the case of the sample does not meet the requirements of Step 3, the water is judged as not conforming to the requirements of the conductivity test.

INSTRUMENTATION AND OPERATING PARAMETERS

The conductivity of the water should be measured using calibrated instruments with resolution of 0.1 µS/cm. The thermometer should have divisions of 0.1 °C and cover the range of 23 to 27 °C. The electrodes should be kept according to the recommendation of the manufacturer of the equipment.

The constant conductivity cell is a factor used as a multiplier for the scale values of conductivity.

Cell Constant: the value must be known in ± 2 %. Generally conductivity cells are constant in the order of 0.1 cm^{-1}.1 cm^{-1} and 2 cm^{-1}. The majority of equipment presents the cell constant defined. You must assess this constant with KCl solution of reference described in Table 1. Normally the check is carried out using only one reference solution; in this case use the reference solution of lower conductivity. However, it is recommended that you periodically measure the conductivity of other patterns and observe the concordance between the reading of the conductivity and the nominal value of each reference solution.

Calibration: according to the manufacturer's instructions. The majority of equipment for multiple scales has a single calibration point, as soon as it is necessary to calibrate

each time they use a different scale. The reading should be obtained between + 0.1 uS/cm of the nominal value of the reference solution.

For calibration of the conductivity meter, use the solutions of references described below.

Solution (0.01 M): weigh exactly 0.7455 g of potassium chloride dry at 105 °C for 2 hours, transfer to 1000 mL volumetric flask and Fill up to volume with water.

Solution B (0.005 M): pipette 50 mL of the Solution to 100 mL volumetric flask and dilute with water.

Solution C (0.001 M): pipette 10 mL of the Solution to 100 mL volumetric flask and dilute with water.

Solution D (0.0005 M): pipette 5 mL of the Solution to 100 mL volumetric flask and dilute with water.

Solution E (0.0001 M): pipette 5 mL of the Solution to 500 mL volumetric flask and dilute with water.

Note 1: *for the preparation of the above solutions always use carbon dioxide free water, i. e. with conductivity less than 0.10 µS. cm -1.*

Note 2: *do not use temperature compensation and keep the reference solutions at 25 °C during the reading.*

Table 1 –Conductivity of solutions of potassium chloride (25°C).

Solution	*Concentration (Mol/L)*	*Conductivity (pS /cm)*
A	0.01	1412
B	0.005	717.5
C	0.001	146.9
D	0.0005	73.9
E	. 001	14.9

PROCEDURE

The procedure described below is established for measures of purified water and water for injectables. Alternatively, the Step 1 can be performed (with appropriate modifications in accordance with item 1 of Step 1) using instrumentation of the type "in line" that has been calibrated properly, whose constant cell have been exactly determined and whose functions of temperature compensation have been disabled. The suitability of such instruments "in line" for quality control tests is also dependent on the location in the system of water. Of course, the positioning of the instrument needs to reflect the quality of the water that will be used.

Step 1

1 Rinse the cell with at least three portions of the sample.

The determination should be carried out in appropriate container or as determining "in line". The value obtained should be less than 1.3 µS/cm, at a temperature of 25.0 °C + 0.1 °C.

3 In Table 2, find the nearest value of temperature and lower than the temperature at which the conductivity was measured. The conductivity value corresponding to this temperature is the limit. (Not interpolate)

4 If the conductivity value measured is not greater than the corresponding value in Table 2, the water meets the requirements for the conductivity. However, if the measured value is greater than that of the table, proceed to determination of agreement With the Step 2.

Table 2 – threshold Values for conductivity in accordance with the temperature (only for conductivity values without temperature compensation).

Temperature (°C)	*Conductivity (pS/cm)*
0	0.6
5	0.8
10	0.9
15	1.0
20	1.1
25	1.3
30	1.4
35	1.5
40	1.7
45	1.8
50	1.9
55	2.1
60	2.2
65	2.4
70	2.5
75	2.7
80	2.7
85	2.7
90	2.7
95	2.9
100	3.1

Step 2

1 Transfer sufficient quantity of water (100 mL or more) for appropriate container and shake the sample. Adjust the (25 ± 1) °C and shake the sample vigorously observing periodically reading the conductivity meter. When the change in conductivity due to the absorption of atmospheric carbon dioxide is less that 0.1 µmS/cm per 5 minutes, record the conductivity.

2 If the conductivity is not greater than 2.1 µs/cm, the water complies with the requirements for the electrical conductivity test. If the conductivity is greater than 2.1 µS/cm, proceed as the Step 3.

Step 3

Perform this test within 5 minutes after the Step 2 with the same sample while maintaining the temperature of the sample (25 ± 1) °C. Add saturated solution of potassium chloride (0.3 mL to 100 mL of sample) and determine the pH with

a precision of 0.1 unit in accordance with pH Determination (5.2.19). Using the Table 3 determine the threshold value for the conductivity in accordance with the pH.

Table 3 – Values of conductivity limits in accordance with the pH (only for samples kept in atmosphere and balanced temperature).

PH	*Conductivity (pS/cm)*
5.0	4.7
5.1	4.1
5.2	3.6
5.3	3.3
5.4	3.0
5.5	2.8
5.6	2.6
5.7	2.5
5.8	2.4
5.9	2.4
6.0	2.4
6.1	2.4
6.2	2.5
6.3	2.4
6.4	2.3
6.5	2.2
6.6	2.1
6.7	2.6
6.8	3.1
6.9	3.8
7.0	4.6

After determined the pH and established the limit according to Table 3, the water meets the test if the conductivity measured in Step 2 is not greater than this limit. If the conductivity is greater or the pH value is outside the range of 5 to 7, the water does not meet the test for conductivity.

WATER ULTRAPURIFICADA

For the water ultrapurificada, in general the conductivity meters or resistivimetros installed in equipment of purification of water have a temperature compensation circuit to 25.0 °C and provide direct reading. Such equipment must be calibrated periodically. The conductivity of the water ultrapurificada must be 0.055 µS/cm to 25.0 °C (resistivity > 18.0 Irradiation) for a specific application.

Alternatively, if the product does not provide a direct reading of the conductivity, proceed as below:

1 Rinse the cell with at least three portions of the sample.

2 Determine both the temperature and the conductivity of the water without automatic temperature compensation. The determination should be carried out in appropriate container or as determining "in line". The obtained value must be less than 0.055 µS/cm, at a temperature of 25.0 °C + 0.1°C.

3 In Table 4, find the value of the temperature more near and lower than the temperature at which the conductivity was measured. The conductivity value corresponding to this temperature is the limit. (Not interpolate)

4 If the conductivity value measured is not greater than the corresponding value in Table 4, the water ultrapurificada meets the requirements for the conductivity.

Table 4 – Limit values for conductivity in accordance with the temperature (only for conductivity values without temperature compensation).

Temperature (°C)	*Conductivity (µS/cm)*
0	0.012
5	0.017
10	0.023
15	0.031
20	0.042
25	0.055
30	0.071
35	0.090
40	0.113
45	0.140
50	0.171
55	0.207
60	0.247
65	0.294
70	0.345
75	0.403
80	0.467
85	0.537
90	0.614
95	0.696
100	0.785

5.2.25 CLEARNESS OF FLUIDS

PROCEDURE

Using tubes of neutral glass, colorless and transparent, with flat bottom and 15 to 25 mm internal diameter, unless indicated differently in the monograph. Introduce, in separate vials, the liquid in exam and the suspension of reference indicated in the monograph, preparing it for occasion of use, as specified in Table 1. The liquid in exam and the suspension of reference must achieve, pipes, a height of 40 mm. Five minutes after the preparation of the suspension of reference, compare the contents of the tubes, observing them, vertically under visible light diffuse and against black background. The diffusion of light must be such that the suspension of reference I is easily distinguished from water and suspension of reference II.

A liquid is considered limpid when, to be examined under the conditions previously described, its transparency corresponds to that of the water or the solvent used, or when their opalescence is not more pronounced than that of the suspension of reference I.

Pattern of opalescence

Dissolve 1 g of hydrazine sulfate in water and make up the volume to 100 mL with the same solvent. Leave in Rest for 4 to 6 hours. Add 25 mL of this solution to a solution containing 2.5 g of methenamine in 25 mL of water. Mix well and leave to rest for 24 hours. This suspension is stable for two months if kept in glass container, with surface free of defects. The suspension should not adhere to the walls of the container and must be vigorously agitated, in original container, before use. For the preparation of standard of opalescence, dilute 15 mL of the suspension to 1000 mL with water. The pattern of opalescence should be prepared at the time of use and can be kept for a maximum of 24 hours.

Table 1 – Preparation of suspensions of reference

Suspension of reference	*I*	*II*	*III*	*IV*
Opalescence standard (mL)	5	10	30	50
Water (mL)	95	90	70	50

5

5.2.26 ALCOHOLOMETRY

Alcoholometry is determining the alcoholic degree or title ethanolic of mixtures of water and ethyl alcohol.

The alcoholic strength by volume of a mixture of water and alcohol is expressed by the number of volumes of ethanol at 20 °C contained in 100 volumes of the mixture at the same temperature. It is expressed in % (v/v).

The alcoholic strength by weight is expressed by the ratio between the mass of ethanol contained in a mixture of water and ethanol and the total mass of this. It is expressed in % (m/m).

The ethyl alcohol contains, at a minimum, 95.1% (v/v) corresponding to 92,55% (m/m) and a maximum of 96.9% (v/v) corresponding to 95.16 % (m/m) of C2H6O at 20 °C. The absolute ethyl alcohol contains at least 99.5% (v/v) corresponding to 99.18% (m/m) of C2H6O at 20 °C. These values can be observed in Tables (Annex D).

ALCOHOL OR ALCOHOMETRY TITRATION DETERMINATION

The alcoholmeter proximate composition is intended for the determination of alcoholic degree of mixtures of water and alcohol indicating only the concentration of alcohol in volume and expressed by their unit of measure, degree Gay-Lussac (°G. L.).

The determinations of the alcoholmeter is accurate only for the mixture of alcohol and water at 20 °C, in which the instrument was graded. If the temperature during the test is less than or greater than 20 °C it becomes necessary to correct the temperature of alcohol to 20 °C.

The determination of the alcoholic degree of mixtures of water in volume is performed by alcoholmeter.

For the determination of alcoholic degree of mixtures of alcohol and water in earth, can be used the method of relative density or checked the graduation in table alcoometricaapos determination by alcoholmeter.

5.2.26 THERMAL ANALYSIS

The thermal analysis is a set of techniques that allow measuring the physico-chemical properties of a substance as a function of temperature. The techniques most commonly used are those that measure the variations of energy or mass of a substance.

THERMOGRAVIMETRY (TG)

The thermogravimetric analysis is the technique of thermal analysis in which the variation in the mass of the sample is determined as a function of temperature, or heating time, using a controlled temperature program.

Apparatus

It consists basically of a termobalanca who is an association between the electric furnace and a high-precision electronic scale in which the substance is inserted into a port- sample under atmosphere specified and controlled temperature program. The device allows heat and simultaneously measuring the mass of analyte. In certain cases, the apparatus can be associated with a system that enables us to detect and analyze volatile products.

Calibration and/or benchmarking of termobalanca. Transfer an adequate amount of calcium oxalate monohydrate SQR in sample. The termobalanca will indicate with great precision and accuracy to its mass. Employ the ratio of heating of 10 ºC/min and heat the sample up to 900 ºC. To finalize the thermal process record: (i) the curve termogravimetrica (TG) by checking the temperature in the abscissa axis (increasing values from left to right) and the mass percentage of the sample in the ordinates axis (increasing values from down to up); (ii) the curve termogravimetrica derivative (DTG), derived from the first curve TG, which enables better define where it began and ended the mass loss. Determine in graph the distance between the initial and final stages of mass curve-temperature, distance that represents the loss in mass of the sample in given temperature range. The losses of pasta declared of the calcium oxalate monohydrate SQR are calculated, estequiometricamente, from the three stages of loss of mass due to successive releases of: a) H2O; b) CO; c) CO2. The verification of the scale of the temperature can be performed using the technique of metal hook fundivel (Ni, Pb, Zn, Al, Ag and Au) according to the manufacturer's instructions.

Procedure

Use the same method described for calibration and/or benchmarking by adding an appropriate amount of sample.

The TG and DTG curves illustrated in Figure 1 indicate a step of weight loss of the sample. In DTG curve, it was observed that among the points ab is the initial level. The loss of mass starts at point b and terminates at point c. Among the points cd is the final level. The interval bc corresponds to interval reactional states. To calculate the loss of mass of the sample in the curve TG, uses If the comparison with the curve DTG for greater precision in location of points b and c. Trace the ramifications of initial and final levels of TG curve plotting using the points b and c. The measured distance corresponds to the loss of mass (Am) of the sample. The projections of the points b and c in the axis of abscissa axs correspond, respectively, to the initial temperature (Ti) and final (Tf) of weight loss. Record the result as a percentage of the ratio m/m.

Note 1: it is necessary to obtain a curve of the blank test (grille under the same experimental conditions using the door-empty sample) before the test sample for subtraction of base line.

Note 2: in the case of the frequent use of the apparatus, perform regularly, checking and/or calibration. Otherwise, perform these operations before each determination.

Note 3: as the atmosphere can affect the results are recorded the flow and composition of gas for each test.

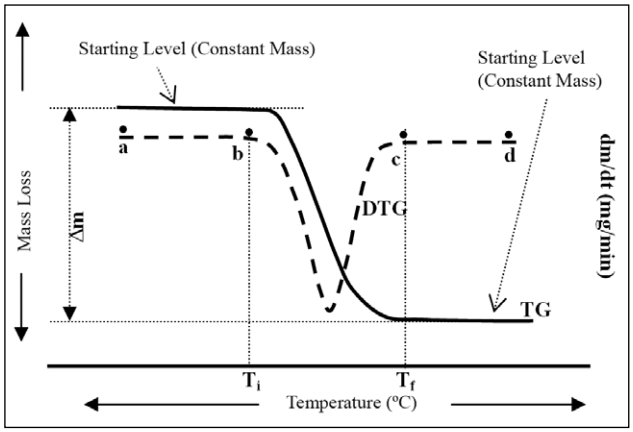

Figure 1– Example of thermogravimetric curve and its measures.

Applications

The determination of the variation of the earth for a substance in certain temperature ranges can be used for evaluation of thermal behavior; determination of moisture content and/or solvents; determination of boiling temperature and sublimation; determination of the temperature of thermal decomposition and determination of ash content.

EXPLORATORY DIFFERENTIAL SCANNING CALORIMETRY (DSC)

The differential scanning calorimetry is a technique that allows to evaluate the energy phenomena, physical and/or chemical produced during the heating (or cooling) of a substance. This technique provides a means to measure the flow of heat differential between the sample and a reference material thermally inert in function of the temperature and/or time of heating under a program

Controlled temperature. The sample and the reference material are maintained at approximately the same temperature during the experiment. You can determine the variations of enthalpy; changes of specific heat and the temperature of events endo and exotermicos. According to the measuring method used, there are two ways: the DSC with power compensation and the DSC with heat flow.

APPARATUS

The DSC with power compensation is composed of a calorimetric cell that contains two ovens, one for the reference material and the other for the sample. With The DSC heat flow is constituted of a calorimetric cell containing a single oven that has a sensor heaty for the reference and sample. The equipment shall include a programming device controlled temperature, one or several thermal detectors and a system of record that can be associated with a data processing system. The determinations are made under specified atmosphere.

Calibration and/or measurement of the self-contained. Calibrate the device for the axis of temperature and heat flow using high purity indium metal or any other material appropriate certificate in accordance with the manufacturer's instructions. For the adjustment of linearity, uses a combination of two metals such as indium and zinc for the measurement of the temperature axis.

PROCEDURE

For a door-sample appropriate transfer a quantity of the sample, accurately known. Set the initial temperature and the end of the test and the reason for heating. Start the grille. After the test, record the curve of differential scanning calorimetry writing in the abscissa axis temperature, or the time (increasing values from left to right) and the flow of heat in the ordinates axis, indicating the direction (endothermic or exothermic). In DSC curves illustrated in Figure 2 shows the variation entalpica between points acd. The point of intersection (b, regarding the extension of the base line with the tangent at the point of greatest slope (inflection point) of the curve, corresponds to the temperature onset (beginning extrapolated from the event, Tonset), employed in events of fusion as the initial temperature of the status change. The end of the thermal event is marked by point c (T_{pico}), however, for purposes of the calculation of the area of the curves is considered the point d (T_{final}). The enthalpy variation (ΔH) the phenomenon is proportional to the area under the curve limited by **acd** points being given the factor of proportionality from the determination of the enthalpy of fusion of a standard substance known (indium, for example) in the same conditions of work. Each term analytical curve is recorded containing the following data: indication of last calibration, size and identity of sample, type of door-sample, reference material, atmosphere (flow and gas composition), heating rate and sensitivity of the calorimetric cell.

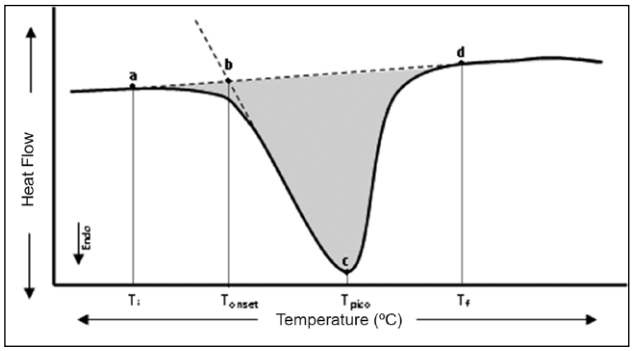

Figure 2 – Example of a typical DSC curve and its measures.

Applications

Assessment of the flow of heat differential on the variations of heat capacity and enthalpy of phase transitions of a substance as a function of temperature can be used for the determination of melting point and range; determination of the temperature of evaporation and sublimation, solidification; determination of the temperature of vitreous transition; evaluation of polymorphism, construction of phase diagram, determination of purity (except those substances, the amorphous polymorphs unstable in experimental temperature range, the compounds that are merging with thermal decomposition and the substances that have lower purity to 95 %).

Determination of purity

The method is based on the fact that the presence of small quantities of impurities in a given material decreases its melting point and extends its range of global fusion. The Figure 3 illustrates this behavior for three hypothetical samples, one of which is the default, and the other two contain small quantities of impurities.

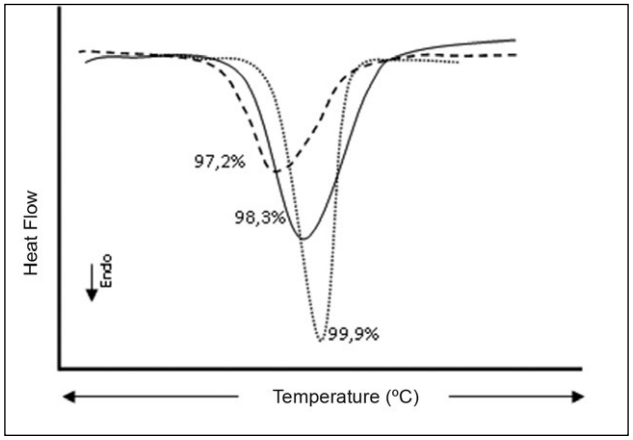

Figure 3 – Example of DSC curves of a hypothetical sample with different levels of purity

Based on the equation of van't Hoff (Equation 1), it is possible the determination of the molar fraction of impurities X (number of moles of impurities by the total number of mols of the sample) considering that there is no solid stage during the melting).

$$X_2 = \frac{(T_o - T_m)\,\Lambda H_f}{RT_o^2}$$ equation 1

Whereas T_m represents the melting temperature of the sample; To is the melting point of the pure substance in degrees Kelvin; R is the gas constant (8.3143 J. K^{-1}. mol^{-1}); ΔH is the heat of fusion of main component expressed in J. mol^{-1}.

When there is no formation of solid phase, the concentration of impurities in the liquid phase at a given temperature during melting is inversely proportional to the fraction melted at this temperature and the decrease of the melting point is directly proportional to the molar fraction of impurity. The graph of the sample temperature (T_s) versus the inverse of the fraction melted (1/F) in temperature Ts results in a straight line with slope equal to the reduction of the melting point ($T_o - T_m$). The theoretical thermal melting point of pure substance can be obtained by extrapolation when 1/F = 0.

$$T_s = T_o - \frac{RT_o^2 X_2\,(1/F)}{\Delta H_f}$$ equation2

Replacing the experimental values obtained for $T_o - T_m$, ΔH_f T_o in equation 1 it is possible to calculate the molar fraction of impurities in the sample.

5.2.28 QUANTITATIVE DETERMINATION OF OSMOLALITY

Osmolality is a practical way that of a total measurement of the contribution of various solutes present in solution by osmotic pressure of the solution. An acceptable approximation of the osmolality in aqueous solution is given by: $\varepsilon_m = \nu m\phi$, if the solute is not ionized, ν= 1; however ν is the total number of ions always present or formed by the lysis of the solution of a molecule of solute; m = molalidade of solution, which is the number of moles of solute per kilogram of solvent; ϕ = osmotic coefficient molar which is quantified the interaction between ions of opposite charge of the solution. It is dependent on the value of m. If the complexity of the solution increases, ϕ starts to be difficult to measure. The unit of osmolality is osmol per kilogram (osmol/kg), but the submultiple miliosmol per kilogram (mosmol/ kg) is normally used.

Another way described, the osmolality is determined by measuring the decrease in freezing point. There is a relationship between the osmolality and the decrease in freezing point ΔT:

$$\varepsilon_m = \Delta T / 1{,}86 \times 1000 \text{ mosmol/kg}$$

EQUIPMENT

The Equipment – Measured with a Freezing Point Osmometer – consists of: refrigerated container for the

measure; system of measurement temperature fitted with a termosensor, with a measuring device of different potentials that can be graduated to the decrease in temperature or directly in osmolality; and must be included a feature to mix the solution.

PROCEDURE

Prepare the solution reference as described in Table 1. Determine the zero of equipment using Water. Calibrate the equipment using the reference solution: pipette 50 to 250 µL of sample to be analyzed; transfer to the measuring cell and start the cooling system. Normally, a device to homogenize is programd to operate the temperature below the expected decrease crioscopica to prevent super cooling. A device indicates when the equilibrium is reached. Before each measurement rinsar measurement cell with the solution to be examined.

Perform the same operation with the test sample. Directly Read the osmolality or calculate by measuring the decrease in freezing point. The test is considered valid when the value found is between two values of the calibration scale.

Table 1 – Information to prepare the reference solution for the calibration of Measured with a Freezing Point Osmometer.

Mass in g of sodium chloride solution per kg of water	*Real Osmolality (mosmol/kg)*	*Optimal Osmolality (mosmol/kg)*	*Molal osmotic Coefficient*	*Cryoscopic Decrease (°C)*
3,087	100	105,67	0,9463	0,186
6,260	200	214,20	0,9337	0,372
9,463	300	323,83	0,9264	0,558
12,684	400	434,07	0,9215	0,744
15,916	500	544,66	0,9180	0,930
19,147	600	655,24	0,9157	1,116
22,380	700	765,86	0,9140	1,302

5.2.29 TESTING PHYSICAL AND PHYSICO CHEMICAL FOR FATS AND OILS

5.2.29.1 DETERMINATION OF THE RELATIVE DENSITY

Proceed as described in Determining the mass density and relative density (5.2.5).

5.2.29.2 DETERMINATION OF THE TEMPERATURE OF MELTING

Proceed as described in *Determining the temperature and range of fusion, Method III* (5.2.2).

5.2.29.3 DETERMINATION OF THE TEMPERATURE DETERMINING

SEPARATION OF FATTY ACIDS

Transfer 75 mL of the potassium hydroxide solution in glycerol (25 g of potassium hydroxide in 100 mL of glycerol) for becher of 1000 mL and heated to 150 °C. Add 50 mL of sample treated as indicated in the monograph specifies and continue the grille under stirring. The temperature should not exceed 150°C. The saponification is given by complete when the mixture present homogeneity, without traces of 0,220. Transfer the mixture to another becher 1000 mL, containing 500 mL of barely simmering water. Together, slowly, 50 mL of sulfuric acid solution 25% (v/v) and heat, stirring continuously, until separation defined phase clear (fatty acids). Wash the stage grease with boiling water in order to free it of sulfuric acid and keep it in becher small, in boiling water bath until decanting water, leaving clear the oily phase. Filter and collect the mixture of fatty acids while still warm in becher dry and desiccation-la at 150 °C for 20 minutes. Transfer the mixture to hot bottle appropriate and keep it in an ice bath until solidification.

To assess the degree of purity of fatty acids separated by the previous procedure, transfer, prior to freezing, 3 mL of the solution of fatty acids dried for test tube and add 15 mL of ethanol. Heat the solution to boiling and add 15 mL of ammonium hydroxide 6 M. The resulting preparation must be clear.

PROCEDURE

Proceed as described in Determining the temperature of freezing (5.2.4).

5.2.29.4 DETERMINATION OF THE INDEX OF REFRAÇÃO

The index of refraction n_{λ}^{t} in a way that the air is equal to the ratio between the sine of the angle of incidence of a ray

shining in the air and the sine of the angle of refraction of the refracted ray in considered. Unless otherwise indicated, the index of refraction is determined at 20 °C ± 0.5°C and in wavelength 589.3 nm, corresponding to the light of the thornback ray D of sodium. In this case, symbol that represents the index of refraction n_D^{20}.

On refractometers chains there is determination of the angle limit. In some devices, the essential part is a prism of index of refraction known, in contact with the liquid under test.

For calibration of the device, use the liquids of reference mentioned in Table 1. The value of the index of refraction of each reference liquid is indicated on its label.

Table 1 – Net of reference in determining the index of refraction.

Reference Liquid	*Δn/Δt (temperature coefficient)*
Trimethylpentane	0.00049
Toluene	0.00056
Methylnaphthalene	0.0048

If used white light for the determination of the index of refraction, the refractometer has a system of compensation. The tool should provide accurate readings up to the third decimal place, at the very least, and you have a device that permits operate the prescribed temperature: the thermometer enables the reading with the approximation of, at least, 0.5 °C.

5.2.29.5 DETERMINATION OF ROTATING POWER

Proceed as described in Determining the rotating power and of the specific optical rotation (5.2.8).

5.2.29.6 DETERMINATION OF WATER

Proceed as described in volumetric Method (5.2.20.1).

5.2.29.7 INDEX OF ACIDITY

The index of acidity, I_A, expressed in milligrams, the required quantity of potassium hydroxide for neutralization of free fatty acids in 1 g of sample.

High Indexes of acidity are suggestive of hydrolysis of esters marked constituents of matter grease. The causes of degradation include chemical treatments members of industrial processes of extraction and purification, bacterial activity, catalytic action (heat, light), storage and inadequate presence of impurities such as moisture, among others.

PROCEDURE

Weigh, placed in 250 mL erlenmeyer flask, approximately 10.0 g or exactly the prescribed quantity (in g) of the test substance. Add 50 mL of a mixture of 96% ethanol and ethyl ether (1:1) v/v. Except when otherwise indicated in specific monograph, the mixture of solvents must be previously eradicated by 0.1 M potassium hydroxide, or sodium hydroxide 0.1 M, in the presence of 0.5 mL of phenolphthalein solution. Heat the sample up to 90 °C if necessary for the dissolution of the same. After complete digestion; holder with potassium hydroxide 0.1 M up to observation of the color pale pink persistent for at least 15 seconds. Carry out the blank test and correct the volume of titrant consumed.

Calculate the I_A according to the equation:

$$I_a = \frac{5{,}610n}{m}$$

Whereas

N = volume (in mL) of potassium hydroxide 0.1 M spent in titration
M = mass of the sample in grams.

5.2.29.8 DETERMINATION OF THE INDEX OF SAPONIFICAÇÃO

The saponification index I_s expresses, in milligrams, the amount of potassium hydroxide required to neutralize the free fatty acid esters and saponificar existing in 1 g of substance.

The I_s provides evidence of tampering of the grease with substances unsaponifiables (mineral oil, for example).

Unless otherwise indicated in the monograph specifies, use the sample quantity indicated in Table 1.

Table 1 – Sample quantity to determine the saponification index.

Expected Value of Is	*Sample quantity (g)*
3 – 10	12 – 15
10 – 40	8 – 12
40 – 60	5 – 8
60 – 100	3 – 5
100 – 200	2.5 – 3
200 – 300	1 – 2
300 – 400	0.5 – 1

Weigh, placed in 250-mL flask, the sample quantity indicated (m), add 25.0 mL of methanolic solution of potassium hydroxide 0.5 M and some boiling stones. Adapt the reflux condenser vertical. Heat in a water bath for 30 min, except in specific indication. Add 1 mL of phenolphthalein solution and titrate immediately, the excess of potassium

hydroxide with 0,5 M hydrochloric acid solution (mL). Log blank test under the same conditions and correct the volume of titrant (n2 mL).

Calculate the saponification index (I_S), using the expression:

$$I_s = \frac{28{,}05\ (n_1 - n_2)}{m}$$

5.2.29.9 DETERMINATION OF THE INDEX OF ESTERS

The index of esters, I_E, expressed the amount of potassium hydroxide, in milligrams, needed for the saponification of esters present in 1 g of sample. The I_E is calculated from the saponification number S and the acidity index would, according to the equation:

$$I_E = I_S - I_A$$

5.2.29.10 DETERMINATION OF IODINE VALUE

The iodine index I_i, expressed in grams, the quantity of iodine susceptible to complexation in 100 g of substance under the conditions described below. Is quantitative measure of the degree of insaturacoes of fatty acids, esterified and free, in the sample. The *Ii value found in the determination is suggestive of the degree of purity of the tested material as well as the presence of contaminants. The replacement of The Method* by *Method B* should be object of a validation.

METHOD A

Unless otherwise indicated in the monograph specifies, use the sample quantity indicated in Table 1.

Table 1 – Sample quantity for determination of iodine value.

Ii expected index	*Sample quantity*
Less Than 20	1.0
20 – 60	0.5 – 0.25
60 – 100	0.25 – 0.15
More Than 100	0.15 – 0.10

In container of 250 mL stoppered lapped, dry, or washed with glacial acetic acid, insert the sample (m g) and dissolve it in 15 mL of chloroform, except in particulars specified in the monograph. Add 25.0 mL of bromide solution of iodine. Cap the container and keep it under the protection of the light for 30 minutes, stirring frequently. After addition of 10 mL of potassium iodide solution to 100 g/L and 100 mL of water and titrate with sodium thiosulphate 0,1 M stirring vigorously, until the yellow color has almost disappeared. Add 5 mL of starch solution and continue the titration by adding the sodium thiosulphate 0,1 M, drop by drop, stirring, until the disappearance of staining (n_2 mL). The blank test should be carried out under the same conditions and without the sample (n_1 mL).

Calculate the index of iodine by expression:

$$I_i = \frac{1{,}269\ (n_2 - n_1)}{m}$$

METHOD B

Unless otherwise indicated, use the sample quantity indicated in Table 2.

Table 2 – Sample quantity for determination of iodine value.

Probable iodine index I	*Mass (g) corresponding to an excess of 150 per cent of ICl*	*Mass (g) corresponding to an excess of 100 per cent of ICl*	*Iodine Chloride Solution (mL)*
<3	10	10	25
3	8.4613	10.5760	25
5	5.0770	6.3460	25
10	2.5384	3.1730	20
20	0.8461	1.5865	20
40	0.6346	0.7935	20
60	0.4321	0.5288	20
80	0.3173	0.3966	20
100	0.2538	0.3173	20
120	0.2115	0.2644	20
140	0.1813	0.2266	20
160	0.1587	0.1983	20
180	0.1410	0.1762	20
200	0.1269	0.1586	20

In container of 250 mL glass-stoppered, previously washed with glacial acetic acid or dry, enter the amount of the sample (m g) and dissolve it in 15 mL of a mixture of equal volumes of cyclohexane and glacial acetic acid, except in contrary indications. If necessary, merge previously the substance (melting point of more than 50 °C). Add, slowly, the volume of solution of chloride of iodine indicated in Table 2. Cap the container and shake, under the light, during 30 min, unless otherwise indicated. Add 10 mL of potassium iodide solution to 100 g/L and 100 mL of water. Titrate with sodium thiosulphate 0,1 M, stirring vigorously, until the yellow color almost disappears. Add 5 mL of starch solution and continue the titration by adding, drop by drop, the sodium thiosulphate 0.1 M until disappearance of staining (n; mL of sodium thiosulphate 0 71 M). Carry out a blank test under the same conditions (n_2 mL of sodium thiosulphate 0,1 M).

Calculate the iodine index using the following expression:

$$I_i = \frac{1{,}269\ (n_2 - n_1)}{m}$$

5

5.2.29.11 DETERMINATION OF THE INDEX OF PEROX

The peroxide index Ip is the number that expresses, in milliequivalents of active oxygen, the amount of hydrogen peroxide in 1000 g of substance.

If the monograph did not indicate the method to be used, perform the Method. The replacement of The Method by Method B is always object of validation.

METHOD A

Weigh 5.00 g of sample, placed in 250 mL erlenmeyer flask with stopper lapped. Add 30 mL of a mixture v/v of glacial acetic acid and chloroform (ratio 3:2). Shake until dissolution of the sample and add 0.5 mL of saturated solution of potassium iodide. Shake1

Min, exactly, and add 30 mL of water. Titrate with sodium thiosulphate 0.01 M, adding, slowly, without ceasing to vigorous stirring until the yellow color has almost disappeared. Add 5 mL of starch solution. Continue the titration stirring vigorously, until disappearance of staining (n1 mL of sodium thiosulphate 0.01 M). Carry out a blank test under the same conditions (n_2 mL of sodium thiosulphate 0.01 M). The blank test does not consume more than 0.1 mL of sodium thiosulphate 0 71 M.

Calculate the index of peroxides by expression:

$$I_i = \frac{1{,}269\ (n_2 - n_1)}{m}$$

METHOD B

Note: operating under the light.

In an erlenmeyer flask, with stopper lapped, introduce 50 Ml of a mixture v/v of glacial acetic acid with trimethylpentane (3:2). Cork and shake until dissolution of the sample (Table 1). Add 0.5 mL of saturated solution of potassium iodide, cork again and let the solution at rest during 60 ± 1S. In this time of rest, shake, at least three times, and then add 30 mL of water. Titrate with sodium thiosulphate solution 0.01 M (v_1 mL), slowly added with vigorous stirring and constant, until almost total disappearance of the yellow color given by the presence of iodine. Add about 0.5 mL of starch solution and continue the titration, without ceasing to stirring, in particular when you are near the equivalence point, to ensure release of iodine from the solvent. Add, drop by drop, the sodium thiosulphate solution until the blue color begins to disappear. If the titration is spent less than 0.5 mL of sodium thiosulphate 0,1 M, repeat the procedure using sodium thiosulphate 0.01 M (v_1 mL) under constant stirring and energetic.

In the case of peroxide value is greater than or equal to 70, and experiencing delay in change of color of the starch indicator 15 to 30 s, shake vigorously until the disappearance of the yellow color. This is due to the tendency of rimethylpentane sobrenadar in aqueous phase and the time required to obtain an appropriate mix between the solvent and the aqueous titrant.

For indexes of peroxide below 150, uses-if sodium thiosulphate 1,01 M can add to the mix a small amount (0.5 to 1.0% (m/m)) ofemulsifier suitable, for delaying the phase separation and decrease the time of release of iodine (for example, polysorbate 60). Make a blank test (v_0 mL). If consumed, more than 0.1 mL of sodium thiosulphate 0.01 M, replace the reagents and repeat the titration. The peroxide index is calculated by the following formula.

$$I_p = \frac{1000\ (v_1 - v_0)c}{m}$$

Whereas:

C = concentration of sodium thiosulphate solution in moles per liter.

Table 1 – Sample quantity for determination of peroxide index

Expected Value of Ip	*Sample quantity (g)*
0 – 12	2.00 – 5.00
12 – 20	1.20 – 2.00
20 – 30	0.80 – 1.20
30 – 50	0.500 – 0.800
50 – 90	0.300 – 0.500

5.2.29.12 DETERMINATION OF HYDROXYL INDEX

The Index of hydroxyl I_{OH} is the number that expresses, in milligrams, the amount of potassium hydroxide required to neutralize acid that combines, by Acylation, with 1 g of substance.

METHOD A

Enter the sample, exactly weighed (g), in accordance with the quantity indicated in Table 1, in a balloon acetylation of 150 mL, except if the monograph specifies is recommended another value. Add the volume of a solution of acetic anhydride indicated and adapt the reflux condenser.

Table 1 – Sample quantity and reagent volume acetilante.

IOH expected	*Sample quantity (g)*	*Volume of the reagent (acetylation) in milliliters*
10 – 100	2.0	5.0
100 – 150	1.5	5.0
150 – 200	1.0	5.0
200 – 250	0.75	5.0
250 – 300	0.6 or 1.20	5.0 or 10
300 – 350	1.0	10.0
350 – 700	0.75	15.0
700 – 950	0.5	15.0

Heat in a water bath for 1 h, taking care to keep the water level of the bath approximately 2.5 cm above the level of the liquid in the flask. Remove the flask and let it cool down. Add 5 mL of water through the upper end of the condenser. If the addition of water originate a turbidity, add pyridine until the disappearance of turbidity and note the volume added. Stir, heat again the flask in a water bath for 10 minutes. Remove the flask and let it cool down. Wash the condenser and the walls of the flask with 5 mL of alcohol, previously inhibited in the presence of phenolphthalein solution. Titrate with alcoholic potassium hydroxide solution 0.5 M, in the presence of 0.2 mL of phenolphthalein solution (n1 mL). Carry out a blank test, under the same conditions (n2 mL).

Calculate the index of hydroxyl using the expression:

$$I_{OH} = \frac{28{,}05(n_2 - n_1)}{m} + I_A$$

I_A = Index of acidity

METHOD B

In erlenmeyer flask and dry stoppered lapped enter the test socket (m g). Add 2.0 mL of reagent of propionic anhydride, cork the flask and shake gently until dissolved. After 2 h of rest, except under contrary indications, remove the stopper from the erlenmeyer flask and transfer its contents to another 500 mL with wide mouth containing 25.0 mL of solution of aniline to 9 g/L in cyclohexane and 30 mL of glacial acetic acid. Shake and after 5 min of rest add 0.05 mL of crystal violet solution. Titrate with 0.1 M perchloric acid until the turning to emerald green (n1 mL). Carry out a blank test under the same conditions (n2 mL).

Calculate the index of hydroxyl using the expression:

$$I_{OH} = \frac{5{,}610\ (n_2 - n_1)}{m} + I_A$$

Whereas

I_A = Index of acidity

The possibility of presence of water, determine the moisture content (y per cent) in the sample according to the specific method. The hydroxyl number is obtained by the equation:

$$I_{OH} = (\text{index found}) - 31.1y$$

5.2.29.13 DETERMINATION OF THE INDEX OF ACETYLATES

The index of acetylation is the quantity of alkali, in milligrams of potassium hydroxide required to neutralize the acetic acid released by the hydrolysis of 1 g of substance acetylated. It is used to establish the degree of presence of alcohol-free substances in greases. It is calculated based on the difference between the indices of saponification of substance acetylated by the technique described below and the substance not acetylated.

PROCEDURE

Transfer 10 g of substance and 20 mL of acetic anhydride for Kjeldahl flask of 200 mL capacity. Adapt Reflux condenser. Support the bottle about asbestos screen in whose center has been cut hole of about 4 cm in diameter and heat on flame of gas jet with a maximum height of 25 mm (avoiding the flame reaches the base of the balloon). Keep boiling regular during 2 hours, cool and transfer the contents of the flask to becher of 1000 mL containing 600 mL of water. Add 0.2 g of powdered pumice stone and boil for 30 minutes. Cool and transfer the mixture to separating funnel, rejecting the lower aqueous layer. Wash the substance acetylated with three or more portions of 50 mL of hot saturated solution of sodium chloride, until the washing solution does not provide more reaction acid to litmus paper. Also, Add 20 mL of hot water to funnel and shake, removing, then, as completely as possible, the aqueous phase. Transfer the substance for porcelain dish, add 1 G of sodium sulphate sprayed and filter through filter paper creped. Determine the index of saponification of the original substance, not acetylated, and the substance acetylated by procedure described and calculate the rate of acetylation by the formula:

$$I_{AC} = \frac{(b - a).1335}{1335 - a}$$

Whereas

A = saponification equivalent of the original substance,

b = index of saponification of substance acetylated.

5.2.29.14 DETERMINATION OF UNSAPONIFIABLE SUBSTANCES

Unsaponifiable Substances are those remaining the reaction of saponification, non-volatile 330 – 105 °C and which were carried in the extraction process of the substance to be tested.

If the monograph specifies do not indicate the procedure, use the Method I. Use of glassware with mouth lapped and degreased.

METHOD I

Add 2.0 – 2.5 g of sample in 250 mL flask. Add 25 mL of ethanolic potassium hydroxide 0.5 M. Attach a reflux condenser to the flask and boil in water bath for 1 hour, shaking. Transfer the contents of the flask to separating funnel, using 50 mL of water and, while the coolant inda is warm, remove, by shaking vigorously, with three quantities of 165 mL of ether peroxide-free. Rinse the flask with the first aliquot of ether. Mix the solutions in ethereal separating funnel containing 20 mL of water. (If the ethereal solutions contain solids in suspension, filter to the separating funnel through a paper filter free of fat. Wash the filter with free ether peroxide). Stir gently, and discard the aqueous phase. Wash the organic fraction, with two portions of 20 mL of water. Then, add three quantities of 20 mL of potassium hydroxide 0.5 M and shake vigorously, in each one of the additions. After each treatment should be performed washing with 20 mL of water. Finally, rinse with successive quantities of 20 mL of water until the aqueous phase does not show alkaline reaction in the presence of phenolphthalein. Transfer the organic fraction to a tared flask, washing the separating funnel with ether peroxide-free. Eliminate the ether and add 3 mL of acetone to the flask. Eliminate the solvent per complete until constant temperature not exceeding 80 °C. Dissolve the contents of the flask in 10 mL of ethanol recently boiled (96 %) and previously. Titrate with sodium hydroxide solution and 0.1 M ethanolic phenolphthalein as the indicator. If the volume of titrating solution spent does not exceed 0.1 mL, the amount of heavy residues, should be taken as unsaponifiable matter. Calculate the unsaponifiable matter as a percentage of the substance to be examined. If the volume of titrant spent exceed 0.1 mL, the amount of heavy residues cannot be taken as the unsaponifiable matter and the test must be repeated.

METHOD II

In a 250-mL flask, docked in system of condensation by reflux, introduce the prescribed quantity (m g) of the sample. Add 50 mL of alcoholic solution of potassium hydroxide 2 M and heat in a water bath, during 1 h under stirring. After cool down the temperature of less than 25 °C, transfer the contents of the flask to separating funnel. Add 100 mL of water. Add 100 mL of ether free of peroxides and stir carefully. Repeat the operation twice more with 100 mL of ethyl ether. Gather the ethereal fractions in another separating funnel containing 40 mL of water. Stir gently for a few minutes and allow to separate the phases. Reject the aqueous phase. Wash the ether phase twice with 40 mL of water each time. Then wash, successively with 40 mL of potassium hydroxide at 30 g/L and with 40 mL of water. Repeat three times this operation. Wash, repeatedly, the ether phase with 40 mL of water each time, until the aqueous phase does not have alkaline reaction to the phenolphthalein. Transfer the ether phase to a tared flask, rinsing the separating funnel with peroxide-free ether. Evaporate the ether to dryness, with the usual precautions. Add 6 mL of acetone to the residue. Eliminate, carefully, the solvent in air current. Dry at 100 – 105 °C, until constant mass, let cool in desiccator and weigh (g). The result is calculated in percentage m/m.

$$\% \text{ of unsaponifiables} = 100a/m$$

Dissolve the residue in 20 mL of alcohol,neutralized previously in the presence of phenolphthalein solution and titrate with alcoholic solution of sodium hydroxide 0.1 M. If the volume of alcoholic solution of sodium hydroxide 0.1 M spent in titration is greater than 0.2 mL, indicates that there was incomplete separation of the two phases and residue obtained cannot be considered unsaponifiables. The test must be repeated.

5.2.29.15 IDENTIFICATION OF FIXED OILS

Identification of vegetable oils by thin-layer chromatography

Stationary Phase: silica gel octadecilsilanizada (RP-18).

Sample Solution. unless specific monograph, dissolve approximately 20 mg (1 drop) of the sample in 3 mL of dichloromethane.

Standard Solution. Dissolve about 20 mg (1 drop) of corn oil in 3 mL of dichloromethane.

Procedure. Apply separately, 1 µmL of each solution on the board. Develop twice the distance of 0.5 cm with ether. Then, develop twice at distances of 8 cm with a mixture of dichloromethane, glacial acetic acid with acetone (2:4:5). Allow the plate to dry the air and nebulized using with solution of phosphomolybdic acid 330 g/L in alcohol. Heat the plate at 120 °C for approximately 3 minutes. Look Into the light of day.

The chromatogram shows stains comparable to those reproduced in Figure 1.

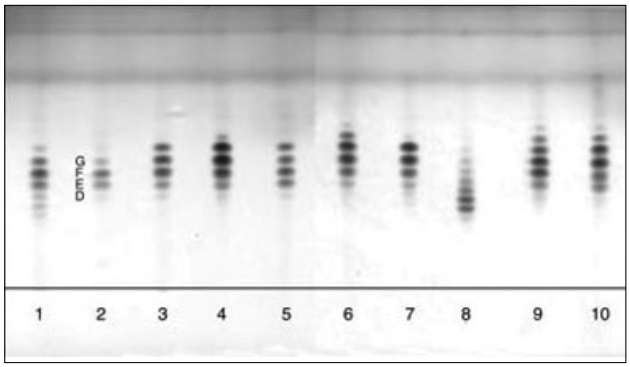

Figure 1 – thin layer chromatography for the identification of fixed oils.

1 Peanut Oil	Soybean Oil
2 Olive Oil	7 Sunflower seed Oil
3 Sesame Oil	8 Canola Oil
4 Corn Oil	9 Canola Oil (free of erucic acid)
5 Oil of almonds	10 Wheat germ Oil

5.2.29.15.2 Alkaline Impurities

Introduce 10 mL of freshly distilled acetone, 0.3 mL of water and 0.05 mL of alcoholic solution of bromophenol blue to 0.4 g/L in test tube. Neutralise, if necessary, with 0.01 M hydrochloric acid or sodium hydroxide 0.01 M. Add 10 mL of the sample, stir and leave to rest. The turning point is indicated by the development of yellow color in the top layer. It is not necessary volume exceeding 1.1 mL of hydrochloric acid 0.01 M.

5.2.29.15.1 Identification of vegetable oils by thin-layer chromatography

Stationary Phase: silica gel octadecilsilanizada (RP-18).

Sample Solution. unless specific monograph, dissolve approximately 20 mg (1 drop) of the sample in 3 mL of dichloromethane.

Standard Solution. Dissolve about 20 mg (1 drop) of corn oil in 3 mL of dichloromethane.

Procedure. Apply separately, 1 µmL of each solution on the board. Develop twice the distance of 0.5 cm with ether. Then, develop twice at distances of 8 cm with a mixture of dichloromethane, glacial acetic acid with acetone (2:4:5). Allow the plate to dry the air and nebulized using with solution of phosphomolybdic acid 330 g/L in alcohol. Heat the plate at 120 °C for approximately 3 minutes. Look Into the light of day.

The chromatogram shows stains comparable to those reproduced in Figure 1.

5.2.29.15.3 Foreign Oils in vegetable oils by thin-layer chromatography

Proceed by thin layer chromatography (5.2.17.1), using kieselguhr plate (G). Impregnate the plate, placing it in a closed chamber containing the required amount of mixture of ethyl ether and liquid paraffin (90:10; v/v) so that the surface of the liquid reaches approximately 5 mm layer of adsorbent. When the mixture of impregnation has traveled, at least, 12 cm layer, remove the plate from the chamber and allow the solvent to evaporate during 5 min. Develop in the same direction of impregnation.

Preparation of the mixture of fatty acids. Heat under reflux for 45 min, 2 g of the sample with 30 mL of alcoholic solution of potassium hydroxide 0.5 M. Join

50 Ml of water and leave to cool. Transfer to separating funnel. Shake three times with 50 mL of diethyl ether each time. Reject the solutions ethereal. Acidify the aqueous phase with hydrochloric acid and shake three times with 50 mL of diethyl ether each time. Gather the ethereal solutions and wash- the three times with 10 mL of water each time. Reject the washings. Add anhydrous sodium sulphate the fraction Ethereal and filter. Evaporate the ether at a temperature lower than 50°C. Use the residue to prepare the solution problem.

The fatty acids, can also be obtained from the saponified solution resulting from the reaction of determination of unsaponifiables.

Sample Solution. Dissolve 40 mg of the mixture of fatty acids obtained from the sample in 4 mL of chloroform.

Standard Solution. Dissolve in 4 mL of chloroform, 40 mg of the mixture of fatty acids obtained from a mixture of 19 volumes of corn oil and 1 volume of canola oil.

Procedure. Fit, separately, on the board, 3 µmL of each solution. Develop the chromatogram with a mixture of glacial acetic acid: water (90:10 v/v) per path of 8 cm. Dry the plate at 110 °C for 10 minutes. Leave to cool. Insert your card, unless otherwise indicated, in Cuba of chromatography saturated iodine vapor. To do this, put iodine in crystallizer, low form, at the bottom of the bowl. After a certain time, spots appear brown or brownish yellow. Remove the plate from Cuba and wait a few minutes. When the background coloring, brown layer disappears, spray with starch solution; appear, then blue stains that, when dry, may pass the chestnuts and returning from new blue after spraying with water. The chromatogram obtained with the sample Solution always shows spots corresponding stains from the chromatogram obtained with the standard Solution: one with Rf close to 0.5 (oleic acid) and another with Rf close to 0.65 (linoleic acid). In certain oils may appear a stain with Rf close to 0.75 (linolenic acid). By comparison with the chromatogram obtained with the standard Solution,

check the absence of stain with Rf 0.25 (erucic acid) on the chromatogram obtained with solution problem.

5.2.29.15.4 Foreign Oils in fixed oils by gas chromatography

When there is no indication in the monograph specifies, use the Method. The search for foreign oils is performed on the methyl esters of fatty acids in the oil analysis, using gas chromatography (5.2.17.5).

METHOD A

This method does not apply to oils containing glycerides of fatty acids with epoxy groups, epoxy, hydro ciclopropilo or ciclopropenilo, nor to that contains a large amount of fatty acids with number of carbon atoms in the chain less than 8, nor those whose acid index is greater than 2.0.

Sample Solution. If the monograph indicate, dry the sample before starting the test. Weigh 1.0 g of the sample, in a balloon mouth lapped 25 mL. Attaching reflux condenser and a device which makes it possible to pass a current of nitrogen inside the balloon. Add 10

Ml of anhydrous methanol and 0.2 mL of the potassium hydroxide solution to 60.0 g/L in methanol. Adapt the condenser and pass a current of nitrogen at a flow rate of approximately 50 mL/min until elimination of air. Stir and heat to boiling. When the preparation becomes clear (usually about 10 min after), heat for 5 more minutes. Cool under running water and transfirir to a separating funnel. Rinse the flask with 5 mL of heptane, add to the contents of the separating funnel and shake. add 10 mL of sodium chloride solution at 200 g/L and shake vigorously. Allow to separate the phases and transfirir the organic phase to a flask containing anhydrous sodium sulphate. Leave at rest and filter.

Standard Solution (a). Prepare 0.50 g mixture of reference substances, as prescribed in the monograph specifies. If the monograph does not indicate the standard solution, use one of which are described in Table 1. Dissolve in heptane and dilute to 50.0 mL with the same solvent. Note: For column chromatography capillary and reason to split it is recommended that the component with long chain mixture in analysis will be added to the mixture of calibration, when the quantitative analysis is performed by calibration curve.

Standard Solution (b). Dilute 1.0 mL of the standard solution (a) and complete to 10.0 mL with heptane.

Standard Solution (c). Prepare 0.50 g of a mixture of methyl esters of fatty acids as indicated in the monograph of the substance under examination. Dissolve in heptane and dilute to 50 mL volumetric flask with the same solvent. Commercial Mixtures of methyl esters of fatty acids, also, can be used.

Conditions Column:

- Material: fused silica, glass or quartz;
- Size: 10 to 30 m in length and 0.2 to 0.8 mm internal diameter;
- Stationary phase: poly (cianopropil) metilfenilmetilsiloxano or macrogol 2.000 (film thickness of 0.1 to 0.5 μmm) or another appropriate stationary phase;

Gas drag: helium or hydrogen for chromatography;

Gas Flow drag: 1.3 mL/min (for columns of 0.32 mm internal diameter);

Splitter Ratio: 1:100 or less, in accordance with the internal diameter of the column in use (1:50 am when diameter is 0.32 mm);

Flame ionization Detector:;

Temperature:

- Column: 160 – 200°C, in agreement with the stationary phase and length (200 °C for a column of 30 m in length, internally coated with macrogol 2.000). If necessary or indicated in the monograph of the Substance under analysis, raising the temperature of the column from 170 to 230°C with heating up ramp of 3 °C per minute (column with macrogol 2.000).
- Injector: 250°C;
- Detector: 250 °C;

Injection Volume: 1 μL;

Sensitivity: The height of the main peak in the chromatogram obtained with reference Solution (a) is 50 to 70% of the full scale of the recorder.

Adequacy of the system when used mixtures of substances reference (Table 2).

Note. For column chromatography capillary and reason of split is recommended that the component with long chain mixture in analysis will be added to the mixture of calibration, when the quantitative analysis is performed by calibration curve.

- Resolution: at the very least, 4 between the peaks of methyl octanoate and methyl Propyl caprate, calculated on the chromatogram obtained with the standard Solution (a);
- Signal/noise ratio: at the very least, 5 for the peak related to methyl caprate, observed in chromatogram obtained by analysis of the standard Solution (b);
- Number of theoretical plates: minimum of 15000, calculated for the peak corresponding to the methyl Isoamyl caproate;.
- Adequacy of the system when they are used to mixtures of substances reference listed in Tables 1 or 3:

Note. For column chromatography capillary and reason of split is recommended that the component with long chain mixture in analysis will be added to the mixture of calibration, when the quantitative analysis is performed by calibration curve.

- Resolution: minimum 1.8 between the peaks of methyl oleate and methyl stearate, calculated on the chromatogram obtained with the standard Solution (a);
- Signal/noise ratio: at the very least, 5 for the peak related to methyl myristate observed in chromatogram obtained by analysis of the standard Solution (b);
- Number of theoretical plates: minimum of 3.000, calculated for the peak corresponding to the methyl stearate.

Evaluation of chromatogram. Avoid conditions of analysis that would allow the emergence of 'masked' peaks (presence of constituents with retention times coming as, for example, the fatty acid linolenic acid and arachidic acid).

Qualitative Analysis

Identify peaks in the chromatogram obtained with the standard Solution (c) (in isothermal conditions of operation or with linear programming of temperature).

When used isothermal conditions of operation, the peaks can be identified by comparing

With the chromatogram obtained from standard Solution (a) and recorded information in Tables 1.2, or 3:

a) Measure the time of reduced retention (t'_R) of each peak obtained from the standard Solution (a). The t'_R the retention time measured in relation to the peak of solvent and not in relation to the time of injection. Draw a straight line through the equation:

Log (t'_R) = f (number of carbon atoms of the chain equivalent)

b) The logarithms of the retention times reduced of unsaturated acids are points of the straight line with values not whole of carbon atoms called 'equivalent length of string'. The equivalent length of string matches the theoretical number of atoms of carbon atoms of saturated fatty acids that would have the same t'_R. For example, the linoleic acid has t'_R as theoretically saturated fatty acid with 18.8 carbon atoms. Identify peaks in the chromatogram obtained with the test solution per calibration curve and by retention time reduced. Chain Lengths are recorded in Table 4. Quantitative Analysis

Generally, the quantification is performed using the method of normalisation, in which the sum of the areas under the peaks of the chromatogram, with the exception of the peak of the solvent, is regarded as being equal to 100 %. Using, preferably, an electronic integrator.

The percentage content of each component is calculated by determining the area under the peak corresponding to the sum of the areas under all the peaks. Do not consider the peaks whose area is less than 0.05 per cent of the total area.

In certain cases, when the chain of fatty acids is less than or equal to twelve carbon atoms, may be indicated correction factors in individual monographs to convert the area under the peaks in percent m/m.

Table 1 – Mixture of substances for calibration.

Mixture of substances	*Composition (% m/m)*
Methyl Laurate	5
Methyl Myristate	5
Methyl palmitate	10
Methyl stearate	20
Lauryl methyl	40
Methyl Oleate	20

Table 2 – Mixture of substances for calibration.

Mixture of substances	*Composition (% m/m)*
Methyl Caproate	10
Methyl Caprilate	10
Methyl Caprate	20
Methyl Laurate	20
Methyl Myristate	40

Table 3 – Mixture of substances for calibration.

Mixture of substances	*Composition (% m/m)*
Methyl Myristate	5
Methyl Palmitate	10
Methyl Stearate	15
Lauryl Methyl	20
Methyl Oleate	20
Methyl Eicosanoate	10
Methyl Behenate	10
Methyl Lignocerate	10

Table 4 – equivalent Length of string values (calculated from the calibration curve and analysis with column of macrogol 20000).

Fatty acid	*Equivalent Chain length*
Caproic acid	6.0
Caprylic Acid	8.0
Capric Acid	10.0
Lauric Acid	12.0
Myristic acid	14.0
Palmitic Acid	16.0
Palmitoleic Acid	16.3
Margaric Acid	17.0
Stearic acid	18.0
Oleic acid	18.3
Linoleic acid	18.8
Gamma-linolenic Acid	19.0
Alpha-linolenic Acid	19.2
Arachidic Acid	0.0
Eicosanoic Acid	20.2
Arachidonic Acid	21.2
Behenic Acid	22.0
Erucic acid	22.2
Acid 12-oxoestearic	22.7
Acid fatty acid ricinoleic.	23.9
Acid 12-hidroxiestearico	23.9
Methyl Lignocerate	24.0
Nervonic Acid	24.2

METHOD B

This method does not apply to oils which contain glycerides of fatty acid with epoxy groups, hidroepoxi, ciclopropilo or ciclopropenilo, nor to oils whose acid index is greater than 2.0.

Problem Solution. Introduce 0.100 g of the sample into centrifuge tube of 10 mL with stopper lapped. Dissolve in 1 mL of heptane and 1 mL of dimetilcarbonato. Shake vigorously, warming the heat brando (50 – 60 °C). Add 1 mL of a solution of sodium 7.5 g/L in anhydrous methanol solution is still hot. Shake vigorously, for approximately 5 minutes. Add 3 mL of distilled water and shake vigorously for about 30 seconds. Centrifuge for 15 min at 4,920 g. Inject 1 µmL of the organic phase.

Standard Solutions and evaluation of chromatograms. In the absence of specific indication in individual monograph, proceed as described in Method A.

Chromatographic Conditions. The chromatography can be performed using:

- Fused silica column of 30 m in length and 0.25 mm internal diameter, coated with macrogol 2.000 (film thickness: 0.25 µmm);
- Gas drag: helium for chromatography, with flow 0.9 mL/min;
- Flame ionization detector;
- Reason for split 1:100

Use the temperature programming represented inTable 1.

Table 1 – Temperature programming for chromatography.

	Time (minutes)	Temperature (°C)
Column	0 – 15	100
	15 – 36	100 → 225
	36 – 61	225
Injector		250
Detector		250

METHOD C

This method does not apply to oils which contain glycerides of fatty acid with epoxy groups, hidroperoxi, aldehyde, ketone, ciclopropilo and ciclopropenilo, as well as the oils with groups conjugated polyunsaturated or with Alkyne groups because of partial or total destruction of these groups.

Solution problem. In conical flask of 25 mL, dissolve 0.10 g of the sample in 2 mL of sodium hydroxide solution (20 g/L in methanol. Adapt the flask to reflux condenser vertical and heat for 30 minutes. Through the condenser, add 2.0 mL of methanolic solution of boron trifluoride and heat for 30 minutes. Through the condenser, add 4 mL of heptane and heat for 5 minutes. Cool the mixture and add 10.0 mL of saturated solution of sodium chloride. Shake for 15 seconds and add a quantity of saturated solution of sodium chloride sufficient to make the upper phase reach the neck of the bottle container. Remove aliquot of 2 mL of the upper phase. Wash three times with 2 mL of water each time and drying with anhydrous sodium sulfate.

Standard Solutions, chromatographic conditions and evaluation of chromatograms. In the absence of any indication in the monograph specifies, proceed as described in The Method.

5.2.29.16 DETERMINATION OF ESTERÓIS IN FIXED OILS

DETACHMENT OF THE FRACTION OF ESTERÓIS

Prepare the unsaponifiable fraction. Separate the sterol fraction of the fixed oil by thin-layer chromatography, using a plate of silica gel G (layer thickness between 0.3 mm and 0.5 mm).

Sample Solution (a). In 150 mL flask, introduce volume of betulin solution at 2 g/L in dichloromethane, which corresponds to approximately 10% of the sterol content of the sample used for the assay (for example, volume of 500 |iL of betulin solution in the case of virgin olive oil, and 1500 µmL in the case of other vegetable oils). If in the monograph is recorded the requirement of calculating the percentage content of each sterol fraction in esterolica, adding the betulin can be omitted. Evaporate to dryness under a stream of nitrogen. Add 5.00 g of the sample

and add 50 mL of potassium hydroxide alcoholic 2 M. Attaching reflux condenser vertical. Heat in a water bath for 1 h, under stirring. Cool until temperature of less than 25 °C and transfer the contents of the flask to a separating funnel, with the aid of 100 mL of water. Shake, with precaution, three times with 100 mL of diethyl ether peroxide-free. Gather the ethereal fractions in another separating funnel with the aid of 40 mL of distilled water. Stir gently for a few minutes. Allow to separate the phases by decantation and reject the aqueous phase. Wash the organic phase several times with 40 mL of water (each time) until the aqueous phase does not present alkaline reaction to phenolphthalein. Transfer the organic fraction to a tared flask, and wash the separating funnel with ethyl ether. Evaporate the ether. Add to residue 6 mL of acetone. Eliminate, carefully, the solvent with current of nitrogen. Dry in an oven at 100 – 105 °C to constant mass. Dissolve the residue with minimum volume of dichloromethane.

Sample Solution (b). Submit 5.00 g of canola oil to the same procedure described for the sample Solution (a) from "Add 50 mL of potassium hydroxide alcoholic 2 M... ".

Problem Solution (c). Submit 5.00 g of sunflower oil to the same procedure described for the Solution problem (a) from "Add 50 mL of potassium hydroxide alcoholic 2 M..".

Standard Solution Dissolve 25 mg of cholesterol and 10 mg of betulin in 1 mL of dichloromethane. Use a different card for each solution problem.

Apply separately, 20 µmL of the standard Solution in the form of band of 20 mm by 3 mm and 0.4 mL of solution problem (a), (b) or (c) in the form of band of 40 mm by 3 mm. Migrate by distance of 18 cm with the mobile phase ether:n-hexane (35:65; v/v). Dry the plates under a stream of nitrogen. Reveal with dichlorofluoroscein solution of 2 g/L in ethanol. Look at 254 nm.

The chromatogram obtained with the standard Solution presents bands corresponding, respectively, to cholesterol and betulin. The chromatograms obtained with the sample Solutions feature Rf bands coming of corresponding to the sterols. For each of the chromatograms scraping the plate region of the corresponding bands of sterols as well as a zone located 2 – 3 mm upwards and downwards of visible areas corresponding to the standard solution. Put these regions in three different erlenmeyer flask of 165 mL. Add to each one, 15 mL of dichloromethane hot and shake. Filter, separately, each solution through a sintered glass filter (40), or by appropriate paper filter. Wash, each filter, three times with 15 mL of dichloromethane. Transfer the filtrate and washing liquids in erlenmeyer flask, weigh. Evaporate to dryness in a current of nitrogen and weigh.

DETERMINATION OF ESTERÓIS

Proceed by gas chromatography (5.2.17.5). The determination should be carried out under the moisture and prepare the solutions at the time of use.

Sample Solution. The sterols separated from the sample by thin-layer chromatography, add 0.02 mL of the mixture, recently prepared, clorotrimetilsilano:/hexamethyl disilazane/trimethyl chlorosilane: anhydrous pyridine (1:3:9; v v) per milligram of residue. Stir gently, until complete dissolution of sterols. Leave to rest in a desiccator with diphosphorus pentaoxide during 30 min. Centrifuge, if necessary, and use the supernatant.

Standard Solution (a). The nine parts of sterols separated from canola oil by thin layer chromatography, join a part of cholesterol. Add 0.02 mL of the mixture, recently prepared, clorotrimetilsila no:/hexamethyl disilazane/trimethyl chlorosilane:anhydrous pyridine (1:3:9; v v) per milligram of residue. Stir gently, until complete dissolution of sterols. Leave to rest in a desiccator with diphosphorus pentaoxide during 30 min. Centrifuge, if necessary, and use the supernatant.

Standard Solution (b). To separate sterols of sunflower oil by thin-layer chromatography, add 0.02 mL of the mixture, recently prepared, clorotrimetilsilano:/hexamethyl disilazane/trimethyl chlorosilane: anhydrous pyridine (1:3:9; v v) per milligram of residue. Stir gently, until complete dissolution of sterols. Leave to rest in a desiccator with diphosphorus pentaoxide during 30 min. Centrifuge, if necessary, and use the supernatant.

Chromatographic Conditions

- Fused silica column 20 to 30 m in length and 0, 2 5 - 0.32 MM in internal diameter, covered with a film of poly[methyl (95) phenyl (5)) siloxane or poly[methyl (94) phenyl (5) vinyl (l)) siloxane phase (the film thickness 0.25 µmm);
- Gas drag: hydrogen gas with a flow of 30 to 50 cm/s or helium with a flow of 20 to 35 cm/s;
- Reason of split (1/50 or 1/100);
- Temperatures: column: 260 °C; injector: 280 °C; detector:290 °C;
- Injection volume: 1 µmL.

Results. The chromatogram obtained with the standard Solution (a) shows four major peaks corresponding, respectively, to cholesterol, campesterol and brassicasterol, p-sitosterol. The chromatogram obtained with the standard Solution (b) presents four major peaks corresponding, respectively, to campesterol, stigmasterol and sitosterol, p-A7-estigmastenol. The relative retention times of different sterols in relation to p-sitosterol are indicated in Table 1.

The peak corresponding to the internal standard (betulin) is clearly separated, the peaks corresponding to the sterols to be quantified.

Table 1 – relative retention Times, of sterols in relation to p-sitosterol, obtained with two different columns.

Sterols	*Poly[methyl (95) phenyl (5) siloxane*	*Poly[methyl (94) Phenyl (5) Vinyl (1) Siloxane*
Cholesterol	0.63	0.67
Brassicasterol	0.71	0.73
24-Metilenocolesterol	0.80	0.82
Campesterol	0.81	0.83
Campestanol	0.82	0.85
Stigmasterol	0.87	0.88
Δ^7-Campesterol	0.92	0.93
$\Delta^{5,23}$-Estigmastadienol	0.95	0.95
Clerosterol	0.96	0.96
β-Sitosterol	1	1
Sitostanol	1.02	1.02
Δ^5-Avenasterol	1.03	1.03
$\Delta^{5,2}$-Stigmasterol	1.08	1.08
A7-Stigmasterol	1.12	1.12
A7-Avenasterol	1.16	1.16
Betulin	1.4	1.6

Examine the chromatogram obtained with the sample Solution. Identify the peaks and calculate the percentage of each sterol fraction of sterols in using the equation:

$$\frac{A}{S} \times 100$$

Whereas

A = area under the peak corresponding to the compound to quantify;
S = The sum of the areas under the peaks corresponding to compounds indicated in Table 1.
If there is no requirement in the monograph, calculate the concentration of each sterol in the sample, in milligrams per 100 grams, using the expression:

$$\frac{A \times m_s \times 100}{A \times m_s}$$

Whereas

A = area under the peak corresponding to the compound to be quantified;
As = area under the peak corresponding to betulin; m = mass of the outlet of the sample for testing, in grams;
m_s = mass, in milligrams, of betulin added

5.2.30 TOTAL ORGANIC CARBON

The determination of total organic carbon (COT) is a sensitive method and unspecific to quantify the carbon atoms connected by covalencia in organic molecules present in a sample. The analysis is used to identify the contamination of water by organic impurities and assist in the control of processes of purification and distribution. Low levels of COT suggest the absence of organic chemical compounds potentially dangerous in the water used in the preparation of drugs. The COT content may be related to the occurrence of endotoxins, the microbial growth and the development of biofilms on the walls of the piping of water distribution systems for pharmaceutical use. The content of COT is independent of the state of oxidation of the organic matter and do not suffer interference from other atoms linked to organizational structure, such as nitrogen and hydrogen. There are several appropriate methods for COT analysis and determinations can occur in line or in the laboratory (off-line).

The methods in general are based on complete oxidation of the organic molecules to carbon dioxide, which is quantified as carbon. Typically, the organic carbon is oxidized by combustion, applying heat, emission of ultraviolet rays or oxidizing agents, such as sodium persulphate. The quantification of carbon dioxide is made by detection of gas produced with IR or by reading the solution conductivity.

The method covered in this chapter is suggestive and the user may adopt any other that is appropriate and accessible to their specific purposes, provided that the limit of quantification is suited to the range of expected reading. Uses a standard solution of easily oxidizable substance, such as sucrose, for example, in a concentration such that the instrumental response obtained corresponds to the limit established for the COT. The method can also be performed with an apparatus installed in-line, that has been properly calibrated and that satisfies the test of conformity of the system.

In Table 1 are shown the average values expected for the main types of water purification.

EQUIPMENT

Consists of an injector, a equipment to decompose the sample, a system to separate the carbon dioxide formed, a detector and a recorder of the electrical signal emitted. The tube of decomposition should be able to generate, at the very least, 0.450 mg/L of organic carbon, for a sample of 1.071 mg/L of sucrose.

The detection limit of the equipment, as specified by the manufacturer, is less than or equal to 0.050 mg of carbon per liter (0.05 ppm). The conformity of the system is checked periodically by means of a solution prepared with a substance of difficult oxidation, such as for example, the 1,4-Benzoquinone. The location of the apparatus is chosen so as to ensure that the results are representative of the

Table 1 – Valores típicos de COT em água

Type of purification	Expected Range of COT (mg/L)
Drinking Water	0.5 To 7.0
Distillation	Approximately 0.10
Deionization	0.05 to 0.50
Reverse Osmosis	0.04 to 0.10
Reverse Osmosis + deionization	0.01 to 0.05
Combined Technologies	0.003 to 0.005
Combined Technologies + UV Oxidation	< 0.002

water used. The reading should be made immediately after the collection of a water sample.

COT WATER (ACOT)

Use water with high purity, which meets the following specifications:

-Conductivity: maximum 0.1 µmS. cm^{-1} at 25 °C;

-Total organic carbon – maximum 100 mg/L.

Depending on the type of equipment used the levels of heavy metals and copper can be critical. Observe the manufacturer's instructions.

Use the water COT as white; in the preparation of standard solutions; compliance solution of the system and in cleaning the equipment. The preparation of the standard solution and the solution of system compliance should be concomitant to the sample.

PREPARATION OF THE GLASS MATERIAL.

Wash carefully, the glass material by means of a process that will eliminate the organic matter. Leave the material immersed in a mixture of equal parts of hydrogen peroxide solution diluted to 30% and dilute nitric acid. Rinse with water COT.

If you use a microsyringe to inject the sample, this must be washed with a mixture of sodium hydroxide solution to 5% with absolute ethyl alcohol (1:1), or in hydrochloric acid to 25 %. Rinse abundantly with water COT.

PREPARATION The SOLUTIONS

White. Prepare the solution of white, or any other solutions needed to set a baseline, or proceed to calibration, according to the manufacturer's instructions. Use the white appropriate to adjust the zero of the apparatus.

Standard Solution. Dissolve the sucrose R, previously dried at a temperature of 105 °C for 3 h, in water COT, so as to obtain a solution containing 1.19 mg of sucrose per liter (0.50 mg of carbon per liter), to check the instrument.

Use solution in water, COT, of potassium hydrogen phthalate, previously dried at 105 °C for 4 h, the concentration determined by the equipment manufacturer, for calibration of the instrument. Preserve the solution, acidifies with H_3PO_4 or concentrated concentrated H2SO4 to pH < 2. To determine organic and inorganic carbon, separately, prepare, also, the standard solution of solution of sodium bicarbonate (dry in a desiccator for at least 18 hours) and sodium carbonate decaidratado (dry 550 – 600 °C for 30 minutes), in proportion to the carbon content of 1:1, in water COT.

The concentration of the standard solution is calculated for purified water, whose limit of COT is 500 ppb. For other types of water, doing the due adequacy.

Compliance Solution Dissolve the system.

1,4 - Benzoquinone in water COT, so as to obtain a solution to 0.75 mg of 1,4-benzoquinone per liter (0.50 mg of carbon per liter).

Sample. Collect the water sample in clean container; dry and with lid, leaving a minimum of air. Take Care not to have any type of contamination. Do not use plastic material. Carry out the analysis as soon as possible, so as to minimize the risk of damage or contamination of the sample.

SYSTEM COMPLIANCE

Carry out the readings (L) of water solutions COT (LC_{Cot}), standard solution (L_{pA}), a solution of conformity of the system (L_{CS}) and register. Calculate the efficiency of the system as a percentage, using the expression:

$$\frac{L_{CS} - L_{Cot}}{L_{Pa} - L_{Cot}} \times 100$$

The system will be as if the value obtained is between 85% and 115% of the theoretical value.

PROCEDURE

Employ the analytical method recommended by the manufacturer of the equipment used. Inject appropriate volume of sample and read the total carbon.

Determine the sample reading (L_{Am}). The sample satisfies the test if LAm is not greater than the $L_{pa} - L_{Cot}$.

$$L_{Am} < L_{Pa} - L_{Cot}.$$

Calculations For different fractions of organic and inorganic carbon, making the reading of total organic carbon, change the configuration of the equipment for the reading of inorganic carbon and calculate the organic carbon by subtraction. Alternatively, you can measure the organic carbon after removal of inorganic carbon and subtract from total carbon. Normally, for waters of high purity the fraction of inorganic carbon is contemptible.

5.3 CHEMICAL METHODS

5.3.1 REACTION OF IDENTIFICATION

5.3.1.1 IONS, GROUPS AND FUNCTIONS

The classic methods of identification of functions or certain chemical groups present in drugs consist in reactions that result in formation of precipitate, colored product, fizz, bleaching of the reagent used or another phenomenon any easily noticeable. These tests are not applicable to mixtures of drugs.

Acetate

1) Heat the sample with equal quantity of oxalic acid; dislodged acid vapors with a characteristic odor of acetic acid.

2) Heat the sample with sulfuric acid SR and ethanol; unlatches ethyl acetate, characteristic odour.

3) Deal neutral solution of the sample with ferric chloride SR; it produces a dark-red color that disappears by addition of mineral acids.

4) Dissolve the sample in water, add 5 drops of nitrate of lanthanum SR, two drops of iodine and 0.1 M

A drop of concentrated solution of ammonia. Heat gently until boiling. After a few minutes form- if blue precipitate or appears intense blue coloration.

Acetylates

Place the sample in a test tube and add three drops of phosphoric acid SR. Close the tube with lid crossed by another test tube less full of water and in whose exterior is deposited a drop of lanthanum nitrate SR. Heat the assembly in a water bath for five minutes (certain substances acetylated flakes if hydrolyzes with difficulty; in this case the mixture should be heated slowly, until boiling, about flame impingement). Transfer the drop lanthanum nitrate SR to a porcelain dish and mix with a drop of iodine SR. Place the edge of the mixing a drop of ammonium hydroxide 2 M. In the area of contact of the two liquids slowly appears blue color that persists for some time.

Alkaloid

Dissolve some milligrams of sample in 5 mL of water, add hydrochloric acid SR up to acidify the solution and then pour 1 mL of potassium iodobismutato- aqueous acetic acid; form-if immediately precipitated orange or red-orange.

Aluminum, ion

1) Add the sample to ammonium hydroxide 6 M; form-if gelatinous white precipitate, insoluble in excess of the same reagent.

2) Add the sample M sodium hydroxide or sodium sulfide SR; form-if gelatinous white precipitate, soluble in excess of the same reagent.

3) The solution of the sample add ammonium hydroxide 5 M until that form turbidity. Add, then three to four drops of freshly prepared solution of quinalizarina 0.05% in sodium hydroxide to 1% (p/v). Heat to boiling, cool and acidify with excess acetic acid 5M; it produces a reddish-violet color.

Primary aromatic Amine

Acidify the sample solution with 2 M hydrochloric acid and add four drops of sodium nitrite SR. After 1 to 2 minutes, add 1 mL of 2-naphthol SR; appears red or orange color intense, trainee-if generally precipitate.

Ammonia and volatile aliphatic amine

Dissolve the sample in a test tube, add magnesium oxide and heat if necessary; dislodged gradually alkali vapors, which darken the role of silver-manganese placed at the top of the tube.

Ammonium ion,

Add the sample excess of sodium hydroxide M cold; occurs detachment of ammonia, characteristic odor, and that changes to blue to red color of litmus paper. The decomposition is accelerated by heating.

Antimony (III), ion

1) Handle the sample solution, strongly acidified with dilute hydrochloric acid (at most 2 M), with hydrogen

sulfide SR; form-if orange precipitate sulfide of antimony, insoluble in ammonium hydroxide 6 M, but soluble in ammonium sulfide SR, sodium hydroxide 2 M and concentrated hydrochloric acid.

2) Dissolve the sample in potassium sodium tartrate SR; after cooling, add, drop by drop, sodium sulfide SR1; form-if red-orange precipitate soluble in sodium hydroxide 2 M.

Arsenic

1) An ammoniacal solution of the sample add sodium sulfide SR and acidify with dilute hydrochloric acid; form-if yellow precipitate, insoluble in hydrochloric acid, but soluble in alkaline solutions.

2) Heat 5 mL of the sample solution strongly HYDROCHLORIDE in water-bath with equal volume of sodium hipofosfito SR; form-if precipitate of brown to black. If we treat The (V), the reduction is slower; the addition of potassium iodide SR exercise catalytic effect.

Barbiturates without nitrogen contents

A methanolic solution of the sample add a few drops of solution with nitrate of cobalt (II) to 10% (p/v) and calcium chloride to 10% (p/v), mix and add, with stirring, some drops of sodium hydroxide 2 M; form- if precipitated blue-violet.

Barium ion,

1) Treat the sample solution with 1 M sulfuric acid; form-if white precipitate, insoluble in hydrochloric and nitric acids.

2) Place the sample in the area of reducing flame; this acquires color yellow-green, which shows blue when seen through green glass.

Benzoate

1) Deal neutral solution of the sample with ferric chloride SR; form-if precipitate dark yellow, soluble in ethyl ether.

2) Acidular moderately concentrated solution of the sample with 1 M sulfuric acid; form-if precipitate of benzoic acid, readily soluble in diethyl ether.

Bicarbonate

1) Treating the sample with mineral acid; 368.4 effervescence with detachment of colorless gas that, when reacting with calcium hydroxide SR, form immediately white precipitate.

2) A cold solution of the sample add phenolphthalein; the solution remains unchanged or is only slightly colored.

Bismuth, ion

Dissolve the sample in slight excess of nitric acid or hydrochloric acid and dilute with water; form-if white precipitate that, treated with hydrogen sulfide, becomes brown; the resulting compound is soluble in hot mixture of equal parts of nitric acid and water, but insoluble in ammonium sulfide SR.

Bisulfite

Treating the sample with 3 M hydrochloric acid; unlatches sulfur dioxide, recognized by its characteristic pungent odour and by darken filter paper moistened with silver nitrate of mercury (I) SR.

Borate

1) A sample solution acidulated with 1/1000 with hydrochloric acid, add a few drops of iodine solution to 0.1% (p/v) and the solution of polyvinyl alcohol to 2% (p/v); produces- if intense green color. The reaction is altered by agents of oxidation or reduction.

2) Treating the sample with sulfuric acid, add methanol and bring the mixture to ignition; it burning with flame of green edges.

Bromide

1) THE sample solution acidified with dilute sulfuric acid SR, add water to chlorine SR; unlatches bromine, which gives brown color the solution; stirring if this with chloroform, the solvent acquires color ranging from red to reddish brown- and the aqueous layer remains colorless.

2) Treat the sample solution with nitric acid SR and nitrate of silver SR; form-if precipitate cheesy white slightly yellowish, insoluble in nitric acid and slightly soluble in ammonium hydroxide 6 M

Calcium ion,

1) Soak the sample with dilute hydrochloric acid and takes it to the zone of the flame; creeper appears reddish orange color transient.

2) Dissolve the sample, add two drops of methyl red SI, neutralize with ammonium hydroxide 6 M, add hydrochloric acid 3 M, drop by drop, until acidular the solution and pour ammonium oxalate SR; form-if

White precipitate of calcium oxalate, insoluble in acetic acid 6 M, but soluble in hydrochloric acid SR.

Soda

1) Treating the sample with mineral acid; 368.4 effervescence, with detachment of colorless gas that, when reacting with calcium hydroxide SR, form immediately white precipitate.

2) A cold solution of soluble sample add phenolphthalein; appears red in color.

Lead, ion

1) Treat the sample solution with 1 M sulfuric acid; form-if white precipitate, insoluble in hydrochloric acid 3 M or 2 M nitric acid, but freely soluble in sodium hydroxide M heated, in ammonium acetate to 10% (p/v) and in excess of sulfuric acid M.

2) Deal with the sample solution, free of mineral acids, with potassium chromate SR; form-if yellow precipitate, insoluble in acetic acid 6 M, but freely soluble in sodium hydroxide M and in nitric acid, the hot.

Cyanide

Treat the sample solution with ferrous sulfate SR, sodium hydroxide SR and ferric chloride SR, heat to boiling and acidular with hydrochloric acid; 368.4 staining or blue precipitate. If the quantity of cyanide present is small, form-if colloidal solution of blue color – green tint.

Citrate

The 15 mL pyridine add some milligrams of sample dissolved or suspended in 1 mL of water, stir, add 5 mL of acetic anhydride to mixture, stir again; red color appears clear.

Chlorate

1) Treat the sample solution with silver nitrate SR in nitric acid medium SR; does not form precipitate. Pour sulfurous acid solution or recent of sodium nitrite SR to this mixture; form-if white precipitate, insoluble in nitric acid SR, but soluble in ammonium hydroxide 6 M.

2) Submit the sample to ignition; form-if chloride, identified by appropriate tests.

3) Treat the dry sample with sulfuric acid; occurs crackle detaching them if gas greenish yellow (for this test using small amount of chlorate, and should- if take extreme care when you run it, because the gas that form decomposes so explosive above 45 °C – use chapel).

Chloride

1) Treat the sample solution, acidified with nitric acid, with silver nitrate SR; form-if white precipitate cheesy, insoluble in nitric acid, but soluble in slight excess of ammonium hydroxide 6 M.

2) Mix the dry sample with equal weight of manganese dioxide, moisten with sulfuric acid SR and warm softly; unlatches chlorine, identified by smell and by production of blue color in role of starch iodated dampened.

Copper (II) ion

1) Treat the sample solution with potassium ferrocyanide SR; form-if precipitate brown-reddish, insoluble in dilute acids, but soluble in ammonium hydroxide.

2) Treat the sample solution with hydrochloric acid and turnings of metallic iron; deposits red film of metallic copper.

3) Treat the sample solution with an excess of ammonium hydroxide 6 M; form-if first precipitate bluish, then solution strongly bluish.

Ester

Add the sample solution of hydroxylamine hydrochloride in 7% (w/v) in methanol and potassium hydroxide solution at 10% (w/v) in ethanol, heat to boiling, cool, acidular with hydrochloric acid SR and add ferric chloride solution; it produces a color bluish-red or red.

Iron

Treating the sample with ammonium sulfide SR; form-if precipitate black, which dissolve in hydrochloric acid 3 M, with detachment of hydrosulphuric gas, characterized by lead acetate paper.

Ferric ion,

1) Treat acid solution of the sample with potassium ferrocyanide SR; form-if precipitate dark blue, which does not dissolve by adding hydrochloric acid SR, but is decomposed by sodium hydroxide 2 M.

2) Treating the sample with ammonium thiocyanate SR; produces- if intense red color that does not disappear with the addition of dilute mineral acids, but can be extracted with ethyl ether, passing the red coloring to the ether layer.

Ferrous Ion

1) Treat the sample solution with potassium ferricyanide SR; form-if precipitate dark blue, insoluble in hydrochloric acid 3 M, but decomposed by sodium hydroxide M.

2) Treat the sample solution with sodium hydroxide M; form-if precipitate greenish white, which quickly turns to green, and then, when shaken, the brown.

Phosphate (or orthophosphate)

1) Deal neutral solution of the sample with silver nitrate SR; form-if yellow precipitate, soluble in nitric acid

2 M or ammonium hydroxide 6 M.

2) Treat nitrate solution of sample with ammonium molybdate SR; form-if yellow precipitate, soluble in ammonium hydroxide 6 M; the reaction is accelerated by heat.

Hipofosfito

1) Heat the sample solution, acidulated with 1/1000 sulfuric acid SR, with cupric sulfate SR; form-if red precipitate.

2) Treat the sample solution with mercuric chloride SR; form-if white precipitate, which takes gray in the presence of excess hipofosfito.

Iodide

1) Treat the sample solution with water of chlorine SR, drop by drop; unlatches iodine, which changes the color of the solution from yellow to red; stirring this solution with chloroform, this acquires violet color.

2) Treat the sample solution acidified with nitric acid SR, with silver nitrate SR; form-if yellow precipitate cheesy, insoluble in nitric acid SR and ammonium hydroxide 6 M

Lactate

Deal with the sample solution, acidulated with 1/1000 sulfuric acid SR, with potassium permanganate SR and heat the mixture; unlatches acetaldehyde, identified by characteristic odour.

Lithium ion,

1) Treat the sample solution moderately concentrated and ALKALIZED with sodium hydroxide SR, with sodium carbonate SR; form-if, by heating, white precipitate, soluble in ammonium chloride SR.

2) Soak the sample with dilute hydrochloric acid and heat in reducing zone of the flame; this acquires intense red color.

Magnesium ion,

1) Treat the sample solution with sodium hydroxide SR; form-if white precipitate, which dissolves with the addition of ammonium chloride SR.

2) Deal with the sample solution, in the presence of ammonium chloride SR, with ammonium carbonate SR; do not be so hasty, but when adding sodium phosphate dibasic

Heptahydrate SR, form-if crystalline precipitate white, insoluble in ammonium hydroxide 6 M.

Mercury

1) Treat the sample solution with hydrogen sulfide SR; form-if precipitate black, insoluble in ammonium sulfide SR and in nitric acid 2 M HNO3.

2) Apply the sample solution, without excess of nitric acid, in copper foil bright; form-if tank which, to be polished, if takes shiny and silver.

Mercury (II), ion

1) Treat the sample solution with sodium hydroxide M; form-if yellow precipitate.

2) Deal neutral solution of the sample with potassium iodide SR; form-if precipitate scarlet, very soluble in excess of reagent.

Mercury (I),ion

1) Treating the sample with sodium hydroxide M; the salt decomposes, giving black color.

2) Treat the sample solution with hydrochloric acid SR; form-if white precipitate, which darkens to be treated with ammonium hydroxide 6 M.

3) Treat the sample solution with potassium iodide SR; form-if yellow precipitate that, with time, you can move on to the green.

Ammonium nitrate

1) Heat the sample with sulfuric acid and metallic copper; vapor dislodged red browns (held in chapel).

2) Treat the sample solution with an equal volume of sulfuric acid, cool the mixture and add 0.5 mL of a solution of ferrous sulfate in 0.5 M; interface 368.4 brown color to purple.

Sodiumnitrate

1) Treating the sample with dilute mineral acids or with acetic acid 5 M; vapor dislodged iiiis (held in chapel).

2) Deal role of starch iodated with sample solution;

The Indicator if cora of blue.

3) Add the sample to the acidified solution of potassium permanganate SR; disappears the color.

Oxalate

1) Deal neutral solution or alkaline sample with calcium chloride SR; form-if white precipitate, insoluble in acetic acid 6 M, but soluble in hydrochloric acid.

2) Deal with hot solution acidified sample with potassium permanganate SR; disappears the color.

Permanganate

1) Deal with the sample solution, acidulated with 1/1000 sulfuric acid SR, with hydrogen peroxide at 3% (p/v) SR; the color disappears when cold.

2) Treat the sample solution, acidulated with 1/1000 sulfuric acid SR, with oxalic acid SR in warmed solution; the color disappears.

Peroxide

Treat the sample solution, slightly acidulated with 1/1000 sulfuric acid SR, with potassium dichromate SR; appears intense blue color. Stir the mixture with an equal volume of diethyl ether and leaving the liquids become separated, the blue color is replaced for the ether layer.

Potassium ion,

1) Deal with alkaline solution of the sample with sodium tetraphenylborate to 1% (p/v); form-if white precipitate.

2) Treat the sample solution with acetic acid and 1 mL of SR cobaltinitrito sodium SR; form-if immediately precipitate yellow or orange yellow, in the absence of ammonium ions.

3) Place the sample solution, acidulated with 1/1000 with hydrochloric acid SR, in reducing zone of the flame; this acquires violet color; the presence of a small quantity of sodium mask to color.

4) Treat the sample solution with perchloric acid SR; form-if white crystalline precipitate.

Silver ion,

1) Treat the sample solution with hydrochloric acid; form-if precipitate cheesy white, insoluble in nitric acid SR, but readily soluble in ammonium hydroxide 6 M

2) Treat the sample solution with ammonium hydroxide 6 M and small quantity of formaldehyde solution; by heating, deposits if mirror of metallic silver on the surface of the container.

Salicylate

1) Treat the diluted solution of the sample with ferric chloride SR; it produces a violet color.

2) Treat moderately concentrated solution of the sample with mineral acid; it is white crystalline precipitate of salicylic acid, which merges between 156 and 160 °C.

Sodium ion,

1) Place the sample solution, acidulated with 1/1000 with hydrochloric acid SR, in reducing zone of the flame; this acquires intense yellow color.

2) Treat the sample solution with hydrochloric acid or nitric and, then, with uranyl acetate and zinc SR; form- if crystalline precipitate yellow-gold, after shaking for a few minutes.

Succinate

1) Deal neutral solution of the sample with ferric chloride SR; form-if precipitate light brown.

2) Deal neutral solution of the sample with silver nitrate SR; form-if white precipitate, readily soluble in ammonium hydroxide 6 M.

Sulphate

1) Treat the sample solution with barium chloride SR; form-if white precipitate, insoluble in hydrochloric acid nitric acid in SR and SR.

2) Treat the sample solution with lead acetate SR; form-if white precipitate, soluble in ammonium acetate SR, but insoluble in hydrochloric acid or nitric SR.

3) Treat the sample solution with hydrochloric acid SR; not that way no precipitate (distinction of thiosulphate).

Sulphite

1) Treating the sample with 3 M hydrochloric acid; unlatches- if sulfur dioxide, recognized by its characteristic pungent odour and by darken filter paper moistened with silver nitrate of mercury (I) SR.

2) Acidify the sample solution with hydrochloric acid SR, warm up with a few drops of potassium permanganate SR and add drops of barium chloride SR; form-if white precipitate.

Tartrate

1) Dissolve some milligrams of the sample in water, acidified with acetic acid SR, add one drop of ferrous sulphate solution at 1% (p/v) and a drop of hydrogen peroxide 3% (p/v); it produces a yellow color fleeting. Add sodium hydroxide 2 M drop by drop; 368.4 intense blue color.

2) Acidify the sample solution with 1 M sulfuric acid, add a few drops of resorcinol 2% (p/v) and add, carefully, sulfuric acid, so as to be formed two layers; warming up on a water-bath for a few minutes, the interface appears red ring.

Thiocyanate

Treat the sample solution with ferric chloride SR; produces-if red color, which does not disappear by adding mineral acids moderately concentrated and can be extracted with ethyl ether, passing the red coloring to the ethereal layer.

Thiosulfate

1) Treat the sample solution with hydrochloric acid; form-if white precipitate, which passes as soon as the yellow, and unclips- if sulfur dioxide, recognized by odor.

2) Treat acetous solution sample with ferric chloride SR; 368.4 dark violet color that disappears quickly.

Xanthine

Treating the sample with two drops of concentrated solution of concentrated hydrogen peroxide and five drops of 2 M hydrochloric acid, and heat to dryness on a water-bath; you get residue yellowish red that, treated with ammonium hydroxide 2 M, changes to red-violet.

Zinc ion,

1) Treat the sample solution with potassium ferrocyanide SR; form-if white precipitate, insoluble in hydrochloric acid 3 M

2) Deal neutral solution or sample alkaline ammonium sulfide with SR; form-if white precipitate.

3) Treat the sample solution with a solution of sodium hydroxide 2 M, drop by drop; form-if white precipitate, flocoso, soluble in excess of sodium hydroxide SR.

5.3.1.2 IDENTIFICATION OF STEROIDS BY THIN-LAYER CHROMATOGRAPHY

PROCEDURE

Prepare chromatoplate using kieselguhr G as support. Enter the chromatoplate in Cuba with the solvent of impregnation and leave develop up to that

The Solvent reaches the top of chromatoplate. Remove the chromatoplate of Cuba and allow the solvent to evaporate. Prepare the sample solution to 0.25% (p/v) and the standard solution of 0.25% (p/v) using, as a solvent, a mixture of 9 volumes of chloroform and 1 volume of methanol. Not TO be that the monograph establish differently, apply on the chromatoplate 2 µmL of the sample solution, 2 µmL of the standard solution and 2 µmL of mixture 1:1 of solutions of the sample and the standard. Develop the chromatogram with the eluent specified in the monograph, leaving it up in the same way that the solvent of impregnation. Remove the chromatoplate of Cuba, evaporate the solvent, heat the chromatoplate at 120 ºC for 15 minutes and nebulized using with solution of sulfuric acid at 10% (v/v) in ethanol at 96 %. Heat to 120 ºC for 10 more minutes, let cool and examine the normal light and ultraviolet light (366 nm). The main spot in the chromatogram obtained with the sample solution will correspond to the main spot obtained in the chromatogram of the standard solution. The main spot resulting from application of the mixture of the sample solutions and standard appears as single and compact.

Solvents of impregnation

I – A mixture of 1 volume of formamide and 9 volumes of acetone

II – A mixture of 1 volume of 1,2-propanediol and 9 volumes of acetone

III – A mixture of 1 volume of liquid paraffin and 9 volumes of petroleum ether boiling range 40 -60 ºC.

Eluents

A – Chloroform
B – a mixture of 3 volumes of toluene and 1 volume of chloroform
C – Toluene
D – Mixture of 4 volumes of cyclohexane and 1 volume of toluene
E – a mixture of equal volumes of cyclohexane and petroleum ether boiling range 40 – 60 ºC
F – Mixture of 2 volumes of glacial acetic acid and 3 volumes of water
G – a mixture of 8 volumes of hexane and 2 volumes of dioxane.

5.3.1.3 SEARCHES OF STEROIDS STRANGERS BY THIN-LAYER CHROMATOGRAPHY

PROCEDURE I

Prepare chromatoplates as described in thin layer chromatography (5.2.17.1), using silica gel G as support. Prepare 3 solutions using, as a solvent, a mixture of 9 volumes of chloroform and 1 volume of methanol in the following concentrations: 1.5% (w/v) of the substance under examination – Solution 1.1.5 % (w/v) of the reference chemical (SQR) corresponding – Solution 2 and 0.03% (w/v) of each one of the following SQR: prednisolone and cortisone acetate – Solution 3. Apply on the chromatoplate 1 µL of each of these solutions, separately, and develop the chromatogram using, as eluent, mixture of 77 volumes of dichloromethane, 15 volumes of ether, 8 volumes of methanol and 1.2 volumes of water. Dry the chromatogram to air, heat to 105 ºC for 10 minutes and nebulized using with solution of blue tetrazolium alkaline SR. The main spot in the chromatogram obtained with Solution 1 corresponds in position, color and intensity, the main spot in the chromatogram obtained with Solution 2. Any secondary stain obtained with the Solution 1 is not more intense than the corresponding spot on the chromatogram obtained with Solution 3.

PROCEDURE II

Proceed to chromatography using silica gel G as support and, as eluent, mixture of 95 volumes of 1,2-dichloroethane, 5 volumes of methanol and 72 volumes of water.

Apply on the chromatoplate, separately, 1µ of each of 3 solutions in a mixture of 9 volumes of chloroform and 1 volume of methanol, as in the Procedure I, With the exception of the Solution 3, which adds acetate desoxicortona SQR.

5.3.1.4 SEARCH FOR RELATED SUBSTANCES SULFONAMIDES BY THIN-LAYER CHROMATOGRAPHY

PROCEDURE I

Proceed to thin layer chromatography (5.2.17.1) using silica gel H as support. Prepare solution of the substance under examination to 1.0% (p/v) using, as a solvent, a mixture of 9 volumes of 96% ethanol and 1 volume of ammonium hydroxide 13.5 M – Solution 1. Prepare solution of sulphanilamide SQR to 0.005% (p/v), using

The Same solvent – Solution 2. Apply separately on the chromatoplate 10 µmL of Solution 1 and Solution

2 . Develop the chromatogram using mixture of 15 volumes of 1-butanol and 3 volumes of ammonium hydroxide M as eluent. Remove the chromatoplate of Cuba, heat to 105 ºC for 10 minutes and nebulized using with solution at 0.1% (w/v) of 4-dimethylaminobenzaldehyde in ethanol 96 %, containing 1% hydrochloric acid (v/v): any spot secondary obtained in the chromatogram with the Solution 1, different from the main spot, is not more intense than that obtained in the chromatogram with the Solution 2.

PROCEDURE II

Proceed to thin layer chromatography (5.2.17.1) using silica gel H as support and a mixture of 20 volumes of chloroform, 2 volumes of methanol and 1 volume of dimethylformamide as mobile phase. Apply on the chromatoplate, separately, 10 µmL of each of the following solutions: 0.25% (w/v) of the substance under examination in a mixture of 9 volumes of ethanol and 1 volume of ammonium hydroxide 13.5 M – Solution 1.0,00125% (p/v) of sulphanilamide SQR in same solvent Solution 1 – Solution 2. Develop the chromatogram, allow to air dry and reveal as prescribed in the Procedure I: any secondary stain obtained with the Solution 1, different from the main spot, is not more intense than that obtained in the chromatogram with the Solution 2.

5.3.1.5 IDENTIFICATION OF PHENOTHYAZINES BY THIN-LAYER CHROMATOGRAPHY

Proceed as described in thin layer chromatography (5.2.17.1). Using kieselguhr G as support. Impregnate the chromatoplate drought, putting it in Cuba Containing a mixture of 10 volumes of 2-phenoxyethanol, 5 volumes of macrogol 300 and 85 volumes of acetone. Leave The Eluent climb at least 17 cm. Remove the chromatoplate of Cuba and use immediately.

Apply on the chromatoplate, separately, 2 µmL of each of the following solutions: 0.2% (w/v) of the substance under examination in chloroform – Solution 1 and 0.2% (w/v) of the reference chemical (SQR) corresponding —
Solution 2, operating in an atmosphere of nitrogen and low light. Develop the chromatogram using, as eluent, mixture of 2 volumes of diethylamine and 100 volumes of petroleum ether boiling range 40 – 60 ºC saturated with 2-phenoxyethanol. Remove the chromatoplate of Cuba, allow to air dry and examine under ultraviolet light with a maximum intensity at 366 nm: notes-if fluorescence, produced in a few minutes. Then, nebulized using chromatoplate with solution of sulfuric acid at 10% (v/v) ethanol and observe the coloration produced: the main spot in the chromatogram obtained with Solution 1, corresponds in position, color and intensity of fluorescence that obtained in the chromatogram with the Solution 2 and has the same stability for a period of at least 20 minutes after nebulization.

5.3.1.6 SEARCH FOR IMPURITIES RELATED TO PHENOTHYAZINES BY THIN-LAYER CHROMATOGRAPHY

PROCEDURE

Prepare chromatoplates using silica gel GF254 as support, operating in an atmosphere of nitrogen and under the light. Prepare solution containing 2.0% (w/v) of the substance under examination in a mixture of 95 volumes of methanol and 5 volumes of diethylamine – Solution 1. Prepare solution 0.01% (w/v) of the substance under examination, using the same solvent – Solution 2. Apply on the chromatoplate, separately, 10µL of each solution freshly prepared. Use mobile phase specified in the monograph. Leave the solvent rises 12 cm above the point of application. Remove the chromatoplate of Cuba, allow to air dry and examine under ultraviolet light (254 nm). Despise any stain on the base line. Any stain secondary obtained in the chromatogram with the Solution 1, except the main spot, is not more intense than the stain obtained with Solution 2, except if the monograph establish differently.

Mobile Phases

A mixture of 80 volumes of cyclohexane, 10 volumes of acetone and 10 volumes of diethylamine B mixture of 85 volumes of hexane, 10 volumes of acetone and 5 volumes of diethylamine C mixture of 15 volumes of 1-butanol and 3 volumes of ammonium hydroxide M

5.3.2 LIMIT TEST FOR INORGANIC IMPURITIES

5.3.2.1 LIMIT TEST FOR CHLORIDES.

Preparation sample: transfer the sample quantity specified in the monograph, or indicated in Table 1, or calculated,

for a Nessler tube (capacity of 50 mL and 22 mm internal diameter), adding a volume of 30 to 40 mL of distilled water. If it is used a solution of the sample, transfer the solution volume specified in the monograph, or calculated, for the Nessler tube and complete the volume to 30 to 40 mL with distilled water. Neutralise, if necessary, with nitrate SR You must employ a sample quantity that enables the use of volume greater than 0.2 mL of hydrochloric acid standard.

5-3 If the volume of standard solution in 1 mL can calculate-if m (mass in grams of the sample) by the formula:

$$m = \frac{354,6}{l}$$

l being the limit of chloride in ppm in raw material.

Standard Preparation: transfer the volume of hydrochloric acid standard (0.01 M HCl SV), indicated in the monograph, or in Table 1, or calculated for a Nessler tube and add a volume of 30 to 40 mL of distilled water.

Procedure: to Nessler tubes containing the standard preparation and preparing sample, add 1 mL of nitric acid SR. If, after acidification, the preparation is not perfectly clear, filter through filter paper free of chloride, transfer the filtrate for the Nessler tube and add 1 mL of silver nitrate SR. Make up the volume to 50 mL with distilled water and mix. Leave at rest, under the light, during 5 minutes. The turbidity of the sample preparation must not be higher than the default.

Being fixes the quantity of sodium chloride (= 3.546 x 10-4 g) in preparing standard, if the limit of chloride in a substance is, for example, 354 ppm, duty-if-to use

Alternatively, proceed as described in ion Chromatography (5.2.17.4.1), using gas chromatograph equipped with anion exchange column and detector by conductivity with chemical suppression.

Table 1 – Limits of impurity chloride and corresponding quantities of raw materials to perform the test considering the constant use of 1.0 mL of the standard solution that contains 3.546 x 10-4 g of chloride

Sample quantity (g)	*Limit of sulfate (ppm)*	*Sample quantity (g)*	*Limit of sulfate (ppm)*
0,10	3546 (= 0,355%)	3,8	93
0,15	2364 (= 0,236%)	4,0	88
0,20	1773 (= 0,180%)	4,2	84
0,25	1418 (= 0,l42%)	4,4	80
0,30	1182 (= 0,120%)	4,6	77
0,35	1013 (= 0,100%)	4,8	74
0,40	886	5,0	71
0,45	788	5,2	68
0,50	709	5,4	65
0,55	645	5,6	63
0,60	591	5,8	61
0,65	545	6,0	59
0,70	506	6,2	57
0,75	473	6,4	55
0,80	443	6,6	53
0,85	417	6,8	52
0,90	394	7,0	50
0,95	373	7,2	49
1,00	354	7,4	48
1,2	295	7,6	46
1,4	253	7,8	45
1,6	221	8,0	44
1,8	197	8,2	43
2,0	177	8,4	42
2,2	161	8,6	41
2,4	148	8,8	40
2,6	136	9,0	39
2,8	126	9,2	38
3,0	118	9,4	37
3,2	111	9,6	37
3,4	104	9,8	36
3,6	98	10,0	35

5.3.2.2. LIMIT TEST FOR SULPHATES

Sample Preparation: transfer the amount of the sample specified in the monograph, or indicated in Table 2, or calculated, for a Nessler tube (capacity of 50 mL and 22 mm internal diameter), adding 30 to 40 mL of distilled water. If it is used a solution of the sample, transfer the solution volume specified in the monograph, or calculated, for the Nessler tube and complete the volume to 30 to 40 mL with distilled water. If necessary, neutralize with dilute hydrochloric acid SR. You can- if, possibly, using acetic acid. If the preparation is not perfectly clear, filter through filter paper free of sulfate. Transfer the filtrate to Nessler tube. You must employ a sample quantity that permits the use of volume greater than 0.2 mL of standard solution of sulfuric acid. 5-3 If the volume of standard solution in 2.5 mL can calculate m (mass in grams of the sample) by the formula:

$$m = \frac{1200,8}{l}$$

Where l is the limit of sulphate in ppm in raw material.

Standard Preparation: transfer the volume of standard sulfuric acid (H2SO4 0.005 M SV) indicated in the monograph, or indicated in Table 2, or calculated for a Nessler tube and add a volume of 30 to 40 mL of distilled water.

Procedure: to Nessler tubes containing the standard preparation and preparing sample, add 1 mL of 3 M hydrochloric acid and 3 mL of barium chloride SR. Complete

The Volume to 165 mL with distilled water. Mix Well. Leave to rest for about 10 minutes. The turbidity of the sample preparation must not be higher than the default.

Being fixed the amount of sulphate (= 1.2008 x 10-3 g), if the limit of sulphate in particular substance is, for example, 500 ppm, should be used 2.4 g of the sample to obtain until the same turbidity standard; if the limit is 151 ppm of sulfate should be used 8 g of the sample and so on.

Alternatively, proceed as described in ion Chromatography (5.2.17.4.1), using gas chromatograph equipped with anion exchange column and detector by conductivity with chemical suppression.

Table 2 – Limits of impurity sulphate and corresponding quantities of raw materials to perform the test considering the constant use of 2.5 mL of the standard solution containing 1.2008 x 10-3 g of sulfate.

Sample quantity (g)	*Limit of sulfate (ppm)*	*Sample quantity (g)*	*Limit of sulfate (ppm)*
0.50	2401 (= 0.240 %)	4.6	261
0.55	2183 (= 0.220 %)	4.8	250
0.60	2001 (= 0.200 %)	5.0	240
0.65	1847 (= 0.185 %)	5.2	231
0.70	1715 (= 0,171 %)	5.4	222
0.75	1601 (= 0.160 %)	5.6	214
0.80	1501 (= 0.150 %)	5.8	207
0.85	1412 (= 0,141 %)	6.0	200
0.90	1334 (= 0,133 %)	6.2	194
0.95	1264 (= 0.126 %)	6.4	187
1.00	1200 (= 0.120 %)	6.6	182
1.2	1001 (= 0.100 %)	6.8	177
1.4	858	7.0	171
1.6	750	7.2	166
1.8	667	7.4	162
2.0	600	7.6	158
2.2	546	7.8	154
2.4	1700	8.0	151
2.6	0462	8.2	146
2.8	429	8.4	143
3.0	400	8.6	139
3.2	375	8.8	136
3.4	353	9.0	133
3.6	333	9.2	130
3.8	316	9.4	127
4.0	300	9.6	125
4.2	286	9.8	122
4.4	273	10.0	120

5.3.2.3 LIMIT TEST FOR HEAVY METALS

The determination of heavy metals can be performed by two methods: limit test for formation of solid particles of sulfides or determination by atomic spectrometry.

The Limit test consists in the formation of solid particles of sulfides of heavy metals, in suspension, and later visual comparison of the intensity of the color in preparations sample and standard in Nessler tube. The test is semi quantitative and enables infer if the sample passes or not, representing the sum of the concentration of contaminants in the sample.

The Method by atomic spectrometry allows quantify each element contaminant in the sample and differentiated limits are established for each element according to their toxicity and the type of pharmaceutical form. Elements such as As, Cd, Pb and Hg, due to high toxicity have lower limits than the others. Due to the greater bioavailability of elements that may be present in substances used in the manufacture of parenteral products, the required limits are lower than those related to oral use.

THE LIMIT TEST METHOD

Special Reagents

Stock Solution of lead nitrate: dissolve, exactly, 159.8 mg of lead nitrate in 100 mL of water added to 1 mL of nitric acid. Dilute with water to 1000 mL and mix. Prepare and store this solution in glass containers free of soluble salts of lead.

Lead standard Solution (10 ppm Pb): on the day of use, dilute 10 mL of the stock solution of lead nitrate to 100 mL with water. Each ml of this solution contains the equivalent of 10 µmg of lead (10 ppm Pb).

Acetate Buffer pH 3.5: dissolve 25.0 g of ammonium acetate in 25 mL of water and add 38 mL of 6 M hydrochloric acid If necessary, adjust the pH to 3.5 with ammonium hydroxide 6 M or 6 M hydrochloric acid Dilute to 100 mL with water and mix.

Preparation of the reagent of thioacetamide: dissolve 4 g of thioacetamide in water and make up the volume to 100 mL. Take 0.2 mL and add 1 mL of the mixture of sodium hydroxide M, 5 mL of water and 20 mL of glycerin. Heat in a water bath for 20 s, cool and use immediately.

METHOD I

Sample Preparation: transfer to suitable pipe sample solution prepared as specified in the monograph and dilute to 25 mL with water, or dissolve and dilute with water to 25 mL sample quantity, in grams, specified in the monograph or calculated according to the equation:

$$2 / (1000l)$$

Whereas

l = Limit of heavy metals in the sample percentage (p/p).

Adjust the pH to between 3.0 and 4.0 M with acetic acid or ammonium hydroxide 6 M using indicator paper of narrow band as external indicator. Dilute with water to about 40 mL and mix.

Standard Preparation: transfer to suitable tube 2 mL of lead standard solution (10 ppm Pb) and dilute to 25 mL with water. Adjust the pH to between 3.0 and 4.0 M with acetic acid or ammonium hydroxide 6 M using indicator paper of narrow band as external indicator. Dilute with water to about 40 mL and mix.

Preparation control: transfer to a third tube volume of sample solution prepared as described in the monograph or in preparation sample and add 2 mL of lead standard solution (10 ppm Pb). Adjust the pH to between 3.0 and 4.0 M with acetic acid or ammonium hydroxide 6 M using indicator paper of narrow band as external indicator. Dilute with water to about 40 mL and mix.

Procedure: each one of the preparations add 2 mL of acetate buffer pH 3.5 and 1.2 mL of thioacetamide. Dilute with water to 165 mL, mix and leave to rest for 2 minutes. After 2 minutes, develop- if-the tone that varies from yellow to black. Observe the preparations from top to bottom, according to the vertical axis of the tube, on a white background. Any color developed in sample preparation is not more intense in default. The test is only valid if the intensity of the color developed in preparation control is equal to or greater than that of the standard.

METHOD II

Sample Preparation: transfer to suitable pipe sample solution prepared as specified in the monograph and dilute to 25 mL with organic solvent (dioxane or acetone, containing, at a minimum, 15% v/v of water), or dissolve and dilute with the same solvent for 25 mL sample quantity, in grams, specified in the monograph or calculated according to the following equation:

$$2 / (1000l)$$

Whereas

l = Limit of heavy metals in the sample percentage (p/p).

Adjust the pH to between 3.0 and 4.0 M with acetic acid or ammonium hydroxide 6 M using indicator paper of narrow band as external indicator. Dilute with water to about 40 mL and mix.

Standard Preparation: transfer to suitable tube 2 mL of lead standard solution (10 ppm Pb) and dilute to 25 mL with the same solvent used to dissolve the sample. Adjust the pH to between 3.0 and 4.0 M with acetic acid or ammonium hydroxide 6 M using indicator paper of narrow band as external indicator. Dilute with the same solvent used to dissolve the sample to approximately 40 mL and mix.

Preparation control: transfer to a third tube volume of sample solution prepared as described in the monograph or in preparation sample and add 2 mL of lead standard solution (10 ppm Pb). Adjust the pH to between 3.0 and 4.0 M with acetic acid or ammonium hydroxide 6 M using indicator paper of narrow band as external indicator. Dilute with the same solvent used to dissolve the sample to approximately 40 mL and mix.

Procedure: each one of the preparations add 2 mL of acetate buffer pH 3.5 and 1.2 mL of thioacetamide. Dilute with water to 165 mL, mix and leave to rest for 2 minutes. After 2 minutes, it will develop the coloration that varies from yellow to black. Observe the preparations from top to bottom, according to the vertical axis of the tube, on a white background. Any color developed in sample preparation is not more intense than the default.

The Test is valid only if the intensity of the color developed in preparation control is equal to or greater than that in default.

METHOD III

Sample Preparation: use the sample quantity, in grams, specified in the monograph or calculated according to the equation:

$$2 / (1000l)$$

Whereas

1 = Limit of heavy metals in the sample percentage (p/p).

Transfer the sample to suitable crucible, add sulfuric acid enough to dampen the substance and incinerate, carefully, under low temperature. Add the carbonized grease 2 mL of nitric acid and 5 drops of sulfuric acid. Warm, with caution, until no more to peel off white fumes. Be Incinerated in muffle furnace at 500 – 600 °C until complete combustion of carbon.

Cool at room temperature, add 4 mL of 6 M hydrochloric acid, cover, digest in a water-bath for 15 minutes, uncover and evaporate in the water-bath, slowly, until dryness. Moisten the residue with 1 drop of hydrochloric acid, add 10 mL of hot water and digest in a water bath for 2 minutes. Alkaline to litmus paper with ammonium hydroxide 6 M added drop by drop. Dilute with water to 25 mL and adjust the pH to between 3.0 and 4.0 with acetic acid M, using indicator paper of narrow band as external indicator. Filter if necessary, wash the crucible and the filter with 10 mL of water and mix. FILTRATE and wash waters in tube suitable for comparison of color. Dilute with water to about 40 mL and mix.

Standard Preparation: transfer to suitable tube 2 mL of lead standard solution (10 ppm Pb) and dilute to 25 mL with the same solvent used to dissolve the sample. Adjust the pH to between 3.0 and 4.0 M with acetic acid or ammonium hydroxide 6 M using indicator paper of narrow band as external indicator. Dilute with the same solvent used to dissolve the sample to approximately 40 mL and mix.

Preparation control: transfer to a third tube volume of sample solution prepared as described in the monograph or in preparation sample and add 2 mL of lead standard solution (10 ppm Pb). Adjust the pH to between 3.0 and 4.0 M with acetic acid or ammonium hydroxide 6 M using indicator paper of narrow band as external indicator. Dilute with the same solvent used to dissolve the sample to approximately 40 mL and mix.

Procedure: each one of the preparations add 2 mL of acetate buffer pH 3.5 and 1.2 mL of thioacetamide. Dilute with water to 165 mL, mix and leave to rest for 2 minutes. After 2 minutes, it will develop the coloration that varies from yellow to preto. Observar preparations from top to bottom, according to the vertical axis of the tube, on a white background. Any color developed in sample preparation is not more intense than that in default. The test is only valid if the intensity of the color developed in preparation control is equal to or greater than that in default.

METHOD IV

Sample Preparation: Weigh, exactly, sample quantity recommended in monograph or calculated according to the equation:

$$2 / (1000l)$$

Whereas

1 = Limit of heavy metals in the sample percentage (p/p).

Transfer to digestion tube borosilicate glass 100 mL and add about 10 mL of nitric acid. Proceed to digestion on a heating plate or block Digester at a temperature of 120 °C for 3 hours. It is recommended to heat the system slowly, to avoid projection of the sample. If there is no evaporation of acid, add another aliquot of 5 mL. If a preparation clear is not obtained, add, after cooling, 2 mL of hydrogen peroxide to 30% (p/p) and heat up to 140°C for a further hour. Cool and dilute, cautiously, with small volume of water. Transfer, with washing, for Nessler tube of 50 mL, without exceeding 25 mL.

Standard Preparation: transfer to suitable tube 2 mL of lead standard solution (10 ppm Pb) and dilute to 25 mL with water. Adjust the pH to between 3.0 and 4.0 M with acetic acid or ammonium hydroxide 6 M using indicator paper of narrow band as external indicator. Dilute with water to about 40 mL and mix.

Preparation control: transfer to a third tube volume of sample solution prepared as described in the monograph or in preparation sample and add 2 mL of lead standard solution (10 ppm Pb). Adjust the pH to between 3.0 and 4.0 M with acetic acid or ammonium hydroxide

6 M using indicator paper of narrow band as external indicator. Dilute with water to about 40 mL and mix.

Procedure: each one of the preparations add 2 mL of acetate buffer pH 3.5 and 1.2 mL of thioacetamide. Dilute with water to 165 mL, mix and leave to rest for 2 minutes.

After 2 minutes, it will develop coloration that varies from yellow to black. Observe the preparations from top to bottom, according to the vertical axis of the tube, on a white background. Any color developed in sample preparation is not more intense than the default. The test is only valid if the intensity of the color developed in preparation control is equal to or greater than that in default.

METHOD V

Sample Preparation: in cases in which the previous methods of sample preparation are not efficient, proceed

As described in Decomposition via wet in closed system or Method of combustion initiated by microwave in pressurized system described in atomic spectrometry Method.

Standard Preparation: transfer to suitable tube 2 mL of lead standard solution (10 ppm Pb) and dilute to 25 mL with water. Adjust the pH to between 3.0 and 4.0 M with acetic acid or ammonium hydroxide 6 M using indicator paper of narrow band as external indicator. Dilute with water to about 40 mL and mix.

Preparation control: transfer to a third tube volume of sample solution prepared as described in the monograph or in preparation sample and add 2 mL of lead standard solution (10 ppm Pb). Adjust the pH to between 3.0 and 4.0 M with acetic acid or ammonium hydroxide 6 M using indicator paper of narrow band as external indicator. Dilute with water to about 40 mL and mix.

Procedure: each one of the preparations add 2 mL of acetate buffer pH 3.5 and 1.2 mL of thioacetamide. Dilute with water to 165 mL, mix and leave to rest for 2 minutes. After 2 minutes, it will develop coloration that varies from yellow to black. Observe the preparations from top to bottom, according to the vertical axis of the tube, on a white background. Any color developed in sample preparation is not more intense than that in default. The test is only valid if the intensity of the color developed in preparation control is equal to or greater than that in default.

METHOD OF ATOMIC SPECTROMETRY

Use atomic spectrometric techniques for determination of As, Cd, Cr, Cu, Hg, Ir, Mn, Mo, Ni, Pb, Pd, Pt, Rh, Ru and V, as atomic Spectrometry (2.5.13). However, different procedures for sample preparation can be applied, as shown in Figure 1.

In the case of substances soluble in water, there is no need of prior decomposition of sample and this can be analyzed directly after dissolution. If it is not soluble in water and present solubility in another solvent, the substance may be analyzed directly after dissolution if there is no incompatibility between the solvent and atomic spectrometry technique used. When none of the conditions above is met, it is recommended that the prior decomposition of the sample. In these cases, two procedures are recommended:

Decomposition via wet in closed system

Weigh exactly, sample quantity between 0.1 and 0.5 g of the sample and add nitric acid as the manufacturer's recommendation and proceed to digestion in closed system with conventional heating or with micro-waves in temperature of 180 °C or higher. In systems that employ conventional heating and microwave, when there is no specification in the monograph, it is recommended to digestion by 240 min and 20 min, respectively.

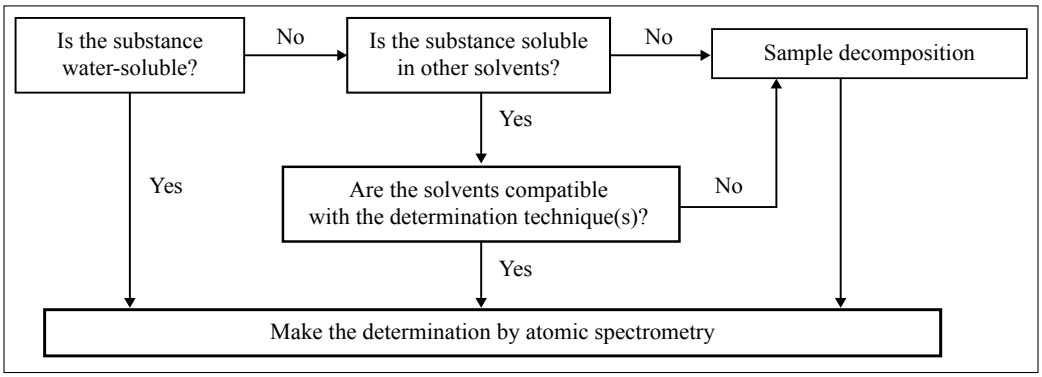

Figure 1 – Sample preparation procedures for the method of atomic spectrometry.

Method of combustion initiated by microwave in pressurized system

Proceed as described in Methods of combustion (5.3.3.3).

In principle, the two procedures of decomposition described may be used interchangeably. However, it is recommended to use the decomposition by track wet due to greater simplicity and processing capacity of samples. Other reagents, such as hydrochloric acid, sulfuric acid, hydrogen peroxide and hydrofluoric acid (cannot be used in bottles of quartz), also, can be used in step of digestion,

depending on the need.

The decomposition by combustion initiated by microwave is recommended in those cases in which the wet digestion is not efficient for organic samples.

The maximum allowable limits for each element are described in Table 1.

5.3.2.4 LIMIT TEST FOR IRON

Standard iron Solution (100 ppm Fe): dissolve 0.8634 g of ferric sulphate ammoniacal dodecaidratado in distilled water in a 1000 mL volumetric flask. Add 5 mL of dilute sulfuric acid SR and make up the volume with distilled water.

Standard Solution of iron (10 ppm Fe): dilute 10 mL of the standard Solution of iron (100 ppm Fe) with distilled water and make up the volume to 100 mL. Standard Solution of iron (2 ppm Fe): dilute 2 mL of the standard Solution of iron (100 ppm Fe) with distilled water and make up the volume to 100 mL.

Table 1 – allowable Limits of impurities in metals and non-metals.

Element	Oral Use Maximum Limit *(µg* g-1)	Parenteral Use Maximum Limit *(µg* g-1)
Arsenic (As)	1.5	0.15
Cadmium (Cd)	0.5	0.05
Lead (Pb)	1.0	0.1
Mercury (Hg)	1.5	0.15
Chromium (Cr)	25	2.5
Copper (Cu)	250	25
Manganese (Mn)	250	25
Molybdenum (Mo)	25	2.5
Nickel (Ni)	25	2.5
Palladium (Pd)	10	1.0
Platinum (Pt)	10	1.0
Vanadium (V)	25	2.5
Iridium (Ir)	The sum of the concentration can not exceed 10	The sum of the concentration can not exceed 10
Ósmio (Os)		
Rhodium (Rh)		
Ruthenium (Ru)		

METHOD I

Sample Preparation: dissolve sample amount of specified in the monograph, or in Table 1, or calculated, in suitable solvent, transfer to Nessler cylinder (capacity of 50 mL and 22 mm internal diameter). Add distilled water, or the solvent indicated in monograph, in sufficient quantity to 40 mL. Add 2 mL of citric acid to 20% (p/v).

5-3 If the volume of the standard Solution of iron (100 ppm Fe) in 1 mL, you can calculate the value of m (mass in grams of the sample) by the formula:

$$m = \frac{100}{l}$$

Where l is the limit of iron in ppm in raw material.

Preparation standard: employ 10 mL of standard Solution of iron (10 ppm of Fe) or 1 mL of the standard Solution of iron (100 ppm Fe), as shown in Table 1, or calculated volume, add distilled water, or the solvent indicated in the monograph, in sufficient quantity to 40 mL. Add 2 mL of citric acid to 20% (p/v).

Procedure: concomitantly, add to tubes containing preparations sample and standard two drops of mercaptoacetic acid. Homogenize, alkaline with ammonium hydroxide, complete the volume to 50 mL with distilled water and mix. Leave to rest for 5 minutes. The pink color produced in preparing sample should not be more intense than in default.

METHOD II

Sample Preparation: dissolve sample amount of specified in the monograph, or calculated, in suitable solvent, or use volume of the sample solution as specified in the monograph. Add 2 mL of 2 M hydrochloric acid and 0.5

mL of bromine water SR. After 5 minutes, remove the excess bromine by air current in chapel (CAUTION! TOXIC REAGENT) and tranfer to Nessler tube (capacity of 50 mL and 22 mm internal diameter).

Preparation standard: submit the volume of standard Solution of iron (2 ppm or 10 ppm of Fe) indicated in the monograph, OR the volume calculated as described in sample preparation.

Procedure: Concomitantly, Add The Tubes containing preparations sample and standard, 3 mL of potassium thiocyanate M, complete the volume to 50 mL, mix and leave to rest for 5 minutes. The coloration produced in preparing sample should not be more intense than in default.

METHOD III

Sample Preparation: transfer to a Nessler tube (capacity of 50 mL and 22 mm internal diameter) sample quantity specified in the monograph, or calculated, or the volume of the sample solution indicated in the monograph. Dilute to 40 mL with distilled water. Add 2 mL of hydrochloric acid M and mix.

Standard Preparation: transfer the volume of standard Solution of iron (10 ppm Fe) indicated in the monograph, or volume calculated for a Nessler tube and proceed as described in sample preparation.

Procedure: add to tubes containing preparations sample and standard 165 mg of crystals of ammonium peroxidissulfato. Add 3 mL of ammonium thiocyanate SR, make up the volume to 50 mL with distilled water and mix thoroughly. The coloration produced in preparing sample should not be more intense than in default.

Being fixed the quantity of iron (= 10-4 g of Fe) in Standard Preparation, if the limit of iron in a given substance is, for example, 1,000 ppm, should be used 0.1 g of the sample to obtain up to the same staining pattern of preparation; if the limit is 200 ppm of iron, should be used 0.5 g of the sample, and so on.

METHOD IV

Alternatively, for determination of iron, proceed to the preparation of the sample solution as described in Limit Test for heavy metals (5.3.2.3) and log the determination by one of atomic Spectrometric techniques (2.5.13).

5.3.2.5 LIMIT TEST FOR ARSENICBY MEANS OF SPECTROPHOTOMETRY

The method is based on the reaction between the arsine (AsH3) released and silver diethyldithiocarbamate that form a complex red; the absorption of radiation can be measured in a spectrophotometer or colorimeter.

Two methods can be employed differing only, in preparation of the sample and the standard. The Method I is, in general, used for inorganic substances, while the Method

Table 1 – Limits of impurity iron and corresponding quantity of raw material to perform the test considering the constant use of 1.0 mL of the standard solution of iron of 100 ppm, which contains 10-4 g of iron, in preparation standard.

Sample quantity (g)	*Limit of iron (ppm)*	*Sample quantity (g)*	*Limit of iron (ppm)*
0.100	1000	0.4	250
0.105	950	0.5	200
0.111	900	0.667	150
0.116	850	1	100
7125	800	1.111	90
0.133	750	1.250	80
0.143	700	1.429	70
0.154	650	1.667	60
0.167	600	2	50
0.182	550	2.5	40
0.200	1700	3.333	30
0.222	450	5	20
0.250	400	10	10
0.285	350	20	5
0.333	300		

II It is used for organic substances.

The System used – Figure 1 – comprises: (a) generator of arsine; (b) and (d) together; (c) unit flipped; (e) absorption tube. Another system adapted that has the essential characteristics of the presented may, possibly, be used.

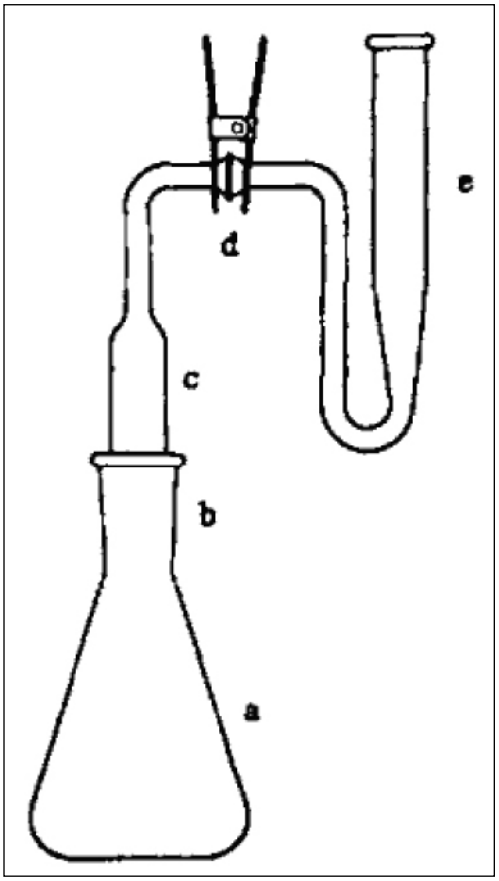

Figure 1 – System for the determination of arsenic by spectrophotometric method.

Stock standard Solution of arsenic: dry arsenic trioxide for 1 hour at 105 °C. Weigh exactly, 132 mg and dissolve in 5 mL of sodium hydroxide solution (1:5) in 1000 mL volumetric flask. Neutralize with 1 M sulfuric acid, and then add more 10 mL of sulfuric acid M. Fill up to volume with water- freshly boiled and cooled.

Standard Solution of arsenic: transfer 1 mL (or 0.5; or 0.25; or 0.1 mL) of the stock standard solution of arsenic to a 100 mL volumetric flask (or 50, or 25, or

OF 10 mL, according to the need of lab)

- Preserve the environment. Add 1 mL of 1 M sulfuric acid and Fill up to volume with water newly-boiled and then cooled down. Mix Well. Keep the solution in glass container and use up to 3 days. Each mL of the solution obtained contains 1 |ag of arsenic.

METHOD I

Sample Preparation: transfer to bottle arsine generator of the quantity of substance specified in the monograph, or the calculated amount.

5-3 If the volume of the standard solution of arsenic in 3 mL you can calculate m (mass in grams of the sample) by the formula:

$$m = 3/l$$

Where l is the limit of arsenic in ppm in raw material.

Dissolve with distilled water by supplementing the volume to 35 mL. Add 20 mL of 1 M sulfuric acid, 2 mL of potassium iodide SR, 0.5 mL of stannous chloride SR strongly acid and 1 mL of isopropyl alcohol. Mix Well. Leave to rest for 30 minutes at ambient temperature. In the unit (c) the apparatus described, put two portions of cotton soaked in saturated solution of lead acetate, leaving between them space of 2 mm. The excess of solution should be eliminated by squeezing if the portions of cotton and drying them at reduced pressure at ambient temperature. The seals (b) and (d) should be lubricated with petroleum jelly and united as in Figure 1.

Standard Preparation: transfer to the bottle arsine generator of 3 mL of the standard Solution of arsenic. Dilute with distilled water to 35 mL. Proceed in the same way as described for the sample preparation.

Procedure: transfer 3 mL of silver diethyldithiocarbamate SR for absorption unit (e) bottles of generators containing preparations sample and standard. Add 3 g of zinc granules (1 mm mesh) the mixture in each flask arsine generator. Immediately join the units (c) and (e) to the bottle generator. Leave in water bath (25 ± 3) °C for 45 minutes. In intervals of 10 minutes stirring, slowly. After this period, transfer the contents of the drive of absorption for cela de 1 CM. Compare the red color produced in preparations sample and standard. The coloration produced in preparing sample should not be more intense than in default. If necessary, determine the absorption spectrophotometer or colorimeter in wavelength between 535 and 540 nm employing silver diethyldithiocarbamate SR as white for the zero adjustment.

METHOD II

This method employs, additionally, hydrogen peroxide in digestion of the sample. With certain substances may cause violent reaction. Thus, it is important to proceed carefully, at all stages. Must- be careful, also, in the presence of compounds

Halogenated, especially when heating the sample with sulfuric acid and, subsequently, add hydrogen peroxide to 26% (v/v). The heater must be more brando preventing that reaches the boiling temperature of the mixture and carbonization to avoid the loss of arsenic.

Sample Preparation: transfer to the bottle generator sample amount of specified in the monograph, or calculated.

5-3 If the volume of the standard solution of arsenic in 3 mL you can calculate m (mass in grams of the sample) by the formula:

$$m = 3/l$$

Where l is the limit of arsenic in ppm in raw material.

Add 5 mL of sulfuric acid and glass beads. If necessary, employ higher amount of acid to dampen the substance completely caring for that

The Volume does not exceed 10 mL. Carry out digestion in chapel preferably using heating plate with temperature not exceeding 120 ºC per time needed to start the digestion. Once started the decomposition of the sample, add dropwise, carefully, concentrated hydrogen peroxide. Expect that the reaction is slow, and then warm up between adding each drop. If there is excess of foam, interrupt the grille. As well as reducing the intensity of the reaction, heat, cautiously, with stirring of the bottle to promote uniform heating. It is necessary to retain the oxidizing conditions during the entire digestion. For both, add small amounts of concentrated hydrogen peroxide whenever the mixture become brown or dark. Destroyed the organic matter, gradually increase the heating temperature allowing the vapors of sulfur trioxide are Loosened and the solution becomes colorless or slightly beige. Cool, add, carefully, 10 mL of distilled water, evaporate until the sulfur trioxide is again popped out and cool. If necessary, repeat the operation, removing traces of hydrogen peroxide. Cool and add 10 mL of distilled water. Wash the flask and dilute with distilled water by supplementing the volume to 35 mL. Proceed as in sample preparation Method I starting by "Add 20 mL of dilute sulfuric acid M.

Standard Preparation: transfer to bottle arsine generator

3 Ml of the standard Solution of arsenic and add 2 mL of sulfuric acid. Mix Well. Add the same volume of concentrated hydrogen peroxide used for sample preparation. Then Proceed in the heating of the solution obtained by forming vapors. Cool and add, carefully, 10 mL of distilled water. Repeat the procedure of heating with over 10 mL, and after, cool again and dilute with distilled water to make 35 mL. Proceed as for the sample preparation.

Procedure: proceed as described in Method I.

Note: antimony is interfering reaction, once that way estibina (SbHJ providing falsely positive result in the development of color with silver diethyldithiocarbamate SR. In these cases, you should compare the preparations at a wave length of 535 and 540 nm, in which the interference of estibina is contemptible.

METHOD OF ATOMIC ABSORPTION SPECTROMETRY WITH HYDRIDE GENERATION

Carry out the preparation sample as described in item Decomposition via wet in closed system – limit Test for heavy metals (5.3.2.3) and determined by atomic absorption Spectrometry with hydride generation (5.2.13.1.2). Proceed in accordance with the manufacturer's specifications employing wavelength of 193.7 nm and resolution of the monochromator (0.5 ± 0.1) nm.

METHOD OF OPTICAL EMISSION SPECTROMETRY WITH INDUCTIVELY COUPLED PLASMA

Carry out the preparation of the sample as described in item Decomposition via wet in closed system – limit Test for heavy metals (5.3.2.3) and determine by optical emission Spectrometry with inductively coupled plasma (5.2.13.2.2). Proceed in accordance with the manufacturer's specifications. It is recommended to use the wavelength of 188.979 to 189.042 nm.

5.3.2.6 LIMIT TEST FOR AMMONIA

Standard Solution of ammonia (2.5 ppm NH3): transfer 1 mL of the solution of ammonium chloride 0,00741% (p/v) to a 10-mL volumetric flask. Fill up to volume with distilled water. Standard Solution of ammonia (1 ppm NH3): dilute 40 mL of the standard solution of 2.5 ppm ammonia (NH3) to 100 mL with distilled water.

Sample Preparation: dissolve the indicated quantity of the substance under examination in 12 mL of distilled water, alkaline, if necessary, with sodium hydroxide 2 M. Transfer to volumetric flask of 15 mL, add 0.3 mL of alkaline solution of tetraiodomercurato (II) potassium and make up the volume with distilled water. Mix Well and leave to rest for 5 minutes. Transfer to a Nessler tube (capacity of 165 mL and internal diameter of 22 mm).

Preparation standard: transfer 10 mL of the standard solution of ammonia (1 ppm of NH3), or the calculated volume, for volumetric flask of 15 mL, add 4.0 mL of distilled water, 0.3 mL of alkaline solution of tetraiodomercurato (II) potassium and complete the volume. Mix Well and leave to rest for 5 minutes. Transfer to a Nessler tube.

Procedure: Compare the color developed in

Preparations. The yellow color produced in preparing sample should not be more intense than in default.

5.3.2.7 LIMIT TEST FOR CALCIUM

Standard alcoholic Solution of calcium (100ppm Ca): weigh, exactly, 2.5 g of calcium carbonate and transfer to 1000 mL volumetric flask with 12 mL of glacial acetic acid. Dissolve and Fill up to volume with distilled water. Transfer, immediately before use, 10 mL of this solution to 100 mL volumetric flask and Fill up to volume with ethanol.

Standard Solution of calcium (10 ppm Ca): weigh, exactly, 0.624 g of calcium carbonate and transfer to volumetric flask of 250 mL with 3 mL of glacial acetic acid. Dissolve and Fill up to volume with distilled water. Transfer, immediately before use, 10 mL of this solution to 1000 mL volumetric flask and Fill up to volume with water.

Sample Preparation: add 1 mL of ammonium oxalate SR a Nessler tube (capacity of 50 mL and 22 mm internal

diameter) containing 0.2 mL of the standard solution of alcoholic calcium (100 ppm Ca). Wait for 1 minute, add mixture of 1 mL of dilute acetic acid and 15 mL of the sample solution prepared as described in the monograph.

Standard Preparation: transfer to a Nessler tube the same quantities of ammonium oxalate SR and the standard solution alcooolica calcium (100 ppm Ca) as sample preparation. Wait for 1 minute, add mixture of 10 mL of the standard solution of calcium (10 ppm Ca), 1 mL of dilute acetic acid and 5 mL of distilled water.

Procedure: Mix the preparations in Nessler tubes. After 15 minutes, the turbidity of the sample preparation should not be more intense than that of the standard.

Alternatively, carry out the preparation of the sample as indicated in the monograph and log the determination of calcium by atomic absorption Spectrometry with flame (5.2.13.1.1) employing flame of type- air acetylene, wavelength of 422.7 nm and resolution of the monochromator (0.7 ± 0.1) nm; or optical emission Spectrometry with inductively coupled plasma (5.2.13.2.2) Employing the wavelength of 393.366 nm.

5

5.3.2.8 LIMIT TEST FOR MAGNESIUM

Sample Preparation: add 0.1 g of sodium tetraborate to 10 mL of the sample solution prepared as specified in the monograph. Adjust the pH between 8.8-9.2 with hydrochloric acid or sodium hydroxide SR SR.

Standard Preparation: add 0.1 g of sodium tetraborate to a mixture of 1 mL of the standard solution of magnesium (10 ppm Mg) and 9 mL of distilled water. Proceed as sample preparation.

Procedure: transfer the sample preparation to a separating funnel and extract 2 times, stirring for 1 minute each time, with 5 mL of a solution of hydroxyquinoline to

0.1% (p/v) in chloroform. Discard the organic phases and add the aqueous phase 0.4 mL of butilamina and 0.1 mL of triethanolamine. Adjust the pH to between 10.5-11.5 if necessary. Add 4 mL of the solution of hydroxyquinoline to 0.1% (p/v) in chloroform and shake for 1 minute. Use the lower phase for comparison. Proceed in the same way with the standard preparation. The coloration produced in preparing sample should not be more intense than in default.

Alternatively, make the determination of magnesium by atomic absorption Spectrometry with flame (5.2.13.1.1) employing flame of type air-acetylene, wavelength of 285.2 nm and resolution of the monochromator (1.2 ± 0.1) nm; or optical emission Spectrometry with inductively coupled plasma (5.2.13.2.2) employing wavelength of 285.213 nm.

5.3.2.9 LIMIT TEST FOR MAGNESIUM AND ALKALI METALS 1855

655 Ml of distilled water add 0.1 g of hydroxylamine hydrochloride, 10 mL of ammonium chloride buffer pH 10.0.1 mL of a solution of zinc sulphate 0.1 M and approximately 15 mg of eriochrome black T. Warming up to, approximately, 40 °C. Titrate with 0.01 M disodium EDTA SV until the violet color change to blue. Add to this solution recommended sample quantity dissolved in 100 mL of distilled water or prepared as described in the monograph. If the color of the solution changes to violet, titrate with 0.01 M disodium EDTA SV until turning to blue. The volume of solution of disodium EDTA 0.01 M SV used in the second titration should not exceed the amount established in the monograph.

5.3.2.10 LIMIT TEST FOR ALUMINUM

Aluminum standard Solution (200 ppm Al): treat a portion of metallic aluminum with 6 M hydrochloric acid at 80 °C for a few minutes. Weigh 100 mg of portion treated and dissolve in a mixture of 10 mL of hydrochloric acid and 2 mL of nitric acid, 80 °C, for approximately 30 minutes. Maintain under heating up to reduce the volume to about 4 mL. Cool to room temperature and add 4 mL of distilled water. Let evaporate to obtain the volume of 2 mL. Cool and transfer the solution quantitatively, using distilled water to 100 mL volumetric flask. Make up the volume with distilled water and mix. Pipette 20 mL of this solution and transfer to another 100 mL volumetric flask. Make up the volume with distilled water and mix.

Aluminum standard Solution (10 ppm Al): transfer, immediately prior to use, 5 mL of the standard Solution of aluminum (Al) 200ppm to 100 mL volumetric flask; complete the volume with distilled water and mix thoroughly.

Aluminum standard Solution (2 ppm Al): transfer, immediately prior to use, 1 mL of the standard Solution of aluminum (Al) 200ppm to 100 mL volumetric flask; complete the volume with distilled water and mix thoroughly.

Solution diluent of nitric acid: transfer 40 mL of nitric acid to 1000 mL volumetric flask; complete the volume with distilled water and mix thoroughly.

METHOD I

Sample Preparation: use the specified quantity of the sample, or calculated, prepared as specified in the monograph.

Standard Preparation: use the specified volume, or calculated, the aluminum standard Solution (10 ppm or 2 ppm).

Procedure: transfer to separation funnels preparations sample and standard and remove with 3 portions (20.20 and

10 mL) of the solution of hydroxyquinoline to 0.5% (p/v) in chloroform. Join the chloroform extracts and dilute to 50 mL with chloroform. Perform a blank preparation using the same solvent. Measure the fluorescence intensity (2.5.15) of sample preparation (Italy), the standard preparation (I2) and the preparation blank (I3) using excitation wavelength of 392 nm and monochromator set at 518 nm. The fluorescence of sample preparation (Italy), deducted the preparation blank (I3) must not be greater than that of the standard preparation (I2), minus the preparation blank (I3).

METHOD II

Sample Preparation: transfer sample amount specified in the monograph, or calculated, for volumetric flask of 100 mL plastic, add 50 mL of distilled water and subjected to ultrasound during 30 minutes. Add 4 mL of nitric acid; complete the volume with distilled water and mix.

Preparations: prepare standard solutions containing 0.01.0.02 and 0.04 ppm of aluminum, immediately before use, by means of a dilution of the aluminum standard Solution (1 ppm Al) with thinner Solution of nitric acid in 100 mL volumetric flask.

Procedure: determine the absorbance of standard preparations and preparing sample by atomic absorption Spectrometry with graphite furnace (5.2.13.1.4) equipped with aluminum hollow-cathode lamp. Adjust

0 Wavelength at 309.3 nm using a resolution of the monochromator (0.7 ± 0.1) nm. Use the Solution diluent of nitric acid as white and carry out the calibration as described in (5.2.13.1.4) Method

1 (Direct Calibration). Determine the concentration of Al in preparing sample in ppm. Calculate the amount of Al in the sample in ppm, by means of multiplying the concentration of sample preparation in ppm per 100/P where P is the mass in grams of the substance used in sample preparation.

METHOD III

Proceed as described in Method II and log the determination by optical emission Spectrometry with

Inductively coupled plasma (5.2.13.2.2). Proceed in accordance with the manufacturer's specifications. It is recommended to use the wavelength of 396.153 nm.

5.3.2.11 LIMIT TEST FOR PHOSPHATES

Standard Solution of phosphate (5 ppm): dissolve 0.716 g of potassium phosphate monobasic in distilled water and make up the volume to 1000 mL. Transfer 10 mL of this solution to a 1000 mL volumetric flask and Fill up to volume with distilled water.

Sample Preparation: transfer the specified quantity of the sample, or calculated, or the volume of the sample solution prepared as described in the monograph for 100 mL volumetric flask and Fill up to volume with suitable solvent. Transfer this solution to becher and add 4 mL of reagent sulfomolibdico, 0.1 mL of stannous chloride SR and homogenize.

Preparation standard: transfer 2 mL of the standard Solution of phosphate (5 ppm) to 100 mL volumetric flask and Fill up to volume with suitable solvent. Continue as described in sample preparation.

Procedure: wait 10 minutes, transfer 20 mL of the contents of the sample and standard preparations for Nessler tubes (capacity of 50 mL and 22 mL of internal diameter) and compare the staining of preparations. The coloration produced in sample preparation is not more intense than the default.

Alternatively, proceed as described in ion Chromatography (5.2.17.4.1) using gas chromatograph equipped with ion exchange column for separation of anions and detector by conductivity with chemical suppression.

5.3.2.12 LIMIT TEST FOR LEAD

Lead diluted standard Solution (1 ppm Pb): dilute exactly measured volume of standard Solution of lead (10ppm Pb) prepared as described in limit Test for heavy metals (5.3.2.3) with 9 volumes of nitric acid at 1% (v/v).

Note: store all the solutions of reagents in containers of borosilicate glass. Rinse all glassware with nitric acid solution in 20% (v/v) and then with distilled water.

Sample Preparation: in the absence of specification in the monograph, prepare the sample solution as follows. Proceed cautiously, because some substances can react with violence when digested with hydrogen peroxide. Transfer 1 g of the sample to appropriate flask, add 5 mL of sulfuric acid, some glass beads and heat in heating plate, in chapel, even evolution of fumes. Can be used other means of adequate heating. If necessary, add excess of sulfuric acid to soak completely the sample not exceeding a total of 10 mL. Add, drop by drop, with careful, concentrated hydrogen peroxide, warming between additions, allowing the reaction to occur. Add the first drops to the few and very slowly mixing carefully to prevent fast reaction and stopping the heating if occur excessive formation of foam. Stir the solution in the flask to allow the reaction of sample adhered on the walls. Add hydrogen peroxide whenever the mixture become brown or dark. Continue the digestion until vapors of sulfur trioxide are Loosened abundantly for that the reaction is complete and the solution becomes colorless. Cool, cautiously, with the addition of 10 mL of distilled water, evaporate again until the sulfur trioxide pops out by complete and cool. Repeat this procedure with 10 mL of distilled water to remove any trace of hydrogen peroxide. Carefully dilute with 10 mL of distilled water and cool.

Note: if, before heating, the sample react very quickly and begin to smolder with 5 mL of sulfuric acid, you should

use 10 mL of sulfuric acid at 50% (v/v) cold and add a few drops of hydrogen peroxide before warming up.

Preparation standard: use specified volume, or calculated from the standard Solution of lead diluted (1 ppm Pb). Refer to the same treatment of sample preparation.

Procedure: transfer the sample preparations and default to a separating funnel using 10 mL of distilled water. Add 6 mL of ammonium citrate SR and 2 mL of hydroxylamine hydrochloride SR1 (for the determination of lead in iron salts use 10 mL of ammonium citrate SR). Add two drops of phenol red to 0.1% (p/v) in ethanol, make alkaline with ammonium hydroxide until red coloring and mix thoroughly. Cool the solution, if necessary, and add 2 mL of potassium cyanide SR. Remove immediately, with portions of 5 mL of solution extractor of dithizone and collect each extract to another separating funnel until the dithizone solution keep their green color. Shake the solution of dithizone combined for 30 seconds with 20 mL of nitric acid to 1% (v/v) and discard the organic phase. Add the acid solution,

5 Ml of the standard solution of dithizone and 4 mL of ammonia cyanide- SR, and shake for 30 seconds. The violet color produced in the organic phase of sample preparation is not more intense than the default.

Alternatively, prepare the sample as described in limit Test for heavy metals (5.3.2.3) and determine lead by one of the techniques of atomic Spectrometry (2.5.13).

5.3.3 CHEMICAL TESTS

5.3.3.1 TITRATIONS BY DIAZOTIZATION

This type of titration is very useful in the analysis of drugs that contain primary aromatic amino group such as the sulfonamides and local anesthetics acid derivatives amino benzoic acid. The titration is performed with volumetric solution of sodium nitrite in acidic medium, providing the diazonium salt of primary aromatic amine.

Are used two methods of quantification.

In Method I, is used starch solution iodated or role of starch iodated as indicator. The excess of nitrous acid converts the iodide to iodine, which in contact with the starch results the characteristic blue color.

In Method II, the end point of the titration is determined potentiometrically. In this method, employ-if platinum electrodes calomel or platinum-platinum with potential difference and adequate sensitivity. After use, it should be to immerse the electrodes for a few seconds in nitric acid SR to which is added 1 mg/mL of ferric chloride, and then rinse with distilled water.

METHOD I

Technique – Weigh, exactly, about 500 mg of sulfonamide or the amount specified in the monograph for other primary aromatic amines. Transfer to 250 mL erlenmeyer flask, and add, with stirring, 100 mL of hydrochloric acid SR for solubilizing the sample. Then, add about 30 mL of water and cool in an ice bath until approximately 15 °C. Holder, under constant stirring, with sodium nitrite solution 0.1 M SV previously standardized with sulphanilamide SQR. Reaches the end point of the titration when a drop of the solution of the erlenmeyer flask form, immediately, a blue coloration with a starch solution iodated SI In touch board or on paper starch iodated SI Dampened. To demonstrate the end of the titration, repeat the proof of touch 2 minutes after the last addition. This must remain positive.

The weight, in mg, of the sample corresponding to each mL of sodium nitrite 0.1 M SV is described in the monograph of each drug.

METHOD II

Technique – Weigh, exactly, about 500 mg of sulfonamide, or the equivalent in weight of the active principle

For the pharmaceutical specialities, or the amount specified in the monograph for other primary aromatic amines. In the case of injecting or other liquid forms should pipette-if quantity equivalent to 500 mg of active principle or the amount specified in the monograph. Transfer to erlenmeyer flask and add 20 mL of dilute hydrochloric acid SR and 165 mL of water. Shake until dissolved. Cool until approximately 15 °C maintaining that temperature in the course of the titration. Add converter suitable when specified. Titrate slowly and under magnetic stirring, with sodium nitrite 0.1 M SV previously standardized with sulphanilamide SQR.

The weight, in mg, the sample that is equivalent to each mL of sodium nitrite 0.1 M SV added is specified in the monograph of each drug.

Note: the tip of the buret should remain slightly above the surface of the solution in order to prevent the oxidation of sodium nitrite. You must shake carefully avoiding the formation of a swirl of air below the surface. When the titration is, approximately, 1 mL of end point calculated, add volumes of 0.1 mL at intervals of at least 1 minute.

5.3.3.2 DETERMINATION OF NITROGEN BY THE KJELDAHL METHOD

The Kjeldahl method described in the form of macro and semimicrotecnica is intended to determination of nitrogen in relatively labile substances as amides and amines. It consists of two stages: (1) catalytic digestion of organic substance in sulfuric acid with the resulting quantitative conversion of nitrogen in ammonium sulphate; (2)

distillation of the digesto alkalinized and volumetric titration of ammonia released in the process.

5.3.3.2.1 Macrodetermination (method I)

Transfer, exactly, about 1 g of sample to 500-mL Kjeldahl flask. Add 10 g of potassium sulphate, 0.5 g of cupric sulfate and 20 mL of sulfuric acid. Tilt the flask about 45o and heat, slowly, maintaining the temperature below the boiling point while there is development of foam. Increase the temperature to boiling lived of acid and continue with the grille for 30 minutes until the mixture becomes clear and acquire light green color. Cool, add 150 mL of water, mix and cool again. Carefully Add 100 mL of sodium hydroxide solution 40% (p/v) allowing the alkali spilling through the wall of the flask and formed independent phase under the acid solution. Add a small amount of granulated zinc; immediately, connect the flask to the bulb of insulation previously attached to the condenser, and immerse the hose

Pickup in 100 mL of boric acid solution 5% (w/v) in 500 mL erlenmeyer flask. Homogenize the mixture in the flask gently swirling and distil until collect into approximately 80% of the volume in the flask. Add about 3 drops of methyl red mixture with methylene blue SI to the erlenmeyer flask and titrate with 0.25 M sulfuric acid SV. Perform blank test and make the necessary corrections. Each mL of sulfuric acid 0.25 M SV is equivalent to 7.003 mg of nitrogen. For samples with low nitrogen content, employ sulfuric acid 0.05 M SV. In this case, each mL is equivalent to 1.401 mg of nitrogen.

In the presence of nitrates or nitrites

Transfer amount exactly weighed sample containing approximately 150 mg of nitrogen for 500-mL Kjeldahl flask and add 25 mL of dilute sulfuric acid containing 1 g of salicylic acid dissolved. Mix and wait for about 30 minutes, stirring frequently. Add 5 g of sodium thiosulphate, mix, then add 0.5 g of cupric sulfate. Proceed as indicated in previous procedure from “Tilt the flask about 45O... “.

When the content of nitrogen in the sample exceeds 10 %, add, previously the digestion, 0.5 to 1.0 g of benzoic acid to facilitate the decomposition of the substance.

5.3.3.2.2 Semimicrodetermination (method II)

Transfer amount exactly heavy substance corresponding to 2 – 3 mg of nitrogen for Kjeldahl flask compatible with the apparatus. Add 1 g of potassium sulphate and 0.1 g of cupric sulphate and, if necessary, wash the solids adhering to the neck with thin jet of water. Add 7 mL of sulfuric acid, and then 1 mL of hydrogen peroxide to 30% (v/v) so that the liquids rainwater run through the wall of the flask. Heat the flask and keep digestion until disappearance of waste carbonization and the preparation blue clear is perfectly clear. Carefully add 70 mL of water and cool. Connect the flask to the distillation apparatus, and through the funnel, add 30 mL of sodium hydroxide solution 40% (p/v) allowing the alkali spilling by the wall of the flask and formed independent phase under the acid solution. Rinse the funnel with water, and immediately begin to distillation. Collect the distillate in 250 mL erlenmeyer flask containing 15 mL of boric acid solution 5% (w/v), enough water to submerge the pickup tube and 3 drops of methyl red mixture with methylene blue. Distil until the distillate volume reaches 80 to 100 mL; remove the collector bottle, wash the walls with small amount of water and titrate with 0.005 M sulfuric acid SV Perform blank test and make the necessary corrections. Each mL of sulfuric acid 0.005 M SV is equivalent to 0.1401 mg of nitrogen.

5.3.3.3 METHOD OF COMBUSTION

The combustion methods consist in decomposition of organic substances in the presence of oxygen, through the oxidation of organic matter for the subsequent step of identification or determination. These methods can be applied in two ways: method of combustion flask at atmospheric pressure and combustion method initiated by microwave in pressurized system.

METHOD OF COMBUSTION FLASK AT ATMOSPHERIC PRESSURE

Apparatus

Comprises a conical flask of refractory resistant borosilicate glass, with internal volume of 500 mL and glass lid flipped. For determination of fluorine, employs-if bottle of quartz. The base of the cover flipped that accompanies the bottle features a prolongation of glass on which is fixed a platinum wire with end composed of a platinum support where it is introduced to the sample (Figure 1).

Solid Samples

Weigh the amount specified in the monograph on piece of filter paper format and appropriate dimensions, fold and attach on the screen of platinum, leaving free a part of the edge. Place inside the bottle the absorbing solution specified and bubbling oxygen in this solution to saturate the inside of the bottle. Do the ignition of the end of the paper (see Note 1) and, without delay, put the lid on the bottle, keeping it in position with firmness to prevent its displacement due to the pressure exerted by the flue gases. Invert the bottle to ensure seal in net cover, taking the precaution of avoiding that incompletely burned material falls in liquid. Complete combustion, shake the bottle until the gases formed in disappear. After 15 to 30 minutes, put small portion of water at the edge of the flask and remove the cover, allowing this water flow to the interior of the flask, washing the walls of the neck. Wash cover, neck, wire and network of platinum with water and add these waters for washing the absorbing solution. The solution obtained according to this procedure is called sample

solution. For the preparation of the white, proceed in the same way, omitting the sample (Note 2).

Liquid Samples

Lullabies small quantity of absorbent cotton in piece of filter paper and weigh, in this device, the specified quantity of the sample, that is absorbed in cotton. After fixing the cotton involved in filter paper the grid of platinum, proceed to combustion as described for solid samples.

Determination of chlorine and bromine

Burn the specified quantity of the substance under examination, employing as absorbing solution 20 mL water plus 1 mL of concentrated hydrogen peroxide and 3 mL of sodium hydroxide 0.1 M. Complete absorption, add 2 drops of bromophenol blue and sufficient quantity of nitric acid 0.1 M to turn the indicator from blue to yellow, incorporating 0.5 mL of excess. If the substance in analysis contains sulfur, add a few drops of barium nitrate 0.005 M. Add 100 mL of ethanol by leveraging the addition to wash the internal walls of the flask, and then 15 drops of diphenyl carbazone indicator. Holder with silver nitrate of mercury (II) 0, 005 M SV until pinky color permanent. Each mL of nitrate of mercury (II) 0.005 M SV is equivalent to 0.3550 mg of chlorine or 0.79904 mg of bromine.

Alternatively, proceed as described in ion Chromatography (5.2.17.4.1), using gas chromatograph equipped with anion exchange column and detector by conductivity with chemical suppression.

Determination of iodine

Burn the specified quantity of the substance under examination in the manner described, employing as absorbent liquid 10 mL water plus 2 mL of sodium hydroxide M. Complete absorption, add 1 mL of a solution of hydrazine hydrate 4 M in water, recap the bottle and shake until the bleaching solution. Then, proceed as described in Determination of chlorine and bromine from "Complete absorption... ". Each mL of nitrate of mercury (II) 0.005 M SV is equivalent to 1.269 mg of iodine.

Alternatively, proceed as described in ion Chromatography (5.2.17.4.1), using gas chromatograph equipped with ion exchange column for separation of anions and detector by conductivity with chemical suppression.

Determination of fluorine

Burn specified quantity of the substance under examination in the manner described, employing as absorbing solution 15 mL of water. The operation is Completed, rinse cover, platinum wire, screen of platinum and walls of the vial (Note 3) with 40 mL of water. Add 0.6 mL of alizarin, and then drop by drop, sodium hydroxide 0.1 M until the color changes from pink to yellow. Add 5 mL of acetate buffer solution pH 3.5 – hydrochloric acid and titrate with silver nitrate of thorium 0.005 M SV until the yellow color change to yellow Rosado. Each mL of thorium nitrate 0.005 M SV is equivalent to 0.380 mg of fluoride. There is difficulty in identifying the turning point, make preliminary test with standardized solution of inorganic fluorine.

Alternatively, proceed as described in ion Chromatography (5.2.17.4.1), using gas chromatograph equipped with ion exchange column for separation of anions and detector by conductivity with chemical suppression.

Determination of sulfur

Burn the specified quantity of the substance under examination in the manner described, employing as absorbing solution 12.5 mL of hydrogen peroxide SR. Complete absorption, add 40 mL of water, leveraging for washing cover, wire and screen of platinum and walls of the vial. Boil the solution for 10 minutes, cool, add 2 mL of acetic acid SR and 20 mL of ethanol. Titrate with 0.01 M barium nitrate SV, using 2 drops of torina SI and 2 drops of Methylthioninium chloride as indicator until the yellow color change to pink. Each mL of barium nitrate 0.01 M SV equals 0.3206 g of sulfur.

Alternatively, proceed as described in ion Chromatography (5.2.17.4.1), using gas chromatograph equipped with anion exchange column and detector by conductivity with chemical suppression.

Notes:

1. It is recommended that the analyst use safety goggles and adequate protection to prevent splinters of bottle the reach in the event of an accident. Currently, there are commercially systems that prevent the ignition manual, employing infrared radiation or electrical current, reducing the risk to the operator.

2. Make sure that the bottles of combustion are clean and free of traces of organic solvents.

3. Substances containing fluorine provide low levels if the combustion is performed in borosilicate glass bottles. Satisfactory results can be obtained in glass bottles-soda free of boron but the yield ideal implies the use of vials of quartz.

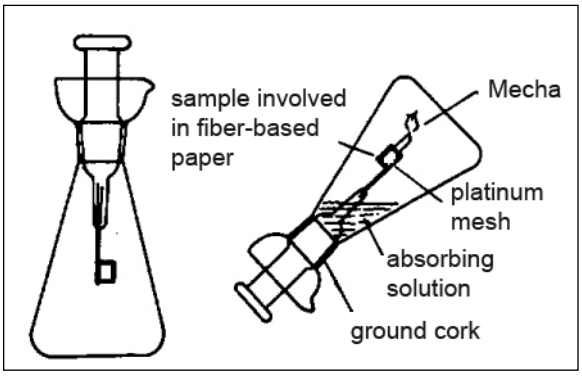

Figure 1 – Bottle of oxygen for determnacaoof sulfur and halogens.

METHOD OF COMBUSTION INITIATED BY MICROS IN PRESSURIZED SYSTEM

Apparatus

Comprises a quartz flask with internal volume of 80 mL and operating pressure of 80 atm. The cover that came with the bottle is of fluorinated polymer, which has a hole to release the gases in the case of the decomposition by track wet, which is used to pressurize

The system with oxygen. A support of quartz for the sample is inserted inside the bottle of decomposition.

Solid Samples

Weigh the specified quantity of substance and pinch in tablet form (approximately 1.2 cm in diameter). Place the sample on the support of quartz containing a piece of filter paper (approximately 10 mg) dampened and ammonium nitrate 6 M. Place inside the bottle the absorbing solution specified and insert the bracket containing the sample and the role inside the bottle. Close the system properly, as per manufacturer's specifications, and pressurize with 20 atm of oxygen. Proceed to the insertion of the bottles of decomposition in the microwave oven and, without delay, place the rotor in the cavity of the oven, starting immediately to irradiation. Started the ignition, using maximum power, irradiation can be continued for a further 5 min, for which the reflux of the absorbing solution happen, allowing complete absorption of analytes in solution. After cooling (20 min), the bottle of decomposition can be opened and the solution transferred to suitable container with the aid of water and measured the known volume, for subsequent identification or determination of the analytes of interest. The solution obtained according to this procedure is called sample solution. For the preparation of the white, proceed in the manner described, omitting the sample.

Determination of chlorine and bromine

Proceed as described in "Determination of chlorine and bromine" on Combustion Flask Method at Atmospheric Pressure.

Determination of iodine

Continue as described in "determination of Iodine" on Combustion Flask Method at Atmospheric Pressure.

Determination of fluorine

Continue as described in "Determination of Fluorine" on Combustion Flask Method at Atmospheric Pressure.

Determination of sulfur

Continue as described in "Determination of Sulfur" on Combustion Flask Method at Atmospheric Pressure.

5.3.3.4 TITULAÇÕES COMPLEXOMÉTRICAS

Complexometry is the analytical method which comprises volumetric titration of metal ions (A) with complexing agent (B). The reaction involved is of the following type:

$$A^{n+} + B^{m-} = AB^{n-m}$$

Many complexing called chelating agents are able to form cyclic structures by means of the simultaneous coordination of various groups with the metal ion. The glacial edetico (ethylenediaminetetraacetic acid, EDTA) is a typical example. This acid is the complexing agent used most often. The EDTA form 1:1 complexes with many metals with oxidation state exceeding +1 being such complexes very soluble in water.

The stability of the complexes with EDTA is dependent on the pH for the various metals. As soon As ideal conditions of pH should be established for the analysis by complexation for each metal.

In complexometry, the turning point can be determined visually or instrumentally. Employ- if complexing indicators that display profound changes of color through coordination with the metal. Typical Examples are: xylenol orange, shimon and negro of eriochrome black T indicator complexometrico acts so competitive with the agent titrant, soon must be moved effectively by this nearby of the equivalence point.

PROCEDURES

Aluminum

Weigh, exactly, the quantity of the substance indicated in the monograph, add 50 mL of water and acidify, if necessary, with minimal amount of hydrochloric acid M, except if the monograph indicate another type of solvent. Add 25 mL of disodium edetate 0.1 M SV and 10 mL of the mixture in equal volumes, ammonium acetate 2 M with acetic acid 2 M. Heat the solution to boiling and keep for 2 minutes. Cool. Add 50 mL of ethanol and 3 mL of freshly prepared solution of dithizone 0.025% (p/v) in ethanol. Titrate the excess of edetate disodium with zinc sulphate 0.1 M SV until color change from blue-green to purple-pink. Each mL of disodium edetate 0.1 M SV is equivalent to 2.698 mg of aluminum.

Bismuth

Weigh, exactly, the quantity of the substance indicated in the monograph and dissolve in minimum quantity of nitric acid 2 M. Add 50 mL of water and concentrated solution of ammonia, dropwise with stirring, until the preparation becomes blurred. Add 0.5 mL of nitric acid. Warm up to 70°C until the disappearance of the turbidity of the preparation. Add a few drops of xylenol orange. Holder, slowly, with edetate disodium 0.05 M SV until color change from violet-pink to yellow. Each mL of disodium edetate 0.05 M SV is equivalent to 10.45 mg of bismuth.

Calcium

Weigh, exactly, the quantity of the substance indicated in the monograph, dissolve in a few milliliters of water and

Acidify with, if necessary, minimum quantity of hydrochloric acid 2 M. Dilute to 100 mL with water. Titrate with 0.05 M disodium edetate SV until approximately 2 mL before the equivalence point provided. Add 4 mL of 10 M sodium hydroxide and drops of shimon HIMSELF. Continue the titration until the color changes from pink to blue intense. Each mL of disodium edetate 0.05 M SV is equivalent to 2.004 mg of calcium.

Lead

Weigh, exactly, the quantity of the substance indicated in the monograph and dissolve in 5 to 10 mL of water, or at minimum quantity of acetic acid 5 M. Dilute to 165 mL with water. Add drops of xylenol orange SI and methenamine enough (approximately 5 g) for solution to purchase violet color. Titrate with edetate disodium SV, 0.05 M or 0.1 M SV, as indicated in the monograph until color change from violet to yellow. Each mL of disodium edetate 0.05 M SV is equivalent to 10.36 mg of lead. Each mL of disodium edetate 0.1 M SV is equivalent to 20.72 mg of lead.

Magnesium

Weigh, exactly, the quantity of the substance indicated in the monograph and dissolve in 5 to 10 mL of water, or at minimum quantity of hydrochloric acid 2 M. Dilute with water to 165 mL. Add 10 mL of ammonium chloride buffer pH 10.0, and some drops of eriochrome black-T. Titrate with edetate disodium 0.05 M or 0.1 M SV SV, as indicated in the monograph, until color change from violet to blue. Each mL of disodium edetate 0.05 M SV is equivalent to 1.215 mg of magnesium. Each mL of disodium edetate 0.1 M SV is equivalent to 2.431 mg of magnesium.

Zinc

Weigh, exactly, the quantity of the substance indicated in the monograph and dissolve in 5 to 10 mL of water, or at minimum quantity of acetic acid 5 M. Dilute to 165 mL with water. Add drops of xylenol orange SI and methenamine enough (approximately 5 g) for solution to purchase violet color. Titrate with edetate disodium 0.05 M or 0.1 M SV SV, as indicated in the monograph, until color change from violet to yellow. Each mL of disodium edetate 0.05 M SV is equivalent to 3.268 mg of zinc. Each mL of disodium edetate 0.1 M SV is equivalent to 6.536 mg of zinc.

5.3.3.5 TITRATIONS IN NON-AQUEOUS MEDIUM

The drugs that are weak acids or bases cannot be quantified in aqueous medium, but can be in a non-aqueous medium.

The titration in a non-aqueous medium is based on the concept of Bronsted-Lowry acid / base, in which the acid is a substance that donates proton and the base is one that receives Proton. Potentially acidic acidic Substances are only in the presence of base to which they can donate proton and vice-versa.

The solvent plays, therefore, very important role in determining the character of an acid / basic substance, since the strength of the acid or base depends on the ability of the solvent to receive or donate protons. The water should be the solvent of choice, due to the easy availability. However, the strongest acid that can exist in aqueous medium is the hidronio ion ($H3O^{+}$) and a stronger base the hydroxide ion (OH^{-}), this is known as leveling effect of solvent. In the determination of acids or bases very weak, the titrant must be a very strong acid or base, respectively, so that the acid-base reaction occurs; however due to the effect of water level is not possible holder such substances in aqueous medium.

Using solvents weakly protofilicos as the acetic acid, it is possible the titration of very weak bases already that the ion acetonio ($CH_3COOH_2^{+}$) is a much stronger acid than the ion hidronio. Acids stronger than the ion hidronio cannot be differentiated in aqueous medium, but can in acetic acid showing that the descending order for the strength of acids is perchloric acid, hydrobromic acid, sulfuric acid, hydrochloric acid and nitric. Similarly, the titration of weak acids is possible with the use of solvents such as n-basic butilamina. The amideto ($CH_3CH_2CH_2CH_2NH^{-}$) is a base much stronger than the hydroxide.

The solvents used in titration in a non-aqueous medium must meet certain requirements: (1) did not react with the substance nor with the titrant; (2) dissolve the substance allowing, at the very least, preparation of solution 0.01 M;

(3) Dissolve the product of titration – if the precipitation is inevitable, the precipitate must be compact and crystalline;

(4) Allow, with ease, the visualization of the end point, this is measured with the use of indicators or potentiometer; (5) be of low cost and easy purification.

For the titration of substances of character (basic heterocyclic amines, nitrogen, quaternary ammonium compounds, alkali salts of organic and inorganic acids and some salts of amines) employ-if solvents of nature relatively neutral or acid, glacial acetic acid being the most used. The acetic anhydride reserves for very weak bases as amides. The mixture of dioxana with acetic acid may occasionally be used to reduce the dielectric constant and consequently less potential for ionization of acids favoring the neutralization reaction. As titrant employs-if, generally, the solution of perchloric acid in acetic acid. Other titulantes useful are perchloric acid in dioxana; acid p-toluenossulfonico (glacial tosico) and glacial fluorsulfonico are generally used with aproticos solvents such as chloroform.

For the determination of salts of fatty halogenated (hydrochloride, hydrobromide and iodidrato) must add-if acetate of mercury; this is not dissociates in solution of acetic acid. The halide ion is a base too weak to react quantitatively with perchloric acid in acetic acid. This ion can be replaced, quantitatively, by ion

Acetate being removed in the form of mercuric complex that cannot be disentangled. The acetate, which is a relatively strong base in acetic acid, can be precisely titrated with perchloric acid.

For the titration of substances that behave as acids (halide acids, acid anhydrides, carboxylic acids, amino acids, enois, imides, phenols, pirrois and sulfonamides) employ as solvent of basic nature or aprotica. In the determination of substances of intermediate acidity is common the use of dimethylformamide. Already in the determination of weak acids employ bases stronger as morpholine, ethylenediamine and n-butilamina. The basic solvent selected, properly, can allow the selective determination of mixture of acids. Two classes of titulantes may be employed for the determination of acidic substances: the alkaline-metal alkoxides, and hydroxides dimonium quaternary. The metoxido of sodium is the most widely used of-metal alkoxides, in a mixture of methanol and toluene or methanol and benzene. The metoxido of lithium in methanol and benzene is used for compounds that form precipitate gelatinous metoxido titrations with sodium. The most widely used among the hydroxides is the tetrabutylammonium. With the quaternary ammonium hydroxides such as tetrabutylammonium hydroxides and trimetilexadecilamonio (in a mixture of benzene and methanol or isopropyl alcohol) there is the advantage that the salt of acid titrated is, in general, soluble in titration.

It is important to protect the solvents for titration of acid substances from excessive exposure to the atmosphere due to the interference of CO2. For this reason, you can employ inert atmosphere or special apparatus during titration. To determine the absorption of CO2 must be the titration of the blank that should not consume more than 0.01 mL of 0.1 M sodium metoxido SV per milliliter of solvent.

The end point of the assay can be determined visually by change of color or potentiometrically. Usually the choice of method is based on pKa of analytes in water. For databases with the pKa of the order of 4, the detection is, in general, by means of indicators; for that the pKa is between 1 and 4, the detection is potentiometric. In this case, the electrode glass / calomel is useful. In acetic acid, such electrode operates in accordance with the predicted theoretically. In the case of calomel electrode as reference, it is advantageous to replace saline bridge of potassium chloride aqueous by lithium perchlorate 0.1 M in glacial acetic acid for titration in solvents acids or by potassium chloride in methanol for titration in basic solvents. The determination of the end point in the quantification of fatty acid whose pKa in water is around 7 can be done with the use of an indicator. For acids with pKa between 7 and 11 it is recommended that potentiometric determination, even that in certain cases for indicators, such as violet azo or the-nitroanilina with less accuracy.

With the use of organic solvents, you should consider the high coefficient of cubical expansion of the majority of those in relation to the water. This is because there is no possibility to occur variation of the content of the titrant in a non-aqueous medium as a function of temperature. You must correct the volume of titrant, by multiplying it by the correction factor below:

$[1 + \text{coefficient of cubical expansion of the solvent } (t_0 - t)]$

Whereas:

t_0 = temperature of standardization of titrant,
t = temperature of use of titrant.

The systems most often used for titration in a non-aqueous medium are listed in Table 1.

Table 1 – Systems for titration in a non-aqueous medium.

Solvent type	*Solvente*[a]	*Indicator*	*Electrodes*
Acid (for titration of bases or salts)	Glacial acetic acid formic acid propionic acid acetic anhydride chloride sulfonila	Alfazurina 2 g metilrosanilin cloret p-naphtolbenzein green malachite red quinaldine	Mercury / mercury acetate Glass / calomel Glass / silver / silver chloride
Relatively neutral (for differential titration of bases)	Ethyl acetate Acetonitrile Alcohols Benzene Chlorobenzene Chloroform Dioxana N-butilamina	P-naphtolbenzein Red methyl methyl orange	Calomel / silver / silver chloride Glass / calomel
Basic (for titration of acids)	Dimethylformamide Ethylenediamine Morpholine	P-the-hidroxiazobenzene nitroanilin thymolphthalein violet azo	Calomel antimony antimony / glass / glass / platinum / calomel calomel
Relatively neutral (for differential titration of acids)	Acetone acetonitrile alcohol tert-butyl 2-butanone isopropilacetone	Bromothymol blue thymol blue p-hidroxyazobenzen violet azo	Antimony / calomel calomel glass / glass / platinab

a) Solvents relatively neutral low dielectric constant, such as benzene, chloroform, or dioxana, can be used along with any solvent acid or base in order to increase the sensitivity of turning points of the titration.

b) The titrant.

Titration of basic substances

Dissolve quantity of substance indicated in the monograph on specified quantity of solvent, or mixture of suitable solvents. In the case of the titration of salts of fatty halogenated, you must add 10 mL of acetate of mercury SR. Add the appropriate indicator, or in the case of potentiometric determination, employ appropriate electrode by titrating with perchloric acid 0.1 M SV in acetic acid. For completion of the blank test, proceed as described by omitting the sample.

If t_0 is different from t correct the volume by:

$$[1 + 0,0011(t_0 - t)]$$

Whereas:

T_0 = temperature at which the titrant was standardized,
t = temperature at which the titration was performed.

Titration of acid substances

Method I – Dissolve quantity of substance specified in the monograph in solvent or mixture of solvents indicated. Add the indicator recommended or, if this is the case, use the appropriate electrode potentiometric determination. Titrate with 0.1 M sodium metoxido SV previously standardized with benzoic acid. Avoid the absorption of carbon dioxide. Log titration in preparation in white. Make the necessary corrections.

Method II – Dissolve quantity of substance indicated in the monograph in solvent or mixture of solvents indicated.

Titrate with tetrabutylammonium hydroxide 0.1 M SV using buret fitted with absorber of carbon dioxide. Determine the end-point potentiometrically. Log titration in preparation in white. Make the necessary corrections.

5.3.3.6 DETERMINATION OF METHOXYL

The technique is intended for the determination of methoxyl groups in organic substances by reaction with hydriodic acid concentrate. The methyl iodide formed is separated by distillation under continuous current of nitrogen or carbon dioxide, flushed and absorbed in solution bromine / acetous. The methyl iodide is converted to iodine, and then titrated with sodium thiosulphate.

APPARATUS

The tool (Figure 1) used in the determination of the methoxyl consists of round-bottom flask with a capacity of 50 mL to which is welded a lateral arm capillary with 1 mm internal diameter for the influx of inert gas drag – nitrogen

or carbon dioxide. The balloon connects with joints grind off material a condenser vertical of 24 cm height and 12 mm external diameter in whose top is displayed curved tube whose capillary end with 3 mm diameter is immersed in wash bottle. The output of the washer pipe consists of approximately 10 mm in diameter that ends in removable pipe diameter of 6 mm immersed in liquid absorbent.

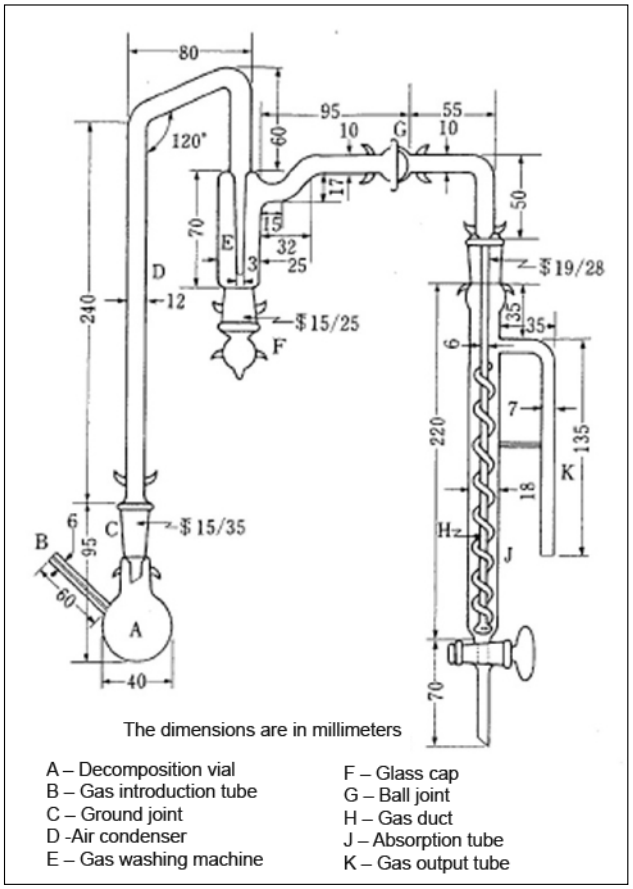

Figure 1 – Apparatus employed in the determination of the methoxyl.

PROCEDURE

Preparation washer: suspend 1 g of red phosphorus in 100 mL of water.

Liquid absorbent: dissolve 15 g of potassium acetate in 150 mL of a mixture of glacial acetic acid and acetic anhydride (9:1). The 145 mL of this solution add 5 mL of bromine. Prepare immediately before use.

Add preparation washer enough to cover half of the gas scrubber. Add 20 mL of the liquid absorbent to absorption tube. Add to the flask sample quantity corresponding to 6.5 mg of methoxy or the quantity indicated in the monograph. Add glass beads and 6 mL of hydriodic acid. Soak the gasket lapped with hydriodic acid and connect to condenser air. Connect the two parts of the apparatus by seal ball using silicone grease to seal. Adjust the influx of gas through the tube B enough to formation of two bubbles per second in gas scrubber E. Heat up gradually by 20 – 30 minutes the balloon up to 150 °C and keep the grille at this temperature for 60 minutes. After cooling the flask until ambient temperature under gas flow, pour the preparation contained in absorption tube in Erlenmeyer flask with a capacity of 500 mL provided with lid flipped containing 10 mL of aqueous solution of sodium acetate trihydrate (1:5). Wash the walls of the tube with water, transfer the wash water to the Erlenmeyer flask and dilute to 200 mL with water. Add formic acid dropwise with stirring until the disappearance of the reddish color of bromine and add more 1 mL of formic acid. Add 3 g of potassium iodide and 15 mL of 1 M sulfuric acid; cap, shake gently and leave to rest for 5 minutes. Titrate the iodine released with sodium thiosulphate 0,1 M SV employing starch as an indicator. Perform titration in preparation blank proceeding in the way described by omitting the sample and make correction if necessary. Each mL of $Na_2S_2O_3$ 0.1 M SV is equivalent to 0.5172 mg of methoxy (CH_3O).

5.3.3.7 DETERMINATION OF SULFUR DIOXIDE

The method comprises the drag of SO2 released on heating the substance in aqueous medium acidified by chain of carbon dioxide followed by the absorption of SO2 in solution of hydrogen peroxide. The sulfuric acid formed in the process is titrated with sodium hydroxide standardized.

APPARATUS

The tool (Figure 1) used in the determination of sulfur dioxide consists of round bottom flask with three pipes with capacity from 1000 to l500 mL. One of The side outlets of the balloon attaches device designed to influx of carbon dioxide. Funnel to add capacity of 100 mL and reflux condenser vertical both provided with gaskets grind off material are coupled to another side outlet and the outlet center, respectively. At the upper end of the condenser is connected the absorption tube D.

PROCEDURE

Transfer to the flask of about 300 mL of water, attach the flask to apparatus and promote slow and uniform influx of carbon dioxide during 15 minutes. Add to absorption tube 20 mL of hydrogen peroxide 3% (p/v) SR previously neutralised with sodium hydroxide 0.1 M using as indicator bromophenol blue. Without interrupting the influx of gas, momentarily remove the funnel, add to the flask, exactly, about 50 g of the sample and 200 mL of water. Add, drop by drop, 50 mL of 6 M hydrochloric acid by funnel and keep at reflux for 45 minutes. Transfer, quantitatively, by washing with water, the liquid contained in absorption tube for 250 mL Erlenmeyer flask and titrate with sodium hydroxide 0.1 M SV employing as indicator bromophenol blue. Perform titration in preparation blank proceeding in the way described by omitting the sample and make correction if necessary. Each mL of 0,1 M sodium hydroxide VS is equivalent to 3.203 mg of sulfur dioxide.

5.3.3.8 DETERMINATION OF ALCOHOL

5.3.3.8.1 Method by distillation

This method should be used in the determination of alcohol in solution containing alcohol, the less that the monograph is specified another method. It is suitable for analysis of the majority of fluid extracts and tinctures.

PROCEDURE

Note

Must be used balloon distiller with capacity from two to four, times, the volume of liquid to be heated.

During all manipulations, take precautions to minimize the loss of alcohol by evaporation.

To prevent the occurrence of violent boiling, add fragments of insoluble material and porous, such as silicon carbonate or glass beads.

The liquids that form too much foam during the distillation should be previously treated with phosphoric acid, sulfuric acid or tannic, until reaction strongly acidic or with slight excess of calcium chloride solution, or small amount of paraffin oil or even vinyl polysiloxane, before starting the distillation.

The distillation rate must be such that it allows the production of distillates speackers. Distillates syrupy should be sharpened by stirring with talc or with calcium carbonate, precipitated and filtered. Adjust the temperature of the filtrate and determine the alcohol content by density.

Method 1

Liquids with less than 30% of alcohol – Transfer to apparatus distiller appropriate, by means of pipette, sample of at least 35 mL of the liquid in which it is being given the alcohol content, record the temperature at which the volume was measured. Add an equal amount of distilled water, and collect a volume of distillate that is approximately 2 mL less than the initial volume of the sample. Adjust the temperature of the distillate that in which it was measured the sample and add enough water to get the initial volume of the sample and mix well. The distillate should be clear or, at most, slightly cloudy. Determine the density of the liquid at 20 ºC. With the result, evaluating the percentage, by volume, of C2H5OH contained in liquid examined, by Tables.

Method 2

Liquids with more than 30% of alcohol – Proceed as indicated in the previous method, with the following modification: dilute the sample with water volume two times higher and

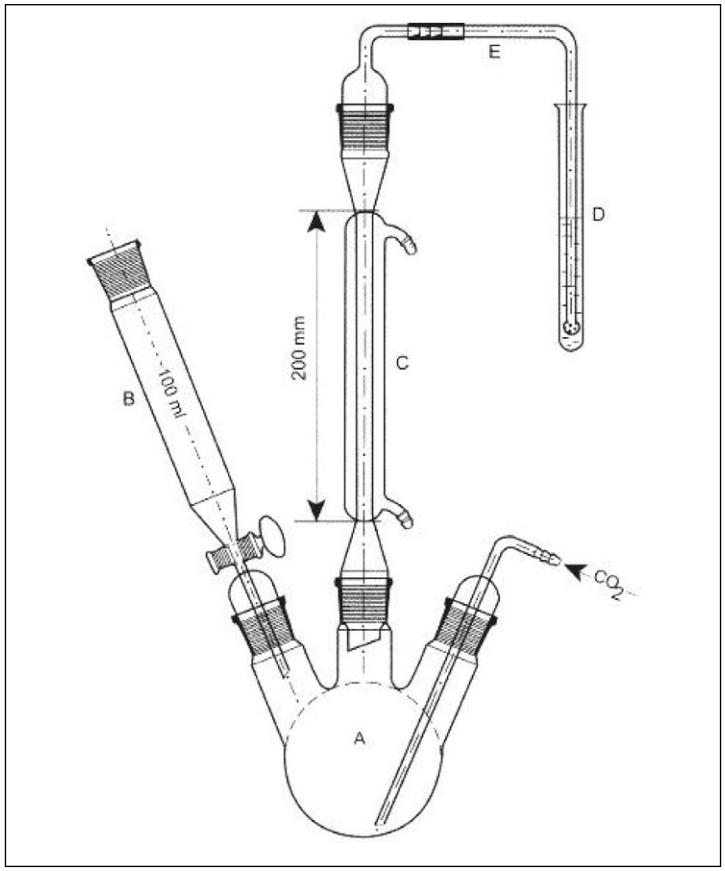

Figure 1 – Apparatus employed in the determination of sulfur dioxide.

Collect distillate volume approximately 2 mL less than two times the initial volume of the sample. Adjust the temperature of the distillate that in which it was measured the sample and complete with water volume equal to twice the volume of the initial sample. Mix and determine the density at 2.0C. The proportion of C2H5OH, in volume, in this distillate, assessed by optical density, is equal to half that of the liquid being examined.

Special Treatments

Acids and Bases – Volatile Liquids containing volatile bases should be treated with dilute sulfuric acid SR, until reaction slightly acidic. If they are present volatile acids, the preparation should be added sodium hydroxide SR until slightly alkaline reaction.

Glycerol – Liquids containing glycerol should be added to such volume of water that the residue, after distillation, contains, at least, 50% of water.

Iodine – Solutions containing free iodine should be treated before distillation with zinc sprayed or membrane pallor with sufficient quantity of sodium thiosulphate solution 10% (p/v) followed by the addition of a few drops of sodium hydroxide SR.

Other volatile substances – Elixirs, tinctures and similar preparations that contain appreciable proportions of volatile substances, in addition to alcohol and water, such as: volatile oils, chloroform, ether, camphor etc., must suffer before distillation, one of the treatments to follow.

1) Liquids with less than 50% of alcohol – Mix the sample to 35 mL, exactly measured, with equal volume of water, in separating funnel, saturating this mixture with sodium chloride. Remove the volatile components, shaking with portion of 25 mL of hexane. Transfer the bottom layer to a second separating funnel and repeat the extraction with two more portions of hexane. Gather the portions of hexane and deal with 3 portions of 10 mL of saturated solution of sodium chloride. Gather solutions salinas and distil collecting distillate volume corresponding to twice the volume of the initial sample.

2) Liquids with more than 50% of alcohol – Measure a sample and dilute with water so that contains approximately 25% of alcohol and that his final volume is approximately 35 mL. Then proceed as indicated for liquids with less than 50% of alcohol, continuing from "saturating this mixture with sodium chloride".

In the preparation of collodion for distillation, using water in place of saturated solution of sodium chloride, previously indicated. If it was not employed in the sample treatment with hexane and the distillate obtained is cloudy (due to the presence of volatile oils present in small proportions), it can be clarified and appropriate for the determination of density, by stirring with approximately 1/5 of its volume of hexane or by filtration through thin layer of talc.

5.3.3.8.2 Method by gas chromatography

Proceed in accordance with the general specifications for gas Chromatography (5.2.17.5). Use efficient tool for quantitative determination of alcohol.

Standard Solution

For liquids containing more than 10% of alcohol, prepare two standard solutions of alcohol in water, so that the concentrations are, respectively, about 5% below standard solution (1) and approximately 5% above (standard solution 2) the alcohol concentration expected in the sample under analysis. Determine the density of each of the standard solutions at 20 ºC (5.2.5) and obtain the exact concentration of C2H5OH by Tables. For liquids containing less than 10% of alcohol, prepare, exactly, two standard solutions of alcohol, so that the concentrations are, respectively, approximately 1% lower and about 1% higher than the concentration, as expected, by diluting with water. Determine the densities of the solutions in the same way as the previous ones.

EQUIPMENT

Under typical conditions, the instrument contains a column of 2 m x 4 mm loaded with macrogol (polyethylene glycol) 1300 to 20% in chromatographic silica calcined. The column is maintained at a temperature of 100 ºC; the injector is equipped with filter for solids and is kept at 160 ºC; as driver uses-if inert gas, such as helium, flowing with flow rate of approximately 60 mL per minute.

PROCEDURE

Proceed with the sample and each of the standard solutions as follows: transfer 25 mL to suitable container stopper lapped, add 1.0 mL of the internal standard (acetone, unless specified differently in the monograph) for each 6% of estimated alcohol in the sample and mix. Add water only if necessary to make the solution. Inject appropriate quantity, in the solution, apparatus. Calculate the ratio between the area under the peak of the alcohol and the area under the peak of the internal standard in the chromatograms. Calculate the percentage of alcohol in the sample by the formula

$$\frac{P_1 (Y - Z + P_2 (Z - X)}{(Z - X)}$$

Whereas

P_1 = percentage of alcohol in standard solution 1,
P_2 = percentage of alcohol in standard solution 2,
X = the ratio between the area under the peak of the alcohol and the area under the peak of the internal standard solution of standard 1,
Y = The ratio between the area under the peak of the alcohol and the area under the peak of the internal standard in the standard solution 2

Z = the ratio between the area under the peak of the alcohol and the area under the peak of the internal standard in the Sample solution.

If the obtained value is outside the range of values included by standard solutions, repeat the procedure using those that provide a range that includes the value of the sample.

5.3.3.9 ANALYSIS OF AMINO ACIDS

The amino acid analysis is performed by means of two steps: hydrolysis of peptide links and evaluation of each amino acid in the hydrolysate resulting from.

Technique for hydrolysis of proteins and peptides isolated

1) Transfer amount of sample containing 4 to 10 mg of protein for test tube of 20 x 150 mm with screw cap and disc of polytetrafluoroethylene previously rinsed with 0.2 M sodium hydroxide, rinsed and dried in an oven.

2) If the sample is solid, add 5 of hydrochloric acid and 5 mL of water. If the sample is liquid, add hydrochloric acid so that the final concentration of hydrochloric acid is 6 M.

3) Remove the oxygen tube by nitrogen flow during 2 – 3 minutes. Close then the tube with disc and screw on cap.

4) Place the tube in a vertical position in oven controlled the 110 °C ± 2°C keeping it for 22 h.

5) Remove the tube from the oven and still vertically, cools it under running water or ice bath.

6) Transfer, quantitatively, the contents of the tube to 10-mL volumetric flask and Fill up to volume with distilled water.

7) If there is any residue or precipitate, remove it by centrifugation and filtration in plate of sintered glass, or membrane filter of 0.45 |am of porosity.

8) Pipette 5.0 mL of the solution, transfer to round-bottom flask and remove the solvent at reduced pressure to, at most, 50 °C.

9) Add the residue in the flask 10 mL of distilled water and re-evaporate. This operation should be repeated two more times, or until the residue does not present odor of hydrochloric acid.

10) Redissolve the dry film formed by hydrolysate in appropriate volume of citrate buffer pH 2.2 (0.20 M in Na+). The resulting solution of amino acids must then be kept in glass bottle, plugged and under refrigeration until the completion of the analysis.

Technique for hydrolysis of samples with low protein content containing carbohydrates and/or lipids

1) Transfer amount of sample containing 10 mg of protein for 150 mL flask round bottom and mouth lapped.

2) Add to medium 40 mL of 6 M hydrochloric acid and some glass beads.

3) Connect reflux condenser and start heating the flask using electric blanket. Keep the suspension under constant boiling and smooth for 24 h.

4) Cool to room temperature and transfer, quantitatively, the content for 50 mL volumetric flask by supplementing the volume with distilled water.

5) Follow the remaining steps as items 7 to 10 of the previous technique.

Mixtures of amino acids in solution (sera) or in pharmaceutical preparations

1) Dilute, properly, the solution with citrate buffer pH 2.2 (0.20 M in Na+) may be analyzed then.

2) If you are in the form of powder, or compressed, solubilizing the sample in hydrochloric acid 0.1 M.

3) Transfer the material to volumetric flask and Fill up to volume with the same buffer above.

4) Filter the solution and keep under refrigeration (4 ºC) to be analyzed.

Technique of hydrolysis with oxidation of cystine and methionine

Due to losses during the acid hydrolysis of proteins, the sulfur amino acids are preferably analyzed by means of respective derivatives oxidised. The oxidation is promoted by performic acid, which converts cystine and cysteine in cysteic acid and methionine in methionine / sulphone, both resistant to conditions of hydrolysis.

1) Prepare the performic acid by adding 1 mL of hydrogen peroxide 30 volumes to 9 mL of formic acid.

2) Gently Swirl the solution to boil then rest for 1 h at room temperature.

3) Cool the performic acid formed in an ice bath.

4) Weigh the sample containing 10 mg of protein in 25 mL round flask and add 2 mL of performic acid ice cream.

5) Keep the mixture in an ice bath during 4 h, if the sample is soluble, or 16 h, if insoluble.

6) Add 0.5 mL of 40% hydrobromic acid to remove The Excess of performic acid.

7) Attach the flask to the rotary evaporator and remove by means of pressure reduced the residual bromine vapors pass by making the solution of sodium hydroxide M.

8) Proceed to hydrolysis as described previously.

Separation and quantitative analysis of amino acids isolated

The separation of amino acids in hydrolysates is normally carried out by ion-exchange chromatography by means of sulfonated polystyrene resins in amino acid analyzers. These apparatus, after the separation, the amino acids 28-32 of chromatography columns form substances of coloring blue/violet by reaction with ninhydrin. The quantitative determination is made spectrophotometrically. The use of autoanalisadores of amino acids, must be followed by the specifications of the respective manufacturers.

5.3.3.10 TEST IODOMÉTRICO OF ANTIBIOTICS

This iodometric test antibiotic is intended for the determination of drugs antibiotics penicilâmicos and of its pharmaceutical products developed, for which the iodometric titration is particularly suitable.

Standard Preparation

Dissolve adequate amount, exactly heavy, reference chemical (SQR), previously desiccated, specified in individual monograph, using the solvent described in Table 1 or as described in the monograph. Dilute, quantitatively, with the same solvent, so as to obtain solution with final concentration known, specified in Table 1 or as described in the monograph. Transfer 2.0 mL of this solution to 250 mL erlenmeyer flask with cover.

Sample Preparation

Unless otherwise specified in individual monograph, dissolve adequate amount, exactly heavy, of the sample, using the solvent described in Table 1 . Dilute, quantitatively, with the same solvent, so as to obtain solution with final concentration known, specified in Table 1. Transfer 2.0 mL of this solution to 250 mL erlenmeyer flask with cover.

PROCEDURE

Inactivation and titration

To each flask containing, respectively, 2.0 mL of standard preparations and sample, add 2 mL of sodium hydroxide 1.0 M, mix with circular movements and leave to rest for 15 minutes. Add 2 Ml of 1.2 M hydrochloric acid, 20.0 mL of 0.005 M iodine SV, cap immediately and leave to rest for 15 minutes. Titrate with sodium thiosulphate 0.01 M SV Near the end point of the titration, add 3 drops of starch and continue with the titration until disappearance of the blue color.

Blank Test

Add 20.0 mL of 0.005 M iodine SV each erlenmeyer flask containing 2.0 mL of standard preparation. If the preparation contains standard amoxicillin or ampicillin, immediately add 0.1 mL of hydrochloric acid 1.2 M. Holder with sodium thiosulphate 0.01 M SV. Near the end point of the titration, add 3 drops of starch and continue

Table 1 – Solvents and final concentrations.

Antibiotic	Solvent *	Final Concentration
Amoxicillin tri-idratada	Water	2.00 MG/mL
Ampicillin	Water	2.50 MG/mL
Ampicillin sodium	Solution 1	2.50 MG/mL
Ampicillin tri-idratada	Water	2.50 MG/mL
Benzathine Benzylpenicillin	Solution 1	4000 U/mL
Potassium Benzilpenicilin	Solution 1	4000 U/mL
Benzylpenicillin procaine	Solution 1	4000 U/mL
Benzylpenicillin sodium	Solution 1	4000 U/mL
Sodium Cloxacillin	Water	2.50 MG/mL
Ciclacilina	Water	2.00 MG/mL
Dicloxacillin sodium	Solution 1	2.50 MG/mL
Phenoxymethylpenicillin potassium	Solution 1	2.50 MG/mL
Feneticilina potassium	Solution 1	2.50 MG/mL
Methicillin sodium	Solution 1	2.50 MG/mL
Nafcillin sodium	Solution 1	2.50 MG/mL
Oxacillin sodium	Solution 1	2.50 MG/mL

* Unless otherwise specified, the Solution 1 is the one defined in section Solutions in microbiological Testing of antibiotics (5.5.3.3), except that the sterilization is not required.

with the titration until disappearance of the blue color. Proceed in a similar way to erlenmeyer flask containing 2.0 mL of sample preparation.

Calculations

Calculate the equivalence factor (F), in micrograms or Unit, for each milliliter of sodium thiosulphate 0.01 M SV consumed by preparing standard according to the equation:

$$F = \frac{2(C_P \, x \, P_P)}{V_b - V_i}$$

Whereas

Cp = concentration in mg/mL, of the chemical substance in the preparation of reference standard;
Pp = power, in |ag/mg or Units/mg, reference chemical;
V_b = volume of titrant, in mL, consumed in Testing
V_i = volume of titrant, in mL, consumed in Inactivation and titration.

5.4 METHODS OF PHARMACOGNOSY

5.4.1 VISUAL EXAMINATION AND INSPECT MICROSCÓPICA

5.4.1.1 VISUAL EXAMINATION, ODOUR AND TASTE

The identity, purity and quality of a plant material must be established through detailed visual examination, both macroscopic and microscopic. Whenever possible, the plant material must be compared with raw material authentic, come from sample perfectly identified in the Pharmacopoeia. The sample that is not similar in color, consistency, odor and flavor should be discarded for not presenting the minimum requirements specified in the monographs. The macroscopic identification of drugs, when whole, is based on order size; color; texture; fracture surface; and the surface appearance of fracture. By virtue of these characteristics of identification are subjective and there are very similar, it is adulterated NECESSARY to carry out, at the same time, analyzes and microscopic physico-chemistry of the sample. The microscopic inspection is indispensable when the material is strikethrough or powder.

Size

Measurements of length, width and thickness must coincide with those cited in the monographs. Fruits and seeds require a small sample equal to ten units and subsequent calculations of the mean and the standard deviation.

Color

Examine the raw material before any treatment, the light of day or under bulbs of wavelength similar to the light of day. The color of the sample should be compared with the reference material.

Surface, texture and fracture

Examine the raw material before any treatment. When necessary, use lens of five up to ten increases. When indicated in the monograph, moisten with water or reagent specified to observe characteristics of the surface of the fracture. Touching the material to check whether it is soft or hard, bend and from the material for obtaining information about the fragility and appearance of the fracture, it is fibrous, smooth, rough, grainy, among others.

Odor

Before checking the odor of the material, make sure that there is no risk. Place a small sample in the palm of your hand or in glass container and inhale slowly and repeatedly. If odour is indistinctive, pressing part of material between the fingers and inhale again. When the monograph indicate toxic material, put a little bit of material crushed in hot water. First, determine the intensity of odour: none; weak; distinct or strong, and then the sensation caused by odor: aromatic; frutoso; musty or stale. When possible, it is important to compare the odor with substance defined, such as, for example, peppermint must have odor similar to menthol and clove, similar to eugenol.

Flavor

Test the taste only when required in the monograph.

5.4.1.2 PREPARATION OF THE MATERIAL FOR ANALYSIS MICROSCÓPICA

SOFTENING OF the MATERIAL

The organs and tissues plant employees typically are dried, and to be observed under the microscope, it is appropriate first softens them upon treatment with hot water. The time required for the softening of each plant organ or its parts varies according to Its texture. When it comes to component newly harvested, only those of firmer need such treatment.

Method of hydration for dry materials

Place the sample in a solution of hydration, prepared with five parts of water, 4 parts of ethanol, a part of glycerin and five drops of commercial detergent for each 200 mL of solution, in a greenhouse at 60 °C for at least 48 hours.

IMPLEMENTATION OF CUTS

Once soft, proceed to preparation of slices of plant organs or parts thereof. The sections may be carried out with the aid of sharp objects such as razor, razor blade or scalpel, for cuts a free hand. In order to be sectioned, include the sample in suitable material, which makes it possible to fix the fragment. Sections of better quality can be obtained with the employment of microtomes. There are basically three types of microtomes: the freezing, used for materials more fragile; the rotary, for cuts in series of material included in paraffin; and the guide, for those more resistant materials, such as branches, parts of stems and roots. In the latter case, a relatively easy method of material preparation to be sectioned consists in its inclusion in macrogol soluble in water or in historesin.

Inclusion of material in paraffin

- Boil the sample in water when dry, to soften and remove the air;
- Dehydrate the sample in a series of ethanol diluted in water: 50.70.80.96% and, finally, in absolute ethanol;
- Transfer the sample to mixture of absolute ethanol and xylene in the ratio 3:1 (v/v) and then for 1:1 and 1:3;
- Transfer the sample to xylene pure;
- Transfer the sample to xylene in paraffin in the proportion 1:1, keeping it in the oven;
- Transfer the sample to paraffin, heated to occur infiltration, keeping it in the oven until it remains deposited at the bottom of the container;
- Emblocar and let cool in pan with cold water;
- Trimming the block for introduction in the microtome;
- Sectioning the material and put in glass slide previously greased with adhesive Haupt or Bissing;
- Put the blades on the hotplate to distend the cuts;
- Desparafinizar;
- Wait for at least one hour prior to 3906.

Inclusion of material in macrogol (polyethylene glycol – PEG 4000 or PEG 6000)

- Boil the sample in water when dry, to soften and remove the air;
- Place the sample in becher, containing macrogol 20% (P/v);
- Mark the becher, from the surface of the liquid, dividing it into five approximately equal parts;
- Leave the material in an oven at 65 °C for 3 to 4 days;
- When the solution evaporate up to 1/5 of its initial volume, transfer the sample to macrogol pure and cast where should stay during 12 to 25 hours, in a greenhouse at 65 °C;
- Remove from the oven, emblocar and let it cool down to ambient temperature;
- Trimming the blocks for introduction in the microtome;
- Sectioning the material to dry;
- Wash the cuts with water and stain.

Inclusion in historesin

There are different brands of historesin embedding on the market, sold in kits, being the methodology for embedment characteristic of each manufacturer. Follow the instruction manual. All contains three main elements: a resin hardener, an agent and an agent accelerator or catalytic converter. The mixing and temperature should follow the specifications, for which there is complete interaction, obtaining final product a polymer. The plant material must be previously fixed and dehydrated. It is suggested that the sections to be paraffin-are immersed in the resin during a night, for which there is complete infiltration. Only after, replace the resin of infiltration by mixture of new portion of resin, hardener agent and agent accelerator. The resin infiltration can be reused by two to four times, and it should then be discarded. The slices are placed on the blades without adhesive. Blush.

METHODS OF COLORING

The staining methods can understand the application of a single dye staining (simple) or from two or three different dyes (coloring composed).

(Some simple Staining dyes that can be used)

- Safranin Solution at 1% (w/v) in ethanol: coloring of cutin, lignin and suberin.
- Solution of Fast Green to 0.5% (w/v) in ethanol: coloration of cellulose.
- Astra Blue to 1% (w/v) in ethanol: coloring of compounds accumulating pectic substances of coverglass medium and wall.
- Acid Phloroglucin SR: coloring of lignin. Color composite (some mixtures of dyes that can be used)
- SafraninaAzul of Astra: colorize the lignin from the cellulose of red and blue. Place the sample in an aqueous solution of safranin to 1% (p/v) for 5 to 25 minutes. Wash twice with distilled water. Put in Astra Blue for 10 to 25 minutes. Wash twice with distilled water. Pass by battery of ethanol at 50 %, 70 %, 90 %, 96 %, and absolute ethanol
- (Twice), xylene. Mount on slides with Canada balsam or synthetic resin.
- SafraninaFast Green: colorize the lignin from the cellulose of red and green. Not desparafinizar the blades. Put in an aqueous solution of safranin to 1% (p/v) for 10 to 20 minutes (or more). Wash in tap water. Put in distilled water for 1 minute. Drain the water from the blade. Put in Fast Green to 0.5% (w/v) in ethanol for 10 to 40 minutes. Wash in tap water. Put in distilled water for 1 minute. Repeat the operation. Drain the water from the blade. Dry in hotplate for 30 minutes. Remove the paraffin with xylene in two exchanges of 5 minutes. Mount on slides with Canada balsam or synthetic resin.

PREPARATION AND ASSEMBLY OF BLADES

The histological sections are assembled, between blade and coverslip was placed, in water, glycerol, potassium

hydroxide at 30% (p/v), chloral hydrate to 50% (p/v), or any other liquid that enables the observation. The glycerol is more used in studies microquimicos mucilage, goma, inulin and aleurone. The potassium hydroxide is agent diafanizador, taking action on protein, starch, fat, resins and colorants. The chloral hydrate, also, is agent diafanizador action and, although more slowly than the alkali hydroxides, has the advantage of not dissolve the calcium oxalate.

Depending on the purpose for which it is designed, you can assemble the cuts in slides for observation immediate or permanent blades said.

In preparations for observation, immediate, once selected and stained, building-if the cuts in a suitable medium, taking care to avoid the formation of air bubbles. If the exam is more prolonged, recommends- if coat the edges of the coverslip was placed in a mourning (seal), which can be nail polish, Canada balsam or alcoholic solution of shellac, to avoid evaporation of the mounting medium, they all applicable with the aid of a brush soft and small.

In the preparations, permanent, once selected and stained, the cuts must be mounted between slide and coverslip was placed, with synthetic resin, Canada balsam or other suitable means. You should keep the mount compressed by means of the application of small weights on the coverslip was placed in position perfectly horizontal and on filter paper, with the purpose of avoiding possible extravasation of mounting medium.

MACERATING FLUID OF FABRICS

Sections of stems, roots, bark or other parts plant or always give accurate idea of the true nature of its cells. The same happens with raw material marketed, when rasurada or powder. To reveal some special features, such as, for example, thickening and pontoacoes, you must employ one of the methods listed for tissue dissociation. In these methods, the structure to be studied is treated with chemical substances capable of dissolving the coverglass medium and, thus, enabling the separation of cells.

Method of tissue dissociation

- Cut the material into small pieces or slices with about 300 nm thick and put in water;
- Remove all air from the material, either by boiling and cooling down quickly;
- Macerate the material in solution of Jeffrey. The time of maceration varies with the nature of the material. Usually the cells begin to separate in approximately 24 hours. If necessary, can be used glass rod of rounded tip for kneading very lightly the material.
- There is difficulty in separation of cells, renew the solution maceradora;
- Wash very well the material with tap water to remove the acid. Pour the mixture with the tissue macerated for a funnel containing filter paper;
- Close the lower opening of the funnel and cover the macerate with aqueous solution of safranin O to 1% (p/v), during long enough for good coloring of the material (15 minutes ago the 6 Hours);
- Open the tip of the funnel and wash again with water, up to remove the excess dye;
- Be dehydrated by adding solutions of ethanol at 50 %, 70 %, 90% and absolute ethanol;
- Remove with tweezers the macerate the filter paper and place in xylene;
- Fit between blade and coverslip was placed, with synthetic resin or Canada balsam;
- Keep the blade in a horizontal position, but do not use weight on the coverslip was placed, because the cells macerated are very fragile.

NOTE THE LEAF EPIDERMIS

Separation of the epidermis with solution of Jeffrey

- To obtain leaf epidermis, sectioning small pieces of sheet and puts them in a solution of Jeffrey diluted in distilled water at 50% (v/v), cap the container and leave for 12 to 48 hours, according to the texture of the sheet (the solution had attacked the mesophyll, leaving the epidermis isolated);
- When the mesophyll is destroyed, wash the samples several times with distilled water.
- Put a sample on blade, divide between the epidermis and put the two parties so that the external faces are facing upward;
- 3906 The material on the blade with ethanolic solution of safranin to 1% (p/v);
- Prepare the blade with glycerin gelatin and seal.
- Separation of the epidermis with chloral hydrate
- Use fragments of leaves of approximately 0.5 cm wide by 0.5 cm long;
- Place the fragments in a test tube, add 5 mL of chloral hydrate, heat in a water bath for about 15 minutes or until the fragments are transparent;
- Transfer the fragments for a blade, taking care that the abaxial side is willing to top;
- Add a drop of chloral hydrate and a drop of ethanol with glycerin and cover with coverslip was placed to prevent dehydration. Seal.

Classification of the stomata and determination of index estomatico

The classification employed, didactically, for determining the types of stomata (Figure 1), is based on form and layout of the cells that surround them. The basic types are:

1) Cells Anomocitico (irregular): the stomata are surrounded by a variable number of cells that do not differ, in general, in any characteristic of other basic cells of the epidermis;

2) Cells Anisocitico (unequal): the stomata are usually surrounded by 3 or 4 cells subsidiaries, being one of them, clearly, smaller than the other.

3) Cells Diacitico (cross-sectional): the stomata are accompanied by 2 cells subsidiaries, whose common walls form a right angle with the cells-guard of stomata;

4) Cells Paracitico (parallel): the stomata present on each side one or several cells subsidiaries parallel to the longitudinal axis of the ostiolo and the guard cells of stomata.

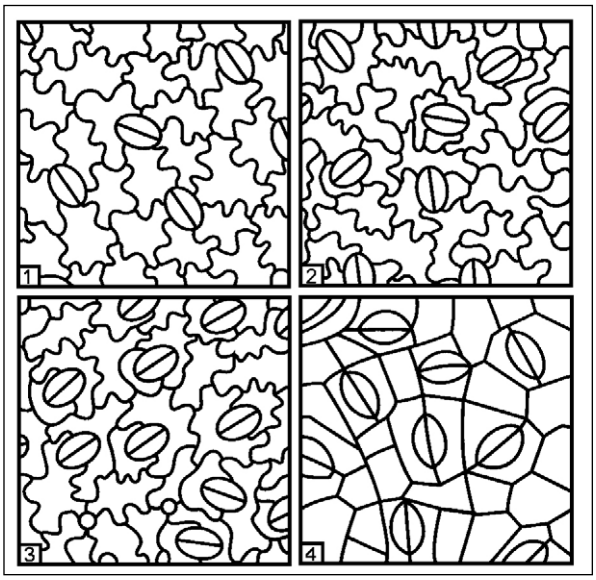

Figure 1 – Types of stomata. 1. Anomocitico; 2. anisocitico; 3. diacitico; 4. paracitico.

Index estomatico

The Index of stomata is calculated according to the equation 100S / (E + S), S being the number of stomata in a particular area of the surface of the sheet and the number of epidermal cells, including the trichomes, existing at the same microscopic field observed. For each sample of leaves, log and calculate the average of at least 10 determinations.

ANALYSIS OF THE POWDER (IDENTIFICATION OF MATTERS- PRESS MARKETED IN POWDER FORM)

- Put 1 or 2 drops of water, or glycerol-ethanol (1:1) or chloral hydrate in a blade;
- Add a little of the powder and mix with a fine brush and soft;
- COVER with coverslip was placed and observe under the microscope.
- Other fluids can be used with the same technique;
- Histochemical reactions or dyes may be used.

REACTION HISTOQUÍMICAS

The reactions can be made with fresh material severed or cut material in microtome and included in paraffin or macrogol, adding a drop of reactive on a blade with a section of the sample.

Starch. Add a drop of iodine SR. The starch acquires stain blue or violet-blue.

Calcium carbonate. Add acetic acid at 6% (p/v) or hydrochloric acid at 7% (p/v). Crystals or deposits of calcium carbonate dissolves slowly, with production of effervescence.

Hidroxiantraquinonas. Add one drop of potassium hydroxide at 5% (p/v). The cells that contain 1,8-diidroxiantraquinonas coram of red.

Inulin. Add 1 drop of 1-naphthol SR and sulfuric acid. Inulin Esferocristais coram to purple-reddish and dissolve.

Lignin. Add 1 drop of acid phloroglucin SR, quickly heat the blade and add a drop of hydrochloric acid to 25% (p/v). The lignin cora to red.

Lipids. Add Sudan III SR or Sudan IV SR for 10 minutes, rinse quickly with ethanol 70% (v/v). Lipids, cutin and suberin coram orange-reddish.

Mucilage. Add 1 drop of ink on dry sample. The mucilage appears as fragments spherically dilated and transparent on a black background. The characterisation, also, may be carried out by adding

1 Drop of thionin SR the dried sample and leave for 15 minutes, followed by washing in ethanol 20% (v/v). The mucilage takes-if reddish-violet (lignin and cellulose coram to blue or violet-blue).

Calcium oxalate. Crystals of calcium oxalate are insoluble in acetic acid at 6% (p/v) and soluble in hydrochloric acid at 7% (p/v), without producing effervescence.

Proteins. Add ninhydrin 0.5% (w/v) in absolute ethanol, and keep at 37 °C for 24 hours. Wash in absolute ethanol and distilled water, add fuchsin pallid SR and leave in contact for 10 to 30 minutes. Wash in water and add sodium bisulphite to 2% (p/v), leave in contact for 1 to 2 minutes. Wash in tap water for 10 to 20 minutes, dehydrate and fit the blade. The proteins coram to red purple. Perform this procedure only with fresh material.

Saponins. Add one drop of sulfuric acid. There is a sequence of color yellow, then red in color, and, finally, violet or blue-green.

Tannins. Add ferric chloride at 10% (p/v) and a small quantity of sodium carbonate, leave in contact for 2 to 3 minutes, rinse with distilled water. The tannins coram to blue-green.

5.4.2 METHODS OF ANALYSIS OF HERBAL DRUGS

5.4.2.1 SAMPLING

The specified sampling procedures take into account three aspects: (a) number of packages that contain the drug; (b) the degree of division of drugs and (c) the quantity of drugs available.

NUMBER OF PACKAGES

Examine the integrity of the containers for packaging and the nature of the drugs contained therein. If there is homogeneity, collect samples as specified in Table 1.

DEGREE OF DIVISION AND QUANTITY OF DRUGS

Consisting of the drug components of dimensions less than 1 cm or when she constitute material finely fragmented or sprayed, employ sampling tool (pipe fitted with closing device on the base). Collect samples from top to bottom and from bottom to top (vertical direction) and laterally (horizontal direction), -0.4 sample of at least 250 g for up to 100 kg of drugs. There are more than 100 kg to be sampled, sampling followed by quarters method selection, generating sample of 250 g at the end of the process.

For drugs with dimensions greater than 1 cm, proceed to manual sampling. Combine the wood samples of each packaging open, taking the precaution of not increasing its degree of fragmentation during manipulation. For quantities of the drug up to 100 kg, the sample should be at least 500 g. There are more than 100 kg of drugs to be sampled, sampling followed by quarters method selection, generating 500 g sample at the end of the process.

In both cases, drugs with dimensions less than or greater than 1 cm, is permissible sample quantities less than those specified above provided that the total quantity of drugs available is less than 10 kg. However, the final sample should not be less than 125 g.

QUARTERS METHOD

Distribute drugs on square area, in four equal parts. With the hand distribute drugs on the area of uniform manner and reject the portions contained in two squares opposites, in one of the diagonals of the square. Join the two remaining portions and repeat the process, if necessary. There is marked difference in dimensions of fragments, perform manual separation and note the approximate percentages of the components of different degrees of division found in the sample.

5.4.2.2 DETERMINATION OF EXTRANEOUS MATTERS

The drugs plants are exempt from fungi, insects and other contamination of animal origin. Unless otherwise indicated, the percentage of foreign elements should not be greater than 2% m/m. Foreign Matter the drug is classified into three types: (a) parts of the body or bodies of which the drug derives, excepted those included in the definition and description of the drug, above the limit of tolerance specified in the monograph; (b) any bodies, portions or products of organisms other than those specified in the definition and description of the drug, in his monograph; and (c) impurities of nature, mineral or organic, non-inherent to drugs.

PROCEDURE

Determine the sample quantity to be subjected to the test as specified below.

- Roots, rhizomes, bark, whole plant and parts airlines: 500g;
- Leaves, flowers, seeds and fruits: 250 g;
- Particulate materials or dispensed (average weight less than 0.5 g/component): 50 g;
- Post: 25 g.

Spoon, by quarters method, the sample quantity specified, from the sample obtained, according to the previously described procedure, and spread it in a thin layer on a flat surface. Separate, manually the foreign material to

Table 1 – Number of packages to be sampled in accordance with the number of existing packaging.

Number of packages	*Number of packages to be sampled*
1 to 3	All
4 to 10	3
1.20	5
2.50	6
5.80	8
8.330	10
More than 100	10% of the total packaging

drugs, initially to the naked eye, and then, with the aid of magnifying glass (five to ten times). Weigh the material separated and determine their percentage based on the weight of the sample under test.

5.4.2.3 DETERMINATION OF WATER IN HERBAL DRUGS

Three methods are employed for the determination of water in herbal drugs: gravimetric method (drying), method azeotropic (distillation with toluene) and volumetric method (Karl Fischer). The first, more technically simple and fast, it is not applicable when the drug contains volatile substances. The others require special equipment and understand more complex technical.

SAMPLE PREPARATION

Reduce by cutting, granulation or fragmentation drugs not sprayed or crushed so as to limit the size of their components, at most, 3 mm thick. Seeds and fruits, even smaller than 3 mm, must be broken. Avoid high-speed mills or other procedures which entail loss of moisture of the sample.

Gravimetric Method

Transfer approximately 2 to 5 g, or the specified, in the monograph, exactly heavy, sample prepared as per previous instructions, for weigh-filter crucible, previously dried under the same conditions to be adopted for the sample, during 30 minutes. Dissicate the sample to 100

Volumetric Method

Proceed as described in Determination of water (5.2.20.1).

Method azeotropic

Proceed as described in Determination of water (5.2.20.2)

5.4.2.4 DETERMINATION OF TOTAL ASH

The total ash ash include physiological and ash- not physiological.

PROCEDURE

Weigh, exactly, about 3 g of the sample sprayed, or the amount specified in the monograph, transfer to crucible (silicon or platinum) previously tared. Distribute the sample evenly in a crucible and ash increasing, gradually, the temperature until, at most, 600 ± 25 °C, until all the coal is eliminated. A temperature gradient (30 minutes at 200°C, 60 minutes at 400°C and 90 minutes at 600°C) can be used. Cool in a desiccator and weigh. In cases in which the coal cannot be eliminated completely, cool the crucible and moisten the residue with about 2 mL of water or saturated solution of ammonium nitrate. Evaporate to dryness on a water-bath and then on hot comal, and incinerate until constant weight. Calculate the percentage of ash in relation to drugs air dry.

5.4.2.5 DETERMINATION OF ASH IN ACID-INSOLUBLE MATTER

Ash insoluble in acid, constitute the residue obtained in boiling of total ash, or sulfated with diluted hydrochloric acid after filtering; washing and incineration. The method is for the determination of silica and constituents silahydrocarbon of drugs.

PROCEDURE

Boil the residue obtained in the determination of total ash for 5 minutes with 25 mL of hydrochloric acid to 7% (w/v) in crucible covered with watch glass. Wash the watch glass with 5 mL of hot water, adding water to wash the crucible. Collect the residue, insoluble in acid, on filter paper, ash-free, washing it with warm water until the filtrate is show. Transfer the filter paper containing the residue into the crucible, original dry on hot plate and ash at about 500 °C until constant weight. Calculate the percentage of ash insoluble in acid in relation to drugs air dry.

5.4.2.6 DETERMINATION OF SULPHATED ASH

PROCEDURE

Warming up to 1939 per 10 minutes a porcelain crucible, let cool in a desiccator and weigh. Put exactly approximately 1.0 g of the drug in tared crucible and moisten the drug with concentrated sulfuric acid and carbonize at Bunsen flame. Moisten again with concentrated sulfuric acid, carbonize and incinerate with gradual warming up to 800 °C. Cool, weigh again, incinerate for 15 more minutes. Repeat this procedure until the difference between two successive weighings is not greater than 0.5.

5.4.2.7 DETERMINATION OF VOLATILE OILS IN HERBAL DRUGS

The content of volatile oils in herbal drugs is determined by distillation process by drag of steam, with the aid of equipment described below.

The equipment (Figure 1), prepared in resistant glass, of appropriate quality, comprises:

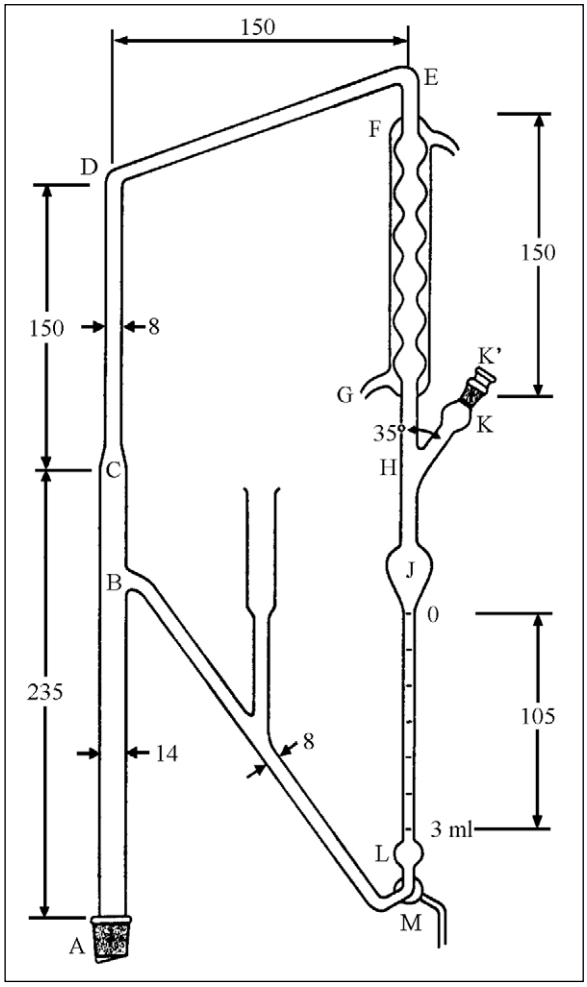

Figure 1 – apparatus for determination of the content of volatile oils in herbal drugs by distillation process by drag of steam.

1) Round bottom flask of 500 mL to 1000 mL capacity, short-necked, fitted with a seal 24/40, female;

2) Condenser, adaptable to the flask by means of a seal flipped 24/40, male, built in single piece of glass, comprising the parties described below, with the respective measures:

2.1) Vertical tube (AC) from 210 to 260 mm in length and 13-15 mm in internal diameter;

2.2) Bent tube, with segments (CD) and (DE) measuring 145-155 mm in length each and internal diameter of 7.8 MM;

2.3) Condenser of balls, Allihn type (FG), of 145-155 mm length and internal diameter of 15 mm on the balls and 8.10 MM in narrowings;

2.4) Lapped seal stopper (14/20) (K') containing hole of about 1 mm in diameter, that plugged a side outlet (K) provided with lapped seal 14/20 female, in the end;

2.5) Tube (GH) of 30-40 mm in length and 7-8 mm in internal diameter, forming the parties (HK) angle (GHK) of 30° to 40°;

2.6) Enlargement in pear shape (J) of 3 mL capacity;

2.7) Tube (JL) fitted with graduated scale of 100-110 mm; 3 mL capacity and subdivided in twentieth of millilitre;

2.8) Enlargement in the form of ball (L) of approximately 2 Ml capacity;

2.9) 3-Way tap;

2.10) Connection pipe (BM) of 7-8 mm in diameter, fitted with safety tube. The insertion point (B) is 20-25 mm above the highest part of the graduated scale;

3) Onte of heat that can be electric heater or gas jet endowed with fine adjustment of flame;

4) Vertical support appropriate.

Before use, the apparatus should be cleaned by repeated washing and successively with acetone, water, chromo and again water. After dry, must be mounted in a protected location of air currents. The graduated scale must be assessed and, if necessary, establish correction factor for each apparatus.

PROCEDURE

Enter in the fluid volume indicated in the monograph and fragments of porous porcelain or glass beads to control boiling. Adapt the condenser to the flask. Remove the stopper lapped (K') and, by opening (K), enter the water until this begins to seep in (B). Using volumetric pipette, introduce xylene, in prescribed quantity, supporting-if the pipette tip on the bottom of the output side (K). Heat the liquid inside the flask until the beginning of the boiling point and distil in reason of 2 to 3 mL per minute, or as prescribed in the monograph.

To determine speed of distillation, drain the water with the aid of three-way tap, until the meniscus is at the level of the reference mark below (Figure 2). Close

The tap and clocking the time required to fill the volume between the dashes of reference upper and lower (3 mL). Open the tap and continue the distillation per 30 minutes. Turn Off the heat, let cool for 10 minutes and do the reading of the volume of xylene in graduated tube.

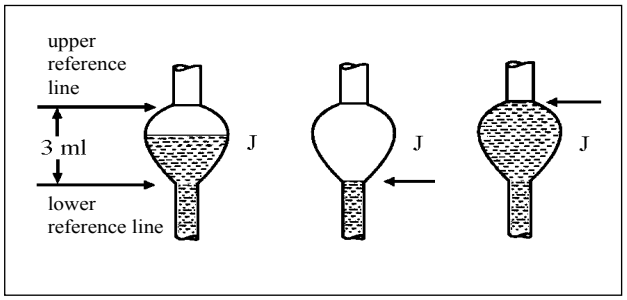

Figure 2 – An indication to determine speed of distillation.

Enter the flask the quantity of drugs prescribed in the monograph and distilled by steam destillation, as described above, by the time and at the speed indicated in the monograph. Complete the operation, let it cool down for 10 minutes and read the volume of essential oil collected in graduated tube. Subtract the reading the volume of xylene previously determined. The difference represents the amount of essential oil contained in the sample. Calculate the result in milliliters of essential oil per 100 g of the drug.

5.4.2.8 DETERMINATION OF FIXED OILS

The determination of fixed oils is based on its solvent extraction which, after evaporated, leaving as a residue oil whose amount is determined by weighing.

If the sample contains high content of water-soluble components (carbohydrates, urea, lactic acid, among others), it is for pre-treatment of the sample in order to avoid interference in the determination of fatty materials. For both, transfer the taking of test to funnel containing filter paper, wash with water and dry the residue at 105 °C for 2 hours.

Employ the Soxhlet apparatus (Figure 1). The equipment, manufactured in resistant glass, of appropriate quality, includes round-bottom flask (A), with 500 mL to 1000 mL capacity, connected to Soxhlet extractor (B) and reflux condenser (C).

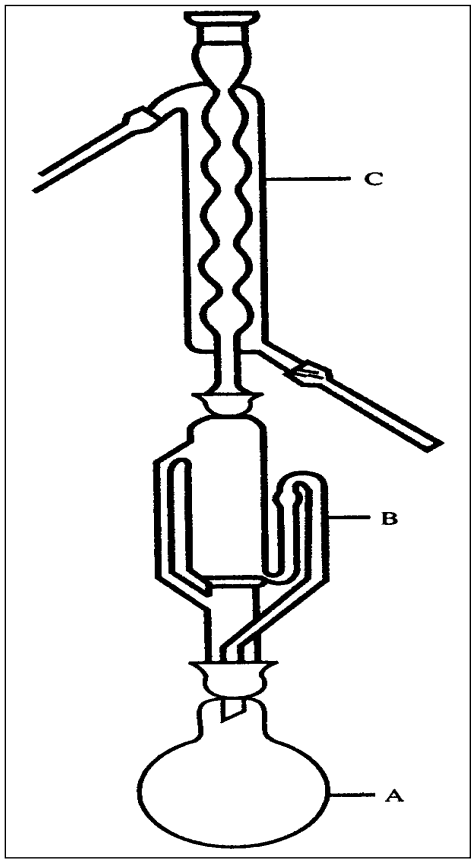

Figure 1 – Soxhlet apparatus.

Before use, the apparatus should be cleaned by repeated washing and successively with acetone, water, chromo and, again, water. After dry, must be mounted in a protected location of air currents.

PROCEDURE

Transfer, exactly, about 10 g of drugs previously desiccated as described in Determination of water in herbal drugs (5.4.2.3), gravimetric Method, and transfer for Soxhlet extractor tool (B), covering it with cotton wool. Weigh the flask (A) clean and dry (containing fragments of porcelain or glass beads) and mount it on the player on water-bath, taking the precaution of ensuring seal in seal flipped the balloon (it is recommended operation in chapel). Transfer to the puller petroleum ether in sufficient quantity to perform three sifonagens and clip the reflux condenser (C). Carry out the extraction under sufficient heating to maintain the solvent simmering during 4 hours.

Complete extraction, wait cool, transfer the contents of the cartridge for mortar porcelain and join amount approximately equal of sand washed and dried. Spray the drug and transfer it again, inside the cartridge, for the puller. Restart and keep the extraction under the above conditions for additional period of 2 hours. Disconnect the flask and evaporate the solvent (preferably by distillation under current of carbon dioxide). Put the balloon to oven to 105 °C, cool and weigh. Repeat the operation until constant weight. Calculate the percentage of fixed oils in

drugs based on mass of heavy drugs and in the mass of oil obtained.

5.4.2.9 DETERMINATION OF 1,8-CINEOLE IN ESSENTIAL OILS

The determination of cineole comprises the determination of the freezing temperature (criometria) composed of molecular combination between cineole and o-cresol-cresineol. Being this temperature proportional to the content of cineole into the compost, it is possible to establish whether your content by analysis of the data recorded in Table 1.

The method is employed in the dosage of cineole in essences of eucalyptus and niaouli. Determinations in other essences are not recommended without proof of prior accuracy in view of some constituents of essential oil solubilized the cresineol (even in essence of eucalyptus, there is the risk of error when the content of alpha-terpineol-fraction is greater than 12.5 %).

Also, Errors arise from the presence of moisture, is in essence or o-cresol. The o-cresol employee must be pure and dry, featuring melting point higher than 30°C. It should be kept in airtight bottle, to be hygroscopic.

PROCEDURE

Dry the sample of essential oil in test, stirring the mixture with sodium sulphate or with calcium chloride, anhydrous, in a test tube or in Erlenmeyer flask fitted with cover flipped. Leave in contact during 24 hours and filter. Transfer to test tube (approximately 15 mm in diameter and 80 mm height) 3.0 g of essential oil, exactly, heavy and add 2.1 g of o-cresol in sobrefusao. Stir the mixture with bulb thermometer (0 – 60 °C, graduated in tenths of a degree) suspended on the tube so that the end of the bulb does not exceed the limit of 5 mm from the base of the tube and without touching in its walls, until induction of crystallization. Note the maximum temperature observed in thermometer during the crystallization. Heat the tube to about 5-10 °C above the temperature reading and insert it into another larger tube (approximately 60 mm in diameter and 100 mm in height) in order to create layer of air. Attach the tube lower inside the other with the aid of plates of cork adapted or by any other means and immerse the assembly in water bath with controlled temperature, keeping the temperature around 5 °C below the freezing point noted previously for the cresineol. Stir the mixture with vertical movements of the thermometer, and the start to the crystallisation (turbidity of the liquid), observe the temperature stabilization. There are fluctuations during the crystallisation, always consider the maximum temperature measured during the period of freezing.

Repeat the determination as often as is necessary for that two successive readings accuse maximum variation of 0.1 °C.

5.4.2.10 DETERMINATION OF THE INDEX OF FOAM

Weigh, exactly, 1 g of plant material reduced the fine dust (mesh of 180 μmm, 2.5.11) and transfer to erlenmeyer flask containing 165 mL of boiling water. Keep boiling under moderate during 30 minutes. Cool, filter to 100 mL volumetric flask. Complete the volume, through the filter, up to 100 mL.

Distribute the decoction obtained in 10 test tubes with lid (16 mm in diameter by 16 cm in height), in successive series of 1.2.3, up to 10 mL, and adjust the volume of the liquid in each tube to 10 mL with water. Cap the tubes and shake them with vertical movements by 15 seconds, with two unrest per second. Leave to rest for 15 minutes and measure the height of the foam.

If the height of the foam of all tubes is less than 1 cm, the index of foam is less than 100.

If, in any one of the tubes, the height of the foam measured is 1 cm, the dilution of the plant material in this tube (A) is the observed index. If this tube is the first or second in the series, it is necessary to make an intermediate dilution, using the same method described previously, to obtain a more accurate result.

If the height of the foam is larger than 1 cm in all pipes, the index of foam is greater than 1000. In this case, the determination must be made with a new series of dilutions of the decoction to obtain an accurate result.

The index of foam is calculated according to the equation 1000/4, being The volume, in ml, of decoction used for preparation of dilution in tube where the foam was observed.

5.4.2.11 DETERMINATION OF SUBSTANCES EXTRAÍVEIS BY ALCOHOL EXTRACT (MEAN)

METHOD A: SOXHLET EXTRACTION BY

Weigh, exactly, about 2 g of the drug cartridge and transfer to the Soxhlet extractor, previously tared and dry. Enter in the flask of the puller 0.2 g of sodium hydroxide and absolute ethanol in sufficient quantity. Remove for 5 hours,

Table 1 – Content of 1,8-cineole in essential oils depending on the temperature of freezing.

Temperature ºC	*0.0*	*0.1*	*0.2*	*0.3*	*04*	*0.5*	*0.6*	*0.7*	*0.8*	*0.9*
24	45.6	45.7	45.9	46.0	46.1	46.3	46.4	46.5	46.6	46.8
25	46.9	47.0	47.2	47.3	47.4	47.6	47.7	47.8	47.9	48.1
26	48.2	48.3	48.5	48.6	48.7	48.9	49.0	49.1	49.2	49.4
27	49.5	49.6	49.8	49.9	50.0	50.2	50.3	50.4	50.5	50.7
28	50.8	50.9	51.1	51.2	51.3	51.5	51.6	51.7	51.8	52.0
29	52.1	52.2	52.4	52.5	52.6	52.8	52.9	53.0	53.1	53.3
30	53.4	53.5	53.7	53.8	53.9	54.1	54.2	54.3	54.4	54.6
31	54.7	54.8	55.0	55.1	55.2	55.4	55.5	55.6	55.7	55.9
32	56.0	56.1	56.3	56.4	56.5	56.7	56.8	56.9	57.0	57.2
33	57.3	57.4	57.6	57.7	57.8	58.0	58.1	58.2	58.3	58.5
34	58.6	58.7	58.9	59.0	59.1	59.3	59.4	59.5	59.6	59.8
35	59.9	60.0	60.2	60.3	60.4	60.6	60.7	60.8	60.9	61.1
36	61.2	61.3	61.5	61.6	61.7	61.9	62.0	62.1	62.2	62.4
37	62.5	62.6	62.8	62.9	63.0	63.2	63.3	63.4	63.5	63.7
38	63.8	63.9	64.1	64.2	64.4	64.5	64.6	64.8	64.9	65.1
39	65.2	65.4	65.5	65.7	65.8	66.0	66.2	66.3	66.5	56.6
40	66.8	67.0	67.2	67.3	67.5	67.7	67.9	68.1	68.2	68.4
41	68.6	68.8	69.0	69.2	69.4	69.6	69.7	69.9	70.1	70.3
42	70.5	70.7	70.9	71.0	71.2	71.4	71.6	71.8	71.9	72.1
43	72.3	72.5	72.7	72.9	73.1	73.3	73.4	73.6	73.8	74.0
44	74.2	74.4	74.6	74.8	75.0	75.2	75.3	75.5	75.7	75.9
45	76.1	76.3	76.5	76.7	76.9	77.1	77.2	77.4	77.6	77.8
46	78.0	78.2	78.4	78.6	78.8	79.0	79.2	79.4	79.6	79.8
47	80.0	80.2	80.4	80.6	80.8	81.1	81.3	81.5	81.7	81.9
48	82.1	82.3	82.5	82.7	82.9	83.2	83.4	83.6	83.8	84.0
49	84.2	84.4	84.6	84.8	85.0	85.3	85.5	85.7	85.9	86.1
50	86.3	86.6	86.8	87.1	87.3	87.6	87.8	88.1	88.3	88.6
51	88.8	89.1	89.3	89.6	89.8	90.1	90.3	90.6	90.8	91.1
52	91.3	91.6	91.8	92.1	92.3	92.6	92.8	93.1	93.3	93.6
53	93.8	94.1	94.3	94.6	94.8	95.1	95.3	95.6	95.8	96.1
54	96.3	96.6	96.9	97.2	97.5	97.8	98.1	98.4	98.7	99.0
55	99.3	99.7	100.0							

remove the cartridge with the residue and dry it in an oven at 105 °C for 30 minutes. Weigh the dry residue and calculate the content of extractable substances by ethanol by difference between the weight of the sample and the weight of the dry residue. Mention the result in relation to drug drought (Determination of water in herbal drugs, 5.4.2.3).

METHOD B: HOT EXTRACTION

Weigh a 250 mL Erlenmeyer flask, with mouth lapped, transfer to it, exactly, about 4.0 g of drugs

Plant dry, finely, sprayed. Add 100 mL of water and weigh them to get the total weight, including the bottle. Cap, shake well and let sit for 1 h. Attach a reflux condenser and heat for 1 h, cool and weigh. After the reflux, correct the original weight with solvent specified in test for the vegetal drug. Mix well and filter, quickly, by means of a dry filter. Transfer 25 mL of the filtrate to a porcelain dish and evaporate to dryness in a water bath. Dry 105 °C for 6 h, cool in a desiccator for 30 min and weigh immediately. Calculate the percentage of extracted materials in mg/g of dry material.

METHOD C: COLD EXTRACTION

Weigh a 250 mL erlenmeyer flask, with mouth lapped and transfer to it, exactly, about 4.0 g of vegetal drug dry, finely pulverized. Macerate, with 100 mL of solvent specified, during 6 h, stirring frequently, and leave to rest for 18 h. Filter, quickly, without losing any quantity of solvent; transfer 25 mL of the filtrate to a porcelain dish and evaporate to dryness on the water-bath. Dry at 105 °C for 6 h, cool in a desiccator for 30 min and immediately weigh. Calculate the percentage of extracted materials in mg/g of dry plant material.

For extractable material with ethanol, use the specified concentration for the solvent in test for each drug plant. For the extractable materials with water, use water

As solvent. Use other solvents, specified in each monograph.

5.4.2.12 DETERMINATION OF THE INDEX OF BITTERNESS

The properties of bitter plant materials are determined by comparing the threshold concentration of the bitterness of an extract with a diluted solution of quinine hydrochloride. The value of the index of bitterness is expressed in terms of units, equivalent to a solution of quinine hydrochloride 0.05% (p/v).

For the extraction of plant material and for cleaning the mouth after each tasting, you should use drinking water as vehicle. The hardness of water rarely has significant influence on the bitterness.

The sensitivity to bitterness can vary from individual to individual, or even for an individual in different situations (fatigue, smoke, food intake). Therefore, the determination of the threshold concentration of the bitterness of the material to be tested with quinine hydrochloride should be made by the same person, within a short space of time. The feeling of bitterness is not perceived by the entire surface of the tongue, but is restricted to the top and side of the base of the tongue. The determination of the threshold concentration of the solution requires training of the analyst. First, it is made the determination of the threshold concentration of quinine hydrochloride and, then, to the material to be tested. Individuals insensitive to feeling bitter induced by a solution containing 0.058 mg of quinine hydrochloride in 10 mL of water are not indicated for the completion of the test.

The preparation of the stock solution of plant material to be tested (ST) must be specified in the relevant monograph. In a series of unique test, the determination always starts with the lowest concentration (less that another order is specified in the monograph) to maintain the sensitivity of the buttons taste buds.

PREPARATION OF SOLUTIONS

Stock Solution and diluted solution of quinine hydrochloride

Dissolve 0.1 g of hydrochloride of quinine in sufficient quantity of potable water to make up to 100 mL. Dilute 5 mL of this solution to 500 mL with water. This standard solution of quinine hydrochloride (SQ) contains 0.01 mg/mL. For the initial test, using nine test tubes for dilution in series, as recorded in Table 1

Stock Solution and diluted solution of plant material

Prepare a stock solution as specified in the monograph (ST). Use 10 test tubes for dilution in series, as shown in Table 2 for the second test.

PROCEDURE

After rinsing the mouth with drinking water, prove 10 mL of dilution, turning it in the mouth, especially near the base of the tongue for 30 seconds. Always start with a less concentrated solution of the series, except when prescribed differently in the monograph. If the sensation of bitterness is no longer felt, remove the solution and wait 1 minute to ensure that there is no sensitivity delayed. Rinse the mouth with water. Wait at least 10 minutes to test the next dilution. The threshold concentration of bitterness is the dilution of lowest concentration at which the material still provokes a feeling of bitterness. After the first series of tests, rinse the mouth with water, until the bitterness is no longer perceived and wait at least 10 minutes before doing the second series of tests.

In this series of tests, to more quickly, it is advisable to ensure that the solution in tube number 5 (containing 5 mL of ST in 10 mL of solution) causes feeling of bitterness. If

Table 1 – dilutions of quinine hydrochloride for the initial test in obtaining the index of bitterness.

	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
SQ (mL)	4,2	4,4	4,6	4,8	5,0	5,2	5,4	5,6	5,8
Drinking Water (mL)	5,8	5,6	5,4	5,2	5,0	4,8	4,6	4,4	4,2
Quinine hydrochloride (10 mg/mL) (c)	0,042	0,044	0,046	0,048	0,050	0,052	0,054	0,056	0,058

Table 2 – dilutions of the stock solution for the second test in obtaining the index of bitterness.

	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
ST (*b*) (mL)	1,00	2,00	3,00	4,00	5,00	6,00	7,00	8,00	9,00	10,0
Water (mL)	9,00	8,00	7,00	6,00	5,00	4,00	3,00	2,00	1,00	-

perceived, find the concentration of the bitterness of the material, proving the dilutions in tubes of numbers 1 to 4. If the solution to the number 5 does not cause feeling of bitterness, find the threshold concentration of bitterness in tubes of numbers 6 to 10.

All the solutions and the water must be at a temperature between 20°C and 25 °C.

The Index of bitterness is calculated according to the equation:

$$V = \frac{2000 \text{ x } c}{(a \text{ x } b)}$$

Whereas

V = Value of bitterness, in units/g;

a = amount of material, in mg/mL, in ST;

B = volume of ST in 10 mL of dilution of concentration threshold of bitterness;

C = amount of quinine hydrochloride, in mg/10 mL in dilution of concentration threshold of bitterness.

5.4.2.13 QUANTITATIVE DETERMINATION OF ACTIVITY HEMOLÍTICA

The hemolytic activity of plant extracts, or a preparation containing saponins, is determined by comparison with the activity of a reference of saponin with hemolytic activity of 1000 units per gram. A suspension of erythrocytes is mixed with equal volumes of a dilution series of the extract. The lowest concentration to cause hemolysis complete is determined after leaving the system at rest for a specific period of time. A similar test is done simultaneously with reference solution of saponin.

PROCEDURE

For the preparation of the suspension of blood, put sodium citrate to 3.65% (p/v) in a bottle with lid until 1/10 of its capacity. Stir to completely wet the walls of the flask and add fresh bovine blood, with new stirring. O Blood, with sodium citrate, thus prepared can be stored for 8 days at a temperature between 2 ºC and 4 ºC.

In 50 mL volumetric flask, dilute, carefully, 1 Ml of blood with sodium citrate in sufficient quantity of phosphate buffer pH 7.4 to complete 165 mL. This suspension of blood diluted (2 %) can be used during the time in which the supernatant liquid stay clear and colorless, being kept cold.

For the reference solution, transfer, exactly, 10 mg of saponin to 100 mL volumetric flask and Fill up to volume with the phosphate buffer pH 7.4. This solution should be freshly prepared. The plant extract and dilutions should be prepared as specified in the monograph, using- if, also, phosphate buffer pH 7.4.

Preliminary Test

Prepare a dilution series of the plant extract with the phosphate buffer pH 7.4 and suspension of blood (2 %), using 4 test tubes as shown in Table 1.

Table 1 – Dilution series of vegetal extract for determination of hemolytic activity.

Tube	*1*	*2*	*3*	*4*
Plant Extract (mL)	0,10	0,20	0,50	1,00
Phosphate Buffer pH 7.4 (mL)	0,90	0,80	0,50	–
Blood Suspension (2%) (mL)	1,00	1,00	1,00	1,00

As soon As the tubes are prepared, reverses them carefully to mix, avoiding the formation of foam. After 30 minutes, stir again and let sit for 6 hours at ambient temperature. Examine the pipes and annotate on which dilution occurred total haemolysis, which will be observed in liquid, clear, red and without deposit of erythrocytes.

If the total haemolysis is observed only on number 4, use the original plant extract directly to the main test.

If the total haemolysis is observed in tubes 3 and 4, dilute twice the original extract with phosphate buffer.

If the total haemolysis is observed in tubes 2.3 and 4, prepare a diluted solution five times, as described above.

If, after 6 hours, all tubes contain a clear liquid and red, prepare a solution diluted 10 times and do the preliminary test, as described above.

If the total haemolysis is not observed in any of the tubes, repeat the preliminary test, using a more concentrated extract.

Main Test

Prepare a dilution series of the plant extract, diluting or not, as in the preliminary test, with phosphate buffer pH 7.4 and suspension of blood (2 %), using 13 test tubes, as specified in Table 2

Table 2 – Serial dilution in the plant extract with phosphate buffer and suspension of blood for the main test.

Pipes	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*
Plant Extract (mL)	0,40	0,45	0,50	0,55	0,60	0,65	0,70	0,75	0,80	0,85	0,90	0,95	1,00
Phosphate Buffer pH 7.4 (mL)	0,60	0,55	0,50	0,45	0,40	0,35	0,30	0,25	0,20	0,15	0,10	0,05	-
Blood Suspension (2%) (mL)	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00

Make the dilutions and assessments as the preliminary test, observing the results after 24 hours. Calculate the quantity of plant material in grams, or proportion in g/mL that produces total haemolysis (b).

Test for saponins

To eliminate the effect of individual variations in the resistance of suspension of blood the solution of saponin, prepare a series of dilutions of saponin in the same manner previously described for the plant extract. Calculate the quantity of saponins (g) that produces total haemolysis (a).

Hemolytic Activity

The hemolytic activity is calculated according to the equation

$$1000\ a / b$$

Whereas

1000 = Hemolytic activity of saponin, in relation to bovine blood;
The = quantity, in grams, of saponin; b = amount, in grams, of plant material.

5.4.2.14 DETERMINATION OF THE INDEX OF INTUMESCÊNCIA

Lead, simultaneously, at least three determinations. Weigh, exactly, 1 g of the drug plant sprayed and put in beaker of 25 mL with lid flipped. The length of the part must be graduated approximately 125 mm and the diameter, internal, close to 16 mm, subdivided into 0.2 mL, marked from 0 to 25 mL, ascending. Add 25 mL of water, or another agent defined, and stir every 10 minutes for one hour. Let the mixture stand for 3 hours at ambient temperature. Measure the volume, in ml, occupied by plant material plus the mucilage or any other material acceded subtracted from the initial volume of the drug. Calculate the average value obtained from the various individual determinations performed and relate to 1 g of plant material.

5.4.3 METHODS OF PREPARATION AND ANALYSIS OF PLANT EXTRACTS

5.4.3.1 METHODS OF PREPARATION OF PLANT EXTRACTS

5.4.3.1.1 Extracts (*extracta fluida*)

The index of intumescence or swelling index is a measurement of the volume occupied by swelling of 1 g of the drug, by adding water or another agent intumescent, under defined conditions.

DEFINITION

Fluid Extracts are liquid preparations in which, except where specified differently, a part of the extract, in mass or volume, corresponds to a part, by mass, of drugs dry, used in its preparation. If necessary, the extracts fluids can be standardized in terms of concentration of the solvent content of constituents or dry residue. If necessary, can be added to preservatives inhibitors of microbial growth.

OBTAINING

The extracts fluids can be obtained by percolation, maceration or by dissolution of dry extracts or soft tissue using as solvent only ethanol, water or ethanol/water mixtures of suitable proportion. If necessary, the extract obtained can be filtered. Whatever the process of obtaining, the extracts fluids present composition and comparable characteristics. The formation of a slight sediment during storage is acceptable, provided that the composition of the extract undergoes no significant changes.

PURITY TESTING

Relative Density (5.2.5). When this is the case, the extracts fluids must comply with the limits prescribed in the monograph.

Determination of ethanol (5.3.3.8). Determine ethanol content in extracts fluids obtained with ethanol or ethanol/ water mixtures. The ethanol content must meet the specified in the monograph.

Determination of methanol and 2-propanol (5.4.3.2.1). Unless specified differently, the extracts fluids should contain no more than 0.05% (v/v) methanol and not more than 0.05% (v/v) of 2-propanol.

Determination of the dry residue (5.4.3.2.2). The dry residue must comply with the specified in the monograph.

PACKAGING AND STORAGE In well-closed containers, under the light.

LABELLING

The label shall contain the following information:

- Botanical nomenclature of the drug that gave origin to the extract;
- If the extract was prepared with fresh plant (where applicable);
- Composition of the solvent and the ethanol content in percentage (v/v) in solvent used in
- Preparation;
- When this is the case the ethanol content in percentage (v/v) in the finished product.
- CONTENT of active principles and/or drug/extract end;
- Name and concentration of antimicrobial preservatives added.

5.4.3.1.2 Soft tissue Extracts (Extracta spissa)

DEFINITION

The extracts are preparations of soft doughy consistency obtained by partial evaporation of the solvent used in its preparation. Are obtained using as solvent only ethanol, water or mixtures ethanol/ water in proper proportion. They have, at least, 70% of dry residue (p/p). The extracts moles can be added preservatives to inhibit microbial growth.

OBTAINING

The soft tissue extracts are obtained from fluid extracts prepared using only water, ethanol or ethanol/water mixtures in proper proportion; after partial evaporation of the solvent. Have at least 70 % of dry residue (p/p).

PURITY TESTING

Dry Residue (5.4.3.2.2). The dry residue must comply with the specified in the monograph.

PACKAGING AND STORAGE In well-closed containers, under the light.

LABELLING

The label shall contain the following information:

- Botanical nomenclature of the drug that gave origin to the extract;
- Name and quantity of inert material used;
- If the extract was prepared with fresh plant (where applicable);
- Composition of the solvent and the ethanol content in percentage (v/v) in liquid extract that gave rise;

CONTENT of active principles and/or drug/extract end;

- Name and concentration of antimicrobial preservatives added.

5.4.3.1.3 Dry Extracts (*Extracta sicca*)

DEFINITION

Dry Extracts are solid preparations obtained by evaporation of the solvent used in its preparation. They have, at least, 95% of dry weight, calculated as a percentage of weight. The dry extracts can be added to inert materials suitable.

The dried extracts standardized have the content of their constituents adjusted by addition of inert materials suitable or by addition of dry extracts obtained with the same drugs used in preparation.

When necessary, the monograph may prescribe testing limit to the solvent used in the preparation.

OBTAINING

Dry Extracts are solid preparations obtained by evaporation or vaporization of the solvent. Regardless of the technique of drying, must be at least 95 % of dry weight, calculated as a percentage of weight.

PURITY TESTING

Dry Residue (5.4.3.2.3). The dry residue must comply with the specified in the monograph.

PACKAGING AND STORAGE In hermetically sealed containers, under the light.

LABELLING

The label must contain:

- Botanical nomenclature of the drug that gave origin to the extract;
- Name and quantity of inert material used;
- If the extract was prepared with fresh plant (where applicable);
- Name of the solvent and the ethanol content in percentage (v/v) in the solvent used in the preparation;
- CONTENT of active principles and/or drug / final extract.

5.4.3.1.3 Dry Extracts (*Extracta sicca*)

DEFINITION

Dry Extracts are solid preparations obtained by evaporation of the solvent used in its preparation. They have, at least, 95% of dry weight, calculated as a percentage of weight. The dry extracts can be added to inert materials suitable.

The dried extracts standardized have the content of their constituents adjusted by addition of inert materials suitable or by addition of dry extracts obtained with the same drugs used in preparation.

When necessary, the monograph may prescribe testing limit to the solvent used in the preparation.

OBTAINING

Dry Extracts are solid preparations obtained by evaporation or vaporization of the solvent. Regardless of the technique of drying, must be at least 95 % of dry weight, calculated as a percentage of weight.

PURITY TESTING

Dry Residue (5.4.3.2.3). The dry residue must comply with the specified in the monograph.

PACKAGING AND STORAGE

In hermetically sealed containers, under the light.

LABELLING

The label must contain:

- Botanical nomenclature of the drug that gave origin to the extract;
- Name and quantity of inert material used;
- If the extract was prepared with fresh plant (where applicable);
- Name of the solvent and the ethanol content in percentage (v/v) in the solvent used in the preparation;
- CONTENT of active principles and/or drug / final extract.

5.4.3.2 METHODS OF ANALYSIS OF PLANT EXTRACTS

5.4.3.2.1 Determination of methanol and 2-propanol in extracts fluids

Carry out the distillation of extract as described in Determination of ethanol (5.3.3.8.1). Examine the distillate by gas Chromatography (5.2.17.5), using gas chromatograph equipped with a flame ionization detector, chromatographic column of glass with 2 m in length and 2 mm in internal diameter, packaged with copolymer of Ethylvinylbenzene / divinylbenzene, particles of 125 µm to 150 µm, and nitrogen as gas chromatography of drag, with a flow of 30 mL/min. Maintain the temperature of the column at 130 °C, the temperature of the injector at 200 °C and the detector temperature at 220 °C.

Internal standard Solution: solution of 1-propanol to 2.5% (v/v) in water.

Sample Solution: add a specified volume of distillate 2 mL of internal standard solution. Dilute to 50 mL with water or ethanol 90% (v/v), adjusting the ethanol content to 10% (v/v).

Standard Solution: prepare 165 mL of a solution containing 2 mL of internal standard solution, 10% ethanol (v/v), 0.05% of 2-propanol (v/v) and 0.05% of anhydrous methanol (v/v).

Procedure: inject separately, 1 µL of the standard Solution and the sample Solution, record the chromatograms and measure the peak areas. Calculate the concentrations of methanol and 2-propanol in relation to sample submitted to

distillation from the responses obtained with the standard Solution and the sample Solution.

5.4.3.2.2 Determination of dry residue in fluid and soft extracts

Transfer 2 mL or 2 g of extract for weigh-filters or Petri dish, measuring approximately 50 mm in diameter and 30 mm height. Evaporate to dryness on a water-bath and dessecar in an oven at 100 – 105 °C for 3 hours. Let cool in desiccator over phosphorus pentoxide and weigh. Calculate the dry residue in percentage on the earth or on the volume.

5.4.3.2.3 Determination of dry residue in dry extracts

Weigh, in Petri plate measuring approximately 50 mm in diameter and 30 mm height, 0.50 g of dry extract finely pulverized. Dessecar in oven at 100 – 105 °C for 3 hours. Let cool in desiccator over phosphorus pentoxide and weigh. Calculate the dry residue in percentage on the earth.

5.5 METHODS BIOLOGICAL, BIOLOGICAL AND MICROBIOLOGICAL TESTS

5.5.1 METHODS BIOLOGICAL

5.5.1.1 DETERMINATION OF HEPARIN ON COAGULATION FACTORS OF

Heparin is given in the form of a complex to antithrombin III (AT) via inhibition of the activity of coagulation Factor Xa. In the reactive mixture is maintained an excess of TA to ensure a constant concentration of complex heparin-TA. The Factor Xa is inhibited by complex heparin-TA and the residual Factor Xa hydrolyses a chromogenic substrate specific peptide releasing a chromophore. The amount of chromophore is inversely proportional to the activity of heparin.

Chromogenic Substrate for Factor Xa: chromogenic substrate specific Factor Xa as: the hydrochloride of N-a-benzoyl-L-isoleucil-L-glutamyl-glicil-L-arginine-4-nitro-anilida. Reestablished in accordance with the manufacturer's instructions.

Dilution Buffer: solution of ketorolac tromethamine to 0,605% (p/v). If necessary, adjust to pH 8.4 with hydrochloric acid.

Problem Solution: dilute the sample with the dilution Buffer in order to obtain a solution that supposedly contains 71 IU of heparin per millilitre.

Reference Solution: dilute the preparation of reference of heparin with the dilution Buffer so as to obtain a solution containing 0.1 IU of heparin per millilitre. The conditions described above are applicable to the microtitre plates. If the test is performed in tubes, adjust the volumes so as to keep the proportions in the mixture. Shortly before the test, put all the solutions at 37 °C in the water bath. Distribute a series of wells, 20 µL of normal human plasma and 20 µL of antithrombin III SR. Join the wells a series of volumes (20 µL, 60 µL, 100 µL and 140 µL) of Solution problem or of the reference Solution and complete the volume of each well with 200 µL using the dilution Buffer (0.02 – 0.08 UI of heparin per milliliter in the reactive mixture final).

METHOD FROM THE POINT OF EQUIVALENCE

Transfer 40 µL to each well to a second series of wells, add 20,00 µL of solution of bovine Factor Xa and incubate at 37 °C for 30 seconds. Add 40 µL of solution of the chromogenic Substrate for Factor Xa to 1 mmol/L and incubated at 37°C for 3 minutes. Stop the reaction by lowering the pH with an appropriate reagent, such as a solution of glacial acetic acid to 20% (v/v) and measure the absorbance at 405 nm (2.5.14). The reaction time is usually of the order of 3 minutes to 15 minutes, but are tolerated certain variations if they allow improving the linearity of the dose/response curve.

METHOD CINÉTICO

Transfer 40 µL to each well to a second series of wells, add 20 µL of solution of bovine Factor Xa and incubate at 37 °C for 30 seconds. Add 40 µL of solution of chromogenic Substrate for Factor Xa to 2 mmol/L, incubate at 37 °C and determine the speed of cleavage of the substrate by taking a continuous reading of the variation of the absorbance at 405 nm (2.5.14) allowing, therefore, calculate the initial speed of cleavage of the substrate. This speed should be proportional to the residual concentration of Factor Xa. Check the validity of the test and calculate the activity of heparin sample by statistical procedures applicable to biological tests (8).

5.5.1.2 DETERMINATION OF VON WILLEBRAND FACTOR HUMAN

The potency of Von Willebrand's Factor is determined by human comparison, in conditions must be given, of its activity in collagen or as ristocetin cofactor with the same activity, and calibrated using a pattern of international reference, in International units, when applicable. The International Unit is the activity of a declared sum of international reference standard for Von Willebrand factor existing in Factor VIII concentrate of coagulation of human blood. The equivalence in international units of international reference standard is indicated by the World Health Organization (WHO).

DETERMINATION OF CONNECTION TO COLLAGEN VASCULAR DISEASES

The connection to collagen is determined by enzyme immunoassay technique in plaques of micro titration, coated with collagen. The method is based on specific binding of Von Willebrand factor the collagen fibers and the subsequent connection of a polyclonal antibody anti – Von Willebrand factor conjugated to an enzyme. After the addition of a chromogenic substrate for the formation of a product quantified spectrophotometrically. Under appropriate conditions, there is a linear relationship between the collagen, Von Willebrand factor and the absorbance indicated.

MATERIALS

Collagen fibrils of collagen: use of native, equine or human, type I or III. To facilitate handling, can be used solutions of collagen.

Diluent of collagen: dissolve 50 g of glucose in water. Adjust the pH to 2.7 to 2.9 with hydrochloric acid M and dilute with water to 1000 mL.

Chloride-phosphate Buffer: dissolve 8 g of sodium chloride, 1.05 g of sodium phosphate, dibasic dihydrate (0.2 g of monobasic sodium phosphate dihydrate (and 0.2 g of potassium chloride in water. Adjust the pH to 7.2 with sodium hydroxide or hydrochloric acid M M. Dilute to 1000 mL with water.

Washing Solution buffered: solution of polysorbate 20 to 0.1% (p/v) in phosphate Buffer of chloride-.

Neutralization reagent: prepare the Buffer- chloride phosphate containing polysorbate 20 to 0.1% (p/v) and bovine serum albumin at 1% (p/v).

Dilution Buffer: prepare the Buffer- chloride phosphate containing polysorbate 20 to 0.1% (p/v) and bovine serum albumin at 5% (p/v).

Conjugation: serum of rabbit anti-human Von Willebrand Factor conjugated to horseradish peroxidase, a marker histochemistry. Follow the manufacturer's recommendations.

Substrate Solution: dissolve, immediately prior to its use, a pill of hydrochloride of o-phenylenediamine and compressed a peroxide carbamide peroxide in 20 mL of water, or use an appropriate volume of oxygenated water. Protect from light.

Microtitration Plates: must have flat bottom polystyrene boards with surface properties optimized for immunoenzyme assay and protein of high capacity connection.

PROCEDURE

Test Solution: reconstitute the preparation to be examined as indicated on the label. Dilute with Buffer for dilution in order to prepare a solution containing approximately 1 IU/ mL of Von Willebrand factor. Prepare two series independent with at least three dilutions through the use of Buffer for dilution.

Reference Solutions: reconstitute the preparation of reference as indicated. Dilute with Buffer for dilution in order to prepare a solution containing approximately

1 IU/mL of Von Willebrand factor. Prepare two series independent with at least three dilutions through the use of Buffer for dilution.

Allow the solution of collagen to reach ambient temperature. Dilute with diluent of collagen in order to obtain a solution containing 30 to 75 mg/mL of collagen. Mix, softly, to produce a uniform suspension of the collagen fibers and then pipette 0.1 mL and transferred to each well of the microplate. Cover the plate with plastic film and incubate at 37 °C for one day to the other. Empty the plate wells coated with collagen by inversion and drain on a paper towel. Add 0.25 mL of washing Solution buffered. Empty the plate wells by inversion and drain on a paper towel, repeating this operation three times. Add to each well, 0.25 mL of reagent neutralisation, cover the plate with plastic film and incubate at 37 °C for 1 hour. The wells of the plate must be emptied by inversion and drain on paper towel. Add 0.25 mL of washing Solution buffered. Empty the plate wells by inversion and drain on a paper towel. Repeat this operation three times.

Add 0.1 mL of each of the Solutions test or reference to the wells. Add 0.1 mL of Buffer for dilution to a series of wells to obtain the negative control. Cover the plate with plastic film and incuba-la at 37 °C for 2 hours. The wells of the plate must be emptied by inversion and drain on paper towel. Add 0.25 mL of washing Solution buffered. Empty the plate wells by inversion and drain on a paper towel, repeating this operation by three times.

Prepare an appropriate dilution of Conjunction with chloride-phosphate Buffer containing bovine serum albumin in 0.5% (w/v) and add 0.1 mL to each well. Cover the plate with plastic film and incubate at 37 °C for 2 hours. Empty the plate wells by inversion and drain on a paper towel. Add 0.25 mL of washing Solution buffered. Empty the plate wells by inversion and drain on a paper towel. Repeat this operation three times.

Add 0.1 mL of substrate Solution to each of the wells and incubated at room temperature for 20 minutes in the dark. Add 0.1 mL of hydrochloric acid M to each of the wells. Measure the absorbance at 492 nm (5.2.14). Using the absorbance values to estimate the power of the preparation to be examined through the employment of statistical procedures applicable to biological tests (8).

The test is valid if the absorbance measurements for the negative controls are greater than 0.05.

5.5.1.3 DETERMINATION OF BLOOD COAGULATION FACTOR II HUMAN

The determination of human coagulation Factor II is performed after specific activation in Factor IIa. The Factor IIa is calculated by comparing its activity on a chromogenic substrate peptide with the same specific activity of international standard or a standard preparation calibrated in International Units (IU). The International Unit of Factor II corresponds to the activity of a given quantity of international standard, which is composed of a concentrated lyophilized Factor II of blood coagulation. The correspondence between the International Unit and the International Standard is established by the World Health Organization.

The method of determining chromogenic includes two successive stages: activation of Factor II by the action of snake venom and the enzymatic cleavage of a chromogenic substrate by Factor Ila who frees a chromophore quantified by spectrophotometry. In terms of appropriate dosing, there is a linear relationship between the activity of Factor IIa and the cleavage of the chromogenic substrate.

REAGENTS

Specific Activator of Factor II from the venom of the viper (Ecarina): protein obtained from the venom of the viper Echis carinatus, specifically the active Factor II. Reconstitute the preparation following the manufacturer's instructions. Once reconstituted, keep at 4 °C and use within 1 month.

Chromogenic Substrate for Factor Ila: chromogenic substrate specific Factor IIa as: hydrochloride of H-D-phenylalanyl-L-pipecolil-L-arginine-4-nitroanilide) as substrate, 4-toluenosulfonil-glicil-prolil-Larginina-4-nitroanilide) as substrate, H-D-Ciclohexilglicil-to-aminobutiril-L-arginine-4-Nitroanilide) as substrate, D-ciclohexilglicil-L-alanilarginina-4-Diacetate-nitroanilide) as substrate. Rebuild following the manufacturer's instructions.

Dilution Buffer: solution containing 0.606) on % (p/v) of ketorolac tromethamine, sodium chloride to 1,753% (p/v), acid edetico to 0.279); as well % (p/v) and bovine serum albumin or human albumin to 0.1% (p/v). If necessary, Adjust the pH to 8.4 with dilute hydrochloric acid.

PROCEDURE

Test Solution: dilute the sample in dilution Buffer so as to obtain a solution containing 0,015 IU of Factor II Per millilitre. Prepare at least three more dilutions of this solution in dilution Buffer.

Standard Solution: dilute the standard dilution Buffer so as to obtain a solution containing 0,015 IU of Factor II per millilitre. Prepare at least three more dilutions of this solution in dilution Buffer. Put all the solutions on a water-bath at 37 °C, shortly before the test. The conditions described apply to the microtitre plates. If the determination is carried out in tubes, adjust the volumes so as to keep the proportions in the mixture. Introduce 25 µL of different dilutions of the sample Solution and the standard Solution, a series of wells of the microtitre plate maintained at 37 °C. Add to each well 125 µL of dilution Buffer and 25 µL-specific Activator of Factor II from the venom of the viper and incubate for exactly 2 minutes. Add to each well 25 µL of chromogenic Substrate for Factor IIa.

Read the speed of variation of the absorbance at 405 nm (2.5.14) and continue for three minutes so get the average speed of variation of the absorbance. If this is not possible a continuous reading, determine the absorbance at 405 nm in consecutive intervals appropriate, for example, from 40 to 40 seconds. Build the linear graph of absorption values as a function of time and calculate the average speed of variation of the absorbance. From the individual values found for each dilution of the standard and the sample, calculate the activity of the sample and check the validity of the determination by usual statistical methods (8).

5.5.1.4 DETERMINATION OF BLOOD COAGULATION FACTOR IX OF HUMAN

Determine if the activity of the sample, comparing the amount of sample required to reduce the clotting time of a mixture of proof which contains substances, in addition to the Factor IX, necessary for the coagulation of the blood; with the amount of a preparation of reference, assessed in international units, necessary to obtain the same effect.

The International Unit corresponds to the activity of a given quantity of International Standard consisting of a concentrated lyophilized Factor IX blood coagulation. The equivalence in international units of international standard is established by the World Health Organization.

Reconstitute, respectively, the sample and the preparation of reference in accordance with label directions and use immediately. When applicable, determine the amount of heparin present and neutralize by adding protamine sulfate (10 µg of protamine sulfate counter 1,0 IU of heparin). Dilute the sample and the preparation of

Reference with imidazole buffer pH 7.3 in order to obtain solutions with 0.5 to 2.0 IU per millilitre. With a mixture of sodium citrate to 3.8 % (p/v) and imidazole buffer pH 7.3 (1:5), prepare a series of dilutions including 1/10.1/20.1/4.1/80. These dilutions should be prepared with precision and are used immediately.

Use, for example, pipes of hatching kept in a water bath at 37 °C. Enter in each tube 0.1 mL of substrate plasma and 0.1 mL of each of the dilutions of the preparation of reference and the sample. Add to each tube 0.1 mL of an appropriate dilution of cephaeline SR or substitute of platelets and 0.1 mL of a suspension of 0.5 g of kaolin mild in 100 mL of sodium chloride 0.9% (p/v) and leave to rest for approximately 10 min, by tilting the pipes regularly. Add to each tube 0.1 mL of calcium chloride solution to 0.74% (p/v). With the aid of a stopwatch, determine the coagulation time, that is, the time interval between the time of the addition of calcium chloride and the first indication

of formation of fibrin that is observed visually or with appropriate tools. Calculate the activity using the statistical procedure applicable to biological tests (8).

To ensure that there is no significant contamination of the substrate by plasma Factor IX, carry out a blank test using, instead of the sample, a corresponding volume of a mixture of sodium citrate to 3.8% (p/v) and imidazole buffer pH 7.3 (1:5). The test is only valid if the clotting time determined for the blank is between 100 and 200 seconds.

5.5.1.5 DETERMINATION OF FACTOR VII THE HUMAN BLOOD COAGULATION

The determination of coagulation Factor VII is performed by determining its biological activity as a cofactor in the activation of Factor X by Factor VIIa/Tissue Factor in the presence of calcium ions and phospholipids. The activity of a preparation of Factor VII is calculated by comparing the respective quantities of preparation and of international standard or a preparation of reference determined in international units that are necessary to obtain a speed of formation of Factor Xa in a reaction medium containing different substances that are involved in the activation of Factor X.

The International Unit of activity of Factor VII corresponds to the activity of a given quantity of international standard that is currently composed of a lyophilized plasma. The correspondence between the International Unit and the International Standard is established by the World Health Organization.

The method of determining chromogenic involves two successive stages: the activation of Factor X, under the action of Factor VIIa, a reactive mixture containing the Tissue Factor/phospholipid-apolipoproteincomplex and the calcium ion and the enzymatic lysis of a chromogenic substrate by Factor Xa which liberates a chromophore quantifiable by spectrophotometry. In

Appropriate conditions of determination, there is a linear relationship between the speed of formation of Factor Xa and the concentration of Factor VII. The following diagram summarizes the principle of determination:

Step 1

$$\text{a) Factor VII} \xrightarrow{\text{Tissue Factor} + Ca^{2+}} \text{Factor VIIa}$$

$$\text{b) Factor X} \xrightarrow{\text{Factor VIIa} + Ca^{2+} + \text{Tissue Factor/phospholipid}} \text{Factor Xa}$$

Step 2

$$\text{a) Chromogenic Substrate} \xrightarrow{\text{Factor Xa}} \text{Peptide + chromophore}$$

The two steps utilize reagents available in the market, originating from various vendors. Although the composition of these reagents may vary slightly, their essential characteristics are described in the specifications that follow.

REAGENTS

The reactive mixture of coagulation factors contains, especially, purified proteins of human origin, or encephalopathy, specifically the X Factor, the thromboplastin and Tissue Factor/phospholipid-apolipoproteincomplex and an activator of Factor VII. These proteins are, partially purified, and does not contain impurities capable of interfering with the activation of Factor VII or Factor X. The X Factor is present in an amount such that its final concentration, outside the activation step is 10 – 350 nmol/L, preferably 14 – 70 nmol/L. The thromboplastin used can be of natural origin (brain of ox or rabbit) or synthetic. The thromboplastin used for determining the time of Quick is diluted 5 to 50 times in a buffer solution so that the final concentration of Ca2+ is of 15-25 nmol/L. The final step of formation of Factor Xa is conducted in a solution containing human serum albumin or bovine serum albumin at a concentration at which no losses occur in adsorption and, conveniently, buffered to pH between 7.3 and 8.0. The Factor VII is the only Factor that limits the formation of Factor Xa in incubation mixture end and none of the constituents of the reactive mixture has the power to induce alone the formation of Factor Xa.

The second step consists in quantifying Factor Xa formed in the previous step, in the midst of a chromogenic substrate specific Factor Xa. This substrate is generally a short peptide derived from 3 to 5 amino acids connected to a reverse split chromophore. The division of this grouping and the substrate peptide promotes a shift of activity cromoforica for a wavelength that allows its quantification by spectrophotometry. The substrate is usually dissolved in water and used at a final concentration of 0.2 – 2 nmol/L. It is possible to understand the inhibitors

Appropriate preventing the continuing training of Factor Xa (addition of iodide).

PROCEDURE

To Reconstitute, separately, the contents of an ampoule of preparation of reference and the sample by adding a quantity of water required and once reconstructed) use them in the space of one hour. Add the preparations reconstituted the quantities of pre-diluent necessary to obtain solutions to 0.5 – 2.0 IU of Factor VII per millilitre.

Prepare the dilutions following the preparation of reference and the sample with an isotonic buffer solution without agent of chelation, containing human serum albumin or bovine to 1% (p/v), and preferably buffered to pH 7.3 – 8.0. Make each one of the two preparations at least three separate dilutions independent, preferably, in duplicate. The concentrations of these dilutions in Factor VII are adjusted so that the final concentration is less than 0,005 IU/mL.

Prepare, also, a control solution containing all the constituents of the reactive mixture with the exception of Factor VII.

All dilutions are prepared in plastic tubes and used during the first hour.

Step 1. TO each of the dilutions, obtained from the preparation of reference and the sample, add an appropriate volume of coagulation reagent pre-heated (or of a mixture of its separate constituents), mix and incubate at 37 °C in plastic tubes or wells of a microplate. The concentration of different constituents during the formation of Factor Xa is as specified in the reagents. Allow to develop the reaction of activation of Factor X during an appropriate time; the end of the reaction happens, preferably before the concentration in Factor Xa has reached its maximum level, so that the dose-response curve presents a satisfactory linearity. The reaction time is also chosen so that the condition of linearity of the production curve of Factor Xa as a function of time is satisfactory. It is generally of the order of 2 to 5 minutes, but are admissible certain variations for allotments improve the linearity of the dose-response curve.

Step 2. Stop the reaction of activation by adding a reactive mixture containing the chromogenic substrate. The speed of lysis of the substrate, which is proportional to the concentration of Factor Xa is determined with the aid of a spectrophotometer by variation of the absorbance at a wavelength appropriate. You can determine if the absorbance, continuously, which allows calculating the initial speed of lysis of the substrate, either by stopping the reaction of hydrolysis at the end of an appropriate time, lowering the pH with an appropriate reagent as the acetic acid to 50% (p/v) or a solution of sodium citrate M at pH 3.0. Adjust the time of hydrolysis so that the condition of linearity of formation of the chromophore as a function of time is satisfactory. This time is usually of the order of 3 to 15 minutes, but are tolerated certain

Variations if they permit improve the linearity of the dose-response curve. Check the validity of the titration and calculate the activity of sample preparation by statistical procedures applicable to biological tests (8).

5.5.1.6 DETERMINATION OF BLOOD COAGULATION FACTOR X OF HUMAN

The determination of the X-Factor of human blood coagulation is performed after specific activation in Factor Xa, which is calculated by comparing their activity in cleave a chromogenic substrate peptide with the same specific activity of International Standard or a preparation of reference calibrated in International Units.

The International Unit of X Factor corresponds to the activity of a given quantity of International Standard that is composed of a lyophilized concentrate X Factor of human blood coagulation.

The correspondence between the International Unit and the International Standard is established by the World Health Organization.

The method of determining chromogenic includes two steps: activation of Factor X under the action of snake venom, followed by enzymatic cleavage of a chromogenic substrate by Factor Xa which liberates a chromophore quantified by spectrophotometry. In terms of appropriate dosing, there is a linear relationship between the activity of Factor Xa and the cleavage of the substrate.

REAGENTS

Specific Activator X Factor coming from the venom of the viper of Russel (RVV): protein obtained from the venom of the viper of Russel (Vipera russelli) which activates, specifically, the Factor X. Reconstitute the preparation by following the manufacturer's instructions. Once reconstituted, keep at 4 °C and use within 1 month.

Chromogenic Substrate for Factor Xa: chromogenic substrate specific Factor Xa as: hydrochloride of N-to-benzyloxycarbonyl-D-arginil-L-glicil-L-arginine-4-nitroanilide) as substrate, the hydrochloride of Nbenzoil-L-isoleucil-L-glutamyl-glicil-L-arginine-4-nitroanilide) as substrate, metanosulfonil- D-leucyl-glicil-L-arginine-4-nitroanilide) as substrate, acetate of methoxycarbonyl-D-cycle-hexilalanil-glicil-L-arginine- 4-nitroanilide) as substrate. Rebuild following the manufacturer's instructions.

Dilution Buffer: solution containing ketorolac tromethamine to 0.37% (p/v), sodium chloride 1.8% (p/v), imidazole to 0.21% (p/v), bromide hexadimetrina to 0.002% (p/v) and bovine serum albumin, or of human albumin to 0.1% (p/v). If necessary, adjust to pH 8.4 with hydrochloric acid.

PROCEDURE

Test Solution: dilute the sample in dilution Buffer so as to obtain a solution containing 0.18 IU of Factor X per millilitre. Prepare at least three more dilutions of this solution in dilution Buffer.

Standard Solution: dilute the preparation standard in dilution Buffer so as to obtain a solution containing 0.18 IU of Factor X per millilitre. Prepare at least three more dilutions of this solution in dilution Buffer.

Shortly before the test, put all the solutions in a water bath at 37 °C.

The conditions described apply to the microtitre plates. If the determination is performed in tubes, adjust the volumes so as to maintain the proportion in mixtures.

Transfer 12.5 µL of different dilutions of the test or the standard Solution to a series of wells of a microtitre plate maintained at 37 °C. Add to each well 25 µL of VVR. Incubate for exactly 90 seconds. Add to each well, 150 ^L chromogenic Substrate for Factor Xa, diluted six times in

dilution Buffer. Read the variation of absorbance at 405 nm (5.2.14) and continue for three minutes in order to obtain the average speed of variation of the absorbance. If this is not possible a continuous reading, determine the absorbance at 405 nm with consecutive intervals appropriate, for example, from 40 to 40 seconds. Build the linear graph of absorbance values as a function of time and calculate the average speed of variation of the absorbance. From the individual values found for each dilution of the standard and the sample, calculate the activity of the sample and check the validity of the measurement by usual statistical methods (8).

5.5.1.7 DETERMINATION OF FACTORVIII THE HUMAN BLOOD COAGULATION, LYOPHILIZED

AND performed by determination of the biological activity of Factor VIII as a cofactor in the activation of Factor X by Factor IX activated (IXa) in the presence of calcium ions and phospholipids. The activity of a preparation of Factor VIII is calculated by comparing the respective quantities of preparation and of International Standard; or of a preparation of reference calibrated in international units that are required to achieve a given speed of formation of Factor Xa in a reaction medium containing different substances that are involved in the activation of Factor X.

The International Unit of Factor VIII activity corresponds to the activity of a given quantity of International Standard that consists in a concentrated lyophilized Factor VIII of human blood coagulation. The equivalence of International Standard with International Units is established by the World Health Organization (WHO). The Factor VIII concentrate human blood coagulation is calibrated in international units in relation to the International Standard. The colorimetric method of benchmarking consists in two successive stages: the activation of Factor X under the action of Factor VIII in a reactive mixture of

Coagulation factors composed of substances purified and the enzymatic cleavage of a chromogenic substrate by Factor Xa that frees a chromophore quantifiable by spectrophotometry. Under appropriate conditions of benchmarking there is a linear relationship between the speed of formation of Factor Xa and the concentration of Factor VIII. The following diagram summarizes the principle of benchmarking:

Step 1

Factor X —(Factor VIII activated; Factor IXa, phospholipid, Ca^{2+})→ Fator Xa

Step 2

Chromogenic substrate —(Factor Xa)→ peptide + chromophore

The two steps utilize reagents which can be obtained commercially. Although the composition of these reagents can be subject to some variation, their essential characteristics are described in these specifications. Can be allowed deviations in relation to such specifications provided that it is demonstrated, through the use of the International Standard, that the results obtained did not differ significantly. The commercial packaging are used according to the manufacturer's instructions; it is important to ensure that the packaging chosen is appropriate.

The sets used must be properly validated, it may be used in this case, the verification of the time of generation of Factor Xa, in order to determine the time required to reach 50% of maximum formation of Factor Xa.

REAGENTS

The reactive mixture of coagulation factors corresponds the purified proteins, of human origin, or encephalopathy, specifically, the X Factor, the Factor IXa and an activator of Factor VIII, generally the thrombin. These proteins are partially purified, preferably, at least 50% and does not contain impurities capable of interfering with the activation of Factor VIII or Factor X. The thrombin can be present in the form of its precursor, prothrombin, provided that their activation in the reactive mixture is fast enough to allow a complete activation and almost instantaneous of Factor VIII in the test. The reactive mixture must contain phospholipids that may be of natural origin (for example: brain, spinal cord and bovine soybean extract) or artificially obtained, being constituted, by approximately 15 to 31% of phosphatidylserine. The final concentration in phospholipids during the training stage of Factor Xa is approximately 10 to 35 ^mol/L. The reactive mixture also contains calcium ions in such quantity that its final concentration is from 5 to 15 mmol/L. The final step of formation of Factor Xa is conducted in a solution that should contain, at a minimum, 1 mg/mL of human albumin, or encephalopathy, conveniently buffered (pH 7.3 to 8.0). The various constituents of the medium reactive are generally gathered in two separate preparations, that should not induce alone the formation of Factor Xa. After reconstitution these two preparations may be satisfied with the condition that no form quantities of Factor Xa in the absence of Factor VIII. The Factor VIII is the only factor that limits the formation of Factor Xa in incubation mixture final. The second step consists in quantifying Factor Xa formed in the previous step in the midst of a chromogenic substrate specific Factor Xa. This substrate is generally a short peptide derived from 3 to 5 amino acids linked to a grouping chromophore. The division of this grouping and the substrate peptide promotes a shift of activity cromoforica for a wavelength that allows its quantification by spectrophotometry. The substrate, usually dissolved in water and used at a final concentration of 0.2 to 2 mmol/L, should contain the appropriate inhibitors the impediment of additional training of Factor Xa and suppress the whole activity trombinica, which allows improving the selectivity of the assay in the presence of Factor Xa.

PROCEDURE

Must be reconstituted all contents of an ampoule of preparation of reference and the sample by adding the amount of water; use immediately. Add quantities of pre-diluents required to obtain solutions between 0.5 to 2.0 IU/mL. The pre-diluent is composed of plasma from a donor bearer of severe hemophilia A, or a reagent prepared artificially, giving equivalent results to those obtained with plasma hemophilia and with the same preparations standard and sample. The pre-diluted solutions should present good stability in addition to the time necessary to determine (at least 30 minutes) to 20 °C, and must be used within 15 minutes. Perform the following dilutions of the standard and the sample preparation by means of an isotonic buffer solution without agent of chelation, and containing 1% human albumin or encephalopathy; the solution may contain, for example, tromethamine or imidazole and is preferably buffered (pH 7.3 to 8.0). Prepare at least three additional independent dilutions, preferably in duplicate. The solutions should be prepared so that the final concentration in Factor VIII, outside the step of formation of Factor Xa, is less than 0.03 IU/mL and preferably to 0.01 IU/mL. Prepare a standard containing all the constituents of the reactive mixture, with the exception of Factor VIII. Prepare the dilutions in plastic tubes and use immediately.

Step 1. TO each of the dilutions pre-heated, obtained from the standard and the sample preparation, add an appropriate volume of coagulation reagent pre-warmed (or of a mixture of its separate constituents), mix and incubate at 37 °C in plastic tubes or wells of a micro-plate. Let the reaction of activation of Factor X during appropriate time; the end of the reaction happens preferably before that the concentration in X Factor has reached its Maximum level, so that the dose-response curve presents a satisfactory linearity. The reaction time is also chosen so that the condition of linearity of the production curve of Factor Xa as a function of time is satisfactory. AND generally of the order of 2 to 5 Minutes ago not being admissible certain variations that make it possible to improve the linearity of the dose-response curve.

Step 2. Stop the reaction of activation by adding a reactive mixture containing the chromogenic substrate. The rate of cleavage of the substrate, which is proportional to the concentration of Factor Xa, is determined in a spectrophotometer, by variation of the absorbance at a wavelength appropriate. Determine the absorbance, continuously, in order to permit the calculation of the initial speed of cleavage of the substrate, either by stopping the reaction of hydrolysis at the end of an appropriate time by lowering the pH, with an appropriate reagent such as acetic acid (50% v/v of $C_2H_4O_2$) or M citrate buffer pH 3.0. Adjust the time of hydrolysis so that the condition of linearity of the formation of the chromophore as a function of time is satisfactory. This time is usually of the order of 3 to 15 minutes, being tolerated certain variations, provided that enable the improvement of the linearity of the dose-response curve. Check the validity of the test and calculate the activity of the sample by statistical procedures applied to biological tests (8).

5.5.1.8 DETERMINATION OF ACTIVATED COAGULATION FACTORS OF

If the sample contains heparin, determine the existing amount and neutralize by adding protamine sulfate (10 µg of protamine sulfate counter 1 IU of heparin). With the tris-sodium chloride pH 7.5, prepare dilutions to 1/10 and 1/100. Place a series of tubes of polystyrene in a water-bath at 37 °C. Enter in each tube 0.1 mL of platelet poor plasma and 0.1 mL of an appropriate dilution of cephaeline SR or a phospholipid preparation which will act as a substitute of platelets. Leave to rest for 60 seconds and add to each tube 0.1 mL of one of the dilutions and for the test tube blank 0.1 mL of buffer solution.

Join, immediately, to each tube 0.1 mL of a solution of calcium chloride to 0.37% (p/v), previously heated to 37 °C, and determine the time interval between the addition of calcium chloride solution and the formation of the clot, this determination is carried out in the 30 minutes that follow the first dilution. The test is only valid if the clotting time test in white is from 200 to 350 seconds.

5.5.1.9 DETERMINATION OF TITLE HAEMAGGLUTININ ANTI-A AND ANTI-B (INDIRECT METHOD)

Prepare a serial dilution in duplicate of the preparation to be examined in a solution of sodium chloride 0.9% (p/v). For each dilution of a series, add volume equal to 5% (v/v) suspension of erythrocytes in group A1. The red blood cells should be washed three times in a solution of sodium chloride. For each dilution of another series add equal volume of 5% (v/v) suspension of red blood cells in group B. The red blood cells should be washed three times in a solution of sodium chloride to 0.9% (p/v). Incubate the dilution series at 37 °C for 30 minutes and then washed three times with 0.9% sodium chloride (p/v). Leave the red blood cells in contact with the polyvalent human antiglobulin reagent for 30 minutes. Without centrifuge, examine each suspension for agglutination in microscope.

5.5.1.10 TECHNIQUES OF AMPLIFICATION OF NUCLEIC ACIDS

INTRODUCTION

The techniques of nucleic acid amplification were established based on two different principles:

a) Amplification of a target sequence of nucleic acids using the polymerase chain reaction (PCR), the ligase chain reaction (LCR), or the isothermal amplification of a sequence of ribonucleic acid (RNA);

b) Amplification of a signal of hybridisation to the deoxyribonucleic acid (DNA) through the employment of the method of branched DNA (bDNA), for example. In this case, the signal amplification is accomplished without submitting the nucleic acid to repetitive cycles of amplification.

In general lines, the PCR method is described as the reference technique. Alternative methods may be used, provided that they meet the requirements of quality and are properly validated.

FIELD OF APPLICATION

Establish the requirements for the preparation of the sample, the amplification of sequences of DNA and specific detection of the product of the PCR reaction. The PCR enables detection and amplification of defined sequences of DNA and RNA (after reverse transcription into complementary DNA – cDNA).

PRINCIPLE OF THE METHOD

The PCR is the foundation of a method that allows the in vitro amplification of specific segments of DNA or RNA. After denaturation of the double chain of DNA in simple chains of DNA, two synthetic oligonucleotide primers, of opposite polarity, if hibridizam with their respective complementary sequences in DNA to be amplified. In this case, the activity of initiators allows it to be supplemented the simple chain of DNA, giving rise to short sequences, biquaternarias surrounding the fragment of DNA to be amplified; thus serving as the starting point of DNA synthesis. Stressing that such process is performed through the action of a thermostable DNA polymerase.

The amplification of DNA occurs in cycles that consist in:

- Denaturation of nucleic acid by heat (target sequence to be amplified) in two chains monoquaternarias;
- Specific hybridization of primers with the sequence to be amplified, under appropriate conditions of reaction;
- Stretching exercises, through the action of DNA polymerase, of initiators connected to each of the two chains simple, at a suitable temperature (favorable to the process of DNA synthesis).

The repeated cycles of denaturation by heat, the hybridization of primers and DNA synthesis gives rise to an exponential amplification of DNA fragment then delimited by initiators.

The specific product of the PCR reaction, known as amplicon, can be detected by means of a variety of methods of appropriate specificity and sensitivity.

The test of Multiplex PCR uses several pairs of primers, intended for simultaneous amplification for different targets of a reaction.

MATERIAL FOR THE TEST

Due to the high sensitivity of PCR, the samples must be protected from the incidence of light and any external contamination. The sampling, storage and transport of the material to be tested shall be developed in conditions that make it possible to minimize the risk of degradation of the sequence to be amplified. In the case of sequences of RNA marked, special precautions must be taken now that the RNA is very sensitive to degradation by Ribonuclease protection assays, as well, to some additives (anticoagulants and preservatives) that can interfere with the tests.

PROCEDURE

Prevention of contaminants

The risk of contamination requires the existence of restricted areas, according to the nature of the materials and technology used. The points to be considered include: the movement of personnel, the work flow, the movement of materials, ventilation systems and decontamination procedures.

You may want to perform a subdivision of the system in areas such as:

- Preparation area (primary site where they manipulate exclusively materials not contained in the matrix, for example, the initiators and tampons);
- Area pre-PCR (where are manipulated the reactive, the samples and the controls);
- Area of amplification (where the amplified material is handled in closed system);
- Detection area post-PCR (single area in which the products of amplification are handled in open system).

Preparation of samples

The preparation of samples consists in extraction or in release of the target sequence to be amplified from the material to be examined. The method used for this purpose must be effective, have reproducibility and compatibility with the attainment of amplification reaction conditions selected. Can be used a variety of physico-chemical methods for extraction and/or enrichment.

Possible additives in the material under analysis may interfere with the PCR method. Must be used the procedures described in the item of Internal Control, with the objective of verifying the absence of inhibition factors in the material to be examined.

As For the models of RNA, precautions must be taken to ensure that there is no activity of type ribonuclease.

Amplification

The amplification of a target sequence by PCR technique requires, at the very least, a pair of primers, the four types of desoxinucleotideos triphosphate (dNTPs), magnesium ions (MgCl2), and a thermostable DNA polymerase for DNA synthesis.

The amplification of the target sequence by PCR is conducted under cyclical conditions defined: temperature profile for denaturation of the double-helix of DNA; annealing and extension of initiators and incubation times at selected temperatures within a range of variation.

Must be considered the following parameters:

- The length and base composition of the initiator and target sequence;
- The type of DNA polymerase, buffer composition and the volume of reaction used in amplification;
- The type of thermal cycler used and the rate of thermal conductivity between the equipment, the tube of reaction and the reaction medium.
- The amplification occurs in cycles that consist in:
- Denaturation of the target sequence of the nucleic acid by heating of the two propellers; simple reaction is heated between 92 – 96 °C;
- Specific annealing of primers to target sequence that will be synthesized, under appropriate conditions of reaction. The temperature is typically of 55 °C, depending on the homology of initiators by target sequence to be amplified, the composition of the initiators and the quantity of bases cytosine and guanine;
- Extension of initiators that are linked to the propellers simple, through the action of DNA
- Thermostable polymerase, to a temperature suitable for the synthesis of DNA. Normally at 72 °C;
- After the end of the baking cycle has-if the cooling at 4 °C and conservation.

Detection

The amplified sequence generated can be identified: by its size, by its sequence, by chemical modification or by a combination of these parameters. The detection and characterization by means of the size can be performed by gel electrophoresis (using plates of agarose gel or polyacrylamide gel, or by capillary electrophoresis), or even by column chromatography (for example, HPLC - High performance liquid chromatography)). The detection and characterization by the composition of the sequence can be performed by hybridization with specific probes complementary to the target sequence or by fragmentation of the amplified material through a restriction enzyme in specific sites of the sequence to be amplified. The characterization by means of chemical modification can be performed by incorporation of a fluoroforo in strings marked and later excitation and detection of fluorescein. They may also be used probes marked that enable a radioisotopic detection later or immunoenzymatic techniques.

EVALUATION AND INTERPRETATION OF RESULTS

The result of a test is only valid if the control (s) positive (s) is (are), unequivocally positive and control (s) negative (s) is (are), unambiguously negative (s). Due to the high sensitivity of the PCR method and the inherent risks of contamination, it is necessary to confirm the positive results by performing the assay in duplicate or, when there is a possibility, with a new aliquot of the sample. The sample was considered positive if at least one of repeated tests show positive result.

5

QUALITY ASSURANCE

Validation of the test system of PCR

The validation program should include the equipment and the PCR method used. How references should be used the recommendations of ICH (The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use: Q2B, Validation of the Analytical Method), or reference equivalent alternative.

It is essential log this validation by biological standards of reference official, properly calibrated by means of International Standards for the target sequences used in the test.

The validation shall include the determination of the threshold of positive response, i. e., the minimum number of sequences marked by unit of volume that can detect at least 95% of the tests. This value depends on several inter-related factors, such as: the volume of the sample extraction and the effectiveness of the method of extraction; transcript of RNA marked in complementary DNA; the procedure of amplification and detection system. To define the detection limit of the system used, it should be considered the threshold of positive response for each sequence to be amplified and the operating characteristics of the test with the respective maximum and minimum limits of positive response.

Quality Control of reagents

All reagents used in crucial methodology implemented in practice should be control object before its use in routine.

The acceptance/rejection should be based on criteria of quality pre-defined.

The initiators are one of the essential components of the PCR method, thus requiring special attention regarding its design; purity and validation of its use in the test. Each new batch of initiators must be controlled and the specificity; effectiveness of amplification and absence of impurities which are inhibitory. The initiators can be modified (for example, by conjugation with a fluoroforo, or an antigen) so as to allow the use of a specific method for the detection of the target sequence to be amplified; provided that those modifications do not inhibit the accuracy and efficiency of amplification of the target sequence.

Test controls

External Controls

To detect possible contamination and ensure adequate sensitivity, it should be included in all PCR assays the following external controls:

- A positive control with a set number of copies of the target sequence, being this number determined, specifically, for each test system and expressed as a multiple of the threshold of positive response of the system in question;

- A negative control consisting of a sample of matrix that has demonstrated to be free of target sequences.

Internal Control

The internal control is formed by nucleotide sequences defined containing binding sites from the initiator. The internal control should be amplified with effectiveness defined and the products must be clearly discernable. This internal control must belong to the same type of nucleic acid (DNA/RNA) of the sample. The internal control is preferably added to the sample before the isolation of the nucleic acid and, therefore, acts as a global control (extraction, reverse transcription, amplification and detection).

External quality Assessment

For each laboratory and each operator, participation in external programs of quality assessment is an important aspect of quality assurance in PCR.

The following section (5.5.1.10.1) is given the title of information.

5.5.1.10.1 Recommendations for validation of techniques of nucleic acid amplification for the detection of RNA of hepatitis c virus (HCV) in mixtures of plasma.

INTRODUCTION

This chapter is provided for information purposes.

The majority of techniques of nucleic acid amplification corresponds to quantitative analytical tests designed to detect their presence. There are some quantitative assays marketed or developed internally by own labs. To detect contamination of the HCV RNA in mixtures of plasma, are suitable the qualitative assays, and can also be considered as limit test for control of impurities. These recommendations are described the methods for validation of techniques for nucleic acid amplification only applicable to qualitative assays for detecting HCV RNA in mixtures of plasma. Therefore, the two validation parameters considered most important are the specificity and the limit of detection. The robustness is also evaluated. However, this document can also be used as a basis for general validation of amplification techniques.

This document is set to analytical technique such as the set of operations performed after nucleic acid extraction, followed by detection of the amplified products. Pointing out that in cases of use of commercial sets, as part of the complete analytical procedure, the considerations of validation documented already carried out by the manufacturer can replace the validation by the operator. However, the performance of all marketed with respect to the use for which it is intended must be demonstrated by the user (ex: limit of detection, robustness and cross-contamination).

SPECIFICITY

Specificity is the ability to assess, unequivocally, the nucleic acid in the presence of components of presence not expected.

The specificity of the analytical procedures of nucleic acid amplification is dependent on the choice of primers, the choice of the probe (for analysis of the final product) and the rigor of the test conditions (for both steps of amplification and detection).

In the design of primers and probes, one of the aspects to be considered is its specificity in detecting HCV RNA; for this fact is appropriate to compare the target sequences with sequences published in databases. For the HCV, primers and probes are, normally, chosen from the areas of the region 5' not codifying (5NCR) of the genome of HCV, composed by 341 nucleotides, which are the most conserved among the different isolates of HCV.

The Amplified product should be identified, unequivocally, by the use of methods such as: amplification with primers vortical, analysis of restriction enzymes, sequencing, or hybridization with specific probe.

For validation of the specificity of the analytical technique, it should be tested, at a minimum, 100 mixtures of plasma negative for HCV RNA, and all the results are negative. The World Health Organization (WHO) has adequate samples of plasmas not reactive.

The capacity of the technique in the detection of all genotypes of HCV will depend on the choice of primers,

the probes and operational parameters. It is desirable that such capacity is demonstrated through the use of a collection of reference preparations characterized.

It has been suggested that the pattern of distribution of the genotypes of HCV in Brazil is similar to that found in many European countries, with the prevalence of types

1 And 3. It is observed a epidemiological behavior of a typical spread exponentially in recent years, probably as a result of blood transfusions. In this context, the genotypes 1 and 3 should be detected at appropriate levels.

LIMIT OF DETECTION

The limit of detection of an individual technique is the smallest quantity of nucleic acid that can be detected, but not necessarily, quantified, with an exact value in the sample.

The amplification process used for the detection of HCV RNA in mixtures of plasmas provides, generally, qualitative results. The number of possible results is limited to two answers: positive or negative. Although it is recommended to determine the limit of detection, for practical reasons, it is determined the threshold of positive response to the techniques of nucleic acid amplification. The threshold of positive response is the minimum number of target sequences per unit volume that can be detected in 95% of the tests. This threshold of positive response is influenced by the distribution of viral genomes in individual samples tested and by factors such as the effectiveness of the enzyme, which may lead to differences of 95% in the positive response thresholds obtained in individual scans.

To determine the threshold of positive response, it is essential to perform technique on different days with a series of dilutions of a reagent of work or the Hepatitis C virus (organic standard of reference), calibrated by comparison with the International Standard of HCV 96/790 WHO, in order to assess between the various tests. Are tested, at a minimum, three series of separate dilutions with a sufficient number of replications of each dilution in order to obtain a total number of 24 results by dilution and allow, as well, the statistical analysis of the results.

For example, in a laboratory tests-if three series of dilutions with eight replicates for each dilution on different days; four dilution series with six replications for each dilution on different days, or six dilution series with four replications for each dilution on different days.

For which the number of dilutions used if hold equal, is indispensable perform a preliminary test (as, for example, logarithmic dilutions in the sample of the mixture

Plasma to obtain a preliminary value of the threshold of positive response, i. e. the highest dilution in which occurs a positive sign).

The distribution of the dilutions can then be performed on the basis of this preliminary value pre-calculated (using, for example, a dilution factor of 0.5 log), or less than a mixture of plasma negative as array of dilution. The content in the HCV RNA that can be detected is of 95% in the tests and can be calculated using an appropriate statistical method.

These results, also, serve to demonstrate the variation of internal test and the variation in several days of the analytical method.

ROBUSTNESS

The robustness of an analytical method is the measure of its capacity to remain unchanged when subjected to small, but deliberate, variations in the operational parameters and provides an indication of the viability of the technique under normal conditions of use. The evaluation of the robustness is one of the aspects to be considered during the development phase. It allows the establishment of the the viability of the technique face deliberate variations in the operational parameters. In the techniques of nucleic acid amplification, small variations in the operational parameters can have a special importance. However, the robustness surrounding this can be demonstrated during the development of the method, when are tested small variations in concentration of reagents (for example: MgCl2, primers, or dNTPs). To demonstrate the robustness during the validation, should consider whether, at the very least, twenty mixtures of plasma (chosen at random) negative for HCV RNA to which is added a concentration, typical end, which corresponds to the threshold of the positive response, previously determined. All the results are positive.

Problems can arise with the robustness in the case of methods that use, in its initial phase, the ultracentrifugation prior to extraction of viral RNA. Therefore to test the robustness of these methods. Are tested, at a minimum, twenty mixtures of plasma containing varying concentrations of HCV RNA, but free of antibodies specific to the HCV All results obtained are positive.

It should demonstrate the absence of cross-contamination by accurate detection of a set of at least 20 samples, alternating samples of mixtures of plasma negative and of mixtures of plasma negative which has been added a high concentration of HCV (at least 102 times, 95% of the threshold of positive response, or, at least, 104 IU/mL.

QUALITY ASSURANCE

The methods of biological tests such as the technique of nucleic acid amplification, can present specific problems that interfere with the validation and interpretation of results.

The procedures must be described precisely in the form of standard operating procedures (SOPs), which should cover:

- Sampling mode (type of containers, etc.);
- Preparation of minimisturas (if applicable);
- Conservation conditions before analysis;

- Exact description of operating conditions (including precautions that should be taken, in order to avoid cross-contamination or destruction of viral RNA) as well as the reagents and reference preparations used;

- Formulas detailed calculation of results, including the statistical evaluation.

The use of an appropriate control (for example, an appropriate dilution of the hepatitis C virus, organic standard of reference; or plasma to which has been added a sample of HCV calibrated by comparison with the International Standard of HCV 96/790 of the WHO) can be considered a stable means of satisfactory control of the system and to ensure and maintain the viability of the technique in each use.

Technical Qualification. For each critical element of the equipment used is created a proper installation and a program of operational qualification. After any modification of a critical equipment (for example, the thermal cyclers) is indispensable reconfirm the acceptability of the technique by proceeding in parallel examination of eight samples of a mixture of plasma to which is added a triple concentration of HCV RNA that corresponds to the threshold of positive response previously determined; all the results obtained are positive.

Qualification of operators. is developed an appropriate program of qualification for the set of operators involved in the test. For this purpose, it is desirable that each operator examine at least eight samples of a mixture of plasma to which was added a triple concentration of HCV RNA that corresponds to the threshold of positive response, previously determined. This test (eight samples) is repeated twice on different days in a total of twenty-four analyzes conducted in three different days. All the results are positive.

5.5.1.11 DETERMINATION OF TITLE ACTIVATOR PRE-CALICREÍNA

The activator of pre-kallikrein (APC) transforms the pre-kallikrein kallikrein in and can be titrated by presenting the ability to split a chromophore of a synthetic peptide substrate, determining if the reaction speed by spectrophotometry. The concentration in APC is calculated by comparison with the standard preparation whose activity is expressed in international units. The International Unit corresponds to the activity of a given quantity of International Standard consisting of enabler of pre-kallikrein lyophilized. The correspondence

Between the International Unit and the International Standard is established by the World Health Organization.

REAGENTS

Buffer A

Tromethamine	6.055 G
Sodium chloride	1.17 G
Hexadimetrina bromide	50 MG
Sodium azide	0.100 G

Dissolve the reagents in water, adjust the pH to 8.0 with 2 M hydrochloric acid and make up to 1000 mL with water.

Buffer B

Tromethamine	6.055 G
Sodium chloride	8.77 G

Dissolve the reagents in water, adjust the pH to 8.0 with 2 M hydrochloric acid and make up to 1000 mL with water.

PREPARATION OF THE SUBSTRATE OF PRE-KALLIKREIN.

The blood or plasma used in the preparation of the pre-kallikrein must be harvested and handled only in plastic or glass materials siliconized so as to avoid the activation of pre-kallikrein resulting in coagulation. Mix 9 volumes of human blood with 1 volume of anticoagulant solution (ACD, CPD or a solution of sodium citrate at 38 g/L) added to 1 mg per ml of ethidium bromide hexadimetrina. Centrifuge at 3600 g for 5 minutes. Separate the plasma and centrifuge at 6000 g for 20 minutes to separate the platelets. Separate the platelet poor plasma and proceed to dialysis against 10 volumes of Buffer A for 20 hours. After dialysis, depositing the plasma in a chromatography column containing two times its volume of DEAE-agarose for ion exchange chromatography previously balanced with Buffer A. Proceed with the elution Buffer (flow rate of 20 mL/cm^2/hour). Collect the eluate by fractions and record the absorbance at 280 nm (**5.2.14**). Gather the fractions containing the first peak of proteins in order to obtain a volume of approximately, 1,2 times the platelet poor plasma.

To check that the substrate does not have active kallikrein, mix 1 volume with 20 volumes of solution of chromogenic substrate that will be used in the determination, previously heated to 37 °C, and keep the mixture at 37 °C for 2 minutes. The substrate is appropriate if the absorbance does not increase more than 0.001 per minute. Add the substrate solution 7 g per liter of sodium chloride and filtered through a membrane (0.45 µm). Freeze the filtrate after partitions it into aliquots and store at -25 °C; you can also freeze the filtrate before conservation. Perform the operations between the chromatography and the freezing of the aliquots on the same day.

TITRATION

Preferably, the titration is carried out in an automatic analyzer enzymatic, 37 °C. Adjust the volume, the concentration of substrates and incubation times so that the reaction speed is linear until at least 35 IU per ml. If necessary, you can dilute the standards, samples and the 7503 pre-kallikrein with Buffer B.

Incubate the patterns or the diluted samples with the substrate pre-kallikrein during 10 minutes; the volume of the standard or sample before dilution does not exceed 1/10 of the total volume of the mixture to be incubated to avoid errors resulting from differences in ionic strength or pH. Incubate the mixture or a part of the mixture with a volume greater than or equal to a solution of a chromogenic substrate specific synthetic, admittedly, for the kallikrein (for example, acetate N-benzoyl-L-prolil-L-phenylalanyl- L-arginine 4- Nitroanilide) as substrate or thiamin hydrochloride D-propyl- L-phenylalanyl-L-arginine 4-nitroanilide) as substrate) and dissolved in Buffer B. Record the variation of the absorbance per minute (AA/min) for 2 to 10 minutes in wavelength appropriate for the substrate used. For each mixture of standard or sample, prepare a white replacing the substrate of pre- kallikrein per Buffer B. Correct the variation of absorbance per minute by subtracting the value obtained with the corresponding white. Plot a calibration curve from the values of the variation of the absorbance per minute, obtained with the standard and their concentrations and determine the content in APC of the sample.

5.5.1.12 QUANTITATIVE DETERMINATION OF HUMAN ANTITHROMBIN III.

The CONTENT of antithrombin III of the sample is determined by comparing their ability to inactivation of thrombin in the presence of an excess of heparin with the capacity of a standard preparation of concentrated antithrombin

III Human concentration in international units. Variable Quantities of sample are added to a thrombin and the residual activity trombinica is determined with a chromogenic substrate appropriate.

The International Unit corresponds to the activity of a given quantity of the International Standard for human antithrombin III concentrate. The equivalence of the International Unit with the International Standard is indicated by the World Health Organization.

PROCEDURE

For the sample and the standard prepare with tris-EDTA buffer pH 8.4 ASB containing 15 IU of heparin per milliliter, two series of three or four independent dilutions ranging between 1.75th and 1.200th from 1 IU/mL. Warm up to 37 °C for 1 to 2 minutes 200 µL of each dilution. Add to each dilution 200 µL of a solution of bovine thrombin containing 2 IU/mL in buffer tris-EDTA ASB of pH 8.4. Mix and keep at 37 °C for exactly 1 minute. Add 500 µL of a substrate

Appropriate chromogenic (for example, D-phenylalanyl-L-pipecolil-L-arginine-4-nitroanilide) as substrate; dissolve this substrate in water to obtain a solution containing 4 mmol/L and diluted with tris-EDTA buffer pH 8.4 ASB without albumin until an appropriate concentration for the test of titration). Determine immediately, absorbance at 405 nm (5.2.14) For at least 30 seconds. Calculate the variation of the absorbance (ΔA/min). You can also use a titration end-point by stopping the reaction with acetic acid and by determining the absorbance at 405 nm. The variation of the absorbance (ΔA/min) is inversely proportional to the activity of antithrombin III human. Check the validity of the test and calculate the activity of the sample by statistical procedures applicable to biological tests (8).

5.5.1.13 QUANTITATIVE DETERMINATION OF ANTI-COMPLEMENTARY EFFECT OF IMMUNOGLOBULIN ACTIVITY

The determination of the activity of the anti-complementary effect (AAC) immunoglobulin is performed by incubation of a sample of immunoglobulin (10 mg) with a certain amount of complement of guinea pigs (20 CH50). The next item is the titration of the remaining complement activity: the anti-complementary effect is expressed by the ratio of supplement consumed, taking the supplement standard as 100 %.

The unit hemolytic complement activity (CH50) is the amount of complement that, in stipulated conditions of reaction, causes the lysis of 2.5 x 108 of a total number of 5 x 108 erythrocytes duly sensitized

REAGENTS

Stock Solution of magnesium and calcium. Dissolve 1.103 g of calcium chloride and 5.083 g of magnesium chloride in water and supplement 25 mL with the same solvent.

Buffer stock Solution of barbitol buffer. Dissolve 207.5 g of sodium chloride and 25.48 g of sodium barbitol buffer in 4000 mL of water and adjust the pH to 7.3 with dilute hydrochloric acid M. Add 12.5 mL of stock Solution of magnesium and calcium and complete 5000 mL with water. Filter by membrane (0.22 µm) and keep at 4 °C in glass container.

Solution of gelatine. Dissolve 12.5 g of gelatine in approximately 800 mL of water, and heat to boiling in water bath. Cool to 20 °C and complete the 10 liters with water. Filter by membrane (0.22 µm) and keep at 4 °C. Use only a clear solution.

Solution citratada. Dissolve 8 g of sodium citrate, 4.2 g of sodium chloride and 20.5 g of glucose in 750 mL of water.

Adjust to pH 6.1 with citric acid solution at 10% (p/v) and make up to 1000 mL with water.

Buffer of gelatine-barbitol buffer. Add 4 volumes of Solution of gelatine 1 volume of stock Solution of buffer barbitol buffer and mix. If necessary, adjust the pH to 7.3 with dilute hydrochloric acid M or M sodium hydroxide and keep at 4 °C. Prepare daily, a new solution.

Sheep Blood stabilized. Collect 1 blood volume of mutton in 1 volume of Solution citratada and mix. Keep the blood stabilized at 4 °C for at least 7 days, and for no more than 28 days. The blood of the lamb or sheep erythrocytes stabilized can be obtained commercially, by various vendors.

Hemolysin. Serum anti-HbS mutton, prepared in rabbit. Such sera can be obtained commercially in various suppliers.

Complement of guinea. Mix the sera obtained from, at least, 10 guinea pigs. Separate serum from blood clots by centrifugation at a temperature around 4 °C. Keep the whey, in small portions, at a temperature below -70 °C.

5

PROCEDURE

Standardization of the solution of erythrocytes of mutton at 5 %.

Separate the red blood cells of mutton by centrifugation of an appropriate volume of sheep blood stabilized; wash the cells, at least three times, with the Buffer gelatin- barbitol buffer and prepare a suspension to 5% (v/v) in the same Wad. Determine the cell concentration by the following method: add 0.2 mL of the suspension in 2.8 mL of water and centrifuguar the lysate for 5 minutes at 1000 g. The cell concentration is adequate if the absorbance (2.5.14) of supernatant, determined at 541 nm, is of 0.62 ± 0.01. Correct the cell concentration by addition of Buffer of gelatine-barbitol buffer, in accordance with the formula:

$$V_f = \frac{V_i \text{ x } A}{0{,}62}$$

Whereas

V_f = final volume,
V = initial volume,
A = absorbance determined at 541 nm for the original suspension.

Once adjusted to the cell concentration, the suspension contains approximately 1 x 109 cells per millilitre.

Titration of hemolysin

Prepare the dilutions of hemolysin in accordance with Table1.

Add 1 mL of a 5% suspension of erythrocytes of mutton in each one of the tubes of serial dilutions of hemolysin from the dilution to 1.75th and mix. Incubate the tubes at 37 °C for 30 minutes. Transfer 0.2 mL of each mixture incubated for dilution of hemolysin for new tubes and add 1.1 mL of the Buffer solution of gelatine-barbitol buffer and 0.2 mL of a dilution of guinea pig complement (for example, 1/150). Perform these manipulations in duplicate.

Table 1 – Dilutions of hemolysin.

Dilution of hemolysin to be prepared	*Buffer of gelatine-barbitol buffer*		*Hemolysin*
	Volume (mL)	*Dilution (1/...)*	*Volume (mL)*
7,5	0,65	não diluído	0,1
10	0,90	não diluído	0,1
75	1,80	7,5	0,2
100	1,80	10	0,2
150	1,00	75	1,0
200	1,00	100	1,0
300	1,00	150	1,0
400	1,00	200	1,0
600	1,00	300	1,0
800	1,00	400	1,0
1200	1,00	600	1,0
1600	1,00	800	1,0
2400	1,00	1200	1,0
3200(*)	1,00	1600	1,0
4800(*)	1,00	2400	1,0

(*)reject 1.0 mL of the mixture..

Prepare three tubes of control cells not between hemolysed transferring to each one of them 1.4 mL Buffer gelatine-barbitol buffer and 0.1 mL of suspension of erythrocytes of mutton to 5 %.

Prepare three tubes of control cells fully between hemolysed transferring to each one 1.4 mL of water and 0.1 mL of suspension of sheep erythrocytes at 5 %.

Incubate all tubes at 37 °C for 60 minutes and centrifuge at 1000 g for 5 minutes. Determine the absorbance (2.5.14) of supernatants at 541 nm and calculate the percentage of hemolysis occurred in each tube, using the formula:

$$\frac{A_c - A_1}{A_b - A_1} \text{ x } 100$$

whereas

A = absorbance of tubes containing the hemolysin dilution,
A_b = Absorbance average of three tubes with total haemolysis,
A_1 = absorbance average of three control tubes without hemolysis.

Construct a graph containing the percentages of hemolises as the ordinate and the inverses of the dilutions of hemolysin in the abscissa axis. Determine the optimal dilution of hemolysin from the graph by choosing a dilution such that an increase in the quantity of hemolysin did not produce a significant variation in the degree of hemolysis. This dilution is considered as containing 1 unit of hemolysis (minimum 1 UHM) in 1.0 mL. For the preparation of the RBCs of mutton sensitized, the dilution of hemolysin corresponding to hemolysis great contains 2 UHM per millilitre.

The titration of hemolysin is valid only if the percentage of total haemolysis is between 50 and 70 %.

If the percentage of total haemolysis can not be determined from the dilution used, repeat the titration using a solution of complement more or less diluted.

Optimal Preparation of erythrocytes of mutton sensitized (system hemolytic).

Prepare an appropriate quantity of hemolysin diluted containing 2 UHM per millilitre and an equal volume of standardized suspension of sheep erythrocytes to 5 %. Add the dilution of hemolysin the standardized suspension of cells and mix. Incubate at 37 ° C for 15 minutes, keeping the temperature from 2 to 8 ° C and use within the period of 6 hours.

Titration of the supplement

Prepare an appropriate dilution of supplement (for example, 1:250) using the Buffer of gelatine-barbitol buffer and perform the titration, in duplicate, in accordance with the information recorded in Table 2.

TO each tube, add 0.2 mL of sensitized sheep erythrocytes, mix well and incubate all tubes at 37 °C for 60 minutes. Cool the tubes in water with ice and centrifuge at 1000 g for 5 minutes. Determine the absorbance of the supernatants at 541 nm and calculate the degree of hemolysis (Y), using the formula:

$$\frac{A_c - A_1}{A_b - A_1} \text{ x } 100$$

Whereas

A_c = absorbance of tubes 1 to 12.
A_b = absorbance average of tubes with 100% hemolysis,
A_1 = average absorbance of the tubes with 0% of hemolysi

Table 2 – Dilution of the supplement.

Number of the tube	*Volume of diluted complement in millilitres (for example, 1:250)*	*Buffer Volume of gelatin-barbitol buffer in milliliters*
1	0.1	1.2
2	0.2	1.1
3	0.3	1.0
4	0.4	0.9
5	0.5	0.8
6	0.6	0.7
7	0.7	0.6
8	0.8	0.5
9	0.9	0.4
10	1.0	0.3
11	1.1	0.2
12	1.2	0.1
3 Control tubes with 0% haemolysis	-	1.3
3 Control tubes with 100% haemolysis	-	1.3 Ml of water

Construct a graph by entering the values in abscissa axs, and the corresponding volume in milliliters of complement diluted in sorted. From points, plot the ideal straight and determine the intercept of the hemolytic dose to 50% of the supplement at point where Y/ (1-Y) = 1.0. Calculate the activity in terms of hemolytic units (CH50/mL) in accordance with the formula:

$$\frac{C_d}{C_a} \text{ x } 5$$

Whereas

C_d = inverse value of dilution of complement,
C_a = volume in ml of diluted supplement that produces 50% of hemolysis,5 = Scale Factor to take into account the number of erythrocytes.

The test is only valid if, between 15 and 85% of hemolysis, the curve obtained is a straight line whose slope is between 0.15 and 0.40, preferably between 0.18 and 0.30.

Determination of activity anti-complementary effect

Dilute the complement of guinea pig titrated with the Plug of gelatine-barbitol buffer so as to obtain 100 CH50/ mL. If necessary, adjust the sample to pH 7.0. For an immunoglobulin containing 50 mg/mL, prepare mixtures of incubation **(Table 3)**

Carry out the determination in the sample and prepare the control of AAC negative and positive from an international standard of human immunoglobulin, in accordance with the instructions provided on the label that accompanies the standard preparation. If the sample does not contain 50 mg/mL of immunoglobulin, adjust the volumes of the

Table 3 – Incubation mixes.

	Sample	Control Complement
Immunoglobulin (50 mg/mL)	0.2 mL	
Buffer of gelatine-barbitol buffer	0.6 mL	0.8 mL
Supplement	0.2 mL	0.2 mL

preparation and the Cap of gelatin- barbitol buffer; for example, pipette 0.33 mL of a preparation that contains 30 mg/mL of immunoglobulin and add 0.47 mL of Buffer of gelatine-barbitol buffer so as to obtain the same total volume of 0.8 mL. Close the tubes and incuba- los at 37 °C for 60 minutes. Add 0.2 mL of each incubation mixture to 9.8 mL of the Buffer solution of gelatin- barbitol buffer to dilute the complement. In each tube, perform titrations of complement as described above to determine the anti-complementary effect residual activity (Table 2). Calculate the activity anti-complementary effect of sample, by reference to the control of the complement considered as 100 %, according to the formula:

$$\frac{a - b}{a} \text{ x } 100$$

In that

A = activity complementary media (CH50/mL) of controls,
B = complementary activity (CH50/mL) of the sample.

The test is only valid if:

- The activities anti-complementary effect found for the control AAC negative and positive control AAC if they lie within the limits indicated on the label that accompanies the preparation standard;

- The complementary activity control of complement (a) is from 80 to 120 CH_{50}/mL.

5.5.1.14 DETERMINATION OF TOTAL PROTEIN

METHOD 1

The proteins in solution absorb the ultraviolet light at a wavelength of 280 nm, due to the presence in their structure of aromatic amino acids (especially tyrosine and tryptophan), property that can be used for the determination of proteins. The use of a buffer as net of compensation can remedy the interference produced in the case of the buffer used for dissolution of protein possess high absorbance, which could compromise the results. At low concentrations, the protein adsorbed on the curve can cause a significant decrease in the protein content of the solution. It is possible to prevent this phenomenon preparing samples of high content or using a non-ionic detergent during the preparation.

Sample Solution. Dissolve an appropriate amount of sample on the cover chosen so as to obtain a solution whose protein concentration lies between 0.2 mg/mL and 2 mg/mL.

Standard Solution. Prepare a solution of the reference substance appropriate matching a protein assay, the same buffer that uses the sample solution and to obtain the same concentration.

Procedure. Keep the sample solution, the standard solution and the coolant temperature compensation the same

throughout the test. Determine the absorbance (5.2.14) of the sample solution and the standard solution at 280 nm in concentration of quartz, using the specific buffer as net of compensation. For the accuracy of the results, the response is linear in the range of protein concentrations the dosing.

Diffusion of light. The diffusion of light by the sample may affect the accuracy of the determination of proteins. If the proteins in solution form particles whose size is of the same order of magnitude of the beam's wavelength of measurement (250 – 300 nm), the diffusion of the light beam is reflected by an increase of the apparent absorbance of the sample. To calculate the contribution of this effect of diffusion in absorbance measured at 280 nm, determine the absorbance of the sample solution in multiple wavelengths (320 nm, 325 nm, 330 nm, 335 nm, 340 nm, 345 nm and 350 nm). Construct a graph of the logarithm of the absorbance measured in function of the logarithm of the respective wavelength and determine, by linear regression analysis, the calibration curve that best fits the different points entered in the chart.

Determine by extrapolation the logarithm of absorbance at 280 nm. The absorbance due to the effect of diffusion is the antilogarithm of this value. Correct the observed values by subtracting the total absorbance at 280 nm of the absorbance due to the effect of diffusion to obtain the value of the absorbance due to protein in solution. It is possible to perform a filtration using a filter of 0.2 µ which does not absorb the proteins, or a clarification by centrifugation, the end of which are reduced the effects of the diffusion of light in the case of solution.

Calculations. Use the corrected values for the calculations. Calculate the protein content of the sample solution (Cu), using the expression:

$$C_u = C_s \left(\frac{A_u}{A_s} \right)$$

In that

C_S = protein content of standard solution;
A_u = value of corrected absorbance of the sample solution;
A_S = absorbance value corrected for the standard solution.

METHOD 2

This method has been designed based on the property that the proteins have to reduce fatty fosfomolibdeniotungstenico contained in the reagent fosfomolibdenio and tungsten; this reaction is chromogenic and is reflected by the existence of a peak absorption at 750 nm.

The Reagent fosfomolibdenio and tungsten react first with the tyrosine residues of the protein. The development of coloration reaches a maximum at the end of 20 – 30 minutes of incubation at ambient temperature; 368.4 then a progressive discoloration. Being the sensitive method to interfering substances, you can use a treatment that produces the precipitation

The protein content of the sample. The greater part of interfering substances reduces the intensity of staining obtained, but some detergents increase-in, slightly. A strong saline concentration may lead to the formation of a precipitate. Given that the intensity of staining obtained may vary according to species considered protein, a protein assay and protein pattern are the same. If necessary, separate the interfering substances of protein in the sample, as is shown below in interfering substances before preparing the sample solution. It is possible to minimize the effect of interfering substances by dilution, provided that the protein content in the dosing if keep high enough to allow an accurate determination.

Use distilled water for the preparation of all buffers and reagents used in this method.

Sample Solution. Dissolve an appropriate amount of sample in buffer specified in order to obtain a concentration not within range covered by calibration curve. The pH of a solution prepared with an appropriate buffer is between 10.0 and 10.5.

Standard Solutions. Dissolve the reference substance corresponding to protein the dosing cap specified. Take samples of this solution and complete them with the same buffer to obtain, at least, five standard solutions of different concentrations ranging between 5 µg/ mL and 100 µg/mL and uniformly distributed on the interval chosen.

Blank Solution. Use the same buffer that was used to prepare the sample Solution and the standard Solutions.

Reagent of copper sulphate Dissolve 100 mg of copper sulphate and 0.2 g of sodium tartrate in distilled water and dilute 50 mL with the same solvent. Dissolve 10 g of anhydrous sodium carbonate in distilled water and dilute 165 mL with the same solvent. Pour, slowly, the solution of sodium carbonate in solution of copper sulphate, mixing always. This solution is used in the 24 hours following its preparation.

Alkaline copper reagent. Prepare a mixture of 1Volume of the reagent of copper sulphate, 2 volumes of sodium lauryl sulphate 5% (p/v) with 1 volume of sodium hydroxide to 3.2% (p/v). Keep this mixture to ambient temperature. The mixture is used in the 2 weeks that follow its preparation.

Reagent fosfomolibdenio and tungstenico diluted. Mix 5 mL of reagent fosfomolibdenio and tungstenico with 55 mL of distilled water. Keep the reagent at ambient temperature in amber glass vial.

Procedure. To 1 mL of each standard Solution, the sample Solution and the blank Solution add 1 mL of alkaline copper reagent and mix. Leave for 10 minutes. Add 0.5 mL of the diluted reagent fosfomolibdenio and tungsten, mix and let stand at room temperature for 30 minutes.

Determine the absorbance (2.5.14) of the solutions at 750 nm, using the blank solution for adjustment of zero.

Calculations. The relationship between the absorbance and the protein content is not linear; however, if the concentration range covered by calibration curve is narrow enough, the curve obtained will be substantially linear. Construct a graph of the absorbance of the standard solutions depending on the protein content of these solutions and determine the calibration curve by linear regression analysis. From the calibration curve and the absorbance of the sample solution, determine the protein content of the sample solution.

Interfering Substances. In this method adds-if sodium desoxycholate and trichloroacetic acid sample to precipitate the proteins and separates them from interfering substances, before dosing. This technique can also be used to concentrate the proteins contained in a solution very diluted. To 1 mL of a solution of the sample add 0.1 mL of sodium desoxycholate to 0.15% (p/v). Stir with a vortex shaker and let stand at room temperature for 10 minutes. Add 0.1 mL of trichloroacetic acid to 72% (p/v). Stir with a vortex shaker and centrifuguar at 3000 g for 30 minutes. Discard the supernatant liquid and eliminate the residual liquid with a pipette. Dissolve the clot in 1 mL of alkaline copper reagent.

METHOD 3

This method was based on the property that the proteins have to shift from 470 nm to 595 nm the absorption maximum of acid blue 90 when connecting to dye. The blue dye acid 90 presents a marked affinity for the residues of arginine and lysine in protein which can cause variations in the response to the determination of different proteins. The protein used as reference substance must, therefore, be the same as the protein to be assayed. There are relatively few interfering substances, but it is preferable to avoid detergents and the analytes in the sample the dosing. Very alkaline Samples may cause interference with the acid reagent.

Use distilled water for the preparation of all buffers and reagents to be used in this method.

Sample Solution. Dissolve an appropriate amount of sample in buffer indicated so as to obtain a concentration not within range covered by calibration curve.

Standard Solutions. Dissolve the reference substance corresponding to protein the dosing cap indicated. Take samples of this solution and complete with the same buffer to obtain at least five standard solutions of protein concentrations ranging from 0.1 mg/mL and 1 mg/mL and uniformly distributed on the interval chosen.

Blank Solution. Use the same buffer that was used to prepare the sample solution and the standard solutions.

Blue reagent acid 90. Dissolve 0.10 g of acid blue 90 in 50 mL of ethanol. Add 100 mL of phosphoric acid, make up to 1000 mL with distilled water and mix. Filter the solution and keep it at room temperature in amber glass vial. 368.4 If a slow precipitation of the dye during storage. The precipitate is removed by filtration prior to using the reagent.

Procedure. At 0.100 mL of each standard Solution, the sample Solution and the blank Solution add 5 mL of reagent acid blue 90. Homogenize the mixture by rotation, avoiding the formation of foam that can create problems of reproducibility. Determine the absorbance (5.2.14) of the standard Solution and the sample Solution at 595 nm, using the blank Solution for zero setting. It Avoids the use of concentration of quartz (silica) already that the dye binds to this material.

Calculations. The relationship between the absorbance and the protein content is not linear. However, if the concentration range covered by calibration curve is narrow enough, the curve obtained will be substantially linear. Construct a graph of the absorbance of the standard Solutions depending on the protein content of these solutions and determine the calibration curve by linear regression analysis. From the calibration curve and the absorbance of the sample Solution determine the protein content of the sample Solution.

METHOD 4

This method, also known as a method of Bicinchoninate (BCA), was drawn up based on the property that the proteins have to reduce the cupric ion (Cu^{2+}) ion the cuprous oxide (Cu^{+}). The reagent of Bicinchoninate serves to detect the ions cuprosos. There are few interfering substances. If there are any interfering substances, it is possible to minimize their effects by dilution, provided that the protein content in the dosing if keep high enough to allow an accurate determination. The technique of precipitation of proteins described in Method 2 can be used to eliminate interfering substances. The intensity of staining obtained by reaction with the reagent may vary from one type of protein to another and, therefore, a protein assay and the reference protein are the same.

Use distilled water for the preparation of all buffers and reagents to be used in this method.

Sample Solution. Dissolve an appropriate amount of sample in buffer indicated so as to obtain a concentration not within range of the concentration

Standard Solutions. Dissolve the reference substance corresponding to protein the dosing cap indicated. Take samples of this solution and complete with the same buffer to obtain at least five standard solutions of concentrations ranging between 10 µg/mL and 1200 µg/mL and uniformly distributed on the interval chosen.

Blank Solution. Use the same buffer that was used to prepare the sample Solution and the standard Solutions.

BCA reagent. Dissolve 10 g of disodium bicinconinato, 20.0 g of sodium carbonate monohydrate, 1.6 g of sodium tartrate, 4 g of sodium hydroxide and 9.5 g of sodium bicarbonate in distilled water. If necessary, adjust the pH

to 11.25 with a solution of sodium hydroxide or sodium bicarbonate. Make up to 1000 mL with distilled water and mix.

Copper reagent-BCA. Mix 1 mL of copper sulphate to 4% (p/v) with 50 mL of reagent BCA.

Procedure. Mix 0.1 mL of each standard Solution, the sample Solution and the blank Solution with 2 mL of copper reagent-BCA. Incubate the solutions at 37 °C for 30 minutes, take note of the time and let cool down to ambient temperature. In the 60 minutes following the incubation period, determine the absorbance (2.5.14) at 562 nm of the standard Solution and the sample Solution at concentration of quartz, using the blank Solution for adjustment of zero. When the temperature of the solutions return to ambient temperature, the staining intensity continues to increase gradually.

Calculations. The relationship between the absorbance and the protein content is not linear. However, if the concentration range covered by calibration curve is narrow enough, the curve obtained will be substantially linear. Register a graph the absorbance of standard solutions as a function of the protein content of these solutions and determine the calibration curve by linear regression analysis. From the calibration curve and the absorbance of the sample solution determine the protein content of the sample solution.

METHOD 5

This method, also known as the biuret method, drawn up on the basis of the property that the proteins have to interact with the cupric ion (Cu^{2+}), in alkaline medium, giving a product of reaction that shows absorbance at 545 nm. The use of this method allows obtaining a minimum deviation between equivalent samples of lgG and albumin. On the contrary, the simultaneous addition of sodium hydroxide and the biuret reagent (in the form of mixture), a homogenization insufficient after the addition of sodium hydroxide or a very long time interval between the addition of sodium hydroxide and the biuret reagent leads to obtaining a higher response with samples of lgG than with samples of albumin. The treatment with trichloroacetic acid used to reduce interference may also allow to quantify protein when its concentration in the sample is less than 0.5 mg/mL.

Use distilled water for the preparation of all buffers and reagents to be used in this method.

Sample Solution. Dissolve an appropriate quantity of the sample in a solution of sodium chloride 0.9% (p/v) so as to obtain a concentration not within range of the concentration of the standard solutions.

Standard Solutions. Dissolve the reference substance corresponding to protein the dosing in sodium chloride solution of 0.9% (p/v). Take samples of this solution and complete with a solution of sodium chloride 0.9% (p/v) in order to obtain at least three standard solutions of concentrations ranging from 0.5 mg/mL and 10 mg/ mL, and uniformly distributed on the interval chosen.

Blank Solution. Use sodium chloride solution of 0.9% (p/v).

Biuret reagent. Dissolve 3.46 g of copper sulphate in 10 mL of hot distilled water and leave to cool (solution A). Dissolve 34.6 g of sodium citrate and 20 g of anhydrous sodium carbonate in 80 mL of hot distilled water and let cool (solution B). Mix the solutions A and B and complete 200 mL with distilled water. This reagent is used within the 6 months following its preparation; it is not used if develop turbidity or precipitate.

Procedure. a volume of sample Solution add an equal volume of sodium hydroxide solution at 6% (p/v) and mix. Add immediately, 0.4 volume (calculated in relation to the sample solution) of biuret reagent and mix briefly. Keep the samples for at least 15 minutes at a temperature of between 15 °C and 25 °C. In the 90 minutes that follow the addition of reagent, determine the absorbance (5.2.14), up to 545 nm, of the standard Solutions and the Solution of the sample, using the blank Solution as net of compensation. If the solutions arise turbidity or precipitate, are not used for the calculation of protein content.

Calculations. The relationship between the absorbance and the protein content is, roughly, linear in the range of concentrations indicated for the standard Solutions. Construct a graph of the absorbance of the standard Solutions depending on the protein content of these solutions and determine the calibration curve by linear regression analysis. Calculate the correlation coefficient for the calibration curve. The system meets if you get a straight line whose correlation coefficient is, at least, 0.99. From the calibration curve and the absorbance of the sample Solution determine the protein content of the sample Solution.

Interfering Substances. It is possible to limit the effect of interfering substances precipitating, as indicated below, the protein in the sample: add 0.1 volume of the trichloroacetic acid solution of 50% (p/v) to 1 volume of the sample Solution, eliminate the supernatant and dissolve the precipitate in a small volume of sodium hydroxide 0.5 M. Use the solution thus obtained to prepare the sample solution.

METHOD 6

This fluorimetric method was elaborated on the basis of a derivation of protein by o-phthalaldehyde which reacts with the primary amines of the protein, that is, the amino acid ^ -terminal and the function-amine of lysine residues. The sensitivity of the assay can be improved by a prior hydrolysis of protein, before adding the o-phthalaldehyde. The hydrolysis liberates the function-amine of constituent amino acids of the protein and which allows it react with the reagent of phthalaldehyde. This method is applicable to small quantities of protein.

The primary amines contained, for example, in buffers of ketorolac tromethamine and in buffers of amino acids react with the phthalaldehyde and are, therefore, to avoid or eliminate. The ammonia in high concentration reacts equally with the phthalaldehyde. The fluorescence resulting from the reaction amina phthalaldehyde can be unstable. The use of automated processes to standardize the method can allow you to improve accuracy and feasibility.

Use distilled water for the preparation of all buffers and reagents for use in this method.

Sample Solution. Dissolve an appropriate amount of sample in a solution of sodium chloride 0.9% (p/v) so as to obtain a concentration not within range of the concentration of the standard Solutions. Adjust the pH of the solution to 8 – 10.5 before joining the reagent of phthalaldehyde.

Standard Solutions. Dissolve the reference substance corresponding to protein assay in solution of sodium chloride 0.9% (p/v). Take samples of this solution and complete with a solution of sodium chloride 0.9% (p/v) in order to obtain at least five standard solutions of concentrations ranging between 10 µg/mL and 200 µg/ mL and uniformly distributed on the interval chosen. Adjust the pH of the solutions for 8-10.5 before adding the reagent of phthalaldehyde.

Blank Solution. Use sodium chloride solution of 0.9% (p/v).

BORATE. Dissolve 61.83 g of boric acid in distilled water and adjust the pH to 10.4 with potassium hydroxide solution. Supplement to 1000 mL with distilled water and mix.

Stock Solution of phthalaldehyde. Dissolve 1.20 g of phthalaldehyde in 1.5 mL methanol, add 100 mL of borate and mix. Add 0.6 mL of solution of ether lauric of macrogol 23 to 30% (p/v) and mix. Keep the solution at room temperature and use within the 3 weeks that follow its preparation.

Phthalaldehyde reagent. TO 5 mL of the stock Solution of phthalaldehyde join 15 µL of 2-mercaptoethanol. Prepare the reagent 30 minutes, at least, before use and within 24 hours after their preparation.

Technique. Mix 10 µL of the sample Solution and of each one of the standard Solutions with 0.1 mL of reagent Phthalaldehyde and let stand at room temperature for 15 minutes. Add 3 mL of sodium hydroxide 0.5 M and mix. Determine the fluorescence intensity (5.2.15)
Samples of the standard Solution and the sample Solution at excitation wavelength of 340 nm and the emission wavelength of 440 nm to 455 nm. Determine the intensity of fluorescence of a sample only once because the irradiation causes a reduction in the intensity of fluorescence.

Calculations. The relationship between the fluorescence intensity and the protein content is linear. Register a graph the fluorescence intensities obtained with the standard Solutions depending on the protein content of these solutions and determine the calibration curve by linear regression analysis. From the calibration curve and the fluorescence intensity of the sample Solution, determine the protein content of the sample Solution.

METHOD 7

This method was developed based on the quantification of proteins by determination of nitrogen. The presence in the sample of other nitrogenous substances can affect the result of the determination of proteins. The techniques used for dosing the nitrogen lead to destruction of the sample during the analysis, but are not limited to the determination of proteins in aqueous medium.

Technique A. Proceed as described for the determination of nitrogen after digestion by sulfuric acid (5.3.3.2) or use instruments available on the market adapted to determination of nitrogen by the Kjeldahl method.

B. There are Technical market instruments adapted for the determination of nitrogen. Most of them use the pyrolysis (combustion of the sample in the presence of oxygen at temperatures close to 1000 °C), which causes the formation of nitrogen monoxide (NO) and other oxides to form NOx from the nitrogen exists in the sample. Certain instruments convert these oxides of nitrogen in nitrogen gas which is quantified by condutimetry thermal. Other blend of nitrogen monoxide (NO) with ozone (O3) to produce nitrogen dioxide excited state (NO2) that emits a light radiation when its decrease and is quantified by chemiluminescence. A reference product, relatively pure, and similar, as to its composition, a protein assay is used to optimize the injection parameters and of pyrolysis and to assess the reproducibility of the analysis.

Calculations. The protein content is estimated by dividing the nitrogen content of the sample by nitrogen content (known) of the protein, which can be determined either from the chemical structure of the protein, either by comparison with a reference substance appropriate

5.5.1.15 QUANTITATIVE DETERMINATION OF ANTI-D HUMAN IMMUNOGLOBULIN

The activity of human immunoglobulin anti-D is assessed by comparison of the quantity required to produce the agglutination of erythrocytes D-positive and of a preparation of reference, calibrated in international units, needed to produce the same effect. The International Unit corresponds to the activity of a given quantity of international reference preparation. The correspondence between international units and the preparation of international reference is indicated by the World Health Organization.

Use a mixture of erythrocytes D-positive, with less than 7 days and kept in appropriate conditions, obtained from, at least, four donors of group OR_1R_1. A an appropriate volume of erythrocytes, washed, previously, three times with a solution of sodium chloride 0.9% (p/v), add an equal

volume of bromelain SR, let stand at 37 °C for 10 minutes. Centrifuge, remove the supernatant and wash three times the erythrocytes with sodium chloride solution of 0.9% (p/v). Suspend 20 volumes of erythrocytes in a mixture of 15 volumes of serum inert, 20 volumes of solution of bovine serum albumin at 30% (p/v) and 45 volumes of sodium chloride solution to 0.9% (p/v). Put the suspension in water with ice under continuous stirring.

With an automatic device of dilution calibrated prepare dilutions of the sample and the preparation of reference in a solution of bovine serum albumin in 0.5% (w/v) and sodium chloride solution of 0.9% (p/v).

Use an appropriate apparatus for automatic analysis continues; keep the temperature in holsters to 15 °C with the exception of spirals of incubation. Aspire to the caskets of admission of the appliance the suspension of erythrocytes with a flow rate of 0.1 mL per minute and a solution of methylcellulose 450 to 0.3% (p/v) with a flow rate of 0.05 mL per minute.

Enter the dilutions of the sample and of the preparation of reference with a flow rate of 0.1 mL per minute for 2 minutes and then the thinner the ratio of 0.1 mL per minute for 4 minutes before entering the next dilution. Introducing air the reason of 0.6 mL per minute. Incubate at 37 °C for 18 minutes and then disperse the spirals by introduction, with a flow rate of 1.6 mL per minute, of a solution of sodium chloride 0.9% (p/v) that contains a wetting agent appropriate (for example, polysorbate 20 at a final concentration of 0.2 g/L) to avoid changing the continuity of bubbles. Leave to settle the agglutinated and decant 2 times, the first time the 0.4 mL per minute and the second time to 0.6 mL per minute. Carry out the analysis of the residue of erythrocytes do not agglutinated with the help of a solution of octoxinol 10 to 0.5% (p/v), potassium ferricyanide to 0.02% (p/v), sodium bicarbonate 0.1% (p/v) and potassium cyanide to 0.005% (p/v), with a flow rate of 2.5 mL per minute. It is introduced a serpentine of delay of 10 minutes to allow the processing of hemoglobin.

Perform the continuous recording of absorbance (2.5.14) hemolysate at a wavelength of 540 to 550 nm.

Determine the concentrations of antibodies for which there is a linear relationship between the concentration and the change in absorbance (AA). Based on the results, construct a calibration curve and use the linear portion of the curve to determine the activity of the sample. Calculate the activity of the sample in international units per milliliter using the formula:

$$\frac{a \times d}{D}$$

In that

A = activity of preparing reference in international units per millilitre to a dilution of 1 in D,
d = dilution factor of the sample that corresponds to a given value of ΔA,
D = dilution factor for the preparation of reference that corresponds to the same value of ΔA.

5.5.1.16 QUANTITATIVE DETERMINATION OF FUNCTION FC IMMUNOGLOBULIN

REAGENTS

Human Blood stabilized. Make a phlebotomy to collect the human blood group O in anticoagulant solution conservative and conserving type ACD. Keep the human blood stabilized at 4 °C, during 3 weeks, maximum.

Phosphate buffered saline Solution, pH 7.2. Dissolve 1.022 g of anhydrous dibasic sodium phosphate, 0.336 g of monobasic sodium phosphate and 8.766 g of sodium chloride in 800 mL of water and make up to 1000 mL with the same solvent.

Stock Solution of magnesium and calcium. Dissolve 1.103 g of calcium chloride and 5.083 g of magnesium chloride in water and supplement 25 mL with the same solvent.

Buffer stock Solution of barbitol buffer. Dissolve 207.5 g of sodium chloride and 25.48 g of sodium barbitol buffer in 4000 mL of water and adjust to pH 7.3 with hydrochloric acid M. Add 12.5 mL of the stock Solution of magnesium and calcium and complete 5000 mL with water. Filter by membrane (0.22 μm) and keep at 4 °C in glass container.

Buffer of albumin-barbitol buffer. Dissolve 0.150 g of bovine serum albumin in 20 mL of stock Solution of buffer of barbitol buffer and make up to 100 mL with water.

Solution of tannic acid. Dissolve 10 mg of tannic acid in 100 mL of phosphate buffered saline Solution of pH 7.2. Prepare immediately before use.

Complement of guinea. Mix the sera obtained from, at least, 10 guinea pigs. Separate the whey of blood clots by centrifugation at a temperature of approximately 4 °C. Keep the whey, in small portions, at a temperature below -70 °C. Immediately before starting the hemolysis by action of the supplement, dilute to 125 – 200 CH_{50} per milliliter with Buffer of albumin-barbitol buffer and, during the test, keep the diluted solution in an ice bath.

Antigen rubella rubella antigen. appropriate for the titrations of hemagglutination inhibition. Title > 256 HA units.

PROCEDURE

Treatment of human erythrocytes with tannic acid. Separate the erythrocytes by centrifugation of an appropriate volume of human blood has stabilised. Wash the erythrocytes, at least three times, with buffered saline Solution of sodium phosphate pH 7.2 and then suspend the 2% (v/v) in phosphate buffered saline Solution, pH 7.2.

Take 71 mL of the Solution of tannic acid and complete 7,5 Ml with phosphate buffered saline Solution of pH 7,2 (Final concentration 1.3 mg/L); mix 1 volume of dilution recently prepared with 1 volume of the suspension of erythrocytes and incubated at 37 °C for 10 minutes. Collect the erythrocytes treated with tannic acid by centrifugation (400 – 800 g, for 10 minutes), discard the supernatant and wash the erythrocytes once with phosphate buffered saline Solution, pH 7.2. Suspend the 1% (v/v) the erythrocytes treated with tannic acid in phosphate buffered saline Solution, pH 7.2.

Addition of antigens to erythrocytes. Take an appropriate volume (v) of erythrocytes treated with tannic acid, add 0.2 mL of antigen of rubella per 1.0 mL of erythrocytes and incubated at 37°C for 30 minutes. Collect the erythrocytes by centrifugation (400-800 g, for 10 minutes) and discard the supernatant, leaving a volume of 200 µL. Add a Buffer volume of albumin- barbitol buffer equal to the volume of the supernatant rejected, shake until suspension of erythrocytes, collect these as described above and would repeat the wash. Complete the remaining volume obtained from 0.2 mL up to three-quarters of vs, thus obtaining the initial volume (vi).

Mix 900 µL of Buffer of albumin-barbitol buffer with 100 µL of v, which is thus reduced to the residual volume and determine the initial absorbance at 541 nm (A). Dilute vr by a factor equal to The Buffer using albumin- barbitol buffer. If, therefore, the final volume adjusted vf = sv x TO human erythrocytes sensitized and a value for of 1.0 ± 0.1 in the case of a dilution to 1/10.

Connection of the antibodies to the erythrocytes with tannic acid and covered in antigen. Prepare in duplicate and, successively, the following solutions using for each solution, separately, a semi-micro cuvette (per example, disposable plates) or a test tube for each solution:

(1) Solutions problem. If necessary, adjust the sample to pH 7 by adding, for example, sodium hydroxide M. Take sample volumes containing, respectively, 30 and 40 mg of immunoglobulin and complement 900 µL with Buffer of albumin-barbitol buffer.

(2) Standard Solution. Prepare the solution as described for the Solution problem from a reference standard for human immunoglobulin.

(3) Testimony of the Complement. 900 µL of Buffer of albumin-barbitol buffer. each cuvette/test tube add 100 µL of human erythrocytes sensitized and mix carefully. Leave to rest for 15 minutes, add 1000 µL of Buffer of albumin-barbitol buffer, collect the erythrocytes by centrifugation (1000 g for 10 minutes) the cuvette/test tube and withdraw 1900 µL of supernatant. Replace this volume with 1900 µL of Buffer of albumin-barbitol buffer and repeat the procedure of washing leaving a final volume of 200 µL. The samples may be preserved in concentration/test tubes closed at 4 °C for 24 hours.

Hemolysis by the action of the complement system. For the determination of hemolysis add 600 |iL Buffer of albumin-barbitol buffer heated to 37 °C to sample, suspend, carefully, the erythrocytes by pipetting them, repeatedly, (at least five times) and place the cuvette in sample of a spectrophotometer with thermostat. After 2 minutes, add 200 µL of Complement of guinea pig diluted to 125 – 200 CH50/mL, mix carefully by pipetting the mixture twice and start immediately after the second pipetting the record of absorbance at 541 nm as a function of time, using the Buffer of albumin-barbitol buffer as net of compensation. Stop recording if the curve of absorbance as a function of time exceed sharply the inflection point.

Determination. Determine the slope (S) of the curve of hemolysis in approximate point of inflection segmenting the curve in the region of greater slope by appropriate intervals (for example, Ta = 1 minute) and calculating S, expressed in AA per minute between the points of intersection between adjacent. The highest value of S corresponds to (Sexp). Also, Determine the absorbance at the start of the curve (The) by extrapolation of the curve, which is almost always linear and parallel to the time axis in the first minutes of the record. Correct S_{exp} in accordance with the formula:

$$S' = \frac{S_{exp}}{A_s}$$

For each preparation, calculate the arithmetic mean of the values of S'. Calculate the index of the function Fc (IFc) from the formula:

$$I_{Fc} = 100 \text{ x } \frac{S' - S'_c}{S'_s - S'_c}$$

S' = arithmetic mean of slope corrected for the sample;
S's = arithmetic mean of slope corrected to the standard;
S'c = arithmetic mean slope corrected for the witness of the complement.

Calculate the index of the function Fc for the sample. The value is not less than that indicated by the manufacturer of the standard.

5.5.2 BIOLOGICAL ASSAYS

5.5.2.1 PIROGÊNIOS

The pyrogen test is based on the measurement of body temperature increase of rabbits, after intravenous injection of sterile solution in analysis.

For products well tolerated by the animals, use a dose that does not exceed 10 mL/kg, injected in time not exceeding 10 minutes.

For products that require preliminary preparation or special conditions of administration, following the recommendations set out in the monograph.

General Conditions

Use rabbits of the same sex, adults, healthy, preferably from the same breed, weighing at least 1.5 kg.

After the selection, keep the animals in individual cages in a room with uniform temperature between 20 and 23 °C free of disturbances that might affects them. The selected temperature may vary up to ± 3 °C.

Perform conditioning for determination of the temperature of the animals, at least once, up to seven days prior to the start of the test. The animals must be conditioned according to the same test procedure only without inoculation of the product. Animals that show elevation of temperature equal to or greater than the 0.5 °C, in relation to the initial temperature, should not be used in the test.

When carrying out the test, use only animals with a temperature equal to or less than 39.8 ºC and that do not differ from one to the other, variation exceeding 1.0 °C.

Record the temperature

Use clinical thermometer calibrated with an accuracy of ± 0.1 °C or any other recording device temperature calibrated to equal sensitivity.

Insert the thermometer in the rectum of the animal at a depth of approximately 6 cm. If used recorder device, which should remain in the rectum during the period of the test, contain the rabbits so that they are in natural position of rest. When you employ clinical thermometer, let him spend the time required (Pre-determined) to reach the maximum temperature, prior to reading.

Material

The syringes, needles and sterile glassware and apirogenicas. The diluents and extracting solutions or washing should, also, be sterile and apirogenicos.

Procedure

Perform the test in an area especially designed for the test, under controlled environmental conditions, free of disturbance that would stress the rabbits. In the two hours preceding and during the test, remove the power supply. Access to water is permitted, but may be restricted during the test.

In up to 40 minutes before the injection of the dose of the product to be tested, record the temperature of each animal through two readings made with an interval of 30 minutes. The average of the two readings will be adopted as control temperature required to evaluate any increase individual temperature following the injection of the sample.

Prepare the product to be tested as specified in the monograph and heat to 37 ± 2°C. For the test of pyrogens of materials for hospital use wash with sterile saline solution, the surfaces of the material that come into contact with the product, injection site or internal tissue of the patient. Perform the procedures ensuring that the solution is not contaminated.

Inject by the ear marginal vein of three rabbits did not less than 0.5 mL and not more than 10 mL of the solution per kg of body weight or the quantity indicated in the monograph. The injection should not last more than 10 minutes, unless in monograph if specify different time.

Record the temperature of each animal in 30-minute intervals for 3 hours after the injection.

Interpretation

Do not consider the declines in temperature submitted by animals during the test. The increase in temperature is determined by the difference between the highest temperature submitted by rabbit during the test and to control its temperature.

If none of the three rabbits present increased individual temperature greater than or equal to 0.5 ºC, in relation to their respective control temperatures, the product complies with the requirements of the test of pyrogens.

If any rabbit present increase of temperature equal to or greater than 0.5 ºC, repeat the test using five other animals.

The product under consideration meets the requirements for the absence of pyrogens if more than three of the eight rabbits submit individual increases temperature greater than or equal to 0.5 ºC, and if the sum of individual increases of all rabbits does not exceed the 3.3 ºC.

5.5.2.2 BACTERIAL ENDOTOXINS

The bacterial endotoxin test is used to detect or quantify endotoxin of gram-negative bacteria present in samples for which the test is recommended. If the aqueous extract of circulating lysate Limulus amoebocyte lysate polyphemus or of Tachypleus tridentatus prepared and characterized as LAL reagent.

There are two techniques with different sensitivity to this test:

1. METHOD OF COAGULATION IN GEL: based on the formation of a clot or gel method (semi-quantitative)

2. Quantitative PHOTOMETRIC METHODS that include:

The METHOD TURBIDIMÉTRICO, (based on the development of turbidity after breakage of an endogenous substrate);

The METHOD CROMOGÊNICO (based on color development after breaking a complex synthetic peptide cromogen).

Any one of these procedures can be performed, unless indicated otherwise in the monograph.

The method of coagulation in gel, determining the end point of the reaction is made from dilutions of the substance under test in direct comparison with parallel dilutions of endotoxin standard. The quantities of endotoxins are expressed in endotoxin units (EU) defined.

Note: EU 1 is equal to 1 IU (international unit).

The LAL reagent (lysate amemocito of Limulus amoebocyte lysate sp.) is prepared for the readings turbidimetricas or colorimetric and these procedures may be used if they meet the requirements of the methods. For its calibration is necessary to prepare a standard curve getting to its linear regression, in which it determines, by interpolation, the concentration of endotoxin of the substance under test.

The procedure includes hatching of endotoxin standard for obtaining a calibration curve and of control solutions with reagent LAL, by pre-determined time and read spectrophotometrically at a wavelength suitable.

In the case of the procedure of the turbidimetric method, the reading is performed immediately after final period of incubation, and the colorimetric procedure the enzymatic reaction is stopped at the end of the pre-determined time by addition of reagent, before the readings. For the procedures kinetic colorimetric ApolipoproteÃ na and the values of

Absorbance measured during the period of the reaction and speed values are determined for those readings.

5

GLASSWARE AND ONE-TIME.

All the glassware must be despirogenisadas in greenhouse using a validated process. Use a minimum time and temperature of 250 °C for 30 minutes. If you use disposable plastic pipettes, as jaws and use only certificates that indicate be free of endotoxins for there shall be no interference in the assay.

PREPARATION OF ENDOTOXIN STANDARD OF REFERENCE AND THE PATTERN OF ENDOTOXIN

The Reference standard endotoxin has a power set of 10,000 endotoxin units (EU) per bottle. Retracing the flask with 5 mL of reagent grade water LAL (free of pyrogen) and shake in vortex intermittently for 30 minutes. Use this concentrated solution (kept in the refrigerator for no more than 14 days) to make serial dilutions. Shake vigorously before use by at least 3 minutes and proceed to serial dilutions, stirring at least 30 seconds before the next dilutions. After the use despise the dilutions due to loss of activity by adsorption. For the preparation of standard endotoxin (follow the guidelines of the supplier, certificates in report of endotoxin).

Preparation for the test

Use reagent LAL with sensitivity declared confirmed.

The validity of the test results for bacterial endotoxin requires a demonstration that the samples, solutions of washes or extracts under test does not inhibit or potentiate the reaction and nor interfere with the test. The validation is performed by means of test of inhibition or potentiation described for each one of the techniques listed. Appropriate negative controls are included. The validation should be repeated if there is change in the origin of the LAL reagent, the method of production or in the formulation of the substance under test.

Sample Preparation

Prepare the sample solution by dissolving the same in LAL reagent grade water. If necessary adjust the pH of the sample solution to the LAL reagent mixture more sample falls in the range of pH 6 to 8. The pH can be justado using a suitable buffer recommended by the supplier. Acids and bases can be prepared using reagent grade water LAL and be validated to be free of endotoxin and interfering factors.

DETERMINATION OF THE MAXIMUM VALID DILUTION (MDV)

The maximum valid dilution dilution is the maximum allowed in the sample analysis where the limit of endotoxin can BE determined. It applies to injections or solutions for parenteral administration in reconstituted or diluted form for administration, amount of drug per weight, if the volume of the shape of the dosage is variable.

The formula for the calculation of the VKM is the following:

$$MDV = \frac{\text{endotoxin limit}}{\lambda}$$

In that:

λ = Is the sensitivity of the reagent labeled LAL

Note: formula used to when the limit of endotoxin drug specified in the monograph is in volume (EU/mL)

When endotoxin limit drug specified in the monograph is in weight (EU/mg) or in unit of drug active (EU/units) the MDV is calculated by the following formula:

$$MDV = \frac{\text{limite de endotoxina x concentração da amostra na solução}}{\lambda}$$

In that:

λ = Is the sensitivity of the reagent labeled LAL

The VKM obtained is the dilution factor limit for the test to be validated.

ESTABLISHMENT OF LIMIT OF ENDOTOXIN

The formula to establish limit of endotoxin to intravenous drug is:

$$LE = \frac{K}{M}$$

In that:

LE is the limit of endotoxin
K is the limit dose human endotoxin per kilo of body weight;
M is equal to the maximum dose of the product per kg of body weight in a period of one hour.
The limit of endotoxin is specified in individual monographs of parenteral drugs in EU/mL, EU/mg or EU/unit of biological activity.

TECHNICAL COAGULATION IN GEL

The technique of coagulation in gel allows the detection and quantification of endotoxin based on the reaction of gelation of LAL reagent

The sensitivity of the LAL labeled is the concentration of endotoxin necessary to cause a gelation of LAL reagent

To ensure the accuracy and validity of the test tests are required to confirm the sensitivity of the LAL labeled as well as tests for verification of interfering factors, as described in the preparation of the sample for testing.

Test for confirmation of sensitivity of LAL

Confirm the sensitivity declared the LAL using at least 01 reagent bottle LAL and prepare a series of dilutions of endotoxin using the reference standard Endotoxin (RSE) or the pattern of Endotoxin (CSE), with geometric ratio equal to 2 to obtain the concentrations of 0.25.0.5 I I, I and 2 s, where I is the sensitivity declared the LAL in EU/mL. Run the test with the four concentrations of standard endotoxin in quadruplicate and include negative controls. The geometric mean concentration of end point whose calculation and interpretation are below must be greater or equal to 0.5 I and less than or equal to 2 I. confirmation of The sensitivity of the LAL assay should be performed for each new batch of LAL.

Calculation and interpretation. The end point of gelation is the last test of the series of descending concentration of endotoxin standard that formed gel. Calculate the geometric mean logarithmic end points of gelation and the antilog of the mean by the formula:

$$\text{Média geométrica da concentração do ponto final} = \text{antilog}\left(\frac{Ee}{f}\right)$$

In that

Ee – is the sum of the log of the concentrations of the end-point dilution series used f – is the number of replications.

The sensitivity of the reagent LAL in EU/mL is calculated by the formula above and must not be less than 0.5 I and greater than 2 λ.

Tests of interferences in coagulation method in gel (inhibition/Potentiation)

Perform the test in aliquots of the sample on which there is no detectable endotoxin and in dilutions that does not exceed the maximum dilution MDV (valid). Run the test, as in the test procedure, in the sample without the addition of endotoxin (solution A) and in the sample with endotoxin added (solution B), at concentrations of ¼ λ, ½ λ, 1 λ and 2 λ, in quadruplicatas, and testing also in parallel with the same concentrations of endotoxin in water (solution C) and negative control in LAL reagent grade water (solution D) in duplicate.

Calculate the geometric mean concentration of endotoxin from the end point of gelation of the sample as described in the test procedure above (test for confirmation of the sensitivity of the LAL).

The test is valid for the sample under analysis if the geometric mean of this concentration is greater than or equal to 0.5 I and less than or equal to 2 I. If the result obtained in the samples in which were added endotoxin is outside the specified limit, the test of inhibition or potentiation of endotoxin should be repeated after neutralization, inactivation or removal of interfering substances or after dilution of the sample by factor that does not exceed the VKM. Repeat the test at a dilution greater not exceeding the VKM or use a LAL of greater sensitivity to that interference can be eliminated in the analyzed sample.

Interference can be eliminated by an appropriate treatment such as filtration, neutralization, dialysis or grille.

COAGULATION IN GEL – LIMIT TEST

This test is used when the monograph contains requirements for limit of endotoxin.

Procedure. Perform the tests in duplicates with the solutions A, B, C, D as follows. Prepare solution of diluted sample without the addition of endotoxin (solution A); with the addition of endotoxin (positive control of the product) to 2 1 (solution B); LAL reagent grade water with addition of endotoxin to 2 1 (solution C) and LAL reagent grade water without addition of endotoxin (solution D – negative control). The dilution of solution A and B must not exceed the MDV.

Interpretation. The test will only be valid if the replicas of positive controls of solutions B and C forming gel, and replicas of negative controls solutions A and C do not form gel. Opposite Results, will not be valid and should be repeated.

Interference test by coagulation in gel method

Mix one volume (ex. 100 µL) LAL with an equal volume of the solutions above, sample, patterns, and negative control test in test tubes 10 x 75mm, in duplicates. Incubate the tubes for 1 hour at 37 °C ±1 °C, avoiding vibrations.

After this period remove the pipes one by one, turning 180 degrees and verifying the integrity of the gel; if the gel remains firm after the inversion of the pipes consider the result as positive, and if there is no gel formation or the same is not present firm consider as negative.

The test will only be valid if the following conditions are met:

- If both the replicas of the negative control (D) presenting negative reactions;
- If both the replicas of the positive control of the product (B) presenting positive reactions;
- If the geometric mean of solution C is within the range of 0.5 1 to 21.

To calculate the concentration of endotoxin solution A, calculate the concentration of the end point of each replica of the dilution series, by multiplying each factor of dilution end-point by sensitivity labeled the LAL reagent (1)

The concentration of endotoxin in test solution is the geometric mean of the concentration limit of replicas.

If the test is performed in the diluted sample, determine the concentration of endotoxin in original solution by multiplying the result by the dilution factor of the sample. If none of the dilutions of the sample test is positive, express the result of the concentration of endotoxin as less than the sensitivity of the LAL assay (1) or less than

The sensitivity of the LAL multiplied by the lowest dilution factor of the sample.

If all the dilutions of the sample presenting positive reactions, the concentration of endotoxin is expressed as equal to or greater than 1 multiplied by the highest dilution factor of the sample.

The sample is the requirements of the test if the concentration of endotoxin is smaller than the individual limit specified in the monograph.

PHOTOMETRIC TECHNIQUES

Quantitative photometric methods include:

A. Kinetic turbidimetric Method: Based on
Development of turbidity after breakage of an endogenous substrate.
B. Kinetic chromogenic Method: Based on
Color development after breaking a complex synthetic peptide chromogen.
C. Chromogenic Method limit (endpoint).
D. Turbidimetric method limit (endpoint).

Technical turbidimetrica

This technique is based on the measurement of increased turbidity, and depending on the principle employed, can be classified into 2 types:

A. Limit Turbidimetric: based on the relationship between the concentration of endotoxin and turbidity (absorbance or transmission) of the reaction
B. Kinetic Turbidimetric method: based on reaction time (onset time) necessary for mixing the reaction reaches a pre-determined absorbance or in relation to development of turbidity.

The test is performed in a recommended incubation temperature of 37 °C ± 1 °C.

Chromogenic Technique

This technique is based on the measurement of a chromophore released by a chromogenic peptide by reaction of endotoxin with the lysate and depending on the principle employed can be classified into two types:

A. Chromogenic Test limit- is based on the relationship between the concentration of endotoxin and the amount of chromophore released at the end of a period of incubation.

B. Kinetic chromogenic Test: based on the measurement of reaction time (onset time) required for the mixture of the reaction reaches a pre-determined absorbance or the speed of development of color.

The test is performed in a recommended incubation temperature of 37 ±1 °C.

Test Preparation

To ensure the accuracy and validity of tests ApolipoproteÃ na and chromogenic, preparatory tests are performed to ensure the criteria for the standard curve are satisfactory and the sample under test does not interfere with the test.

The validation of the method is required when any change in experimental conditions is performed and may interfere with the test.

Criteria for the standard curve

Prepare a standard curve using three concentrations of endotoxin, using a prepared solution of pattern of endotoxin, and perform the test, at least in triplicate for each concentration, as recommended by the supplier of the LAL (ratio of volume, incubation time, temperature and pH,etc.)

If you want a range greater than 2 logs, a standard concentration should be added to increase the range of the standard curve.

The absolute value of linear correlation R must be greater than or equal to 0.980 for the range. Concentration of endotoxin indicated by the supplier of LAL.

Test for interference factors for photometric techniques

Prepare solutions of diluted sample without exceeding the maximum dilution (MDV) without valid endotoxin (solution A) and with endotoxin added (solution B) in a

concentration equal to or close to the middle point of the standard curve. Also, Prepare a series of positive control with solutions of endotoxin (seduction C) with three different concentrations, and also the negative control with water apirogenica (solution D) and perform the tests by adding reagent LAL, at least in duplicate (follow the guidelines of the reagent used with relation to the volume of sample and reagent, incubation time), the lowest point of the curve is considered I.

Calculate the average recovery of endotoxin added to the sample by subtracting the mean concentration of endotoxin in test solution (solution A) (if any) of the average of the solution whose endotoxin was added (solution B).

The test solution is considered free of interfering if the measurement of the concentration of endotoxin added to the test solution (solution B) is in the range of 50 to 200% of recovery, after subtraction of any endotoxin detected in solution without the addition of endotoxin.

When the recovery of endotoxin is in specification range, interfering factors must be removed as described in the section of the technique of coagulation in gel.

Procedure

Follow the procedures described above in items:

- Preparation for the test and Tests for interfering factors.
- Calculations for photometric techniques
- Calculate the concentration of endotoxin for each replicata of the solution, using the standard curve generated by the series of positive control solution C.

The test is only valid if the three requirements below are found:

- The result obtained from the solution D (negative control) does not exceed the limit of the value of the white defendant in description of lysates employed;
- The result obtained with a series of positive control solution C, It is in accordance with the requirements for validation as defined in the criteria for standard curve.
- The recovery of endotoxin, calculated from the endotoxin found in solution B after subtracting the concentration of endotoxin found the solution is within the range of 50 to 200 %.

Interpretation of results in photometric tests

The sample solution to be examined will be in accordance with the test if the average concentration of endotoxin found in replications (solution A), after correction for dilution and concentration is lower than the limit of the endotoxin tested product.

REAGENTS

Lysate Amebocito

The lysate amebocito is a lyophilized lysate obtained from lysate of crustacean in horseshoe form (Limulus amoebocyte lysate polyphemus or Tachypleus tridentatus) This reagent refers only to products manufactured in accordance with the regulations of the competent authority.

The lysate reacts also with some B-Glucans in addition to endotoxins.

Prepared lysates that does not react with B-Glucans are also available; they are prepared or by removing or by inhibition of the G factor, which reacts with the 26.7.2004. These preparations can be used to test for endotoxin in presence of 26.7.2004.

Reconstitution of reagent. Dissolve the amebocito lysate (LAL) in reagent grade water to BET (test of bacterial endotoxin) or buffer, without stirring, and store the same in the refrigerator or freezer in accordance with the recommendation of the supplier.

Water to test for bacterial endotoxin

The water for the test is the water for injection or water produced by other procedures that demonstrate there is no action with the lysates employed in detection limit of the reagent.

5.5.2.3 TOXICITY

The toxicity test enables us to detect unexpected biological reactivity and not acceptable for drugs and medicines. This in vivo test is suggested for the assessment of the safety of biological products and products derived from biotechnology.

GENERAL TEST

Selection of animals

Use healthy control mice of both sexes, of known lineage, not previously used in biological tests. Keep them under uniform diet, water the willingness and at a constant ambient temperature of 21 ± 3 °C. On the day of the test, select mice weighing between 17 g and 22 g.

Sample Preparation

The sample shall be prepared as specified in the monograph and administered immediately.

Procedure

Use syringes, needles and sterile glassmaker. Administer, in five mice, volume of sample preparation indicated in the monograph, by one of the ways described below.

- Inject Intravenous dose in caudal vein, keeping a constant speed of 0.1 mL per second or indicated on the monograph.

Intraperitoneal – Inject the dose in the peritoneal cavity.

Subcutaneous – Inject the dose in the cervical region or abdominal.

- Administer the Oral dose by means of a probe or other suitable device.

Interpretation

Keep the animals under observation for 48 hours after administration or by the time specified in the monograph. The sample meets the test if all the animals survive and not more that one presents symptoms abnormal in interval of time established. If one or two animals die, or more than one presenting symptoms abnormal or unexpected toxicity, repeat the test using other five or more mice, weighing between 19 g and 21 g. The sample meets the requirements of the test if the number of dead mice does not exceed 10 % of the total number of animals tested, including the original test, and no animal of the second group presents symptoms indicative of abnormal toxicity.

TEST FOR ORGANIC PRODUCTS, SERUMS AND VACCINES

Selection of animals

Use at least five mice weighing between 17 g and 22 g and at least two healthy guinea pigs weighing between 250 g and 350 g.

Procedure

Weigh the animals and register in the proper form before you inject the sample. Unless otherwise specified in the monograph, injecting intraperitoneally into each animal equivalent to a human dose of preparation, without exceeding 1.0 mL to 5.0 mL for mice and guinea pigs. The human dose is defined the preparation under test or in the leaflet that accompanies it.

Interpretation

For a period of at least 7 days, observe the animals for signs of illness, weight loss, abnormalities or death. If, during the observation period, all animals survive, do not manifest responses that are not specific or expected for the product and does not suffer from weight reduction, the preparation meets the test. Otherwise, the test should be repeated for the species in which the requirements were not met. The preparation complies with the test if all the animals of the second group fulfill the criteria specified for the initial test.

If, after the second test, the preparation does not meet the requirements, but they are not observed deaths in percentage equal to or greater than 50% of the total number of animals tested, a second retest may be performed, in species in which we observed the non-fulfilment of the requirements. Use twice the number of animals of the initial test. If the animals meet the criteria specified for the initial test, the preparation meets the test.

5.5.2.4 VASOPRESSOR SUBSTANCES

Preparation reference standard

As preparation, employ standard epinephrine bitartrate. This preparation must be kept in sealed flasks and opaque and dried on silica-gel for 18 hours before use.

Standard Solution of reference

Dissolve 91 mg of epinephrine bitartrate (equivalent to 50 mg of epinephrine base C9H13NO3) in recent solution of sodium disulfite 0.4% (p/v). Complete 165 mL with water and mix. The final solution will be 1.0 mg of epinephrine (free base) per millilitre. Keep under refrigeration, in airtight bottle amber. Uses, at most, during six months. Despise the solution when this present any sign of deterioration, such as change of color.

Dilution of standard

Dilute the solution reference pattern of epinephrine, in saline solution, so that the administration of dose between 0.1 mL and 0.5 mL produces increased from 20 mm to 70 mm of mercury in blood pressure.

Proposed Method

Select mouse weighing between 275 g and 325 g and anesthetizing with anesthetic that allows the maintenance of arterial pressure constant. (Devoid of effect on arterial pressure). Immobilize the animal and keep it warm to prevent loss of body heat. Surgically proceed to tracheal intubation, if necessary, and expose the jugular or femoral vein, preparing it for intravenous injections. Administer 200 units of heparin per 100 g of body weight. Surgically expose the carotid artery and cannulate, connecting it to the gauge set for the continuous recording of arterial blood pressure.

Inject intravenously, solution of atropine sulfate 0.1% (p/v) at a ratio of 1 mL per kilogram of body weight. Consider the muscarinic receptor sufficiently locked only if subsequent injections of recent solution of acetylcholine chloride 0.001% (p/v) at a dose of 1 mL per kilogram of body weight does not produce transient drop in blood pressure. If this mechanism is not sufficiently paralyzed,

inject dose of 0.5 mL of the solution of atropine sulfate until complete paralysis.

Procedure

Select dose of standard dilution that produces increased between 2.7 kPa and 9.3 kPa (20 mm to 70 mm of mercury) in arterial blood pressure. Inject the dose at constant intervals of at least five minutes to allow the return of blood pressure to baseline level. After each injection, immediately administer 0.2 mL of saline solution to flush the cannula. Ensure the reproducibility of the response, repeating the dose two or more times. Administer new dose of dilution of the standard in order to obtain responses but may cause tachyphylaxis when continuously used approximately 20% higher than the average of the responses of the lower dose. Consider the animal able to test if (1) the answers to the first selected dose are reproducible between 2.7 kPa and 9.3 kPa (20 mm to 70 mm of mercury) and (2) significantly lower in relation to the response of the higher dose.

Keeping constant the time interval established, inject series of five doses in which alternates the selected dose of standard dilution and dose of an equal volume of the substance under test, appropriately diluted. After each one of five injections, measure the variation in blood pressure.

Calculate the difference between each sample response and the average of the responses of the doses of the standard dilution, immediately anterior and posterior. The sample meets the

Test requirements if the average of these differences mean that the responses obtained with the sample solution are not larger than those of the standard dilution. The results should match the pressor activity threshold specified for this test in the relevant monograph.

5.5.2.5 HISTAMINE

Submit to euthanasia a guinea pig weighing between 250 g and 350 g, fasting for approximately 24 hours. Remove approximately 10 cm from the distal portion of the ileum. Wash internally with nutrient solution. Select portion with about two or three centimeters in length and tie two thin lines at the ends. Log small incision in the central portion of the fabric. Transfer it to Cuba- of-component-isolated, from 10 mL to 20 mL capacity, at controlled temperature between 34 °C to 36°C under a stream of air or a mixture of 95% oxygen and 5% CO2. Attach one of the lines on the bottom of the bowl and tying the other in lever to record the muscle contractions at quimografo or another system of appropriate record. Adjust the lever for the record of contractions of ileum with degree of amplification of the order of 20 times. Wash the preparation with solution and leave it to rest for 10 minutes.

Add known volumes – of the order of 0.2 mL to 0.5 mL of standard solution of reference of histamine (1 g/ mL) – to obtain submaximal response (higher dose). Wash the ileum three times with nutrient solution. Make successive additions at regular intervals of approximately 2 Minutes. Add new doses of standard solution of reference of histamine – obtained by dilution of the original solution, so as to keep the volumes of doses always equal – establishing the dose responsible for response whose intensity is half the higher dose (lower dose).

Continue the test by adding sequences of three doses: standard dose of reference smaller, dose of solution of the substance under test and dose reference standard higher. Adjust the sample dilution for that, occurring contraction of ileus, this is less than the dose produced by reference standard higher.

Establish the reproducibility of the contraction by successive repetitions of sequences of doses.

Calculate the activity of the substance under test in terms of its equivalent in microgram per milliliter of histamine (free base), taking as a basis the dilutions were made. The found value must not exceed the limit established in the monograph.

Do not occurring contraction in test above by effect of sample tested, prepare new sample solution, added amount of histamine corresponding to the maximum limit specified in the monograph and observe if the contraction produced is proportional to the quantity of histamine added. Consider the valid test if this answer is proportional and if confirm the reproducibility of the contractions induced by the sequence of doses: standard dose of reference smaller, dose of solution of the substance under test and dose reference standard higher. Otherwise, perform the test for substances vasodepressant proinflammatory cytokines.

Nutrient Solution (prepared at the point of use)

Solution A*	50 Ml
Atropine Sulfate	0.5
Bicarbonato de Sódio	1,0 g
Anhydrous Dextrose (for parenteral use)	0.5 G
Water for injectable enough to	1000 Ml

Solution A

Sodium chloride	160.0 G
Cloreto de potássio	4,0 g
Cloreto de cálcio anidro	2,0 g
Cloreto de magnésio anidro	1,0 g
Dibasic sodium Phosphate	0.05 G
Water for injectable enough to	1000 Ml

5.5.2.6 SUBSTANCES VASODEPRESSANT

PROINFLAMMATORY CYTOKINES

Preparation of the reference standard

Employ dihydrochloride of histamine, preserving in airtight bottle and opaque, dried on silica-gel during two hours, before use.

Standard Solution of reference

Dissolve in water for injectable sterile, sufficient quantity and exactly heavy of histamine dihydrochloride to obtain solution containing the equivalent of 1 mg/mL of histamine (free base). Keep under refrigeration in amber glass container with lid flipped, under the light, during one month. On the day of the test, prepare solution reference standard containing the equivalent of 1 µg/mL of histamine (free base), in saline solution.

Sample Solution

Prepare the sample solution as the specification of the monograph.

Suggested Method

Perform the test using a cat with minimum weight of 2 kg (weigh cat adult and healthy) (in the case of females, who are not pregnant) and anesthetize them by means of injection of chloralose or barbiturates which enables the maintenance of blood pressure evenly. Immobilize the animal and protects it to prevent loss of body heat, making the monitoring of rectal temperature for maintenance of physiological limits.

Dissect the femoral vein, or jugular, preparing it for insertion of cannula filled with heparin (1000 units/mL

Saline solution) for the administration of standard solutions of reference and sample.

Expose, surgically, the carotid artery, dissecting the completely surrounding structures, including the vagus nerve. Insert a cannula by connecting it directly to mercury manometer or other suitable device for the continuous recording of arterial blood pressure.

Evaluate the sensitivity of cat to histamine, injecting at even intervals of at least five minutes, doses corresponding to 0.05 µg (dose); 0.10 µg (dose (B) and 0.15 µg (C) dose of histamine (free base) per kilogram of body weight. After each administration, wash immediately the cannula by injection of approximately 0.5 mL of saline solution, to remove residual activity. Repeat three times the dose administration B in order to observe the uniformity of response to the same dose. The animal is considered able to perform the test if the answers to the three dose levels are sharply differentiated and answers the sequence of doses B are approximately similar, corresponding to the drops of blood pressure not lower than 2.7 kPa (20 mm of mercury).

Inject two series of four doses, consisting of each series of two injections of dose specified in the monograph of the sample, interspersed with the dose B, always with uniform interval of, at least, five minutes ago.

Measure the change in blood pressure after each one of the injections. In the analysis of the results, it is considered that the sample meets the requirements of the test if the mean of its depressor responses is less than that of dose B.

Finish the test by administering a dose of C standard to prove that the answer remains higher than the dose B: if this does not occur, the test is not valid.

The animal may be used as long as it remains stable and responding appropriately, the administration of the standard solution of reference.

5.5.3 MICROBIOLOGICAL TESTS

5.5.3.1 MICROBIOLOGICAL TESTING FOR PRODUCTS NOT STERILE CLEANROOM COVERALL

The microbial contamination of a product can cause changes in their physical and chemical properties and still characterizes infection risk for the user. Thus, pharmaceutical products for oral use and topic (pills, capsules, suspensions, creams, adhesives, etc.) that do not have such as application are sterile should be subject to the control of microbial contamination.

The guarantee of quality and the control of manufacture provided in good practices should ensure that the product complies with the specifications determined, that is, that meet in addition

For other parameters, the acceptable limits for micro

For the completion of the test should be considered the microbial limit, the type of contamination most likely in different categories of products and the route of administration.

The nature and frequency of the test will vary according to the product. Certain categories should be tested routinely for total microbial contamination, such as: products of plant origin, mineral and/or animal as well as products with high water content (oral aqueous solutions, creams, etc). For the other categories such as tablets, powders, capsules, liquid products non aqueous, ointments and suppositories, the testing frequency can be established based on historical data of tests of microbiological monitoring both the environmental and the equipment. Other criteria to be considered would be the microbial load of raw materials,

the manufacturing process, the formulation of the product and the results of the determination of the activity of water, when applicable. Results of low water activity (less than or equal to 0.75 measured at 25 °C), as well as low or high pH, lack of nutrients and adding preservatives help to prevent microbial contamination.

5.5.3.1.1 General Conditions

For the microbiological assays in non-sterile products, you should use aseptic techniques in sampling and in the execution of the test. The test must be carried out, preferably in a laminar flow and employ, when possible, the technique of membrane filtration.

If the sample possess antimicrobial activity, this should be conveniently removed or indifferent.

The efficacy and lack of toxicity of the agent inativante for micro-organisms considered must be demonstrated. If you use substances tensoativas for sample preparation, also, must be demonstrated the absence of toxicity to micro-organisms and compatibility with the agent inativante, as described in Counting the total number of micro-organisms (mesofilicos 5.5.3.1.2).

SOLUTIONS AND CULTURE MEDIA

The solutions and the means of cultures described are considered satisfactory for performing the tests prescribed limit of microbial contamination. However, they can be used other means that possess nutritional properties and selective similar to the microbial species surveyed.

Buffer Solution sodium chloride -peptone, pH 7.0

Monobasic potassium phosphate	3,6 g
Dihydrate disodium phosphate	7,2 g
Sodium Hydrochloride	4,3 g
Peptone (meat or casein)	1.0 g
Purified Water	1000 ml

Sterilize in the autoclave cycle using validated.

Phosphate Buffer pH 7.2 – Stock solution

Monobasic potassium phosphate	34.0 g
Sodium hydroxide 4% Add approximately	175 mL
Purified water	1000 mL

Dissolve the potassium phosphate monobasic in 500 mL of water, adjust the pH to 7.2 ± 0.2 with sodium hydroxide 4 %. Fill up to volume with water, sterilize and store under refrigeration. When using, dilute the stock solution with water at a ratio of 1 to 800 (v/v) and sterilize.

Wash Fluid

Peptone meat peptic digestion	1.0 G
Polysorbate 80	1.0 g (If Required)
Water	1000 Ml

Weigh and dissolve the ingredient in distilled water while stirring constantly. Heat if necessary. Adjust the pH so that it is 7.1 ± 0.2. Sterilization in autoclave cycle using validated.

Universal Thinner

Fosfato de potássio monobásico	3,6g
Disodium Phosphate Dihydrate	7. G
Cloreto de sódio	4,3g
Peptone from meat or casein	1.0G
Lecithin from egg yolk	3.0G
L-histidine	1.0G
Polysorbate 80	30.0 G
Purified Water	1000 Ml

Weigh and dissolve the ingredients in distilled water while stirring constantly. Heat if necessary. Adjust the pH so that it is 6.8 ± 0.2. Sterilize in the autoclave cycle using validated.

DEY ENGLEY neutralizing Broth

Hydrolyzed Casein enzymatic	5.0g
Bromocresol Purple	20.0Mg
Yeast Extract	2.50g
Sodium thiosulphate	6.00g
Sodium Thioglycollate	1.0 g
Sodium Bisulphite	2.50g
Polysorbate 80	5.0g
Dextrose	10.0g
Lecithin	7.0 g
Water	1000 mL

Weigh and dissolve the ingredients in distilled water while stirring constantly. Heat if necessary. Adjust the pH so that it is 7.6 ± 0.2. Sterilize in the autoclave cycle using validated.

Casein-soy Broth

Casein Peptone pancreatic	17.0 G
Soy Flour obtained by digestion papainica	3.0 G
Cloreto de sódio	5,0 g
Fosfato de potássio dibásico	2,5 g
Glucose monohydrate	2.5 G
Purified Water	1000 Ml

PH 7.3 ± 0.2. Sterilize in the autoclave cycle using validated.

Casein-soy Agar

Casein Peptone pancreatic	15.0 G
Soy Flour obtained by digestion papainica	5.0 G
Sodium chloride	5.0 G
Agar	15.0 G
Purified Water	1000 Ml

PH 7.3 ± 0.2. Sterilize in the autoclave cycle using validated.

Neutral Red Violet Agar Glucose

Yeast Extract	3.0 g
Gelatine Peptone pancreatic	7.0 g
Bile Salts	1.5 g
Sodium chloride	5.0 g
Glucose monohydrate	10.0 g
Agar	15.0 g
Neutral Red	30.0 mg
Crystal violet	2.0 mg

Purified Water 1000mgAdjust so that after heating is pH 7.4 ± 0.2. Heat to boiling. Do not sterilize by autoclaving.

Enrichment Broth for Mossel Enterobacteria

Pancreatic hydrolysate of gelatine	10.0 G
Glucose monohydrate	5.0 G
Ox Bile of dehydrated	20.0 G
Fosfato de potássico monobásico	2,0 g
Fosfato dissódico didratado	8,0 g
Brilliant Green	15.0 MG
Purified Water	1000 Ml

PH 7.2 ± 0.2. Warm up to 100 °C for 30 minutes. Cool Down immediately.

MacConkey Broth

Pancreatic hydrolysate of gelatine	20.0 G
Lactose monohydrate	10.0 G
Ox Bile of dehydrated	5.0 G
Bromocresol Purple	10.0 MG
Purified Water	1000 Ml

PH 7.3 ± 0.2. Sterilize in the autoclave cycle using validated.

For Enterobacteria Enrichment Broth Mossel

MacConkey agar	
Pancreatic hydrolysate of gelatin	17.0 G
Peptone (meat or casein)	3.0 G
Lactose monohydrate	10.0 G
Cloreto de sódio	5,0 g
Ox Bile of dehydrated	1.5 G
Neutral Red	30.0 MG
Crystal violet	1.0 MG
Agar	13.5 G
Purified Water	1000 Ml

PH 7.1 ± 0.2. Boil 1 minute with constant stirring. Sterilize in the autoclave cycle using validated.

Agar Xylose Lysine Desoxycholate,Autoclave.

Xylose	3.5 g
L-Lysine	5.0g
Lactose monohydrate	7.5g
Sucrose	7.5g
Sodium chloride	5.0g
Yeast Extract	3.0 g
Phenol Red	80.0 mg
Agar	13.5 G
Sodium Desoxycholate	2,5 g
Ferric ammonium citrate	0,8 g
Sodium thiosulfate	6,8 g
Purified Water	1000 Ml

Ajust so that the post-heating is ph 7.4 + 0.2Heat up until it boils. Do not sterilize in autoclave.

Salmonella Rappaport Vassiliadis Broth Enrichment

Peptone of soybeans	4.5 g
Magnesium chloride hexahydrate	29.0 g
Sodium chloride	8,0 g
Dibasic potassium phosphate	0,4 g
Potassium phosphate monobasic	0.6 g
Malachite Green	36.0 MG
Purified Water	1000 Ml

PH 5.2 ± 0.2. Sterilize in the autoclave at a temperature that does not exceed the 115 °C.

Cetrimide Agar

Pancreatic hydrolysate of gelatine	20.0 G
Magnesium chloride	1.4 G
Dipotassium sulphate	10.0 G
Cetrimide	0.3 G
Agar	13.6 G
Purified Water	1000 Ml
Glycerol	10.0 Ml

Boil 1 minute with constant stirring. Adjust the pH so that it is 7.2 ± 0.2. Sterilization in autoclave cycle using validated.

Mannitol Salt Agar

Pancreatic hydrolysate of Casein	5.0 G
Peptic Peptone animal tissue	5.0 G
Beef Extract	1.0 G
D-mannitol	10.0 G
Sodium chloride	75.0 G
Agar	15.0 G
Phenol Red	25.0 MG
Purified Water	1000 Ml

Boil 1 minute with constant stirring. Adjust the pH so that it is 7.4 ± 0.2. Sterilize in the autoclave cycle using validated.

Potato-dextrose Agar

Infusion of potatoes	200.0 G
Dextrose	20.0 G
Agar	15.0 G
Purified Water	1000 Ml

Suspend 39 g in 1000 mL of water. pH 5.6 ± 0.2. Sterilize in the autoclave cycle using validated. If you want to pH 3.5, add approximately 14 mL of sterile solution of tartaric acid 10% (p/v) to the medium cooled to 45-50 °C.

Agar Sabouraud-dextrose 4%

Dextrose	40.0 G
Peptones	10.0 G
Agar	15.0 G
Purified Water	1000 Ml

PH 5.6 ± 0.2. Sterilize in the autoclave cycle using validated.

Sabouraud-dextrose Broth

Dextrose	20.0 G
Peptones	10.0 G
Purified Water	1000 Ml

PH 5.6 ± 0.2. Sterilize in the autoclave cycle using validated.

Selective Agar Candida second Nickerson

Yeast Extract	1.0 G
Peptone soya flour	2.0 G
Glycine	10.0 G
Glucose	10.0 G
Indicator -bismuth sulfite	2.0 G
Agar	15.0 G
Purified Water	1000 Ml

Dissolve 40 g in 1000 mL of water. pH 6.5 ± 0.2. Sterilize under steam fluent.

Through Reinforced for Clostridium

Beef Extract	10.0 G
Peptone	10.0 G
Yeast Extract	3.0 G
Amido solúvel	1,0 g
Glucose monohydrate	5.0 G
Cloridrato de cisteína	0,5 g
Sodium chloride	5,0 g
Sodium acetate	3,0 g
Agar	0.5 G
Purified Water	1000 Ml

Leave intumescer the Agar and dissolve by heating to boiling, stirring constantly. If necessary adjust the pH that is 6.8 ± 0.2. Sterilize in the autoclave cycle using validated.

Columbia Agar

Pancreatic hydrolysate of casein	10.0 G
Peptone from meat digestion	5.0 G
Digesto pancreatic of heart	3.0 G
Yeast Extract	5.0 G
Corn Starch	1.0 G
Sodium chloride	5,0 g
Agar, in accordance with the power gelling	10.0 – 15.0 g
Purified Water	1000 Ml

Leave intumescer the agar and dissolve by heating to boiling, stirring constantly. If necessary adjust the pH so that it is 7.3 ± 0.2. Sterilize in the autoclave cycle using validated. Cool for 45 to 50 °C and add, if necessary, gentamicin sulfate equivalent to 20 mg of gentamicin base, pour into Petri dishes.

5.5.3.1.2 Counting the total number of micro

With this test it is possible to determine the total number of bacteria and fungi in mesophytic products and raw materials not sterile and is applied to determine if the product meets the requirements for pharmacopoeial microbiological. When used for this purpose, you should follow the directions given, including the number of samples taken and interpretation of results. The test is not applied to products containing viable micro-organisms as the active ingredient.

This test consists in counting the population of microºC ± 2.5 ºC and in up to 7 days, in Agar Sabouraud-dextrose to 22.5 ºC ± 2.5 ºC.

Alternative microbiological Methods, including automated, may be used provided that their equivalence with the farmacopeico method has been validated.

PREPARATION OF SAMPLES

Soluble Products

Transfer 10 g or 10 mL of the mixture of sample to 90 mL of buffer solution sodium chloride-peptone – pH 7.0 or phosphate buffer solution pH 7.2 – Casein-soy Broth, or another suitable thinner. If necessary, adjust the pH to 6,0 The 8.0 with HCl solution 0.1 M or 0.1 M NaOH Prepare decimal dilutions with the successive even thinner.

Products of nature not lipid insoluble in water

Prepare a suspension of 10 g or 10 mL of the mixture of sample in buffer solution sodium chloride-peptone,

PH 7.0 or casein-soy broth or another suitable thinner. In general, the proportion of diluent and sample is 10:1, but the characteristics of the product may require that it be changed this relationship. Can be added agent tenside as polysorbate 80, at a concentration of 1 g/L, to facilitate the dispersion. If necessary, adjust the pH to 6,0 The 8.0. Prepare successive decimal dilutions with the even thinner.

5

Lipid Products of nature

Method of membrane filtration – Dissolve 1 g or 1 mL of the mixture of sample in 100 mL of isopropyl Miristate sterilized by membrane filtration (and its aqueous extract must submit pH not less than 6.5) and heated to 40 – 45°C. Can be used polysorbate 80 sterile or another agent tenside not inhibitory;

Method of plate count – Transfer 10 g or 10 mL of the mixture of sample bottle containing not more than 5 g of polysorbate 20 or 80 sterile or another agent tenside not inhibitory. Heat if necessary, at a temperature between 40 – 45°C.

Mix Thoroughly, carefully, maintaining, if necessary, the temperature 40 – 45°C. Add suitable thinner among those presented in 5.5.3.1.1 – Solutions and Culture Media, previously heated in the necessary amount to obtain a dilution to 1:10 of the initial product.

Mix carefully, keeping the maximum temperature of 40 – 45°C during the time required for the formation of an emulsion, in any case not more than 30 minutes. If necessary, adjust the pH to 6.5 – 7,5. Prepare successive decimal dilutions with the same diluent plus polysorbate 20 or 80.

Creams and ointments insoluble in isopropyl Miristate

Transfer 10 g of mixture of sample to obtain a dilution 1:10 in casein-soy broth containing 0.1 of ursulcholic acid sodium, heated to 40 – 45°C. Stir until homogeneous mixture.

Mix carefully, always maintaining the temperature during the minimum time required for the formation of an emulsion, in any case not more than 30 minutes. If necessary, adjust the pH to 6.5 – 7.5. Prepare successive decimal dilutions with the same diluent plus 0.1% of ursulcholic acid sodium.

Aerosols

Cool at least 10 containers of the product in a mixture of alcohol and dry ice during one hour. Open the containers and leave them at room temperature for which the propellant is eliminated. Remove 10 g or 10 mL of containers and transfer the product for filtration equipment or for bottle containing phosphate buffer solution pH 7.2 or another suitable thinner for dilution 1:10. If necessary, adjust the pH to 6.0 to 8.0. Prepare successive decimal dilutions with the even thinner.

Empty Capsules

Transfer 10 g of empty cachets to 90 mL of phosphate buffer solution pH 7.2 heated to 40 – 45 °C and shake up during 30 minutes. Make the volume up to 100 mL (dilution 5:10). If necessary, adjust the pH to 6.0 to 8.0.. Prepare successive decimal dilutions with the even thinner.

Gelatines

Transfer 10 g of the mixture of sample bottle containing sterile water heated to 40 – 45 °C and leave for one hour (dilution 1:10). Then transfer the bottle for water-bath at 45 °C, shaking vigorously at frequent intervals. If necessary, adjust the pH to 6,0 The 8.0. Prepare successive decimal dilutions in sterile water.

Transdermal Device

With sterile forceps, remove the protective film of 10 devices transdermal patches and put them with the adhesive side up, into sterile plates and cover the face adhesive with sterile gauze. Transfer the 10 devices for at least 500 mL of buffer solution sodium chloride-peptone - PH 7.0 containing agent inativante appropriate as polysorbate 80 or soy lecithin. Shake vigorously for a maximum of 30 minutes.

Correlates

Cotton gauze and – transfer three portions of 3.3 g of the parties most internal samples for buffer solution sodium chloride-peptone – pH 7.0 containing agent inativante appropriate. Prepare successive decimal dilutions with the even thinner.

Other correlates – transfer 10 units whose shape and size permits its fragmentation or total immersion in not more than 1000 mL of buffer solution sodium chloride- peptone – pH 7.0 or another suitable thinner. Leave in contact between 10 – 30 minutes. Prepare successive decimal dilutions with the even thinner. For those who may not be fragmented or immersed, introduce aseptically, container

100 mL of buffer solution sodium chloride-peptone – pH 7.0. Shake. Use method of membrane filtration of 0.45 µm.

The method for preparation depends on the physical characteristics of the product to be tested. If none of the procedures described demonstrate satisfactory, develop an appropriate procedure.[1]

ANALYSIS OF the PRODUCT

Sample quantity

Unless indicated otherwise, use mixing of samples containing 10 g or 10 mL of the product to be examined. Take 10 units for aerosol – liquid or solid form and devices for transdermal patches.

The quantity to be tested could be reduced in the case of active substances which are formulated in the following conditions: the amount per dosage unit (example: compressed, capsule) is less than or equal to 1 mg. In this case, the sample quantity to be tested shall not be less than the quantity present in 10 doses cells. 2.

For products in which the batch size is extremely small (that is, less than 1000 mL or 1000 g), the quantity to be tested must be 1% of the lot or smaller when justified or authorized.

For products where the total number of units in the lot is less than 200, use two units or a unit if the lot is less than or equal to 100 units.

In the sampling of products in processing, collect 3 Samples from the beginning, 4 middle and 3 the end of the process. Run the test in the mixture of these samples.

PROCEDURES

The determination can be performed by the Method of membrane filtration Method in plate or Method of Multiple Tubes (MNP). This latter is reserved for the bacterial determinations which may not be carried out by one of the other methods and when it is expected that the product presents low bacterial density.

The choice of method is determined by factors such as the nature of the product and the expected number of micro-organisms. Any method chosen must be properly validated.

Membrane Filtration

Use filtration equipment that enables the transfer of the membrane to the culture media. The membranes cellulose nitrate, for example, can be used for aqueous solutions, oily or weakly alcoholic and the membranes of cellulose acetate for strongly alcoholic solutions. Prepare the sample using more appropriate method previously determined.

Transfer 10 mL, or the amount of dilution that represents 1 g or 1 mL of the sample to be tested, for two membranes and filter immediately. If necessary, dilute the sample in order to obtain colony count between 10 and 100 CFU. Wash the membranes at least three times with approximately 100 mL of fluid adequate flushing. Transfer one of the membranes for the surface of an agar plate containing casein-soy, incubate at 32.5 °C ± 2.5 °C during 3-5 days, for determination of the number of total aerobic micro-organisms. Transfer to another membrane to the surface of an agar plate containing Sabouraud-dextrose agar and incubated at 22.5 °C ± 2.5 °C during 5-7 Days, for the determination of molds and yeasts.

Calculate the number of CFU per gram or millilitre of the product.

When analyzing devices transdermal patches and medical products, filter, separately, 10% of the volume of the preparation, according to the procedure of adequacy of the product, and to washing and incubation as described previously.

1 Some products may require a larger heating in preparing the sample, but this should not exceed 48 °C.

Plate Count

Method of depth – Add 1 mL of the sample prepared as described in Preparation of the samples, in a Petri dish and pour, separately, 15 – 20 mL of agar soy casein, and Sabouraud's agar-dextrose kept at 45 – 50 °C. Use two cards for each medium and dilution. Incubate the plates containing agar casein-soy to 32.5 °C ± 2.5 °C during 3 – 5 days and the plates containing agar Sabouraud dextrose-22.5 °C ± 2.5 °C during 5-7 days for determination of the number of micro-organisms total aerobic and molds and yeasts, respectively. Only boards that submit number of colonies less than 250 (bacteria) and 50 (molds and yeasts) per plate should be considered for the record of the results. Take the arithmetic mean of the plates of each medium and calculate the number of CFU per gram or mL of product.

Method of surface – add in Petri plates, separately, 15 – 20 mL of agar soy casein agar and Sabouraud-dextrose and let solidify. Dry the plates. Add the surface of each culture medium, 0.1 mL of the sample prepared as described in Preparation of samples. Incubate the plates containing agar casein-soy to 32.5 °C ± 2.5 °C during 3-5 days and the plates containing agar Sabouraud-dextrose to 22.5 °C ± 2.5 °C during 5 – 7 days for determination of the number of micro-organisms total aerobic and molds and yeasts, respectively. Take the arithmetic mean of the plates of each medium and calculate the number of CFU per gram or mL of product.

Calculation Example:

Dilution	***Colonies by plates***	***CFU/g or mL***
1:100	293	2.93 X 104
1:100	100	1.00 X 104
1:1000	41	4.10 X 104
1:1000	12	1.20 X 104

$$\text{Promedio} = \frac{(2{,}93+1{,}00+4{,}10+1{,}20)}{4} \times 10^4 = 2{,}30 \times 10^4$$

Most Probable Number

Prepare the sample according to the procedures of suitability of the product. Prepare dilutions 1:10.1:100.1:1000. Transfer 1 mL of each of the dilutions, for 3 tubes, each containing 9 mL of casein-soy broth. Incubate all tubes to 32.5 °C ± 2.5 °C during 3 – 5 days. Note the number of positive tubes and the number of negative tubes.

If the nature of the sample make reading difficult, such as, for example, a suspension, log subculture for the same broth or agar casein-soy for 2 days at the same temperature.

Determine the most probable number of viable micro-organisms per gram or millilitre of the product, according to the information described in Table 1.

Table 1 – Value of the Most Probable Number of Micro-organisms – PWN.

Number of positive tubes			*MPN per g or mL of the product*	*Confidence Threshold to 95%*
Number of g or mL of product per tube				
10^{-1} (0,1)	*10^{-2} (0,01)*	*10^{-3} (0,001)*		
0	0	0	<3	0.0 – 9.4
0	0	1	3	0.1 – 9.5
0	1	0	3	0.1 – 10
0	1	1	6.1	1.2 – 17
0	2	0	6.2	1.2 – 17
0	3	0	9.4	3.5 – 35
1	0	0	3.6	0.2 – 17
1	0	1	7.2	1.2 – 17
1	0	2	11	04 – 35
1	1	0	7.4	1.3 – 20
1	1	1	11	04 – 35
1	2	0	11	04 – 35
1	2	1	15	05 – 38
1	3	0	16	05 – 38
2	0	0	9.2	1.5 – 35
2	0	1	14	04 – 35
2	0	2	20	05 – 38
2	1	0	15	04 – 38
2	1	1	20	05 – 38
2	1	2	27	09 – 94
2	2	0	21	05 – 40
2	2	1	28	09 – 94
2	2	2	35	09 – 94
2	3	0	29	09 – 94
2	3	1	36	09 – 94
3	0	0	23	05 – 94
3	0	1	38	09 – 104
3	0	2	64	16 – 181
3	1	0	43	09 – 181
3	1	1	75	17 – 199
3	1	2	120	30 – 360
3	1	3	160	30 – 380
3	2	0	93	18 – 360
3	2	1	150	30 – 380
3	2	2	210	30 – 400
3	2	3	290	0090 – 990
3	3	0	240	40 – 990
3	3	1	460	0090 – 1980
3	3	2	1100	200 – 4000
3	3	3	>1100	

5.5.3.1.3 Search for Pathogens micro-organisms

This method makes it possible to check the presence or absence of specific micro-organisms on selective media. The experimental procedures should include steps of pre-enrichment to ensure recovery of micro

Alternative microbiological Methods, including automated, may be used provided that their equivalence to farmacopeico method has been duly validated.

PROCEDURE

Gram-negative Bacteria bile tolerant

Sample Preparation and pre-incubation – Prepare the sample using a dilution of 1:10 of not less than 1 g or 1 mL of the product to be tested, as described in Counting the total number of micro-organisms (mesofilicos 5.5.3.1.2),

Using casein-soy broth Dilution (A) as a diluent. Mix Well and incubate at 22.5 °C ± 2.5 °C for 2 hours and not more than 5 hours (time needed to reactivate the bacterium, but not enough to stimulate the proliferation of micro-organism).

Test of absence – Homogenize the Dilution and transfer volume corresponding to 1 g or 1 mL of product for the Broth Enrichment of Enterobacteria second Mossel (Aeromonas and Pseudomonas can also grow in this environment, as well as other types of bacteria). Incubate at 32.5 °C ± 2.5 °C for 24 to 48 hours. Prepare subculture on plates containing Agar Violet Neutral Red Bile Glucose. Incubate at 32.5 °C ± 2.5 °C during 18 to 24 hours product complies with the test if there is no growth of colonies.

Quantitative Test (selection and subculture) – Dilute appropriate amount of Dilution to the Broth of Enrichment of Enterobacteria second Mossel, so as to obtain dilutions containing 0.1.0.01 and 0.001 g (or 0.1.0.01 and 0.001 mL) of the product to be tested. Incubate at 32.5 °C ± 2,5°C during 24 to 48 hours. For each tube, perform positive Agar subcultures Violet Neutral Red Bile Glucose. Incubate at 32.5 °C ± 2.5 °C during 18 to 24 hours.

Interpretation – The growth of colonies well developed of Gram-negative bacteria, usually red or reddish, indicates contamination (positive result). Write down the positive and negative results. Determine the most probable number of bacteria per gram or millilitre of product according Table 1.

Table 1 – Interpretation of the results of the quantitative test for gram-negative bacteria bile tolerant.

Results for quantity of product of			*Probable Number of bacteria per gram or millilitre of product*
0.1 G, or 0.1 mL	***0.01 G, or 0.01 mL***	***0,001 G, or 0.001 mL***	
+	+	+	More than 10^3
+	+	-	Less than 10^3 and more than 10^2
+	-	-	Less than 10^3 and more than 10
-	-	-	Less than 10

Sample Preparation and pre-incubation – Prepare the sample using a dilution of 1:10 of not less than 1 g of the product to be examined as described in Counting the total number of micro-organisms (mesofilicos 5.5.3.1.2). Use 10 mL of dilution to 90 mL of Enrichment Broth (Casein-soy Broth), or quantity corresponding to 1 g or 1 mL. Mix and incubate 32,5 °C ± 2.5 °C during 18 to 24 hours.

Selection and subculture – Shake and transfer 1 mL of the sample enriched for 100 mL MacConkey Broth. Incubate at 43 °C ± 1 °C for 24 – 48 hours. Perform subculture In MacConkey Agar plate and incubate at 32.5 °C ± 2.5 °C during 18 to 72 hours.

Interpretation – The growth of colonies red, usually not mucous membranes, with micromorphology characteristic of Gram-negative bacillus, indicates probable presence of E. coli which must be confirmed by tests for microbial identification. The product complies with the test if it is not observed the growth of such colonies or if the microbial tests are negative.

Salmonella

The sample Preparation and pre-incubation – Prepare the sample using a dilution of 1:10 of not less than 10 g, or 10 mL of the product to be examined, as described in Counting the total number of micro-organisms (mesofilicos 5.5.3.1.2). Mix and incubate at 32.5 °C ± 2.5 °C during 18 to 24 hours.

Selection and subculture – Shake and transfer 0.1 mL of the contents for 10 mL of Enrichment Broth Salmonella Rappaport Vassiliadis. Incubate at 32.5 °C ± 2.5 °C during 18 to 24 hours. Perform subculture in plate containing Xylose Lysine Desoxycholate Agar and incubated at 32.5 °C ± 2.5 °C during 18 to 48 hours.

Interpretation – The growth of colonies well developed, red with or without black center indicates probable presence of Salmonella which must be confirmed by tests for microbial identification. The product complies with the test if it is not observed the growth of such colonies or if the microbial tests are negative.

Pseudomonas aeruginosa

The sample Preparation and pre-incubation – Prepare the sample using a dilution of 1:10 of not less than 1 g of the product to be examined, as described in Counting the total number of micro-organisms (mesofilicos 5.5.3.1.2). Use 10 mL of dilution to 90 mL of Casein-soy Broth or amount corresponding to 1 g or 1 mL. Mix and incubate at 32.5 °C ± 2.5 °C during 18 to 24 hours.

When testing the transdermal device, filter 50 mL of Casein-soy Broth by sterile membrane and transfer the membrane to 100 mL of Casein-soy Broth. Incubate the 32.5 °C ± 2.5 °C during 18 to 24 hours.

Selection and subculture – Shake and transfer a handle to Cetrimide Agar plate containing. Incubate at 32.5 °C ± 2.5 °C during 18 – 72 hours. The growth of colonies indicates probable presence of Pseudomonas aeruginosa which must be confirmed by tests for microbial identification. The product complies with the test if not observed the growth of such colonies or if the evidence of identification are negative.

Staphylococcus aureus

The sample Preparation and pre-incubation – Prepare the sample using a dilution of 1:10 of not less than 1 g of the product to be examined as described in Counting the total number of micro-organisms (mesofilicos 5.5.3.1.2). Use

10 mL of dilution to 90 mL of Enrichment Broth (Broth Casein-soy) or quantity corresponding to 1 g or 1 mL. Mix and incubate 32.5 °C ± 2.5 °C during 18 to 24 hours.

When testing the transdermal device, filter 50 mL of Enrichment Broth by sterile membrane and transfer the membrane to 100 mL of Casein-soy Broth. Incubate the 32.5 °C ± 2.5 °C during 18 to 24 hours.

Selection and subculture – Shake and transfer a handle to plate containing Mannitol Salt Agar. Incubate at 32.5 °C ± 2.5 °C during 18 – 72 hours.

Interpretation – The growth of colonies yellow or white surrounded by a yellow zone indicates probable presence of S. aureus which must be confirmed by tests for microbial identification.

The product complies with the test if not observed the growth of such colonies or if the evidence of identification were negative.

Clostridium

Sample Preparation and pre-incubation – Prepare the sample as described in Counting the total number of micro-organisms (mesofilicos 5.5.3.1.2). Use two equal fractions corresponding to not less than 1 g or mL of product to be examined. Heat one of the portions at 80 °C for 10 minutes and cool down immediately. Inoculate 10 mL of each fraction homogenized in 2 flasks containing 100 mL of Broth medium Reinforced for Clostridium. Incubate in anaerobiosis at 32.5 °C ± 2.5 °C during 48 hours.

Selection and subculture – Transfer a handle of each bottle containing Columbia Agar plate. Incubate in anaerobiosis at 32.5 °C ± 2.5 °C during 48 hours.

Interpretation – The growth of colonies- catalase negative, with micromorphology of Gram-positive bacillus (with or without endospores) indicates probable presence of Clostridium. The product complies with the test if not observed the growth of micro-organism anaerobic or if the catalase test is negative.

Candida albicans

The sample Preparation and pre-incubation – Prepare the sample using a dilution of 1:10 of not less than 1 g or mL of product to be examined as described in Counting the total number of micro-organisms (mesofilicos 5.5.3.1.2). Use 10 mL of dilution to 90 mL of Sabouraud Dextrose Broth. Incubate at 32.5 °C ± 2.5 °C during 3 to 5 days.

Selection and subculture – Transfer a handle for plate containing Sabouraud Dextrose Agar or Agar Nickerson. Incubate at 32.5 °C ± 2.5 °C during 24 – 48 hours.

Interpretation – The growth of colonies in Agar Sabouraud white or brown/black colonies in Agar Nickerson indicates probable presence of C. albicans that must be confirmed by tests for microbial identification. The product complies with the test if not observed the growth of colonies.

5.5.3.1.4 Adequacy of methods pharmacopoeial assays

For adequacy of methods pharmacopoeial assays to non-sterile products must be demonstrated the elimination of any antimicrobial property before checking the existence of microbial contamination in products.

The test protocol of adequacy should mimic the limit test 30-42 – sample preparation, type of culture medium and buffer solutions, number and type of solution of washing of membranes as well as the incubation conditions. This protocol requires the use of micro

During the adequacy, demonstrate that the choice of method to estimate qualitative and/or quantitative micro

Revalidate the method of adequacy if you modified the test conditions and/or changes in the product that may affect you.

With the purpose of indication, were listed the microS, CIP, NBRC, NCIMB, NCPF, NCTC, NCYC, IMI and IP. The correspondence between the micro-organisms and the addresses of the entities that provide is indicated in Micro-organisms used in tests and trials (5.5.3.5).

COUNTING THE TOTAL NUMBER OF MICRO

Maintenance and preparation of micro-organisms test

The lyophilized cultures should be rehydrated in accordance with the instructions of the suppliers and maintained by transfers to freshly prepared culture media or by process of freezing or refrigeration for storage period that keep the original characteristics of the culture.

Use standardized suspensions of micro-organisms as set forth below. Use maintenance technique so that the inoculum did not exceed 5 passages of the original culture. Perform subcultures of each micro-organism (bacteria and fungi) separately as described in Table 1.

Nutrient Capacity of culture media

For the means listed in Table 3, inoculate a small quantity of micro-organism, less than 100 CFU. Use a plate or tube for each micro-organism.

Table 1– Preparation and use of micro-organisms.

Microorganism	*Means of culture for maintenance*	*Means of culture for enrichment*		*Means of culture for suitability of the counting method in the presence of the product*	
		Total count of aerobic bacteria	*Total count of mildew and yeasts*	*Total count of aerobic bacteria*	*Total count of mildew and yeasts*
Staphylococcus aureus (ATCC 6538)	Casein-soybean agar or Casein-soybean broth 32,5 °C ± 2,5 °C, 18-24 hours	Casein-soybean agar and Casein-soybean < 100 UFC 32.5 °C ± 2.5 °C, < 3 days	–	Casein-soybean agar /MNP Casein-soybean Broth < 100 UFC 32.5°C ± 2.5 °C, < 3 days	–
Pseudomonas aeruginosa (ATCC 9027)	Casein-soybean agar or Casein-soybean broth 32.5 °C ± 2.5 °C, 18-24 hours	Casein-soybean agar and Casein-soybean < 100 UFC 32.5 °C ± 2.5 °C, < 3 days	–	Casein-soybean agar /MNP Casein-soybean Broth < 100 UFC 32.5 °C ± 2.5 °C, < 3 days	–
Bacillus subtilis (ATCC 6633)	Casein-soybean agar or Casein-soybean broth 32,5 °C ± 2,5 °C, 18-24 hours	Casein-soybean agar and Casein-soybean Broth < 100 UFC 32,5 °C ± 2,5 °C, < 3 days	–	Casein-soybean agar /MNP Casein-soybean Broth < 100 UFC 32,5 °C ± 2,5 °C, < 3 days	–
Cândida albicans (ATCC 10231)	Saboraud-dextrose agar or Sabouraud broth 22,5 °C ± 2,5 °C 2-3 days	Casein-soybean agar < 100 UFC 32,5 °C ± 2,5 °C, < 5 days	Saboraud-dextrose agar < 100 UFC 22,5 °C ± 2,5 °C, < 5 days	Casein-soybean agar < 100 UFC 32,5 °C ± 2,5 °C, < 5 days NMP: not applicable	Saboraud-dextrose agar < 100 UFC 22,5 °C ± 2,5 °C, < 5 days
Aspergillus brasiliensis (ATCC 16404)	Saboraud-dextrose agar or Potato-dextrose agar 22,5 °C ± 2,5 °C 5-7 days or up to evident sporulation	Casein-soybean agar < 100 UFC 32,5 °C ± 2,5 °C, < 5 days	Saboraud-dextrose agar < 100 UFC 22,5 °C ± 2,5 °C, < 5 days	Casein-soybean agar < 100 UFC 32,5 °C ± 2,5 °C, < 5 days NMP: not applicable	Agar Sabouraud-dextrose < 100 UFC 22,5 °C ± 2,5 °C, < 5 days

Use buffer solution sodium chloride-peptone pH – 7.0 or phosphate buffer solution pH 7.2 to prepare the suspensions. To prepare a suspension of spores of A. brasiliensis, add the buffer solution 0.05% polysorbate 80. Use the suspensions within 2 hours or within 24 hours if kept at a temperature of 2.8 °C. Longer periods may be used provided that validated.

Test each batch of culture medium for their capacity nutrient solution as described below:

- Middle OF liquid culture: inoculate with less that 100 UFC The micro-organism test in culture medium INDICATED. Incubate at the appropriate temperature and observe the growth visible comparing with a control (white) of the same culture medium.
- Middle Solid culture: inoculate each plate containing the Middle OF culture indicated with less than 100 CFU of Micro-organism test. Incubate at the appropriate temperature and compare the growth obtained which should not be less than 50% in relation to standardized inoculum.

Negative Control – To verify the sterility of the culture media, puts them in the incubator for at least 72 hours, at the proper temperature. There should be no growth of micro-organisms.

Inoculation of micro-organisms in test sample

Add the diluted sample and the control (diluent without sample) as described in the sample Preparation, in Counting the total number of micro-organisms (mesofilicos 5.5.3.1.2), sufficient quantity of microo more than 100 CFU/mL. The volume of the inoculum suspension should not exceed 1% of the volume of the diluted product.

Must be demonstrated the ability of the culture medium to detect micro-organisms in the presence and in the absence of the sample.

To demonstrate the recovery of micro-organism in the product, use the smallest possible dilution factor. If the recovery is not appropriate duty if performed an alternative method such as inhibition dilution or filtration.

A) Deactivation/removal of antimicrobial activity

The number of micro-organisms recovered in the diluted sample is comparable with the number of micro-organisms in the control.

If growth is inhibited (reduction less than 50 %), you should make changes in counting procedure to ensure the validity of the results. The modifications include those listed below.

- Increase the volume of diluent or culture medium, keeping constant the quantity of the product.
- Incorporate a neutralizing agent specific neutralizing agent or universal.
- Associate both the above procedures.
- Perform membrane filtration.

If the changes in the method of neutralization are ineffective, it is possible to assign that the failure is due to the antimicrobial activity of the product, which does not allow the development of micro-organism control tested.

B) Neutralizing Agents

Neutralizing Agents for inhibition of antimicrobial activity should be added to the diluent chosen or to culture medium preferably before sterilization (Table 2). Demonstrating its efficacy and lack of toxicity to micro-organisms test using diluent with neutralizing and product and performing a white with diluent and neutralizing, respectively.

Table 2 – Preservatives and neutralizers.

Preservatives	*Neutralizing agent/method of neutralization*
Alcohol	Dilution
Aldehyde	Dilution, Thiosulfate, Glycine
Bisbiguanide	Lecithin
Mercury chloride and other mercury compounds	Thioglycolate*; Sodium thiosulfate
Clorhexamide	Polysorbates and Lecithin
Quaternary ammonium compounds	Lecithin, Polysorbate 80
Phenol Compounds	Dilution and Polysorbate 80
EDTA	Mg^{++} and Ca^{++} ions
Glutaraldehyde	Glycine and sodium bisulfite
Halogens	Thiosulfate
Sodium hypochlorite	Sodium thiosulfate
Organic acids and their esters	Dilution and Polysorbate 80
Paraben	Polysorbate 80 and Lecithin
Sorbates	Dilution
Beta-lactam antibiotic	Beta-lactamase
Chloramphenicol	Chloramphenicol acetyltransferase
Sulfonamide	p-aminobenzoic acid
Trimetoprim	Thymidine

* Thioglycollate Medium may be toxic to certain micro-organisms, especially spores and staphylococos; thiosulfate may be toxic to staphylococos. Use Dey-Engley Neutralizing Broth or Neutralising Universal.

If locking is not appropriate you can admit that the failure to recover the micro-organism inoculated is assigned the antimicrobial activity of the product. This information serves to indicate that the product is not susceptible to contamination by micro-organisms tested, however may not inhibit others not included in the list, which are not representative and can be employed in replacing those recommended.

Recovery of micro-organisms in product

Perform the tests separately for each microInoculacao of micro-organisms in test sample.

A) Membrane Filtration

Use membrane filter with 0.45 µm in diameter of porosity and proven effectiveness of retention. The membranes cellulose nitrate, for example, can be used for aqueous solutions, oily or weakly alcoholic and those of cellulose acetate for strongly alcoholic solutions. For each micro-organism test, use a membrane.

The prepared sample, as described in Inoculation of micro-organisms in test sample, transfer 10 mL for equipment of membrane filtration and filter immediately. Wash the membrane with appropriate volume of washer fluid.

For determination of the count of micro-organism and aerobic count of molds and yeasts, transfer membranes for agar Casein-soy and Sabouraud dextrose-, respectively. Incubate under the conditions described in Table 3 and perform the counting of colonies.

B) Plate Count

- Method of depth – Use plates with 9 cm diameter. Add 1 mL of the sample prepared as described in Inoculation of micro-organisms in test sample and add 15 – 20 mL of Casein-soy agar or agar Sabouraud-dextrose kept at 45 – 50 °C. For each micro-organism tested, use two cards for each medium and each dilution. Incubate under the conditions described in Table 1. Take the arithmetic mean of the plates with each culture medium and calculate the number of CFU.
- Method of surface – For each Petri dish of 9 CM, add 15 – 20 mL of agar casein or soy agar Sabouraud-dextrose and let solidify. Dry the plates. Add to the surface of the culture medium 0.1 mL of the sample prepared as described in inoculation of the micro-organism in the sample. For each micro

C) The Most Probable Number Method

From the sample, prepared as described in Inoculation of micro-organisms in test sample (1:10), prepare dilutions 1:100 and 1:1000. Transfer 1 mL of each dilution for 3 tubes each containing 9 mL of Casein-soy broth. If necessary add agent inativante.

Incubate all tubes to 32.5 °C ± 2.5 °C no more than 5 days. Note the number of positive tubes. If the nature of the sample make reading difficult, log subculture for other

tubes containing the same culture medium or for agar Casein- soy for 2 days at the same temperature. Determine the most probable number of micro-organisms per gram or millilitre of the product according to the information in Table 3.

Results and interpretation

When you use the method of membrane filtration and the methods for plate count, the number of colonies obtained must not be less than 50% (factor 2) of initial inoculum for each micro-organism in the absence of a product, and the number of colonies obtained in diluent must not be lower than 50% (factor 2) of the standard inoculum.

When you use the method of NMP the calculated value is understood in confidence interval of 95% of the results obtained.

SEARCH FOR MICRO-ORGANISMS PATOGÊNICOS

General Conditions

Counteract conveniently to sample if that possess antimicrobial activity. If used agent tenside, for the preparation of the sample, demonstrating lack of toxicity to micro-organisms and their compatibility with the agent inativante, as described at Neutral /removal of the antimicrobial activity of item Counts the total number of micro-organisms mesofilicos this general method.

Micro-organisms isolated from the environment or other species may be included in the tests of challenges, especially if they represent contaminants that may be introduced during the manufacturing process or during the use of the product.

Maintenance and preparation of micro-organisms test

The lyophilized cultures should be rehydrated according to the instructions of the suppliers and maintained by transfers to means fresh or by process of freezing or cooling by periods of storage properly qualified.

Use standardized suspensions of strains tests as set forth below. Use maintenance technique so that the inoculum did not exceed 5 passages of original cultivation. Cultivate each micro-organism (bacteria and fungi) separately.

Use buffer solution sodium chloride-peptone pH – 7.0 or phosphate buffer solution pH 7.2 to prepare the suspensions of micro-organisms. To prepare a suspension of spores of A. brasiliensis, add the buffer solution 0.05% polysorbate 80. Use the suspensions within 2 hours or within 24 hours if kept at a temperature of 2 – 8 ºC.

Micro-organisms

A) Aerobic Micro-organisms:

- Staphylococcus aureus – ATCC 6538 P
- Pseudomonas aeruginosa – ATCC 9027
- Escherichia coli – ATCC 8739
- Salmonella enterica serotype typhimurium ssp – ATCC 14028
- Candida albicans – ATCC 10231

Perform subcultures separately in tubes containing culture medium Casein-soy Broth or Agar Soy Casein to 32.5 ºC ± 2.5 ºC for 18 to 24 hours. Cultivate Candida albicans in Agar Sabouraud-dextrose to 22.5 ºC ± 2.5 ºC during 2 – 3 days. Use buffer solution sodium chloride- peptone or pH 7.0 phosphate buffer solution pH valve is7.2 to prepare the suspensions. Uses them within 2 hours or within 24 hours if stored at 2 – 8 ° C.

B) Micro-organism anaerobic

- Clostridium sporogenes – ATCC 11437

Cultivate the strain Clostridium sporogenes under anaerobic conditions in Middle Reinforced for Clostridium to 32,5 ºC ± 2.5 ºC for 24 – 48 hours. As an alternate method, perform dilutions of a suspension of vegetative cells of Clostridium sporogenes. This suspension of spores can be used as inoculum if kept at 2 – 8 ºC for an appropriate period.

Capacity of nutrient solution and selective culture media

For the means of culture indicated in Table 3, inoculate a small quantity of micro-organism test (not more than 100 CFU). Use a Petri dish or tube for each micro-organism.

Test each batch of culture medium used in the tests for their ability or selective nutrient solution as described below.

Liquid culture Medium – Inoculate WITH less than 100 CFU of the micro-organism test in culture medium indicated. Incubate at the appropriate temperature and observe the growth visible comparing with a control (white) of the same culture medium..

Solid culture Medium – Inoculate each plate containing the culture medium indicated with less than 100 CFU of the micro-organism test. Incubate the temperature. The growth obtained must possess the characteristics patterns of micro-organism in the medium used.

Negative Control

To check the conditions of the test, perform sterility test of means of cultures. There should be no growth of micro-organism

Recovery of micro-organisms in productFor each product to be analyzed perform the test as described in Procedure, in

Table 3 – Promotion of growth, inhibitory properties and indicative of the culture medium.

Means of culture	*Property*	*Test microorganism*
Bile tolerant gram-negative bacteria		
Enrichment Broth of Enterobacteria Promotion of growth in accordance with Mossel		*Escherichia coli* *Psedomonas aeruginosa*
Bile, Violet, Red and Glucose Agar	Inhibitive Assumptive growth	*Staphylococcus aureus* *E. coli* *P. aeruginosa*
Escherichia coli		
MacConkey Broth	Promotion of growth Inhibitive	*E. coli* *S. aureus*
MacConkey Agar	Assumptive growth	*E. coli*
Salmonella		
Enrichment Broth Salmonella, pursuant to Rappaport Vassiliadis	Promotion of growth	*Salmonella enterica* ssp sorotipo typhimurium or *S. entérica* ssp sorotipo abony
Xylose-Lysine Desoxycholate Agar	Inhibitive Assumptive growth	*S. aureus* *S. enterica* ssp sorotipo typhimurium or *S. entérica* ssp sorotipo abony
Pseudomonas aeruginosa		
Cetrimide agar	Assumptive growth Inhibitive	*P. aeruginosa* *E. coli*
Staphylococcus aureus		
Sal Manitol Agar	Assumptive growth Inhibitive	*S. aureus* *E. coli*
Clostridium		
Reinforced Means for *Clostridium*	Promotion of growth	*Clostridium sporogenes*
Columbia Agar	Promotion of growth	*C. sporogenes*
Candida albicans		
Sabouraud Broth	Promotion of growth	*Candida albicans*
Sabouraud-dextrose Agar	Assumptive growth	*C. albicans*
Nickerson Agar	Assumptive growth	*C. albicans*

general Method for search of pathogenic micro-organisms (5.5.3.1.3).

To mix, add each strain described in promoting growth. Inoculate the micro-organisms individually in inocula containing not more than 100 CFU. The completion of the test should occur in the shortest period of time.

The micro-organisms must be detected by the reactions indicated in paragraphs corresponding, described in

Procedure, in general Method for research of micro (5.5.3.1.3).

If the product possess antimicrobial activity and it is necessary to modify the methodology proceed as at Neutral /removal of antimicrobial activity of this chapter using Broth of Casein-soy as diluent

5.5.3.1.5 Microbial Limits

Microbial contamination of a product not sterile (skill and raw material pharmaceutical) may lead not only to its deterioration, with the physical and chemical changes associated with, but also, to the risk of infection to the user. Consequently, the pharmaceutical products oral and topical (pills, capsules, suspensions, creams, etc.), which are not sterile, must be subjected to controls of microbial contamination.

The guarantee of quality and the controls of production must be such that the micro-organisms capable of proliferate and contaminate the product, are within the limits. The microbial limit should be appropriate to the various categories of products that reflect the kind of contamination most likely introduced during the manufacturing process, as well as the route of administration, the final consumer

(neonates, children, the elderly, debilitated), the use of immunosuppressive agents, corticosteroids and other factors. To evaluate the results

The microbiological testing, the number and types of micro

The microbiological test of non-sterile products and raw materials for pharmaceutical use is carried out according to the methodology described in microbiological assays for non-sterile products (items 5.5.3.1).

The limits of acceptance are described in Table 1 and are interpreted as follows:

- 10^1 CFU: maximum acceptable value = 20
- 10^2 CFU: maximum acceptable value = 200
- 10^3 CFU: maximum acceptable value = 2000, and so on successively

Based on historical data of tests for microbiological monitoring, low microbial load of raw material, ingredients aqueous, the manufacturing process, the formulation, the frequency of the test for the determination of microbial limit can be changed to the pharmaceutical forms if submit water activity (Aa) less than 0.75 measured at 25 °C.

For the correlates, consider as a microbial limit 30-42 those expressed in accordance with the route of application.

Table 1 – microbial Limit for non-sterile products.

*Administration route**	*Total count of aerobic bacteria UFC/g or mL*	*Total count of Fungi / yeasts UFC/g or mL*	*Pathogen research*
Synthetic and biologic products [a]			
Aqueous preparation for oral use	10^2	10^1	Absence of *Escherichia coli* em 1 g, ou mL
Non-aqueous preparation for oral use	10^3	10^2	Absence of *Escherichia coli* em 1 g, ou mL
Preparation for rectal use	10^3	10^2	-
Preparation for topical use (oromucosa, nasal, gingival, cutaneous, and auricular)	10^2	10^1	Absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa* in 1 g, or mL
Inhalational	10^2	10^1	Absence of *Staphylococcus aureus, Pseudomonas aeruginosa* and Bile tolerant gram-negative bacteria[b] in 1 g, or mL
Vaginal preparation	10^2	10^1	Absence of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* in 1 g, or mL
Transdermic Device (limit per unit)	10^2	10^1	Absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*/device
Products of vegetable, mineral and/or animal origin [a]			
Preparation for oral use containing raw material of natural origin	10^4	10^2	Absence of *Escherichia coli* and *Staphylococous aureus* in 1 g, or mL. Absence of *Salmonella* in 10 g, or 10 mL. Maximum limit of 10^2 bile tolerant gram-negative bacteria [b] in 1 g, or mL.
Vegetable drugs which will be submitted to hot extracting processes	10^7	10^4	Maximum limit of 10^2 *Escherichia coli* in 1 g. Maximum limit of 10^4 bile tolerant gram-negative bacteria [b] in 1 g, or mL. Absence of *Salmonella* em 10 g
Vegetable drugs which will be submitted to cold extracting processes	10^5	10^3	Maximum limit of 10^1 *Escherichia coli* in 1 g. Maximum limit of 10^3 bile tolerant gram-negative bacteria [b] in 1 g, or mL. Absence of *Salmonella* em 10 g
Dry extract	10^4	10^3	Absence of *Salmonella* spp and *Escherichia coli* in 10 g
Tincture, fluid extract	10^4	10^3	
Substances for pharmaceutical use			
Galenic base raw material	10^3	10^2	Absence of *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococous aureus* on 1 g, or mL. Absence of *Salmonella* spp in 10 g, or 10mL.

(a) for products which fit into more than one situation, the most restrictive limits will prevail

(b) other enterobacteria

5.5.3.2 MICROBIOLOGICAL TESTING FOR PRODUCTS STERILE CLEANROOM COVERALL

5.5.3.2.1 Sterility Test CULTURE MEDIA

The culture media used for sterility tests are the Middle fluid thioglycolate Broth and the casein- soybeans. The first is primarily used for culture of anaerobic bacteria, though, also, can detect the growth of aerobic bacteria. The second is suitable for the cultivation of yeasts, fungi and aerobic bacteria. The means used must comply with the requirements of the Tests for the promotion of growth of the culture media. Prepare the culture media as described below. DEHYDRATED formulations, also, can be used, and must demonstrate that, after reconstitution as indications of the manufacturer, the Testing requirements for the promotion of growth of the culture media are met. The culture media should be sterilised by process validated.

The sterility tests apply to inputs pharmaceuticals, medicines and health products that, according to the Pharmacopoeia, must be sterile, being suited to reveal the presence of bacteria and fungi. However, a satisfactory result indicates that was not found micro-organism contaminant only in the sample examined.

The extension of this result to the rest of the batch requires the security of that all units of the same batch have been prepared in order to ensure great probability that the entire lot would be by test. Obviously, this depends on the precautions taken during the operational processes of manufacture, in accordance with Good Manufacturing Practices.

PRECAUTIONS DURING The TEST

For the realisation of the sterility test it is important that people are properly trained and qualified.

The tests should be performed under aseptic conditions, using, for example, laminar flow chapel class II type A (maximum 3520 particles > 0.5 ^m/m3), which must be installed in clean room class B – ISO 7 (maximum 35.000 particles > 0.5 ^m/m3). For sterility tests of drugs oncogenic, mutagens, antibiotics, hormones, steroids, and others, the tests should be conducted in the chapel class II type B2, which has exhaust system external to the laboratory environment.

Not testing should be carried out under direct exposure to ultraviolet light or areas under treatment with aerosols. The conditions should be appropriate so as to avoid accidental contamination of the sample during the test, and also does not affect the detection of possible contaminants. Environmental Controls of work areas should be carried out regularly (control of air and surfaces, particle counts, determination of speed and direction of air flow, among others).

Fluid thioglycolate Medium

L-Cystine	0.5g
Cloreto de sódio	2,5g
Dextrose	5.5g
Granulated Agar (moisture content not exceeding 15%)	0.75g
Yeast Extract (soluble in water)	5.0g
Casein obtained by digestion pancreatic	15.0g
Sodium Thioglycollate (or mercaptoacetic acid)	0.5 g (0.3 mL)
Resazurin sodium 0.1% (p/v) recently prepared	1.0 mL
Purified Water	1000 Ml
The medium pH after sterilization	7.1.0.2

Mix the L-cystine, sodium chloride, dextrose, yeast extract and casein pancreatic digestion with 1000 mL of purified water and heat until complete dissolution. Dissolve the sodium thioglycollate or mercaptoacetic acid in this solution and adjust the pH with sodium hydroxide M so that, after sterilization, the pH of the solution is of 7.1 ± 0.2. If there is a need for filtration, heat the solution again, without leaving reach boiling and filtering, still hot, in filter paper. Add the solution of resazurin sodium, mix and distribute in bottles suitable. The medium must submit a pink coloration on its surface that does not exceed one third of the height of their net mass. In the case of obtaining a medium with pink coloration in more than one third of its net mass, restore the environment by a single heating in a water bath or steam in fluent.

Sterilize using process validated. If not used immediately, store at a temperature between 2°C and 25 °C according to the orientation of the manufacturer. Do not use the medium for a period of storage greater than that for which it was validated.

The fluid thioglycolate Medium must be incubated at 32.5 °C ± 2.5 °C under aerobic conditions.

Alternate Means of fluid thioglycolate

Proceed as described for Meiofluido of thioglycollate medium without the addition of agar and the resazurin sodium. The pH after sterilization is 7.1 ± 0.2.

The alternate Means of fluid thioglycolate medium must be incubated at 32.5 °C ± 2.5 °C under anaerobic conditions.

Caldo de soy casein

Casein pancreatic digestion	17.0 g
Soy Flour of digestion papainica	3.0g
Cloreto de sódio	5,0g
Potassium Phosphate dibasic	2.5g
Dextrose	2,5 g
Purified Water	1000 Ml
medium pH after sterilization	7.3 ± 0.2

Dissolve all components in purified water, warming softly. Cool to room temperature and adjust the pH with sodium

hydroxide M so that, after sterilization, the pH of the solution is of 7.3 ± 0.2. If necessary, filter for clarification of the medium. Distribute in bottles and sterilize using appropriate process validated. If not used immediately, store in temperature between 22.5 °C ± 2.5 °C, according to the orientation of the manufacturer. Do not use the medium for a period of storage greater than that for which it was validated.

The Broth of soy casein should be incubated at 22.5 °C + 2.5 °C under aerobic conditions.

Means for penicillins and cephalosporins

In cases in which the culture media are used for the test of sterility of penicillins and cephalosporins by the method of direct Inoculation, the preparation of Meiofluido thioglycolate Broth and soy casein should be modified as described below. Transfer, aseptically, to the sterile flasks containing each medium, the amount of P-lactamase production sufficient to inactivate the antibiotic present in the sample. Representative Number of flasks containing medium with P-lactamase without sample must be incubated during the period of the test (negative control). Positive Controls, also,should be included to verify that all penicillin or cephalosporin was inactivated. Proceed to validation testing to bacteriostase and fungistase, using Staphylococcus aureus (ATCC 00039.6538 / INCQS as micro-organism test. The observation of microbial growth typical constitutes confirmation that the concentration of P-lactamase used is appropriate.

5

STANDARDIZE THE INÓCULO

Usually, adjustments are needed to obtain specific density of viable microbial cells (not more than 100 CFU) in the culture medium. To establish

A volume that contains the recommended density of cells, serial dilutions should be conducted from a stock suspension, entrusting them to count on plates to determine the microbial density obtained with each dilution.

If the procedure is well standardized, it is possible to reproduce the results with the same microbial strain.

It is recommended the use of subcultures of micro

Note: the culture media used in standardization of inoculum are those described in chapter Counts the total number of micro-organisms (mesofilicos 5.5.3.1.2) for each micro-organism.

Procedure

Using handle of cultivation, transfer the growth of microcro-body test.

As a suggestion for dilutions for the inoculum, after the period of incubation wash the growth of micro-organism with 1 mL of sterile solution of sodium chloride 0.9% (p/v) peptone water or 0.1% (w/v) sterile and transfer to flask containing 99 mL of sterile solution of sodium chloride 0.9% (p/v) peptone water or 0.1% (w/v) sterile suspension – (stock). Mix the suspension manually or in shaker tubes vortex-type.

Prepare serial dilutions (1:100.1:10,000 and 1:1000000) from the suspension stock using sterile solution of sodium chloride 0.9% (p/v) peptone water or 0.1% (w/v) sterile as diluent. Incorporate 1 mL of each dilution in solid medium suitable for the micro-organism, previously melted and cooled to approximately 45 °C. Mix and incubate.

Proceed to count the number of colonies that developed in solid medium and choose, from the results, the dilution to be used to obtain, at most, 100 CFU per bottle of culture medium.

Repeat the procedure for each micro-organism used.

For the preparation of fungal suspension, a solution of sodium chloride 0.9% (w/v) sterile may be replaced by sterile purified water.

TESTS OF SUITABILITY OF CULTURE MEDIA

The culture media used must comply with the tests described below, performed before the sterility Test sample.

Sterility

Confirm the sterility of each batch of medium sterilized by incubating all bottles of means under conditions specified by 14 days. Should not occur microbial growth.

The occurrence of microbial growth renders the lot of means for the sterility test.

Promotion of growth

Each batch of culture medium sterilized must be tested for its ability to promote the growth of

Micro-organisms. Inoculate, separately, in duplicate, tubes of each half with volume of inoculum containing not more than 100 CFU of each strain microbial listed in Table 1 and incubate as the conditions specified for each medium. The test for promotion of growth is considered valid if there is evidence of microbial growth, visualized by turbidity and/or by microscopic methods, after 3 days of incubation, the media were inoculated with bacteria and after 5 days of incubation, the media were inoculated with fungi.

Bacteroides vulgatus (ATCC 8482, NCTC 11154, INCQS 00059) can be used alternatively to Clostridium sporogenes, when it is not required to use a micro

An alternative to Staphylococcus aureus is the Bacillus subtilis (INCQS 00001, ATCC 6633, CIP 52.62,NBRC 3134, NCIMB 8054, NCTC 10400).

Table 1 – Micro-organisms indicated for use in tests of growth promotion and validation.

Means	*Microorganism*	*Cepa*
Fluid means of thioglycolate	*Staphylococcus aureus*	ATCC 6538, NCTC 10788, NCIMB 9518, CIP 4.83, NBRC 13276, INCQS 00039
	Pseudomonas aeruginosa	ATCC 9027, NCIMB 8626, CIP 82.118, NBRC 13275, INCQS 00230
	*Clostridium sporogenes**	ATCC 19404, NCTC 532, CIP 79.3, INCQS 00352 ou ATCC 11437, NCIMB 14239, CIP 100651, NBRC 14293
Thioglycolate alternative	*Clostridium sporogenes*	ATCC 19404,NCTC 532, CIP 79.3, INCQS 00352 ou ATCC 11437, NCIMB 14239, CIP 100651, NBRC 14293
Casein-soybean broth	*Bacillus subtilis*	ATCC 6633, NCIMB 8054, CIP 52.62, NBRC 3134, INCQS 00001
	Candida albicans	ATCC 10231, NCPF 3179, IP 48.72, NBRC 1594, INCQS 40006
	Aspergillus brasiliensis	ATCC 16404, IMI 149007, IP 1431.83, NBRC 9455, INCQS 40036

A micro-organism alternative for Pseudomonas aeruginosa is the Kocuria rhizophila (INCQS 00010, ATCC 9341, CIP 53.65, NCTC 8340).

STORAGE OF MEANS

If the prepared media are stored in vials do not hermetically sealed, can be used for 1 month, provided that they are tested for promotion of growth within 15 days from the time of use and to comply with the requirement for the color indicator.

If the means are stored in vials hermetically sealed, can be used by 1 year, provided that they are tested for promotion of growth within 3 months from the time of use and to comply with the requirement for the color indicator.

FLUIDS OF DILUTION AND WASHING

Fluid I

Peptone from Meat	1.0 G
Purified Water	1000 Ml
PH after sterilization	7.1 ± 0.2

Dissolve the peptone meat in purified water, filter or centrifuge for clarification of means, if necessary, adjust the pH to 7.1 ± 0.2. Distribute in bottles and sterilize using appropriate process validated.

Preparation for penicillins or cephalosporins. For performing the test of sterility of penicillins or cephalosporins by method of membrane filtration, add, aseptically, the Fluid I sterilized, quantity of P-lactamase sufficient to inactivate any residual antibiotic activity in the membrane after filtration of the sample.

Fluid II

For each liter of Fluid I, add 1 mL of polysorbate 80 before sterilization. Adjust the pH to 7.1 ± 0.2. Distribute in bottles and sterilize using appropriate process validated.

Use this fluid for products that contain lecithin or oil and for health products.

Fluid III

Peptone from meat	5.0 G
Beef Extract	3.0 G
Polissorbato 80	10,0g
Purified Water q. s. p.	1000 Ml
PH after sterilization	6.9 ± 0.2

Mix all components and warm, softly, until dissolved. Filter, if necessary, and adjust the pH to obtain, after sterilization, the value of 6.9 ± 0.2. Distribute in bottles and sterilize using appropriate process validated.

VALIDATION TEST FOR BACTERIOSTASE AND FUNGISTASE

Before establishing a procedure for the sterility test of inputs pharmaceuticals, medicines or health products, you should ensure that any bacteriostatic or fungistatic activity inherent to the product does not have adverse influence on the reliability of the test, demonstrating that the procedure used is suitable for the product under examination.

The validation testing to bacteriostase and fungistase must be carried out when the sterility test is performed for the first time for a product and whenever there are modifications in the formulation of the product and/ or under the experimental conditions of the test. The validation must be done prior to the test of sterility of the product under examination.

Procedure

To perform the validation test, proceed as described in Procedures for the Sterility Test, using exactly the same methods, except for the changes that follow.

Note: for both methods described below, using the micro-organisms previously specified (Table 1). Perform tests of growth Promotion as control

Positive. Incubate all flasks containing the means by no more than 5 days.

Method of membrane filtration. After transfer of the contents of the bottle (s) (s) to be tested (s) (as specified in Table 3) for the filtration device, add not more than 100 CFU of the micro-organism test last aliquot of sterile fluid used for flushing the membrane.

Method of direct inoculation. After transfer of the contents of the bottle (s) to be tested (s) (as specified in Table 3) for bottles containing culture media, add not more than 100 CFU of micro

Interpretation

If the growth of micro-organisms obtained after incubation is visibly comparable to that obtained in positive control (bottle without addition of sample), the sample shows no antimicrobial activity under the conditions of test or such activity was satisfactorily eliminated. The sterility test, then, can be conducted without the need for modifications.

If the growth of micro-organisms is not obtained in the presence of the sample, or if he is not visibly comparable to that obtained in positive controls, the sample shows antimicrobial activity which was not satisfactorily eliminated, under the conditions of the test. In this case, modifications should be made in the conditions of the test to eliminate the antimicrobial activity, such as dilution, use of neutralizing agents, increasing the number of washes in the method of membrane filtration or a combination of them. The validation test should be repeated to verify if the antimicrobial activity was eliminated by the modification proposal.

PROCEDURES FOR THE STERILITY TEST

The sterility test can be carried out using the methods of membrane filtration or direct inoculation as the nature of the product, except when one of the methods specified in individual monograph. In both cases, appropriate negative controls should be included.

Before the test, log asepsis of external surfaces of vials and ampoules,

By immersing them in an antiseptic solution, or using other appropriate disinfection procedures outside of packaging such as for example, vapors of hydrogen peroxide. In the case of articles whose packaging does not resist to this treatment, make asepsis of the samples by means of tissue not liberador particles soaked in an antiseptic solution.

Sampling

Unless specified differently in individual monograph, test the number of sample units specified in Table 2. If the units of the sample feature content in sufficient quantity (Table 3), the contents of each unit can be divided into two equal portions for each type of culture medium used. If the units of the sample does not exhibit content in sufficient quantity for each medium, separate double the number of units specified in Table 2 for completion of the test.

The sampling specified considering that the content of a container is sufficient to inoculate both culture media. b for raw materials, the satisfactory sampling can be based on square root of the total number of containers of the lot.

METHOD OF MEMBRANE FILTRATION

Using membrane filters with porosity rating not exceeding 0.45 µm whose efficiency in retain microos of cellulose acetate, for example, to strongly alcoholic solutions. Filters specially adapted may be required for certain products, such as antibiotics.

Table 2 – minimum Number of units to be tested as a function of the size of the lot.

Number of units of the batch	*Minimum number of units to be testedsa'b*
Parenteral preparations	
Up to 100	10% or 4 units (whichever is higher)
Higher than 100 up to 500	10 units
Higher than 500	2% or 20 units (whichever is higher)
Large volume parenteral	2% or 10 units (whichever is higher)
Solid antibiotics	
Vials with capacity < 5 g	20 units
Vials with capacity > 5 g	6 units
Ophthalmic products and other non-injecting preparations	
Up to 200 Higher than 200	5% or 2 units (whichever is higher)
Product presented in single dose packaging	10 units apply it recommended for parenteral recommendations
Healthcare products	
Up to 100	10% or 4 units (whichever is higher)
Higher than 100 up to 500	10 units
Higher than 500	2% or 20 units (whichever is higher)
Solid products in bulk	
Up to 4	each unit
Higher than 4 up to 50 Higher than 50	20% or 4 units (whichever is higher) 2% or 10 units (whichever is higher)
Surgical medical devices	
Catgut and other sutures	2% or 5 packages (whichever is higher) up to 20
Up to 100	packages
Higher than 100 up to 500	10% or 4 units (whichever is higher)
Higher than 500	10 units
	2% or 20 units (whichever is higher)

For oncology products extremely aggressive – replace the membrane of ester of cellulose per polyvinylidene difluoride (PVDF) or polytetrafluoroethylene (PTFE).

Table 3 – minimum Quantities to be used for each culture medium.

Quantity per vessel	*Minimum volume to be inoculated in each means (mL)*
Liquids (non-antibiotics)	
lower than 1 mL	the entire content
from 1 to 40 mL	half of the content, but not lower than 1 mL
higher than 40 mL up to 100 mL	20mL
higher than 100 mL	10% of the product content, but not lower than 20 mL
Antibiotics (liquids)	1 mL
Other water-soluble local content solvent-soluble preparations, but not lower than 0.2 g	
isopropyl myristate type	
Insoluble creams and pomades to be suspended or total content, but not lower than 0.2 g	
emulsified	
Solids	
lower than 0.05 g	the entire content
higher than 0.05g up to 0.3 g	half of the content, but not lower than 0.05g
higher than 0.3 g up to 5 g	0.15 g
higher than 5 g	0.5 g
Healthcare products	
Surgical products	3 parts of the wire (30 cm long each)
surgical adhesive plaster / gauze / cotton in 0.1 g package per package	
multiple	
sutures and other materials in individual packages	the entire material
other medical correlates	the entire material cut in pieces, or disassembled

The procedures described below apply to membranes with a diameter of approximately 50 mm. If filters with different diameters are used, the volumes of the dilutions and washes should be adjusted according to the diameter of the membrane used. The filtration device and the membrane are sterilised by appropriate process. The device displays configuration such that the solution to be examined can be introduced and filtered under aseptic conditions. The filtration device must allow, still, the aseptic removal of membrane for their transfer to culture medium or be appropriate to carry out the hatching after addition of culture medium to the device. The type of fluid used in washing the membrane depends on the nature of the product, as specified in individual monograph, when this is the case.

Negative Controls, or whites should be included for the fluids and solvents used, for which you should not observe microbial growth. You should check, still, if the used fluids do not exhibit antimicrobial activity under the conditions of the test.

Miscible Liquids aqueous vehicles

Transfer small amount of sterile diluent, such as the Fluid I, for the membrane and filter. The diluent may contain neutralizing agents and or inativantes, as in the case of Ntibioticos.

For the membrane content of containers to be tested or the appropriate dilution (previously defined in validation Testing to bacteriostase and fungistase) in quantities of not less than those recommended in Tables 2 And 3 and filter immediately. If the product proves antimicrobial activity, wash the membrane, at least three times by filtering, each time, the volume of diluent sterile established in validation Testing to bacteriostase and fungistase. The amount of wash fluid used must not be more than five portions of 200 mL, even if during the validation test has been demonstrated that such cycle of washes do not completely eliminates the antimicrobial activity. Transfer the entire membrane for media selected. Use the same volumes of means employed in validation testing. Incubate the means for at least 14 days.

Oils and oily solutions

Use, for each culture medium sample quantity specified in Tables 2 and 3. Oils and oily solutions of low viscosity can be filtered without dilution through the membrane dry. Viscous Oils should be diluted in sterile suitable solvent such as, for example, myristate isoproprila, provided that demonstrated not possess antimicrobial activity under the conditions of the test. Leave the oil penetrate the membrane, filter using vacuum gradually. Wash the membrane with at least three portions of Fluid III. Proceed as described for miscible Liquids in aqueous vehicles.

Polishes and creams

Use, for each culture medium, sample quantity specified in Tables 2 and 3. Polishes of oily base and emulsions of water-in-oil type can be diluted to 1% in suitable solvent

(isopropyl Miristate, or another) as described in previous item, warming up, if necessary, to 40 °C (in exceptional cases, heat up to 44 °C). Filter, as quickly as possible, and continue as described in Oils and oily solutions. In the case of the use of isopropyl Miristate as diluent, provided that demonstrated not possess antimicrobial activity under the conditions of the test, this must be sterilized before use, by membrane filtration, and its aqueous extract must submit pH not less than 6.5.

Soluble Solids (no antibiotics)

Use, for each culture medium, sample quantity specified in Tables 2 and 3. Dissolve the product in adequate fluid, such as the Fluid I, and proceed as described for miscible Liquids in aqueous vehicles.

Solid preparations for injectables (no antibiotics)

Reconstitute the product as described on the label and proceed as described for miscible Liquids in aqueous vehicles, or Oils and oily solutions, depending on the case. If necessary, can be used an excess of diluent to assist in rebuilding and filtration of the product.

Antibiotics solids for injectable preparations

For packages with less than 5 g withdraw, aseptically, of each of the 20 bottles recommended, about 0.3 g of the sample and dissolve in 200 mL of Fluid I and mix. Alternatively, reconstitute the product as described on the label, transfer the equivalent, in liquid, 0.3 g of the sample and dilute to 200 mL with Fluid I. For packaging with 5 g or more transfer, aseptically, each six containers, 1 g of the sample to appropriate flask, dissolve in 200 mL of Fluid I and mix.

Alternatively, reconstitute the six bottles of the product as recommended by the manufacturer, transfer amount of liquid, equivalent to 1 g of the sample, for appropriate flask, dilute to 200 mL with Fluid I and mix. Proceed as described for miscible Liquids in aqueous vehicles.

Aerosols sterile

For liquid products pressurized, freeze the contents in a mixture of ethanol and dry ice to at least -20 °C, for approximately 1 hour. If possible, before the opening of the container, leaving the propellant escape and transferred aseptically content for appropriate sterile vial. Add 100 mL of Fluid II and mix gently. Proceed as described for miscible Liquids in aqueous vehicles or Oils and oily solutions, as the case may be.

Syringes already filled with or without attached needle

Expel the contents of each syringe directly over the membrane (s) or in separate vials and then filter. Proceed as described for miscible Liquids in aqueous vehicles.

Sterile Devices

Pass aseptically a Fluid volume II no less than 10% of the volume of each unit of total devices to be tested as set out in Tables 2 and 3. Collect the fluid into a suitable container sterile and proceed as described for miscible liquids in vehicles aqueous or aqueous solutions of oils and oily solutions, as the case may be. In the case of empty syringes, sterile, remove the sterile diluent container through the sterile needle, if you are attached, or by means of a sterile needle coupled to proceed to trial, and expel the contents in a sterile container. Proceed as indicated previously.

METHOD OF INOCULANT DIRECTLY IN CULTURE MEDIUM

Direct Transfer, and aseptically, for the means of culture, the amount of product specified in Tables 2 and 3, such that the volume of the product is not greater than 10% of the volume of the culture medium, unless specified differently in individual monograph or in this section. If the sample present antimicrobial activity, perform test after neutralization of the activity with a neutralising substance or by appropriate dilution in sufficient quantity of culture medium. When it is necessary the use of large volumes of product, you can- if working with culture medium concentrated, prepared taking into account the dilution following the addition of the product. If the container behave, the focused means can be added directly to the sample.

Liquids

Transfer the indicated volume of each sample as Table 3 to tubes containing the means fluid thioglycolate broth and casein-soy, using sterile pipette or syringe and needle sterile. Mix the liquid with the means, without aerate excessively. Incubate under conditions specified for each medium during 14 days.

Oily Liquid

Using culture medium containing emulsifying agent in appropriate concentration which has shown to be adequate in validation, for example, polysorbate 80 to 1% (p/v).

Polishes and creams

Prepare sample dilution to 10% using a suitable emulsifying agent added to a sterile diluent as the Fluid I. Transfer the diluted sample for culture media without emulsifier. Incubate the inoculated media for, at least, 14 days. Observe the media during the entire period of incubation. Stir gently, the bottles of culture medium containing oil, daily, during the entire period of incubation. The flasks containing liquid Medium of thioglycollate or any other similar means shall be agitated so as not to impair the anaerobic conditions.

Solid

Transfer the sample quantity specified in Tables

2 and 3 or prepare a solution or suspension of the product by adding volume not exceeding 20 mL of diluent to sterile container. Transfer the material thus obtained for 200 mL of fluid thioglycollate Medium. In The same way, transfer the same amount of material for 200 mL of casein-soy Broth and mix. Proceed as described for Liquids.

Catgut and other surgical sutures

For each medium, use the sample quantity specified in Tables 2 and 3. Open the packaging aseptically and remove three portions of wire for each culture medium. These portions must be withdrawn from the beginning, middle and end and having 30 cm in length. Cover each part of the wire with sufficient volume of media (20 mL to 150 mL).

Purified Cotton, gauze, bandages and related material

Each pack of cotton, gauze roll bandage or gauze in to be analyzed, withdraw, with sterile instruments, two portions of 0.1 g to 0.5 g of the parties more inside the sample. For materials in individual packaging, such as wad of gauze, remove two individual portions of 0.25 g to 0.5 g, or two total units, in the case of small units (ex: bandages smaller than 25 mm to 75 mm). Transfer a portion to tube with 40 mL of fluid thioglycolate Medium and another for tubes with 40 mL of casein-soy Broth. Proceed as described for Liquids.

Parenteral Appliances

For apparatus of shapes and dimensions that allow its immersion in volume of medium which does not exceed 1000 mL, make your immersion using the quantities specified in Tables 2 and 3 and proceed as described in Liquids. For very large appliances, make the immersion of parts that come in contact with the patient in volume of sufficient means for immersion of all the parties. For catheters whose lumens, internal and external, must be sterile, pass the means within the lumen or fill the lumen with the environment and promote the immersion of the whole device.

OBSERVATIONS AND INTERPRETATION OF RESULTS

During the incubation period and until the end, examine the means as the macroscopic evidence of microbial growth. If the sample under examination causes turbidity of the culture media, in order to prevent the observation of microbial growth, transfer appropriate portions of each vial (not less than 1 mL) for

New bottles of same means 14 days after the beginning of incubation. Incubate the original bottles and flasks new for an additional period of not less than 4 days. If, at the end of the incubation period, there is no evidence of microbial growth, the sample under examination complies with the requirement of sterility. If evidenced growth of micro-organisms, the sample does not comply with the requirement of sterility, not be that if showing failure during the execution of the test such as, for example, contamination not related with the product under examination.

The sterility test can be considered invalid if one or more of the following conditions are met.

a) The data of microbiological monitoring of the area of carrying out the test demonstrate failure;

b) A review of the analytical procedures used during the test reveals failure;

c) Microbial growth is observed in negative controls;

d) After the identification of the micro-organism (s) isolated (s) from the test, the growth of this kind (s) may be assigned, unmistakeably, failures related to the material used and/or the techniques used in the implementation of the sterility test.

If it is considered to be invalid, the sterility test should be repeated with the same number of units of the initial test. If, after repeating the test, is not observed microbial growth, the sample complies with the requirement of sterility. If observed microbial growth after repeating the test, the sample under examination does not comply with the requirement of sterility.

Conventional microbiological Techniques/biochemical are generally satisfactory for identification of micro test, the growth of this (s) kind (s) can be assigned unambiguously to failures with respect to the material and/or technique used in the procedure of the test for sterility, may be necessary to use more sensitive techniques to demonstrate that the micro-organism isolated in product is identical to the isolate in materials or in the environment. While the techniques of microbiological / biochemical identification of routine can demonstrate that 2 isolates are not identical, these methods may not be sufficiently sensitive or reliable to provide unequivocal evidence that two isolates are from the same source. Molecular Methods can be employed to determine if two micro-organisms belong to the same clone and have origin in common.

FITTING THE STERILITY TEST To PARENTERAL PREPARATIONS, OFTÁLMICAS AND OTHER PREPARATIONS NOT INJETÁVEIS WITH REQUEST FOR STERILITY.

By employing the technique of membrane filtration, use, whenever possible, the entire contents of the container, but not less than the amount indicated in Tables 2 and 3, diluting, when necessary, to approximately 100 mL with a sterile solution, such as the adequate Fluid I.

By employing the technique of direct inoculation, use the quantities indicated in Tables 2 and 3, unless otherwise authorized and justified. The tests for bacteria and fungi are performed with the same unit of the sample under

examination. When the volume or quantity in a single container is insufficient for the completion of the test, the content of two or more containers are used to inoculate the different means.

FITTING THE STERILITY TEST The PHARMACEUTIC PRODUCTS RADIOACTIVE

Due to the rapid radioactive decay, it is not practicable to delay release of some pharmaceutical products radioactive per account the sterility test.

In such cases, the results of the tests for sterility only provide retrospective confirmatory evidence for the assurance of sterility, and therefore depend on the initial methods established in manufacturing and validation procedures / certification.

5.5.3.3 MICROBIOLOGICAL ASSAY OF ANTIBIOTICS

Determines if the power or activity of a product containing antibiotic by comparing the dose that inhibits the growth of a micro-organism liable in relation to the dose of a standard substance or preparation of biological reference of antibiotic that produces similar inhibition.

INTERNATIONAL UNIT AND PREPARING STANDARD

International Unit is the specific activity contained in a quantity (mass) of organic Standard International or Preparation of International Biological Reference. The equivalent quantity of units for international use is established, where necessary, by the World Health Organization.

International Chemical Reference Substances not present units of biological activity defined. When are necessary biological tests, the power of these products is in terms of mass equivalent to the pure substance.

The number of units, or the equivalent mass of the pure substance, in micrograms, contained in 1 mg of antibiotic substance, is indicated in the monograph of each of the products included in the Pharmacopoeia.

For the microbiological assays registered in the Pharmacopoeia, Preparations Primary Standard (Standards) are the International Standards and Preparations of Reference established by the World Health Organization and the European Pharmacopoeia or the Patterns and Preparations of

Reference Brazilians. Other preparations, use appropriate international chain, in which the power has been determined in relation to the standard preparations of the World Health Organization, have legal value identical.

It is recommended that they be prepared and employees working patterns (secondary); however, it is essential that the power has been determined by appropriate number of comparative tests in relation to a primary standard or farmacopeico, validated by appropriate statistical analysis and that the data and results are archived at the disposal of the competent surveillance for the same period of validity of the tested products.

For the assay of lots of antibiotic substances for which there are national Standard Preparations, Syrians by international organizations, it is mandatory the use of these preparations.

SOLUTIONS

Solution 1 (potassium phosphate buffer at 1 %, sterile pH.

6.0) - Dissolve 2.0 g of potassium phosphate dibasic and 8 g of potassium phosphate monobasic phosphates in purified water sufficient to dilute to 1000 mL. Sterilize the solution for 20 minutes in an autoclave at 121 °C and, if necessary, adjust the pH to 5.9 – 6.1 with phosphoric acid 6 M or potassium hydroxide 10 M.

Solution 2 (0.1 M potassium phosphate buffer, pH, sterile 8.0) - Dissolve 16.73 g of potassium phosphate dibasic and 0.523 g of potassium phosphate monobasic in purified water sufficient to 1000 mL. Sterilize the solution for 20 minutes in an autoclave at 121 °C and, if necessary, adjust the pH to 7.9 – 8.1 with phosphoric acid 6 M or potassium hydroxide 10 M.

Solution 3 (0.1 M potassium phosphate buffer, sterile, pH 4,5) – Dissolve 13.6 g of potassium phosphate monobasic in purified water sufficient to 1000 mL. Sterilize the solution for 20 minutes in an autoclave at 121 °C and, if necessary, adjust the pH to 4.4 – 4.5 with phosphoric acid 6 M or potassium hydroxide 10 M

Solution 4 (potassium phosphate buffer at 10 %, sterile, pH 6,0) – Dissolve 20.0 g of potassium phosphate dibasic and 80.0 g of potassium phosphate monobasic in purified water sufficient to 1000 mL. Sterilize the solution for 20 minutes in an autoclave at 121 °C and, if necessary, adjust the pH 5.9 – 6.1 with phosphoric acid 6 M or potassium hydroxide 10 M.

Solution 5 (potassium phosphate buffer 0.2 M, sterile, pH 10.5) – Dissolve 35.0 g of potassium phosphate dibasic and add 2.0 mL of potassium hydroxide 10 M in purified water sufficient to dilute to 1000 mL. Sterilize the solution for 20 minutes in an autoclave at 121 °C and, if necessary, adjust the pH to 10.4 – 10.6 with 6 M phosphoric acid or potassium hydroxide 10 M

Solution 6 (hydrochloric acid did not produce any signals 0.1 M) – Dilute 10,0 Ml of 1.0 M hydrochloric acid in sufficient methanol to 1000 mL.

Solution 7 (solution of isopropyl alcohol to 80 %) – Dilute 800 mL of isopropyl alcohol in purified water sufficient for 1000 mL.

Solution 8 (0.1 M potassium phosphate buffer, pH, sterile 7,0) Dissolve 13.6 g of potassium phosphate dibasic and 3.97 g of potassium phosphate monobasic in purified water sufficient to 1000 mL. Sterilize the solution for 20 minutes in an autoclave at 121 °C and, if necessary, adjust the pH to 6.8 – 7.2 with phosphoric acid 6 M or potassium hydroxide 10 M.

CULTURE MEDIA

Employees can be dehydrated culture media, available in trade that, to be reconstituted with purified water, according to the manufacturer's specifications, possess the same composition as the means produced with the ingredients individually indicated for their achievement.

Culture Medium no. 1 – Dissolve 6.0 g of peptone drought, 4.0 G of casein pancreatic digestion. 3.0 G of yeast extract, 1.0 g of dextrose and 15.0 g of agar in purified water sufficient to dilute to 1000 mL. The pH after sterilization, should be 6.6.

Culture Medium no. 2 – Dissolve 6.0 g of peptone drought, 3.0 g of yeast extract, 1.5 g of meat extract and 15.0 g of agar in purified water sufficient to dilute to 1000 mL. The pH after sterilization, should be 6.6.

Culture Medium no. 3 – Dissolve 5.0 g of peptone drought, 1.5 G yeast extract, 1.5 g of beef extract, 2.5 G of sodium chloride, 1.0 g of dextrose, 3.68 g of potassium phosphate dibasic and 1.32 g of potassium phosphate monobasic, in purified water sufficient to 1000 mL. The pH after sterilization, should be 7.0.

Culture Medium no. 4 – Dissolve 6.0 g of peptone drought, 3.0 g of yeast extract, 1.5 g of beef extract, 1.0 g of D-glucose and 15.0 g of agar in purified water sufficient to 1000 mL. The pH after sterilization, should be 6.6.

Culture Medium no. 5 – Use the culture medium no. 2, however, the pH after sterilization, should be 7.8.

Culture Medium no. 6 – Dissolve 40.0 g of dextrose and 10.0 G of peptone drought in purified water sufficient to 1000 mL. The pH after sterilization, should be 5.6.

Culture Medium no. 7 – Use the culture medium no. 1, sterilized and cooled to 50 °C. Prepare aqueous solution containing 10 mg of neomycin per mL and sterilized by filtration through a membrane with a porosity of 0.22 ^m. Add, aseptically, sterile solution of neomycin sulphate, to obtain final concentration with power of 100 µg of neomycin per mL of medium.

Culture Medium no. 8 – Use the culture medium no. 2, however, the pH after sterilization, should be adjusted to 5.8 – 6.0.

Culture Medium no. 9 – Dissolve 17.0 g of casein pancreatic digestion, 3.0 g of soybean of digestion papainica, 5.0 G of sodium chloride, 2.5 g of potassium phosphate dibasic, 2.5 g of dextrose and 20.0 g of agar in purified water sufficient to 1000 mL. The pH after sterilization, should be 7.3.

Culture Medium no. 10 – Use the culture medium no. 9, adding, however, instead of 20.0 g, 12.0 g of agar and 10.0 mL of polysorbate 80 (this latter added after warming up the means to dissolve the agar, diluting immediately, with water to make 1000 mL). The pH after sterilization, should be 7.3.

Culture Medium no. 11 – Use the culture medium at 1, but the pH after sterilization, should be adjusted to 8.0.

Culture Medium no. 12 – Prepare as the culture medium no. 1, adding, however, 300 mg of manganese sulphate monohydrate (MnSO4. H2O) for each 1000 mL of medium.

Culture Medium no. 13 – Dissolve 10.0 g of peptone drought and 20.0 g of dextrose in purified water sufficient to 1000 mL. The pH after sterilization, should be 5.6.

Culture Medium at no. 14 – Dissolve 10.0 g of glycerol, l0.6 g of peptone drought, 10.60 g of meat extract and 3.0 g of sodium chloride in purified water sufficient to dilute to 1000 mL. The pH after sterilization, should be 7.0.

Culture Medium no. 15 – Prepare as the culture medium no. 14, adding, however, 17.0 g of agar for each 1000 mL of medium.

Culture Medium no. 16 – Dissolve 15.0 g of casein pancreatic digestion, 5.0 g of soybean papainica digestion, 5 g of sodium chloride and 15.0 g of agar in purified water sufficient for 1000 mL. The pH after sterilization, should be 7.3.

Culture Medium no. 17 – Dissolve 17.0 g of casein pancreatic digestion, 3.0 g of peptone soybeans, 2.5 g of dextrose. 5.0 G of sodium chloride and 2.5 g of potassium phosphate dibasic in purified water sufficient to dilute to 1000 mL. The pH after sterilization, should be 7.3.

Culture Medium no. 18 – Use the culture medium no. 11, but, after heating the solution to dissolve the ingredients, add 20.0 mL of polysorbate 80. The pH after sterilization should be 8.0.

Culture Medium no. 19 – Dissolve 9.4 g of peptone drought, 4.7 g of yeast extract, 2.4 g of beef extract, 15.0 g of sodium chloride, 10.0 g of dextrose and 23.5 g agar in purified water sufficient to dilute to 1000 mL. The pH after sterilization, should be 6.1.

Culture Medium no. 20 – Dissolve 40.0 g of dextrose, 10.0 g of peptone drought, 15.0 g of agar and 0.05 g of clorafenicol (in power) in purified water sufficient to 1000 mL. The pH after sterilization, should be 5.6.

Culture Medium no. 21 – Use the culture medium no. 20, sterilized and cooled to 50 °C. Add, aseptically, 2.0 Ml of sterile solution of cicloeximida for each 100 mL of molten agar. Prepare solution containing 10.0 mg of cicloeximida per mL, in purified water, sterilize by filtration through a membrane with a porosity of 0.22 µm.

Culture Medium no. 22 – Dissolve 15.0 g of peptone drought,

5,0 G of soya meal digestion papainica, 4.0 g of sodium chloride, 0.2 g of sodium sulfite, 0.7 g of L-cystine,

5,5 G dextrose and 15.0 g of agar, in purified water sufisterilizacao, should be 7.0.

PREPARATION OF THE INÓCULO

Micro-organisms recommended

•Staphylococcus aureus (ATCC 6538p)
• Micrococcus luteus (ATCC 7468)
• Kocuria rhizophila (ATCC 9341)
• Staphylococcus epidermidis (ATCC 12228)
• Saccharomyces cerevisiae (ATCC 9763)
• Bordetella bronchiseptica (ATCC 4617)
• Bacilius cereus var. mycoides (ATCC 11778)
• Bacillus subtilis (ATCC 6633)
• Klebsiella pneumoniae (ATCC Typing by)
• Escherichia coli (ATCC 10536)
• Enterococcus hirae (ATCC 10541)
• Micrococcus luteus (ATCC 10240)
• Microsporum gypseum (ATCC 14683)
• Saccharomyces cerevisiae (ATCC 2601)
• Micrococcus luteus (ATCC 14452)
• Pseudomonas aeruginosa (ATCC 25619)
• Mycobacterium smegmatis (ATCC 607)

With the purpose of indication, were listed the micromicroMicro-organisms employed in tests and trials (5.5.3.5).

Procedure 1 – Staphylococcus aureus, Micrococcus luteus, Kocuria rhizophila, Staphylococcus epidermidis, Bordetella bronchiseptica, Bacillus subtilis, Klebsiella pneumoniae, Escherichia coli and Pseudomonas aeruginosa.

Preparation of suspension – Transfer the micro-organism from a stock culture to tubes containing 10 mL of culture medium no. 1 tilted. Incubate the tube at 32 – 35 °C, by 24 Hours. After incubation, wash the growth of microisiologica sterile.

Standardization of the suspension – Dilute the suspension prepared with sterile saline solution, so as to obtain the transmittance of 25% at a wavelength of 580 nm, employing suitable spectrophotometer and test tubes with a diameter of 13 mm as Cuba for absorption. Determine the amount of suspension to be added to each 100 mL of agar or nutrient broth to produce zones of inhibition clear and defined or satisfactory relationship dose- response in turbidimetric method. The suspensions of micro-organisms undergoing the procedure 1 may

BE stored at a temperature of 4°C, respectively, for the following periods: 1 week, 2 weeks, 2 weeks,

2 Weeks, 2 weeks, 6 months, 1 week, 2 weeks and 2 weeks.

Micrococcus luteus ATCC 14452. Log as shown in Procedure 1. Employ, however, in tube with half tilted and in Roux, culture medium no. 7, by incubating the bottle per period of 48 hours. The suspension can be stored for two weeks, at a temperature not exceeding 4 °C.

Procedure 2 – Bacillus subtillis.

Log as shown in Procedure 1. In the preparation of the suspension, however, employ culture medium no. 12, whose incubation period is 5 days. On the standardization of suspension, proceed to thermal shock and standardize the suspension as follows: centrifuge and decant the supernatant liquid. Resuspend the pellet with 50 to 70 mL of sterile saline solution and heat the suspension for 30 minutes at 70°C. Perform tests on boards, to ensure the viability of spores and determine the amount that should be added to each 100 mL of medium, to obtain appropriate zones of inhibition. The suspension may be stored for 6 months, at a temperature not exceeding 4 °C.

Procedure 3 – Bacillus cereus.

Log as shown in Procedure 1. However, incubate the tube with the micro-organism for a week. On the standardization of the suspension, proceed to thermal shock and standardize the suspension as follows: warm up the suspension for 30 minutes, at 80 °C. Wash three times the spore suspension with 20 to 25 mL of sterile water. Resuspend the micro-organisms in 50 to 70 mL of sterile water and promote new thermal shock for 30 minutes at 70°C. Perform tests on plates to ensure the viability of spores and determine the amount of which shall be added to each 100 mL of agar, to obtain appropriate zones of inhibition. The suspension may be stored for 6 months, the temperature does not exceed 4 °C.

Procedure 4 – Microsporum gypseum.

Incubate for the micro-organism, for 6 to 8 weeks to 25 °C, in bottles of 3 liter Erlenmeyer flask containing 200 mL of culture medium no. 6. Check the growth by sporulation. When sporulation is 80% or more, collect the conidia of mycelial layer with sterile spatula or other suitable instrument. The conidia are on top of the floating layer. Keep the conidia in 50 mL of saline solution. Determine, experimentally, the quantity of conidia for the test. The suspension can be stored for two months, the temperature does not exceed 4 °C.

Procedure 5 – Enterococcus hirae.

Transfer the micro-organism in a culture medium stock no. 33 and incubate for 16 to 18 hours at 37 °C. Determine, experimentally, the n for the test. Keep this culture under refrigeration for a period not exceeding 24 hours.

Procedure 6 – Saccharomyces cerevisiae (ATCC 9763).

Keep the micro-organism in tubes containing 10 mL of culture medium no. 19 tilted. Incubate the tubes at 32 – 35 °C, during 24 hours. Inoculate 100 mL of nutrient broth – Culture medium no. 13 – and incubate for 16 to 18 hours at 37 °C. Standardize the suspension as described in Procedure 1. The suspension may be stored, for 4 weeks, the temperature not exceeding 4 °C.

Procedure 7 – Saccharomyces cerevisiae (ATCC 9763 and ATCC 2601).

Follow the indicated in Procedure 1. Incubate overnight, however, the tube tilted with the culture medium no. 19.30 °C, the last for a period of 48 hours. The suspension may be stored, for 4 weeks, the temperature not exceeding 4 °C.

Procedure 8 – Mycobacterium smegmatis.

Keep the micro-organism in tubes with half tilted containing 10 mL of culture medium no. 16 and log weekly samplings. Incubate the tube at 37 °C for 48 hours. Using 3 mL of sterile saline solution, transfer the crops that grew the agar slant to erlenmeyer flask of 500 mL, containing 100 mL of culture medium no. 14 and 50 g of glass beads. Shake the culture by rotation speed of 130 cycles per minute, a radius of 3.5 cm and a temperature of 27 °C, for a period of five days. Determine the amount of suspension to be added to each 100 mL of agar per test medium in plates. The suspension may be stored, for two weeks, the temperature not exceeding 4 °C.

* The micro-organisms can be used in conditions that ensure maximum 5 passages of culture of origin.

ON DRYING OF SUBSTANCES ANTIBIÓTICAS

Use for desiccation of the patterns, the following procedures, and recommended in accordance with the information described in Tables 2 in 5.5.3.3.1 and 5.5.3.3.2 .

Method 1

Transfer sufficient quantity of standard for weigh-keyed tared filter cover flipped. Weigh the flask and place it in the oven under reduced pressure, thereby tilting the lid over the mouth of the bottle to ensure that remains open during drying. Dessecar at 60 °C, under pressure of 0.67 kPa or less, during three hours. The process is Completed, enter air dried in an oven, submitting to agent desiccant such as sulfuric acid or silica-gel. Refit the cover and place the weighed filter in desiccator containing desiccant agent such as phosphorus pentoxide or silica-gel. Let it cool down to room temperature and weigh, calculating the percentage loss of mass of the pattern.

Method 2

Proceed as described in Method 1. Employ, however, weighs-keyed tared filter cover with capillary tube of internal diameter of the order of 0.20 to 0.25 mm, and dessecar without removing the cover.

Method 3

Proceed as described in Method 1. Dessecar, however the sample to 110 °C, under pressure of 0.67 kPa or less, during three hours.

Method 4

Proceed as described in Method 1. Dessecar, however the sample to 40 °C, under pressure of 0.67 kPa or less, during two hours.

Method 5

Proceed as described in Method 1. Dessecar, however the sample to 100 °C, under pressure of 0.67 kPa or less, during four hours.

Method 6

Proceed as described in Method 1. Dessecar, however the sample to 40 °C, under pressure of 0.67 kPa or less, during three hours.

Method 7

Proceed as described in Method 1. Dessecar, however the sample 25 °C, under pressure of 0.67 kPa or less, for three hours.

Method 8

The antibiotic substance is not subjected to desiccation.

PROCEDURE

All material must be suitable for the intended use and shall be thoroughly cleaned after each use, to remove any traces of antibiotics. The material must remain covered when not in use. All glassware used in contact with the micro-organism must be sterilized in a greenhouse at a temperature between 200 ºC and 220 ºC for 2 hours. In dilution of the standard solution and the sample employ measuring flasks, pipettes or equipment carefully calibrated.

5.5.3.3.1 Microbiological Testing by diffusion in agar

PROCEDURE

For each antibiotic listed in Table 1, check the culture medium (according to the relationship of culture media), the quantity of medium to be used in base layer and layer in inoculated and the micro-organism to test. The volume of inoculum to be added to each 100 mL of culture medium must be determined experimentally.

However, as initial reference, suggests that quantity of inoculum to be added per 100 mL of medium.

Prepare the base layer through the addition of appropriate quantity of molten agar in Petri dishes which should be, specially selected, have flat bottom, having dimensions of 20 x 100 mm and cover of suitable material. Distribute evenly in the agar plates, which should be placed on a level surface to the middle layer has uniform depth. Put the lid on each plate at the side of this; if used cover non-porous, leaving it slightly ajar to avoid the accumulation of condensed moisture from the agar layer hot. After the hardening of the agar, capping the plates. To prepare the layer inoculated – surface, add the volume of inoculum determined for the appropriate amount of culture medium that has been melted and cooled between 46 ºC and 48 ºC. Shake the bottle, by rotation, in order to obtain homogeneous suspension and add the indicated quantity of inoculated medium in each Petri dish, containing the base layer does not inoculated. Spread evenly to coat, capping plates and allow your hardening on flat surface. After the hardening of the medium, place six cylinders of stainless steel, with outer diameter of 8 mm ± 0.1 mm, internal diameter 6 mm ± 0.1 mm and length of 10 mm ± 0.1 mm, on the surface of the agar inoculated, so that they may form between si angle of 60° and with radius of 2.8 cm. Also, can be used cylinders made of glass, porcelain or aluminum and sterile conditions already described. In place of the cylinders, can be drilled in the middle, with drilling wells of sterile, 5 to 8 mm in diameter. They may also be used paper disks, made with paper of appropriate quality or molds of stainless steel. When are used paper disks, these must be sterile.

Preparation of standard solution to work, the sample and standard curve

The preparation of samples of antibiotics is indicated in the monograph.

The concentrations of the antibiotics used in the test shall be in geometric progression; for example, for the preparation of dilution series in the ratio 2:1 or another experimentally determined provided that it is proven to the linear relationship between the logarithm of the concentration of the antibiotic and the diameter of the inhibition zone.

In Table 2 is indicated for each antibiotic, the preparation of the standard solution of work and the standard curve, comprising:

a) Conditions of desiccation, as described in item Desiccation of antibiotic substances (5.5.3.3);

b) Initial solvent for dissolving the antibiotic, if necessary, and even which concentration is used;

c) Solution for dilution until the concentration of work, as described in Solutions;

d) Concentration of working solution, expressed in weight, or International Units per mL of solution;

e) PERIOD of validity of the standard solution of work under refrigeration;

f) Solution employed for dilution of working solution, on the occasion of the preparation of the standard curve, as Solutions;

g) Concentration ranges suggested, in weight or International Units per mL, inside of which can be found the appropriate concentrations for the standard curve.

Procedure for straight, parallel design (3 x 3 or 2.2)

Employ, in at least six Petri plates. Have the standard solutions and sample, in each plate, with 3 test concentrations for 3 x 3 (low, medium and high) or 2 concentrations for test 2 x 2 (high and low). The solutions must be distributed in such a way that the solutions of the standard and sample preparation are staggered at layer inoculated (high concentration and low) to avoid overlap of inhibition halos.

Procedure for a randomized 5 x 1

For the standard curve, using a total of 12 cards, 3 for each of the standard solutions (Pj, P2, P4, P5), except for the average concentration of the curve (P3) that is included in all the plates. In each set of 3 plates, use 3 cylinders for the average concentration (P3) and toggle 3 cylinders for low concentration (Pj) and so forth with the other standard solutions. This way, you get 36 inhibition halos for concentration (P3) and 9 inhibition halos for each of the other four concentrations of curve.

For each sample, employ 3 plates, where will be placed 3 cylinders to the average concentration of the standard (P3) and 3 with the sample solution prepared in the same concentration of the standard (A3).

Apply 0.2 mL of the solutions in the cylinders or in molds of stainless steel by means of pipette or other calibrated instrument. When you used the system of wells, the volume of liquid applied should be sufficient to fill them completely.

After performing the procedures appropriate for the chosen study design, the plates are incubated at the indicated temperature, whose variation should not exceed ± 0.5 °C, during a period of 16 to 18 hours. Then measure the

Tabela 1 – Ensaio microbiológico por difusão em ágar.

Antibiótico	*Micro-organismo*	*Meio de cultura*		*Volume (mL) do meio aplicado nas camadas*		*Volume do inóculo mL/100 mL*	*Temperatura de incubação (ºC)*
		Base	*Superfície*	*Base*	*Superfície*		
Amoxicilina	*Kocuria rhizophila* (ATCC 9341)	11	11	21	4	0,5	32 a 35
Ampicilina	*Kocuria rhizophila* (ATCC 9341)	11	11	21	4	0,5	32 a 35
Anfomicina	*Micrococcus luteus* resistente a neomicina (ATCC 14452)	2	1	21	4	0,5	36 a 38
Anfotericina B	*Saccharomyces cerevisiae* (ATCC 9763)	-	19	-	8	1,0	29 a 31
Bacitracina	*Micrococcus luteus* (ATCC 7468)	2	1	21	4	0,3	32 a 35
Bacitracina	*Micrococcus luteus* (ATCC 10240)	2	1	21	4	0,3	32 a 35
Benzilpenicilina	*Staphylococcus aureus* (ATCC 6538p)	2	1	21	4	1,0	32 a 35
Bleomicina	*Mycobacterium smegmatis* (ATCC 607)	15	15	10	6	1,0	32 a 35
Canamicina	*Bacillus subtilis* (ATCC 6633)	5	5	21	4	(1)	36 a 38
Carbenicilina	*Pseudomonas aeruginosa* (ATCC 25619)	9	10	21	4	(1)	36 a 38
Cefacetrila	*Staphylococcus aureus* (ATCC 6538p)	2	1	21	4	0,5	32 a 35
Cefadroxila	*Staphylococcus aureus* (ATCC 6538p)	2	1	21	4	0,05	36 a 38
Cefalexina	*Staphylococcus aureus* (ATCC 6538p)	2	1	21	4	0,05	32 a 35
Cefaloglicina	*Staphylococcus aureus* (ATCC 6538p)	2	1	21	4	0,2	32 a 35
Cefaloridina	*Staphylococcus aureus* (ATCC 6538p)	2	1	21	4	0,1	32 a 35
Cefalotina	*Staphylococcus aureus* (ATCC 6538p)	2	1	21	4	0,1	32 a 35
Cefapirina	*Staphylococcus aureus* (ATCC 6538p)	2	1	21	4	0,08	32 a 35
Cefazolina	*Staphylococcus aureus* (ATCC 6538p)	2	1	21	4	0,05	32 a 35
Cefoxitina	*Staphylococcus aureus* (ATCC 6538p)	2	1	21	5	0,1	32 a 35
Cefradina	*Staphylococcus aureus* (ATCC 6538p)	2	1	21	4	0,05	32 a 35
Ciclacilina	*Kocuria rhizophila* (ATCC 9341)	11	11	21	4	0,5	36 a 38
Ciclosserina	*Staphylococcus aureus* (ATCC 6538p)	2	1	10	4	0,04	29 a 31
Clindamicina	*Kocuria rhizophila* (ATCC 9341)	11	11	21	4	1,5	36 a 38
Cloranfenicol	*Kocuria rhizophila* (ATCC 9341)	1	1	21	4	2,0	32 a 35
Cloxacilina	*Staphylococcus aureus* (ATCC 6538p)	2	1	21	4	0,1	32 a 35
Colistina	*Bordetella bronchiseptica* (ATCC 4617)	9	10	21	4	0,1	36 a 38
Dactinomicina	*Bacillus subtilis* (ATCC 6633)	5	5	10	4	(1)	36 a 38
Dicloxacilina	*Staphylococcus aureus* (ATCC 6538p)	2	1	21	4	0,1	32 a 35
Diidroestreptomicina	*Bacillus subtilis* (ATCC 6633)	5	5	21	4	(1)	36 a 38
Eritromicina	*Kocuria rhizophila* (ATCC 9341)	11	11	21	4	1,5	32 a 35
Estreptomicina	*Bacillus subtilis* (ATCC 6633)	5	5	21	4	(1)	36 a 38
Feneticilina	*Kocuria rhizophila* (ATCC 9341)	11	11	21	4	0,5	32 a 35
Fenoximetilpenicilina	*Staphylococcus aureus* (ATCC 6538p)	2	1	21	4	1,0	32 a 35

Tabela 1 (conclusão)

Antibiótico	*Micro-organismo*	*Meio de cultura*		*Volume (mL) do meio aplicado nas camadas*		*Volume do inóculo mL/100 mL*	*Temperatura de incubação (ºC)*
		Base	*Superfície*	*Base*	*Superfície*		
Griseofulvina	*Microsporrum gypseum* (ATCC 14683)	20	21	6	4	(1)	29 a 31 durante 48 horas
Mitomicina	*Bacillus subtilis* (ATCC 6633)0,4	8	8	10	4	0,5	36 a 38
Neomicina	*Staphylococcus aureus* (ATCC 6538p)	11	11	21	4	1,0	32 a 35
Neomicina	*Staphylococcus epidermides* (ATCC 12228)	11	11	21	4	1,0	36 a 38
Nistatina	*Saccharomyces cerevisiae* (ATCC 2601)	-	19	-	8	1,0	29 a 31
Novobiocona	*Staphylococcus epidermides* (ATCC 12228)	2	1	21	4	4,0	34 a 36
Oxacilina	*Staphylococcus aureus* (ATCC 6538p)	2	1	21	4	0,3	32 a 35
Paromomicina	*Staphylococcus epidermides* (ATCC 12228)	11	11	21	4	2,0	36 a 38
Polimixina B	*Bordetella bronchiseptica (ATCC 4617)*	9	10	21	4	0,1	36 a 38
Rifampicina	*Bacillus subtilis* (ATCC 6633)	2	2	21	4	0,1	29 a 31
Rifampicina	*Staphylococcus aureus* (ATCC 6538p)	2	2	21	4	0,1	36 a 38
Sisomicina	*Staphylococcus epidermides* (ATCC 12228)	11	11	21	4	0,03	36 a 38
Vancomicina	*Bacillus subtilis* (ATCC 6633)	8	8	10	4	(1)	36 a 38

(1) Determinar a quantidade de inóculo, na ocasião do ensaio, através de difusão em placas.

Tabela 2 – Preparação da solução padrão de trabalho e da curva padrão.

Antibiótico	*a. Condição de dessecação* (5.5.3.3)	*b. Solvente inicial*	*c. Solução para diluição* (5.5.3.3)	*d. Concentração da solução de trabalho (/mL)*	*e. Prazo de validade da solução sob refrigeração*	*f. Solução para diluição* (5.5.3.3)	*g. Dose mediana (µg ou U.I./mL)*
Amoxicilina	8	-	Água estéril	1 mg	7 dias	2	0,05 a 0,2 µg[7]
Ampicilina	8	-	Água estéril	0,1 mg	7 dias	2	0,05 a 0,2 µg[7]
Anfomicina[8]	1	-	2	0,1 mg	14 dias	2	5 a 20 µg
Anfotericina B	1	-	Dimetilsulfóxido	1 mg[1]	Usar no mesmo dia	5	0,5 a 2 µ[7]
Bacitracina	1	-	HCl 0,01 M	100 U.I.	Usar no mesmo dia	1	1 a 4 U.I.
Benzilpenicilina	8	-	1	1 000 U.I.	4 dias	1	0,2 a 2 U.I.
Bleomicina	7	-	8	2 U.I.	14 dias	8	0,01 a 0,2 U.I.
Canamicina B	8	-	2	1 mg	30 dias	2	0,5 a 2 µg
Carbenicilina	8	-	1	1 mg	14 dias	1	10 a 40 µg
Cefacetrila	8	-	1	1 mg	7 dias	1	5 a 20 µg
Cefadroxila	8	-	1	1 mg	Usar no mesmo dia	1	10 a 40 µg
Cefalexina	8	-	1	1 mg	7 dias	1	10 a 40 µg
Cefaloridina	1	-	1	1 mg	5 dias	1	0,5 a 2 µg
Cefaloglicina	8	-	Água estéril	100 µg	7 dias	3	5 a 20 µg
Cefalotina	1	-	1	1 mg	5 dias	1	0,5 a 2 µg
Cefapirina	8	-	1	1 mg	3 dias	1	0,5 a 2 µg
Cefazolina	8	10 000 µg por mL na *solução 4*	1	1 mg	5 dias	1	0,5 a 2 µg
Cefoxitina	8	-	1	1 mg	Usar no mesmo dia	1	10 a 40 µg
Ciclacilina	8	-	Água estéril	1 mg	1 dia	2	0,5 a 2 µg[7]
Cefradina	8	-	1	1 mg	5 dias	1	5 a 20 µg
Ciclosserina	1	-	Água estéril	1 mg	30 dias	1	20 a 80 µg
Clindamicina	8	-	Água estéril	1 mg	30 dias	2	0,5 a 2 µg
Cloranfenicol	8	10 000 µg por mL em *álcool etílico*	1	1 mg	30 dias	1	20 a 80 µg
Cloxacilina	8	-	1	1 mg	7 dias	1	2 a 8 µg
Colistina	1	10 000 µg por mL em *álcool etílico*	4	1 mg	14 dias	4	0,5 a 2 µg
Dactinomicina	1	10 000 µg por mL em *álcool metílico*	2	1 mg	90 dias	2	0,5 a 2 µg
Dicloxacilina	8	-	1	1 mg	7 dias	1	2,5 a 10 µg
Diidroestreptomicina	5	-	2	1 mg	30 dias	2	0,5 a 2 µg
Eritromicina[5]	1	10 000 µg por mL em *álcool metílico*	2	1 mg	14 dias	2	0,5 a 2 µg
Estreptomicina	1	-	2	1 mg	30 dias	2	0,5 a 2 µg
Feneticilina	8	-	Água estéril	1 000 U.I.	7 dias	2	0,05 a 0,2 U.I.
Fenoximetilpenicilina	8	-	1	100 U.I.	4 dias	1	0,2 a 2 U.I.

Tabela 2 (conclusão)

Antibiótico	*a. Condição de dessecação* (5.5.3.3)	*b. Solvente inicial*	*c. Solução para diluição* (5.5.3.3)	*d. Concentração da solução de trabalho (/mL)*	*e. Prazo de validade da solução sob refrigeração*	*f. Solução para diluição* (5.5.3.3)	*g. Dose mediana (µg ou U.I./mL)*
Gentamicina	3	-	2	1 mg	30 dias	2	0,5 a 2 µg
Grisoefulvina	8	-	Dimetilformamida	1 mg[4]	90 dias	2	2 a 10 µg
Mitomicina	8	-	1	1 mg	14 dias	1	0,5 a 2 µg
Neomicina	1	-	2	1 mg	14 dias	2	5 a 20 µg (*S. aureus*)
Neomicina	1	-	2	1 mg	14 dias	2	0,5 a 2 µg (*S. epidermidis*)
Nistatina	4	-	Dimetilformamida	1 000 U.I.[2]	Usar no mesmo dia	4	10 a 40 U.I.[7]
Novobiocina	5	10 000 µg por mL em *álcool etílico*	2	1 mg	5 dias	4	0,2 a 1 µg
Oxacilina	8	-	1	1 mg	3 dias	1	2 a 10 µg
Paromomicina	1	-	2	1 mg	21 dias	2	0,5 a 2 µg
Polimixina B	1	*Água estéril*[3]	4	10 000 U.I.	14 dias	4	200 a 800 U.I.
Rifampicina	8	-	Álcool metílico	1 mg	1 dia	1	2 a 10 µg
Sisomicina[6]	8	-	2	1 mg	14 dias	2	0,05 a 0,2 µg
Vancomicina	1	-	Água estéril	1 mg	7 dias	2	5 a 20 µg

1 Dilute aliquots of working solution with dimethylsulfoxide, to obtain concentration between 10 and 40 mg per mL as the points of the standard curve.

2 Dilute aliquots of working solution with dimethylformamide to obtain concentrations between 10 and 40 units per mL as the points of the standard curve.

3 Add 2 mL of sterile water for each 5 mg of standard.

4 Dilute aliquots of working solution with dimethylformamide to obtain concentrations between 40 and 200 mg per mL as the points of the standard curve.

5 When you employ erythromycin in the form of for estolate, hydrolyzing the working solution, in a water bath at 60 °C for 2 hours.

6 Sisomycin is hygroscopic, take precautions during weighing. The pattern of work to stay at 20 °C, in an atmosphere of nitrogen.

7 Prepare concomitantly with the solutions of the standard and sample. The dilutions of the sample must contain the same amount of dimethylformamide that the dilutions of the standard.

8 The standard solution of work must stay overnight at ambient temperature for complete dissolution.

diameter of the inhibition halos employing suitable device to measure, such as vernier caliper, or optical projector that has accuracy of 0.1 mm or less.

For some micro-organisms, the procedure can be improved if the prepared plates remain at ambient temperature for a period of 30 minutes to 2 hours before the incubation period, which occurs in the diffusion of the antibiotic for the medium.

5.5.3.3.2 Microbiological Testing by turbidimetry

PROCEDURE

Preparation of working solution, the sample and the standard curve

The preparation of samples of antibiotics is indicated in the monograph.

In Table 2, presented below, there is no indication, for each antibiotic, the preparation of the standard solution of work and the standard curve, comprising:

a) Conditions of desiccation, as described in item Desiccation of antibiotic substances (5.5.3.3);

b) Initial solvent for dissolving the antibiotic, if necessary, and even which concentration is used;

c) Solution for the dilution of the antibiotic until the concentration of work as Solutions;

d) Concentration of working solution, expressed in weight or International Units per mL of solution.

e) PERIOD of validity of the standard solution of work under refrigeration;

f) Solution employed for dilution of working solution, on the occasion of the preparation of the standard curve, as Solutions;

g) Range of concentration, in weight or International Units per mL, within which the appropriate concentrations for the standard curve can be found.

Employ for each antibiotic the micro-organism and the nutritive broth listed in Table 1. Determine experimentally the volume of inoculum to be added to 100 mL of broth from the quantity suggested as initial reference. The inoculated medium must be prepared and used immediately.

Procedure for straight, parallel design (3 x 3 or 2 x 2)

Distribute, in tubes identical, equal volume of each one of the solutions of the standard and the sample. Add to each tube equal volume of nutrient broth inoculated via, for example, 1 mL of antibiotic solution and 9 mL of medium (0.1 mL of solution for gramicidina and tyrothricin). At least 18 tubes are used for testing by parallel straight 3 x 3 and 12 tubes for testing by parallel straight 2 x 2. The number of replicas per concentration in each test should be sufficient to ensure that the statistical accuracy specified in the monograph, but you must perform, at a minimum, three tubes for each concentration of the standard and the sample. It may be necessary to conduct the test with greater number of doses of the standard and the sample or repeat it and combine the results to obtain the required accuracy. The doses used must be in geometric progression.

Procedure for delineating curve 5 x 1

For the experimental design was a 5 x 1, prepare dilutions which represent 5 concentrations of standard (P15 P2, P3, P4 and P5) and 1 concentration

The sample (A_3). The sample solution must match the same dilution of the standard that corresponds to the average concentration of the curve (P3). Employ at least three tubes for each concentration of the standard and the sample. In This way, at least eighteen tubes are required for testing.

After performing the procedures appropriate for the chosen study design, inoculate the culture medium recommended with known amount of suspension of the micro-organism sensitive to the antibiotic, so that, after incubation of approximately four hours, the bacterial turbidity in the midst is easily measured and keep correlation between the dose and the response of the substance under examination.

In Table 1 are described the antibiotics to be analyzed by turbidimetric method with description of the micro-organism, culture medium, volume of standardized inoculum suggested as initial reference and incubation temperature for each case.

Incubate in a water bath for 3 to 4 hours, taking the precaution of ensuring adequate temperature and uniform for all tubes. Adequate time must be verified by observation of growth in tube containing the average concentration (P3) used in the test. After the incubation period, stop the mu1tiplicacao of micro-organisms by the addition of 0.5 mL of formaldehyde solution to 12 per cent, in each tube.

Determine the absorbance for each tube in a spectrophotometer at a wavelength of 530 nm. Standardize the appliance in absorbance through the white containing the same amount of nutrient broth and formaldehyde at 12 %.

In routine tests, when the linearity of the system was proven by appropriate number of experiments using the test of three points (3 x 3), can be used to test two points (2 x 2). Will be accepted, also, the experimental design was a 5 x 1, officially adopted by other pharmacopoeias of international

Table 1 – microbiological Test by turbidimetry.

Antibiotic	*Micro-organism*	*Broth Nutrient*	*Volume of inoculum mL/100 mL*	*Incubation Temperature (°C)*
Amikacin	Staphylococcus aureus (ATCC 6538p)	3	0.1	37
Kanamycin	Staphylococcus aureus (ATCC 6538p)	3	0.2	37
Candicidina	Saccharomyces cerevisiae (ATCC 9763)	13	0.2	28
Capreomycin	Klebsiella pneumoniae (ATCC Typing by)	3	0.05	37
Cycloserine	Staphylococcus aureus (ATCC 6538p)	3	0.4	37
Chloramphenicol	Escherichia coli (ATCC 10536,)	3	0.7	37
Chlortetracycline	Staphylococcus aureus (ATCC 6538p)	3	0.1	36
Demeclociclina	Staphylococcus aureus (ATCC 6538p)	3	0.1	37
Dihydrostreptomycin	Klebsiella pneumoniae (ATCC 10031,)	3	0.1	37
Doxicilina	Staphylococcus aureus (ATCC 6538p)	3	0.1	37
Spectinomycin	Escherichia coli (ATCC 10536)	3	0.1	37
Streptomycin	Klebsiella pneumoniae (ATCC Typing by)	3	0.1	37
Gramicidina	Enterococcus hirae (ATCC 10541)	3	1.0	37
Lincomycin	Staphylococcus aureus (ATCC 6538p)	3	0.1	37
Minocycline	Staphylococcus aureus (ATCC 6538p)	3	0.2	37
Oxytetracycline	Staphylococcus aureus (ATCC 6538p)	3	0.1	37
Rolitetraciclina	Staphylococcus aureus (ATCC 6538p)	3	0.1	37
Tetracycline	Staphylococcus aureus (ATCC 6538p)	3	0.1	37
Tyrothricin	Enterococcus hirae (ATCC 10541)	3	1.0	37
Tobramycin	Staphylococcus aureus (ATCC 6538p)	3	0.15	37

use chain. However, in the event of controversy or dispute, should be applied the test of three points.

Calculation of power

From the results, calculate the power of the sample and its confidence limits, by means of statistical method described in standard statistical Procedures applicable to biological assays – tests indirect quantitative (8.5).

Confidence Interval (CI)

The accuracy of a test is determined by the interval of confidence which ensures that the true power is within the specified limits.

In the absence of CI in the monograph of the product, it is recommended that confidence limits upper and lower of 5% or less, in relation to power calculated, being accepted threshold values of up to 10 %.

5.5.3.4 ANTIMICROBIAL EFFECTIVENESS TEST

OBJECTIVE

Ensure the effectiveness of antimicrobial preservatives added to pharmaceutical products.

Antimicrobial Preservatives are substances added in non-sterile pharmaceutical forms with the purpose of

Table 2 – Preparation of the standard solution and the standard curve – turbidimetric Method.

Antibiotic	*A. Condition of dissection (5.5.3.3)*	*B. Initial Solvent*	*C. Solution for dilution (5.5.3.3)*	*D. Concentration of working solution (/mL)*	*And. Period of validity of the solution under refrigeration*	*F. Solution for dilution (5.5.3.3)*	*G. Range of concentration (/mL)*
Amikacin	8	-	Sterile Water	1 MG	14 Days	Sterile Water	6 To 14]ug
Kanamycin	8	-	Sterile Water	1 MG	30 Days	Sterile Water	6 To 14]ug
Candimicina1	6	-	Dimethyl Sulfoxide	1 MG	Use the same day	Sterile Water	0.02 To 0.14]ug3
Capreomycin	5	-	Sterile Water	1 MG	7 Days	Sterile Water	6.591]ug
Cycloserine	1	-	Sterile Water	1 MG	30 Days	Sterile Water	2.80]ug
Chloramphenicol	8	1.000 |_IG per mL in ethyl alcohol	1	1 MG	30 Days	1	1 To 4 |ag
Chlortetracycline	8	-	HC1 0.01M	1 MG	4 Days	Sterile Water	0.03 To 0.09]ug
Demectociclina	1	-	HC1 0.1 M	1 MG	4 Days	Sterile Water	0.06 To 0.14]ug
Dihydrostreptomycin	5	-	Sterile Water	1 MG	30 Days	Sterile Water	2.60]ug
Doxycycline	8	-	HC10,1 M	1 MG	5 Days	Sterile Water	0.06 To 0.14]ug
Spectinomycin	8	-	Sterile Water	1 MG	30 Days	Sterile Water	2.60]ug
Streptomycin	1	-	Sterile Water	1 MG	30 Days	Sterile Water	2.60]ug
Gramicidina	1	-	Ethyl Alcohol 95%	1 MG	30 Days	Ethyl Alcohol 95%	0.02 To 0.08]ug
Lincomycin	8	-	Sterile Water	1 MG	30 Days	Sterile Water	0.3 To 0.8]ug
Minocycline	8	-	HC10,1 M	1 MG	2 Days	Sterile Water	0.06 To 0.12]ug
Oxytetracycline	8	-	HC10,1 M	1 MG	4 Days	Sterile Water	0.16 To 0.32]ug
Rolitetraciclina	1	-	Sterile Water	1 MG	1 Day	Sterile Water	0.16 To 0.32]ug
Tetracycline	8	-	HC10,1 M	1 MG	1 Day	Sterile Water	0.16 To 0.32]ug
Tyrothricin2	1	-	Ethyl Alcohol 95%	1 MG	30 Days	Ethyl Alcohol 95%	0.02 To 0.08]ug
Tobramycin	8	-	Sterile Water	1 MG	14 Days	Sterile Water	1 To 4 |ag

1 In the candicidina, employ sterile equipment in all the steps.

2 For the assay of tyrothricin, employing the standard solution of labor and the dose-response curve of gramicidina.

3 Prepare, simultaneously, the standard solutions and sample.

protecting them from any microbial growth. For the sterile pharmaceutical forms, packed in cartons of multiple doses, the antimicrobial preservatives are added to inhibit the growth of micro-organisms contaminants during the repeated use of individual doses.

The amount of preservative used in a formulation should be the minimum necessary for the protection of the product without harming the patient or consumer.

The antimicrobial efficacy, it is inherent to the product or due to the addition of preservatives, needs to be demonstrated for topical products; multiple-dose oral products; ophthalmic; otological; nasal fluids; dialysis; irrigation, etc.

The test and the criteria apply to the product in the way it is found in the market.

MICRO-ORGANISMS USED

- Candida albicans ATCC 10231
- Aspergillus niger ATCC 16404
- Escherichia coli ATCC 8739
- Pseudomonas aeruginosa ATCC 9027
- Staphylococcus aureus ATCC 6538

The micro-organisms used in the test should not have more than 5 passages counted from the culture ATCC original. A passage is defined as the transfer of a culture established for a sterile culture medium.

In the case of cultures maintained by techniques of freezing, each cycle of freezing and thawing; reactivation is considered a pass.

The lyophilized cultures received from ATCC should be reconstituted according to the instructions supplied with the equipment.

Retrieve the material in culture medium liquid or solid. The conditions for the preparation of culture are recorded in Table 1.

If the recovery of the micro-organism if der in liquid culture medium after incubation, centrifuge and discard the supernatant. Suspend the pellet with a 1/20 dilution of culture medium for maintaining sterile and add an equal volume of glycerol solution sterile 20% v/v in water.

If the recovery of the micro-organism if der in a solid culture medium, transfer the growth of surface to the culture medium of maintaining sterile fluid, plus 10% of sterile glycerol.

5

Table 1 – Conditions for reconstitution of the strains.

Micro-organism	*Culture Medium*	*Incubation Temperature*		*Incubation Time of inoculum*	*Incubation Time for microbial recovery*
Escherichia coli ATCC 8739	Soybean-Casein Digest Broth / Soybean-Casein Digest Agar	32.5	°C â± 2.5°C	18 – 24 hours	3 – 5 days
Pseudomonas aeruginosa ATCC 9027	Soybean-Casein Digest Broth / Soybean-Casein Digest Agar	32.5	°C â± 2.5°C	18 – 24 hours	3 – 5 days
Staphylococcus aureus ATCC 6538	Soybean-Casein Digest Broth / Soybean-Casein Digest Agar	32.5	°C â± 2.5°C	18 – 24 hours	3 – 5 days
Candida albicans ATCC 10231	Sabouraud Dextrose Broth / Sabouraud Dextrose Agar	22.5	°C â± 2.5°C	44 – 52 hours	3 – 5 days
Aspergillus niger ATCC 16404	Sabouraud Dextrose Broth / Sabouraud Dextrose Agar	22.5	°C â± 2.5°C	6 – 10 days	3 – 7 days

In both cases, exempt small aliquots of suspension in sterile cryogenic tubes, suitable for freezing of micro-organisms.

Store the cryo tubes in liquid nitrogen or ultrafreezer (no more than -50 °C).

This culture inventory can be used to inoculate a series of work culture.

CULTURE MEDIA USED

All culture media used in the test must be tested for the ability of growth

PREPARATION OF THE INÓCULO

From the culture stock, inoculate the surface of solid culture medium specified in Table 1.

To collect the growth of bacteria and yeasts, use sterile saline solution. Collect the suspension obtained in a tube or bottle sterile appropriate and add sufficient amount of sterile saline solution to obtain a concentration of 1 x 108 CFU/mL.

To collect the growth of A. niger, use sterile saline solution containing 0.05% of polysorbate 80. Collect the suspension obtained in a tube or bottle sterile appropriate and add sufficient amount of sterile saline solution to obtain a concentration of 1 x 108 CFU/mL.

Alternatively, the culture inventory can be inoculated in liquid medium (Table 1), incubated and subsequently centrifuged. Discard the supernatant and suspend the sediment with sufficient quantity of sterile saline solution to obtain a concentration of 1 x 108 CFU /mL.

Refrigerate the suspensions if you do not use them in a period of 2 hours.

Determine the number of CFU's /mL of each suspension by turbidimetry or plate count, checking the conditions of time and temperature of incubation and the incubation time for microbial recovery described in Table 1, with the aim of confirming the initial count in UFC. These values will be used to calibrate the size of the inoculum to be used in contamination of the product under test.

The suspension of bacteria and yeasts should be used in 24 hours. The suspension of molds can be used for up to 7 days if kept under refrigeration.

PROCEDURE

When the type of packaging allow the introduction of the suspension of micro-organisms and when its content is sufficient for the completion of all the steps, conduct the test in 5 original packaging of the product to be tested. Otherwise, transfer the contents of a or more original packaging for a bottle with lid, previously sterilized and of adequate size to contain the required amount of sample for the achievement of all the stages of the test.

Inoculate each original packaging or sterile bottle with lid, with each of the micro-organisms required.

The concentration of the inoculum used should be sufficient to obtain a final concentration on product between 1 x 105 and 1 x 106 CFU's per g or mL – apply to categories 1.2 and 3 (see Table 2 – column "Product Type").

For category 4, the concentration of the inoculum should be sufficient to obtain a final concentration on product between 1 x 103 and 1 x 104 CFU's per g or mL.

The volume of inoculum to be introduced should be between 0.5% and 1.0% in relation to the volume (liquid sample) or weight (solid sample or semissolida) product total.

Incubate the inoculated samples in an oven with a temperature between 22.5 °C ± 2.5°C.

Be Sampled each package or bottle with sample inoculated in intervals of 7.14 and 28 days.

Determine the method of plating, the number of Colony Forming Units (CFU's) of each sample, the initial time and in each time interval specified.

A specific neutralizing agent (s) for the condom (s) present in the formulation of the product, as determined in the validation study, must (m) be incorporated (s) on the boards of count or the dilution of sample prepared for the plating.

Calculate the concentration of each micro-organism (CFU/mL) present in the sample, compare with the count in the initial time and expressing the change in terms of logarithmic reductions.

PRODUCT CATEGORY AND CRITERIA FOR ANTIMICROBIAL EFFECTIVENESS

For the purpose with the test, the products were separated into 4 categories according to Table 2.

The requirements for the effectiveness of antimicrobial preservative are met if they meet the criteria established for each category as shown in Table 2.

5.5.3.5 MICRO-ORGANISMS USED IN TESTS AND TRIALS

The micro-organisms listed in Table 1. They are indicated for trials and tests recommended in Pharmacopoeia.

Main suppliers of cultures of micro-organisms:

Table 2 – Categories of products and criteria for antimicrobial effectiveness.

Type of product	*Micro-organism*	*7 Day*	*14° day*	*28° day*
Category 1 – Injectables, other Parenteral emulsions, Otological nasal products, sterile ophthalmic, consisting of base or aqueous vehicle	Bacteria	There must Reduction of 1 log The no. of CFU's Initially Inoculated	There must Reduction of 3 logs The no. of CFU's Initially Inoculated	The count does not Should increase in Relation to 14° day
	Molds and Yeasts	There should be no increase in the no. of CFU's initially inoculated	There should be no increase in the no. of CFU's initially inoculated	There should be no increase in the no. of CFU's initially inoculated
Category 2 – Products for topical use. consisting of base, or aqueous vehicle, nasal products not sterile and emulsions, including those applied in Mucous membranes	Bacteria		Should there be reduction of 2 logs of no. of CFU's initially inoculated	There should be no increase in the count in relation to 14° day
	Molds and Yeasts		There should be no increase in the no. of CFU's initially inoculated	There should be no increase in the no. of CFU's initially inoculated
Category 3 – oral Products consisting of base or aqueous vehicle, except antacids	Bacteria		Should there be reduction of 1 log no. of CFU's initially inoculated	The count should not increase in relation to the 14TH day.
	Molds and Yeasts		There should be no increase in the no. of CFU's initially inoculated	There should be no increase in the no. of CFU's initially inoculated
Category 4 – Antacids consisting of aqueous base	Bacteria		There should be no increase in the no. of CFU's initially inoculated	There should be no increase in the no. of CFU's initially inoculated
	Molds and Yeasts		There should be no increase in the no. of CFU's initially inoculated	There should be no increase in the no. of CFU's initially inoculated

Note. The "no increase" of no. of CFU's inoculated is defined as no more than 0.5 log10 units larger than the value obtained in advance.

ATCC American Type Culture Collection
http://www.atcc.org

CIP Collection de l'Institut Pasteur
http://www.pasteur.fr/ip/index.jsp

IMI United Kingdom National Culture Collection (UKNCC)
http://www.cabi.org
Email: cultures@cabi.org

INCQS Instituto Nacional de Controle de Qualidade em Saúde
Departamento de Microbiologia - Laboratório de Materiais de Referência
Av. Brasil, 4365, Manguinhos, Rio de Janeiro, RJ, Brasil, CEP: 21.040-900
http://www.incqs.fiocruz.br
Email: colecao@incqs.fiocruz.br

NCIMB National Collection of Industrial Bacteria
http://www.ncimb.com
Email:enquiries@ncimb.com

NBRC NITE Biological Resource Center
http://www.nbrc.nite.go.jp
Email: collection@nbrc.nite.go.jp

NCPF National Collection of Pathogenic Fungi
http://www.hpacultures.or.uk
Email: hpacultures@hpa.org.uk

NCTC National Collection of Type Cultures
http://www.hpacultures.or.uk
Email: hpacultures@hpa.org.uk

NCYC National Collection of Yeast Cultures
http://www.ncyc.co.uk
Email: ncyc@ncyc.co.uk

Table 1 – Micro-organisms used in tests and trials.

Micro-organism	*ATCC*	*CIP*	*INCQS*	*NBRC*	*NCIMB*	*NCTC*	*NCPF*	*NCYC*	*IMI*	*IP*
Molds and yeasts										
Aspergillus brasiliensis	16404	-	-	9455	-	-	2275	-	149007	1431.83
Candida albicans	10231	-	40006	1594	-	-	3179	1363	-	48.72
Microsporrum gypseum	14683	-	40005	-	-	-	-	-	-	
Saccharomyces cerevisiae	2601	-	40001	-	-	-	-	-	-	-
Saccharomyces cerevisiae	9763	1432.83	40002	-	-	10716	-	87	-	-
Bactérias										
Bacillus atrophaens	9372	-	-	-	-	-	-	-	-	-
Bacillus cereus var. mycoides	11778	64.52	003	-	-	10230	-	-	-	-
Bacillus pumilis	27142	77.25	-	-	10692	10327	-	-	-	-
Bacillus subtilis	6633	52.62	001	3134	8054	10400	-	-	-	-
Bacteroides vulgatus	8482	103717	059	-	-	11154	-	-	-	-
Bordetella bronchiseptica	4617	53.157	023	-	-	8347	-	-	-	-
Clostridium sporogenes	19404	79.3	-	-	532	532	-	-	-	-
Clostridium sporogenes	11437	-	060	-	-	-	-	-	-	-
Enterococcus hirae	10541	-	019	-	-	-	-	-	-	-
Escherichia coli	8739	53.126	-	3972	8545	12923	-	-	-	-
Escherichia coli	10536	54.127	031	-	8879	10418	-	-	-	-
Geobacillus stearothermophilus	7953	-	-	-	-	-	-	-	-	-
Klebsiella pneumoniae	10031	53.153	030	-	9111	7427	-	-	-	-
Kocuria rhizophila	9341	53.65	010	-	-	8340	-	-	-	-
Micrococcus luteus	7468	-	009	-	-	-	-	-	-	-
Micrococcus luteus	10240	53.160	011		8166	7743	-	-	-	-
Micrococcus luteus resistente a neomicina	14452	-	012	-	10418	-	-	-	-	-
Mycobacterium smegmatis	607	-	021	-	-	-	-	-	-	-
Peudomonas aeruginosa	9027	82.118	-	13275	8626	-	-	-	-	-
Peudomonas aeruginosa	25619	-	026	-	-	-	-	-	-	-
Salmonella enterica subsp entérica	-	80.39	-	100797	-	6017	-	-	-	-
Staphylococcus aureus	6538p	53.156	013	-	8625	7447	-	-	-	-
Staphylococcus aureus	6538	4.83	039	13276	9518	10788	-	-	-	-
Staplylococcus epidermides	12228	68.21	016	-	8853	-	-	-	-	-

5.6 METHODS IMUNOQUÍMICOS

The immunochemical methods are based on a connection selective, reversible and non-covalent between antigens and antibodies. These methods are used to detect or dosing antigens and antibodies. The detection or determination of antigen-antibody complex can be accomplished by several techniques. The requirements of this method applies to the immunochemical methods used, in the case of marked reagents or not. The results of immunochemical methods depend on the conditions of the experiment; the nature and quality of the reagents employed. It is essential measure the components of an immunological assay and use International Reference Preparations for immunoassay whenever available. The reagents necessary for many of immunochemical methods are available on the market in the form of assemblies that include reagents (especially the antigen or the antibody) and material intended for the in vitro evaluation of a substance; as well as the necessary instructions for its proper use. The sets should be used according to the manufacturer's instructions, and it is important to ensure that they are suitable for analysis of the sample, particularly as regards the selectivity and sensitivity. The requirements concerning the sets for immunoassay are supplied by the World Health Organization (Series of Reports Techniques 658,1981).

METHODS THAT ARE USED ANTIGENS, OR ANTIBODIES MARKED

The techniques that use substances marked shall employ appropriate markers, such as enzymes and radioisotopes. When the marker is a radioisotope we call the radioimmunological method employs a technique of testing. All the techniques performed with radioactive substances must be made in accordance with national and international laws to protect against the risk of radiation.

METHODS THAT ARE USED ANTIGENS, ANTIBODIES OR NOT MARKED

Methods of Immunoprecipitation. methods of immunoprecipitation include the reactions of flocculation and precipitation. When a solution of an antigen is mixed with antibodies corresponding, in appropriate conditions, reagents form aggregates flocculants or precipitants. The relationship between the quantities of reagents corresponding to shorter time of flocculation, or the precipitation more accentuated flame-if relationship great. This is typically achieved in the presence of equivalent amounts of antigen and antibody. The immunoprecipitation can be assessed visually, or by measuring the dispersion of light.

Methods Immunochemical Apolipoproteã Na. You can get an increase in the sensitivity of the method by the use of particles coated with antibodies, or of antigens (por example, latex).

In the methods of flocculation using- if, generally, successive dilutions of one of the reagents while in-diffusion method (ID), the dilution is achieved by diffusion in a gel. Are obtained gradients of concentration of one, or two reagents in order to create the gel zones in which the proportions of reagents favor the precipitation. While the methods of flocculation are performed in test tubes, the methods of immunodiffusion can be performed using-if different media, such as: measuring cylinders, plates, slides, tinas, or cameras. It is called immunoprecipitation simple when the antigen reacts only with its corresponding antibody; it is complex when using several reagents serologically relatives; and multiple, when using multiple reagents serologically unrelated. The method of simple diffusion is established a concentration gradient for only one of the reagents broadcast from an external source into the gel that contains the reagent corresponding to a relatively low concentration.

Radial immuno Diffusion Simple (IDRS). is a simple quantitative immunodiffusion technique. When establishing the balance between the internal and external reagents, the area of the circular zone of precipitation, originated from the external reagent, is directly proportional to the concentration of antigen applied and inversely proportional to the concentration of antibodies in gel.

Methods of Dissemination Double. The gradients of concentration are established for two reagents. Both the antigen and the antibody spreads from separate locations in a gel initially neutral immunological point of view. Double immunodiffusion methods are used to compare, qualitatively, several antigens in relation to an appropriate antibody or vice-versa. The comparison is based on the presence or absence of interaction between the patterns of precipitation. IS It possible to distinguish between reactions of identity, not identity, or partial identity between antigens and antibodies.

Methods of immunoelectrophoresis. The immunoelectrophoresis (IE) is a qualitative technique of two associated methods: gel electrophoresis, followed by immunodiffusion. The counterimmunoelectrophoresis is a modification of the immunoelectrophoresis (IE), adapted to the qualitative and quantitative analysis.

A first time is held a classical electrophoresis. A range of gel that contains the fractions to analyze, separated by electrophoresis, is subsequently cropped and transferred to another card. This new card is then subjected to a second electrophoresis in a direction perpendicular to the previous track, through the use of a gel that contains a relatively low content in antibodies corresponding to the antigen. For a given concentration of antibodies and thickness of the gel, the relationship between the area of each one of the peaks of precipitation and the amount of antigen corresponding is linear.

Counter-immunoelectrophoresis. is a quantitative method that enables fast establish gradients of concentration of antigens and antibodies in external electric field dependent on their different loads. The dilutions of the standard

and the sample must be organized in a row of cavities in gel. A known quantity of reagent is placed in a queue corresponding opposite of cavities.

The Title of the substance to be assayed can be considered as the highest dilution in which it is observed a line of precipitation. There are variants of counterimmunoelectrophoresis and imunoeletrodoseamento. Other techniques are associated with the separation of the antigen by molecular size and serologic properties. The visualization and characterization of lines of immunoprecipitation can be carried out by colorations, selective or not selective, by fluorescence, by enzyme markers, isotopic markers, or by other appropriate techniques. The colorations are selective, usually used for characterization of non-protein substances in precipitates.

In translucent gels, such as agar or agarose, the line of precipitation becomes clearly visible in gel, provided that the concentration of each of the reagents is appropriate.

VALIDATION OF THE METHOD

Validation Criteria.

A quantitative immunochemical method shall be valid only if:

a) The antigen or the antibody does not discriminate, significantly, the standard the substance under analysis. In the case of a reagent marked, the corresponding reagent should not distinguish, in a significant way, the labelled substance of not marked;

b) The method is not influenced by the matrix of the test, that is, all the components of the sample under analysis, or its excipients, which may vary from one sample to another. These may include high concentrations of other proteins, salts, preservatives in high concentrations or exercise a proteolytic activity of contamination;

c) The limit of quantification is below the acceptability criteria indicated in individual monograph;

d) The accuracy of the assay is such that the variation of results meets the requirements laid down in individual monograph;

e) Not systematic errors related to the order in which the test is performed.

Validation Methods

For these criteria to be verified, the validation includes the following elements:

a) The test must be performed at least in triplicate;

b) The test must include at least three different dilutions of the standard and three different dilutions of the sample with a presumed activity similar to that of the standard preparation;

c) The distribution of the samples must be carried out at random;

d) If the sample is present in serum, or if it is mixed with other constituents, the standard should be prepared in the same way;

e) The test should include a measure of non-specific binding of the reagent marked;

f) For Radioimmunological assays with displacement:

a_1) should be given the maximum connection (zero displacement);

b_1) dilutions must cover the full range of responses to the values closest to the connection does not specify the maximum connection, preferably both for the sample as for the standard.

STATISTICAL CALCULATION

For analysis of the results, the response curves of the sample and the standard can be analyzed by statistical procedures applicable to biological tests (8). The parallelism not significant indicates that antigen or antibody distinguishes between the sample and standard implies the invalidation of the result. We imunodoseamentos with displacement, the values of non-specific binding and the maximum displacement to a high concentration of the sample or standard should not be significantly different. The differences may reflect effects due to array, either by inhibition of connection or degradation of the marker.

5.7 PHYSICAL METHODS APPLIED TO MATERIALS AND SURGICAL HOSPITAL

5.7.1 RESISTANCE TO TRACTION

The determination of the resistance to traction of surgical sutures must be carried out in an environment with constant moisture and temperature. The relative humidity should be 60 – 80 per cent and the temperature 20 – 25°C.

EQUIPMENT

In the determination of the resistance to traction of surgical sutures the equipment must have electric motor that apply the suture in analysis rate of constant load per unit of time.

EQUIPMENT INCLINED-PLANE

Specifications

The fasteners must be of the type of roll with flat surfaces for the fixing of sutures. The diameter of the roll should be from 1.8 cm to 1.9 cm and the flat surfaces must have, at a minimum, 2.5 cm in length. The distance between the fasteners should be 1.25 cm. The friction of the carriage of cargo must allow the penalty incritora slide up to 2.5% of the capacity to record when there is no sample.

The speed of inclination of the plane must be regulated so as to be required 20 seconds from the start of the test to ensure that the maximum slope of 30° is reached.

PROCEDURE

Determine the tensile strength of surgical sutures with the same care required for the preliminary test for the determination of the diameter. Adjust the weight of the car to which, at the time the rupture, the position of Âº is between 20 and 80% of the ability to record.

Direct Drive

Place the suture in equipment securing one of the ends and passing the free end by another fastener. Apply on this last a voltage equivalent to 1/4 of the minimum strength required for the suture in test and tighten the fastener. Adjust the zero point Âº in the graph and connect the equipment; note the reading and evaluating the resistance. Despise the determination every time that the suture rupture in near point of fasteners.

Traction on-no

Determine the resistance to traction on it running in surgical suture in test a surgeon (Figure 1) on a segment of 5 cm length of a flexible rubber pipe of 6.5 mm in internal diameter and 8.1 mm in outer diameter. Place the suture in equipment so that the node is equidistant from each of the fasteners. Adjust the zero point Âº in the graph and connect the equipment; note the reading and evaluating the resistance. Despise the determination every time that the suture rupture in near point of fasteners.

Implementation of the surgical

To make a surgical, proceed as follows:

a) Hold the tips of surgical suture, one in each hand;

b) Place the tip that is located on the left hand on the tip of the right hand forming a circle;

c) Enter the tip overlying the circle;

d) Repeat the operation;

e) Attach the flexible hose;

f) Place the tip of the right hand on the tip of the left forming a second circle;

g) Close the node.

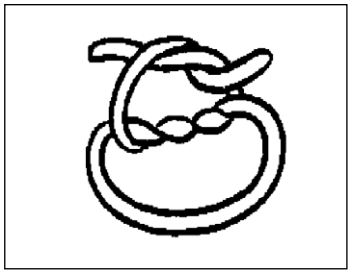

Figure 1 – surgical.

Results

The results should meet the described in Tables 1.2 and 3 – The respective monographs.

5.7.2 DIAMETER OF SUTURES

The determination of the diameter of surgical sutures must be carried out in an environment with constant temperature and humidity. The relative humidity should be 60 – 80 per center and the temperature between 20 – 25 °C. The weights for pre-tension for determination of diameter of Multifilamentary wires are recorded in Table 1.

APPARATUS

The clock dial gauge used to determine the diameter of sutures is of type “dead weight”, mechanical or electronic and is equipped with a display of direct reading, digital or printed output reading. The resolution of scale is at least 0.002 mm and the shoe of support must have approximately 12.70 mm ± 0.02 mm in diameter. The shoe support and moving parts connected to it must apply a total load of 210 g ± 3 g sample.

For surgical sutures of number 9-0 and minors, remove the additional weight of the shoe so that the total weight of the sample does not exceed 60 g.

The shoe and the base of the equipment must submit parallelism and flatness of 0.005 mm.

PROCEDURE

The diameter of surgical sutures of natural origin, wrapped without liquid preservative is determined after his permanence during 4 hours, at the very least, in an atmosphere with the humidity and temperature previously specified. The sutures wrapped with liquid preservative are subjected to the test, immediately after its removal from the liquid without previous drying.

Table 1 – Weights for pre-tension for determination of diameter of Multifilamentary wires.

Diâmetro		*Massa (g)*	
Número conforme sistema métrico	*Número cirúrgico*	*Suturas absorvíveis*	*Suturas não absorvíveis*
0,01	12-0	-	-
0,1	11-0	-	-
0,2	10-0	12,5	12
0,3	9-0	25	27
0,4	8-0	35	38
0,5	7-0	70	69
0,7	6-0	125	125
1,0	5-0	340	250
1,5	4-0	475	375
2	3-0	885	600
3	2-0	1340	900
3,5	0	1950	1350
4	1	2540	1700
5	2	3175	2200
6	3 e 4	3645	3050
7	5	-	3850
8	6	-	4550
9	7	-	5650

Multifilamentary Sutures

For the determination of the diameter of surgical sutures Multifilamentary, measurements should be made keeping-the tensioned with the aid of a pulley system attached to a table, as shown in Figure 1 and by proceeding as follows:

a) FIX one end of the suture through a retaining clip;

b) At the other end free, put a weight with mass according to Table 1. Obs. care must be taken not to distort the suture;

c) Position the suture on the dial indicator so that pass through the center of the circular base and, with the aid of the lever, lowering the foot mobile rod slowly until the entire load is applied;

d) Measure the diameter of the suture in three points, approximately 1/4.1/2 and 3/4 of its total length;

e) In the case of sutures locked of diameters greater than the surgical number 3-0, perform two measurements perpendicular between themselves at each point.

Monofilament Sutures

For determining the diameter of sutures monofilamen-sions, it should be carried out as follows:

a) Perform the measurement in sutures in dry form or with fluid, immediately after its removal from the packaging without previous drying;

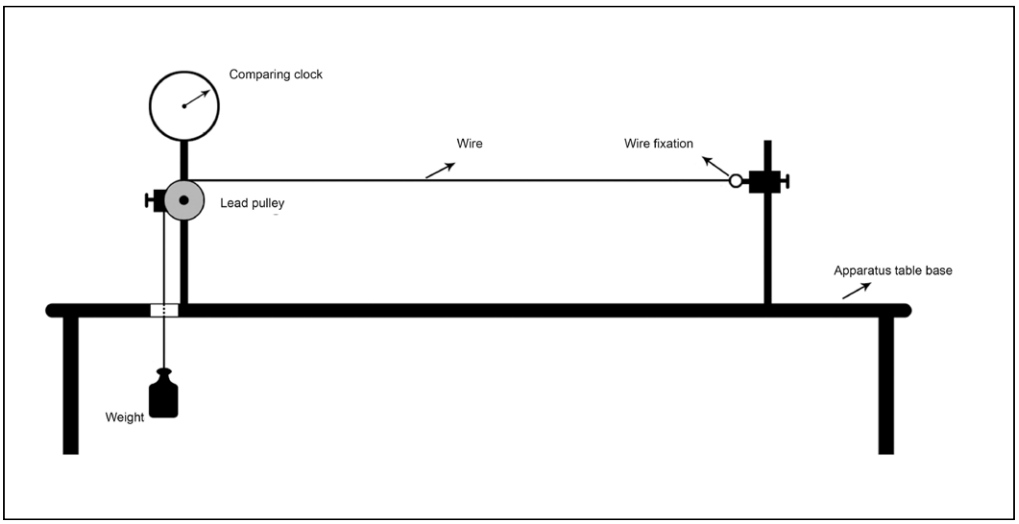

Figure 1 – table Model suggested for measuring diameter of Multifilamentary sutures

b) Position the suture on the dial indicator, between the fixed base and the base of the movable locking;

c) Lower the lever slowly so that the entire load is under the suture;

d) Measure the diameter of the suture in three points, approximately 1/4.1/2 and 3/4 of its total length.

Result

The average of the measurements performed on the sutures should be between the limits of tables 1.2, or 3 of their respective monographs.

The individual values must be understood between the averages of the limits to the numbers surgical, immediately, inferior and posterior to the analyzed.

5.7.3 RESISTANCE TO NEEDLE ENCASTOAMENTO

The purpose of this test is to assess the fixing of yarn needles for sutures in affected.

APPARATUS

Use a machine universal drive equipped with electric motor that apply rate of constant load per unit of time.

The load cell used must be compatible with the traction force required for the verification.

PROCEDURE

Attach the needle into one of the fasteners of the equipment so that the part set is free and aligned with the direction in which it will apply the strength by mobile holder. Measure the force required to desencastoar suture the needle.

Results

The results must be evaluated considering the Table 1.

Note: the evaluation of the resistance to encastoamento should consider both the individual limits for the wires and the limits to the average of five wires of batch analyzed. If one of the results of individual limit, and not more than one, does not meet the minimum limits for individual values, repeat the test with ten more wires. The requirement of the test will be met if none of the 10 samples is below the limits described.

Table 1 – Limits of resistance of the inlay needle in relation to surgical number.

Number Surgical	*Number as metric system*			*Minimum Limits of resistance*			
	Absorbable		*Not Absorbable*	*Media*		*Individual*	
	Natural	*Synthetic*		*Kgf*	*N*	*Kgf*	*N*
11	-	0.1	0.1	0.007	0.07	0.005	0.05
10-0	-	0.2	0.2	0.014	0.14	0.010	0.10
9-0	0.4	0.3	0.3	0.021	0.21	0.015	0.15
8-0	0.5	0.4	0.4	0.05	0.49	7025	0.25
7-0	0.7	0.5	0.5	0.08	0.78	0.045	0.44
(6-0)	1	0.7	0.7	0.17	1.67	0.08	0.78
5-0	1.5	1.0	1.0	0.23	226	0.11	1.08
4-0	2	1.5	1.5	0.45	4.41	0.23	2.26
3-0	3	2	2	0.68	6.67	0.34	3.33
2-0	3.5	3	3	1.10	10.79	0.45	4.41
0	4	3.5	3.5	1.50	14.71	0.45	4.41
1	5	4.0	4.0	1.80	17.65	0.60	5.88
2	6	5	5	1.80	17.65	0.70	6.86
3	7	6	6	2.00	19.61	0.90	8.83
4	8	6	6	2.00	19.61	0.90	8.83
> 5	-	> 7	> 7	2.20	21.57	1.10	10.79

5.7.4 ABSORPTION DETERMINATION

For the carrying out of the tests for the *Absorption determination, remove the cotton from its original packaging and condition it for* at least 4 hours, in standard atmosphere of 65% ±2% of relative humidity to 21 ºC ±1.1 ºC.

PROCEDURE

Use basket, which weigh up to 3 g, consisting of copper wire of approximately 0.4 mm in diameter, in the form of a cylinder of approximately 5 cm in diameter and 8 cm deep, with spaces of about 2 cm between the wires. Transfer portions of absorbent cotton, exactly, about 1 g ±0.05 g, of five different parts of the package, through tugs and not of slices of sample. Place the portions combined in basket and weigh. Hold the basket side by approximately 12 mm above the surface of the water at 25 ºC ±1 ºC and dropping in the same. Determine, preferably by use of a timer, the time in seconds required for complete submersion.

Remove the basket from the water, let it drain for 10 seconds in the same horizontal position, then place it immediately in a tared container and covered and weigh. Calculate the mass of water absorbed from the earth of the basket of test and mass of absorbent cotton.

5.7.5 DETERMINATION OF THE LENGTH OF THE FIBER

For the carrying out of the tests for the Determination of the *length of the fiber,* remove the cotton from its original packaging and condition it for at least 4 hours, in standard atmosphere of 65% ±2% of relative humidity at 21 ºC ±1.1 ºC.

This procedure applies to appliance tab duplex cotton fiber Suter-Webb. With changes in procedure, two Baer tabs can be applied arranged one behind the other or applied to a Johannsen or other similar device.

APPARATUS

The Tab consists of two banks of combs rigidly mounted side by side on a common basis. Each bank of combs consists of at least 12 individual combs spaced by 3.2 mm, one behind the other and mounted in docked mode for that, to the extent that they are approximated during the dispensing process and no longer needed, they can be loose to fall below the work plan. Each comb has a simple series of teeth precisely aligned and well sharp, of 12 mm length, consisting in needles of 0.38 mm in diameter. The teeth are spaced 62 mm to 25 mm for a length of approximately 50 mm.

The accessories consist of forceps separator grate depressor fibers, fibers, flat dish depressor fibers and dishes covered with velvet. The forceps tab consists of two pieces of brass, of 75 mm in length, approximately, fitted on one side and slightly curved, thus exhibiting a format of nozzle for picking up the fibers that are outside and near the surfaces of the combs. Usually, one of the ends gatherers has a leather cushion or other fibrous material. The end catcher has approximately 19 mm in width.

The grid depressor fibers consist in a series of metal rods spaced by 3.2 mm, so that they can be placed between the combs for pressing the fibers down between the teeth. The flat dish depressor fibers consists in a dish of polished metal, approximately 25 mm by 50 mm, with a rounded protrusion or handle at top surface through which the dish can be planed on the fibers as they are placed on the surface of the dishes covered with velvet. The dishes covered with velvet, on which the fibers may be placed in order, they are aluminum plates of approximately 100 mm by 225 mm and 2.4 mm thick, covered on both sides by high quality velvet, preferably black.

SELECTION OF COTTON

After unfolding the cotton, prepare a representative sample by outlet, from a package containing 225 g to 450 g, of 32 samples (each one with approximately 75 mg) well distributed along the piece, being 16 withdrawals of a half lengthwise and the rest of the other half. Avoid the edges of the piece and take particular care, while ensuring that the portions are removed by taking into account the thickness of the piece. To prevent the selection of only long or short fiber, remove all the fibers of each sample and not to let the same pass through the fingers.

Bundles of, at most, 112.5 g, weigh 8 samples and packages weighing between 112.5 g and 225 g, weigh 16 samples, all well distributed.

Mix the samples to pairs, indiscriminately, and combine each pair pulling and curling gently in your fingers. Then split lengthwise each pair combined into two approximately equal parts and use a part in subsequent blending (the other party can be discarded or reserved for any other tests or controls).

Repeat the process described in the previous paragraph with the halves of successive series forked up which would result in only one sample. Gently, have in a position parallel to the fibers of the final sample, pulling and winding- the fingers. Retain all the fibers, including, as far as possible, the handicapped and the masses of fibers locked, discarding only the fragments of immature seeds with fibers and foreign material non-fibrous as petioles, leaves and fragments of tegument.

From the final sample described in the previous paragraph, separate longitudinally a sample of 75 mg ±2 mg, exactly heavy. Retain the residue for any test necessary.

PROCEDURE

Using the grid depressor of fibers, carefully insert the weighed sample in a bank of combs of separator of cotton, so that it extends through the combs at angles approximately straight.

With the forceps tab, hold, by free ends, a small portion of the fibers that extends through the comb teeth closest to the operator; gently strip it of the combs and transfer it to the tips of the teeth of the second bank, pouring the fibers parallel to each other, linearly and approximately at right angles to the faces of the combs, releasing so close to the front face of the comb as possible. Using the grid depressor, carefully pressing the fibers transferred downwards, in the teeth of the combs. Continue the operation until all the fibers are transferred to the second bank of combs. During this transfer of fibers, dropping the combs of first bank successively when and while all the protruding fibers are removed.

Turn the equipment to 180º and transfer the cotton fibers back to the first bank of combs the manner described in the preceding paragraph.

Be very careful to flatten the ends of the fibers during both transfers, by furnishing them as soon as possible to the front surface of the proximal comb. Such planing can involve the withdrawal of Fibers isolated from both sides, front and distal, banks of combs and the deposit them again in main beam of the combs.

Turn the equipment back to 180º. Dropping successive combs, if necessary, to expose the ends of longer fibers. It may be necessary to deposit again some fibers isolated. Using forceps, remove the few fibers more salient. In This way, continue to withdraw successively the protruding fibers remaining back to front face of proximal comb. Dropping this comb and repeat the series of operations in the same manner until all the fibers have been removed. Not To seriously disrupt the sample and therefore vitiate the fractionation in groups, pull several times (eight to ten) between each pair of combs.

Put the tugging on the dishes covered by velvet in parallel to each other, so righteously as possible, with the ends as clearly defined as possible and with the distal parts arranged in a straight line, pressing them down gently with the flat dish depressor fibers before releasing the tug of forceps. Employ at least 50 and at most 100 tugs for fractionating the sample.

Grouping all the fibers that have length of 12.5 mm or more and weigh the group up to tenths of 1 milligram. The same way, grouping all the fibers that have length of 6.25 mm or less and weigh the same way. Finally, grouping the remaining fiber lengths, intermediaries and weigh. The sum of three weights must not differ from the initial weight of the sample by more than 3 mg. Divide the mass of each of the first two groups by the mass of the sample to obtain the percentage by weight of fiber in two wavelength ranges.

6 CONTAINERS FOR MEDICINES AND CORRELATES

6.1 GLASS CONTAINERS

CLASSIFICATION

Glass type I. neutral Glass borosilicate type, non-alkaline, high thermal resistance, mechanical and hydrolytic with alkalinity of up to 1.0 mL of H2SO4 0.01 *M* (bottle test of crushed glass). For the packaging of medicinal products; application for intravascular and parenteral use.

Type II Glass. Glass type alkaline sodium / soda, hydrolytic resistance high, resulting from the appropriate treatment of the inner surface of the glass type III, so that your alkalinity is at maximum 0.7 mL of H2SO4 0.01 *M* for bottles up to 100 mL and 0.2 mL of H2SO4 0.01 *M* for capacity above 100 mL (test in glass bottle integer). For the packaging of solutions for parenteral use; neutral and acidic, which does not have its pH changed.

Glass type III. Glass type alkaline sodium / soda, hydrolytic resistance average, but with a good mechanical resistance, without any surface treatment, with maximum alkalinity of 8.5 mL of H2SO4 0.01 *M* (bottle test of crushed glass). Intended For the preparation of solutions for topical use and oral cancer; it can be used for parenteral solutions, when approved by stability tests.

Glass type NP (non-parenteral). Glass type alkaline sodium / soda, hydrolytic resistance low and high alkalinity, maximum of 15 mL of H2SO4 0.01 *M* (bottle test of crushed glass). Indicated to the packaging of products not parenteral, i. e., for topical use and oral.

6.1.1 RESISTANCE HIDROLÍTICA OR ALKALINITY

Test that quantifies the intensity of the chemical reaction between the water and the alkali-elements existing in glass, especially sodium and potassium. This resistance determines the classification of the type of glass.

EQUIPMENT, MATERIALS AND REAGENTS

- Autoclave with control of temperature of 121 ºC ±1.0 ºC, equipped with thermometer, pressure gauge, valve HCl Solution to 0.01 M.
- Safety and shelf for sustaining of, at a minimum, 12 bottles. Ball Mill with hard steel restrictor and polished steel balls or mortar tempered steel with the specifications in Figure 1.

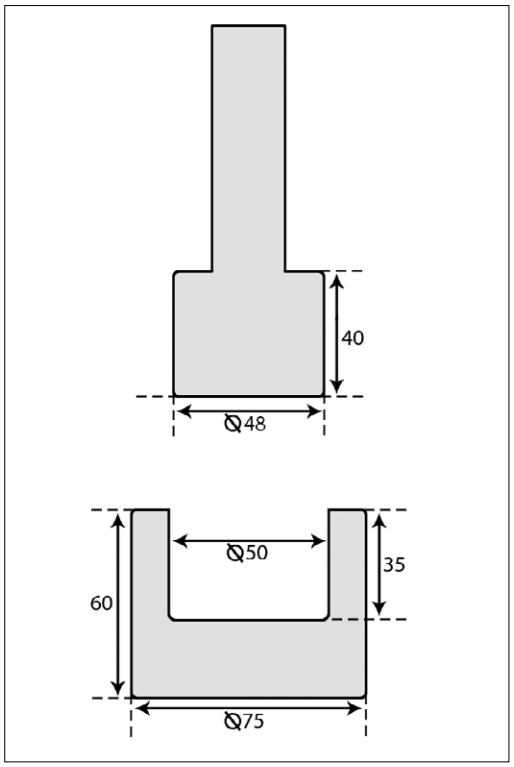

Figure 1 – Mortar and pestle for glass pulverizationOven for drying with temperature of 140 ºC;

- Precision Scale with two decimal places;
- Set of sieves in stainless steel, no. 20, no. 40 and no. 50, with a diameter of 20.3 cm (8 “), including the silencer and the cover;
- Ima;
- Becher or aluminum paper;
- 250 Ml Erlenmeyer flask.
- Desiccator;
- And Buret buret titration;
- Graduated cylinder 100 mL;
- DISTILLED or deionized Water, with maximum conductivity of 0.15 µS/cm (or 6.67 MQ/cm) at 25 ºC;
- Methyl red Solution (24 mg in 100 mL of water);
- Acetone PA;
- Solution of H2SO4 to 0.01 *M*;
- Solution of HCl a 0,01 *M*.

THE TEST PROCEDURE IN GLASS Bottle GROUND

Wash, at least six bottles, randomly chosen, with doubly distilled water or deionized water, and dry them in a current of air dry and clean.

If necessary, cut the bottles and transfer and crush of 30 to 40 g of glass using the ball mill or mortar. Pass the crushed glass by sieve no. 20 and transfer the portion retained on the sieve again for the ball mill or mortar. Repeat the operations of milling and passages of the fragments by sieve until, at least, 2/3 of the material has passed through sieve no. 20. Combine all portions of crushed glass that passed through sieve no. 20 and pass through sieve no. 40. Crush the portion retained on the sieve and repeat the operation.

Combine the portions of crushed glass that passed through sieve no. 40 and transfer to mounted assembly of sieves no. 40 and no. 50. Shake horizontally by 5 minutes. Collect 12.0 g of crushed glass that has passed through sieve no. 40 but not passed through sieve no. 50 and store in a desiccator to be used in the test.

Spread the sample of crushed glass on a piece of paper and satin pass the magnet to remove possible fragments of iron that may have been introduced during the procedure of milling.

Transfer the sample to 250 mL erlenmeyer flask and rinse the glass particles with 6 portions of 30 mL of acetone PA, shaking for about 30 seconds for each procedure, and carefully decant the acetone. After washing, the sample must be free of blocks of glass powder and the surface of the grain must be practically free from the grip of fine particles. Dry the material for 20 minutes at 140ºC.

The sample should be tested up to 48 hours after the drying and in this case, must be kept in a desiccator.

Weigh out 10.0 g of crushed glass, transfer to 250 mL erlenmeyer flask, previously prepared with doubly distilled water or deionized water in a water bath at 90 ºC for at least 24 hours or at 121 ºC for 1 hour, and add 50 mL of distilled or deionized water.

As *white,* use 250 mL erlenmeyer flask, previously prepared in doubly distilled water or deionized water in a bath at 90 ºC for at least 24 hours or at 121 ºC for 1 hour, and add 50 mL of distilled or deionized water.

Close the erlenmeyer flasks with the use of becher inverted or aluminum paper, previously rinsed with distilled or deionized water.

Puts them in the autoclave and submit them to the following treatment:

- Promote the increase of the temperature of the autoclave after the closing of the exhaust valve, between 19 to 23 minutes, until it reaches 121 ºC ±1 °C;
- Keep at a temperature of 121 ºC ±1 ºC for 30 minutes;
- Discharge the pressure in a period of 38 to 46 minutes, until it reaches the atmospheric pressure.

Removing the bottles and cools them, immediately, in water mains. After cooling, decant the water from the erlenmeyer flask and wash the crushed glass with 4 portions of 15 mL of distilled or deionized water. Add 5 drops of methyl red solution and titrate immediately with sulfuric acid 1,01 M If the expected volume of solution that will be used in the titration is less than 10 mL, use a buret.

Record the volume of sulfuric acid used in the titration and correct the value in relation to the volume of the *white.*

Limits

Maximum value of alkalinity for the glass bottle type Is 1.0 mL of H2SO4 0.01 *M* to 10 g of crushed glass.

The value of maximum alkalinity for the glass bottle type III is 8.5 mL of H2SO4 0.01 *M* to 10 g of crushed glass.

The value of maximum alkalinity for the glass bottle type NP is 15 mL of H2SO4 0.01 *M* to 10 g of crushed glass.

THE TEST PROCEDURE IN GLASS BOTTLE FULL

Wash bottles, randomly chosen, with doubly distilled water or deionized water and dry them in a current of air dry and clean. Add volume of distilled or deionized water corresponding to 90% of the total capacity of the vial, determined as described in *Total Volumetric Capacity* (6.1.3).

Close the bottles with aluminum paper previously rinsed with distilled or deionized water and puts them in an autoclave. Submit them to the following treatment:

- Heat the autoclave at 100 ºC, with the exhaust valve open, for 10 minutes;
- Promote the increase of the temperature of the autoclave after the closing of the exhaust valve at 1 ºC/min, until it reaches 121 ºC ±1 °C;
- Maintain the temperature of 121 ºC ±1 ºC for 60 minutes;
- Lower the temperature in 0.5 °C/min, until it reaches 100 ºC, discharging the pressure until you reach the atmospheric pressure;
- Open the autoclave only after reaching the temperature of 95 °C;
- Transfer the bottles for water-bath at 80 ºC. Add cold water; taking care to avoid contamination of the extraction solution, being that the cooling time should not exceed 30 minutes.

After cooling, combine the solution for extraction of each one of the flasks. Measure the volume as recorded in Table 1 and transfer to 250 mL erlenmeyer flask.

As *white,* use 250 mL erlenmeyer flask and add the same volume of distilled water or deionized water.

Table 1 – The Volume of extraction solution in accordance with the total volumetric capacity of the container.

Volumetric Capacity The vial (mL)	*Volume of solution OF extraction (mL)*
< 3	25.0
From 3 to 30	50.0
Of 3.330	100.0
> 100	100.0

Add 5 drops of methyl red solution for each 25 mL of extraction solution and titrate immediately with hydrochloric acid 0.01 M, using a buret. Record the volume of 0.01 *M hydrochloric acid* used in the titration and correct the value in relation to the volume of the *white.*

Limits

The value of maximum alkalinity must not exceed the values given in Table 2.

Table 2 – maximum alkalinity in accordance with the type of glass and the volumetric capacity of the bottle.

Volumetric Capacity of bottle (mL)	***Maximum Volume of 0.01 M HCl (mL) to 100 mL of a solution of extraction***	
	Types I and II	***Type III***
< 1	2.0	20.0
From 1 to 2	1.8	17.6
From 2 to 5	1.3	13.2
5 To 10	1.0	10.2
10 To 20	0.80	8.1
From 20 to 50	0.60	6.1
50 To 100	0.50	4.8
100 To 200	0.40	3.8
200 To 500	0.30	2.9
> 1700	0.20	2.2

PROCEDURE THE TEST OF ATTACK OF WATER At 121 ºC – TO QUALIFY The GLASS OF TYPE II.

Rinse 3 or more bottles, randomly chosen, with doubly distilled water or deionized water for 2 times and dry- los in air current clean and dry. Add volume of distilled or deionized water corresponding to 90% of the total capacity of the vial, determined as described in *Total Volumetric Capacity* (6.1.3). Close the bottles with the use of becher inverted or aluminum paper, previously washed with distilled water, or deionized water.

Puts them in the autoclave and submit them to the following treatment:

- Promote the increase of the temperature of the autoclave after the closing of the exhaust valve, between 19 to 23 minutes, until it reaches 121 ºC ±1 °C;
- Keep at a temperature of 121 ºC ±1 ºC for 60 minutes;
- Discharge the pressure in a period of 38 to 46 minutes, until it reaches the atmospheric pressure.

Combine the solution volume of extraction of several bottles, in graduated cylinder and transfer 100.0 mL to 250 mL erlenmeyer flask. Add 5 drops of methyl red solution and titrate immediately with sulfuric acid 1,01 M Complete the titration within 60 minutes after opening the autoclave. Record the volume of sulfuric acid used in the titration and correct the value in relation to the volume of the *white* (100 mL of doubly distilled water or deionized water at the same temperature and with the same quantity of TI).

Limits

The value of maximum alkalinity for the glass bottle type Is 0.7 mL of H2SO4 0.01 *M* for bottles with up to 100 mL volumetric capacity.

The value of maximum alkalinity for the glass bottle type Is 0.2 mL of H2SO4 0.01 *M* for bottles with more than 100 mL volumetric capacity.

6.1.2 ARSENIUM

EQUIPMENT, MATERIALS AND REAGENTS

- Autoclave with temperature control from 121 ºC ±1.0 ºC, equipped with thermometer, pressure gauge, safety valve and shelf for sustaining of, at the very least, Flasks;
- OVEN for drying with temperature of 140 ºC;
- Becher or aluminum paper;
- 250 Ml erlenmeyer flask.
- Graduated cylinder 100 mL;
- DISTILLED or deionized water, with maximum conductivity of 0.15 µS/cm (or 6.67 MQ/cm) to 25 ºC.

PROCEDURE

Wash bottles, randomly chosen, with doubly distilled water, or deionized water and dry them in a current of air dry and clean. Add volume of distilled or deionized water corresponding to 90% of the total capacity of the vial, determined as described in *Total Volumetric Capacity* (6.1.3). Close the bottles with aluminum paper previously rinsed with distilled or deionized water and puts them in an autoclave. Submit them to the following treatment:

- Heat the autoclave at 100 ºC, with the exhaust valve open, for 10 minutes;
- Promote the increase of the temperature of the autoclave after the closing of the exhaust valve at 1 ºC/min, until it reaches 121 ºC ±1 °C;
- Keep at a temperature of 121 ºC ±1 ºC for 60 minutes;
- Lower the temperature in 0.5 °C/min, until it reaches 100 ºC, discharging the pressure until you reach the atmospheric pressure;
- Open the autoclave only after reaching the temperature of 95 °C;

Transfer the bottles for water-bath at 80 ºC. Add cold water, carefully, to avoid contamination of the extraction solution, being that the cooling time should not exceed 30 minutes.

After cooling, combine the extraction solution of each one of the flasks to obtain 35 mL and transfer to 250 mL erlenmeyer flask.

Proceed as described for *limit Tests of arsenic*. Limit for arsenic is 1 µg/g.

6.1.3 TOTAL VOLUMETRIC CAPACITY

Test for determining the volume of liquid product that the bottle may contain, when full, up to the upper surface of the termination.

EQUIPMENT AND MATERIALS

- Balance with minimum resolution of 0.1 g;
- Thermometer of 0 ºC to 100 ºC, with resolution of 0.5 ºC;
- Doubly distilled water.

PROCEDURE

Select six units at random. Weigh the scales with the dry bottle and empty.

Fill the flask with distilled water until the sealing surface of the termination (region of closing the bottle, also called neck, *finish,* or finishing), maintaining the external surface completely dry, being that for ampoules filling should be performed until the height of the point Figure 1.

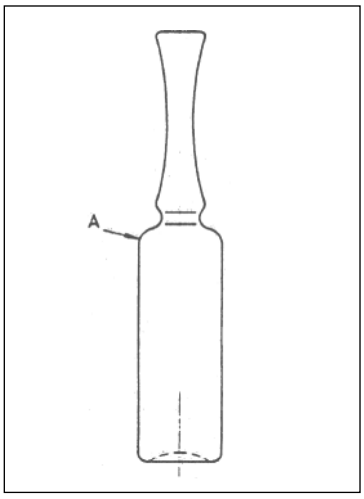

Figure 1 – Filling the volume of ampoules (up to a point).

Determine the water temperature – during the execution of the test, ensure that the temperature of the water has no variation above 1 ºC.

Weigh the bottle filled and determine the mass of water it contains.

Calculate the volume of the flask by dividing the mass of the water by its density, at the test temperature, with the use of the data recorded in Table 1 for distilled water.

Table 1 – Density of distilled water as a function of temperature.

Temperature (ºC)	*Water Density (g/mL)*	*Temperature (ºC)*	*Water Density (g/mL)*
10	0.99839	23	0.99660
11	0.99832	24	0.99638
12	0.99823	25	0.99617
13	0.99814	26	0.99593
14	0.99804	27	0.99569
15	0.99893	28	0.99544
16	0.99780	29	0.99518
17	0.99766	30	0.99491
18	0.99751	31	0.99464
19	0.99735	32	0.99435
20	0.99718	33	0.99406
21	0.99700	34	0.99375
22	0.99680	35	0.99345

RESULTS

The results expressed in mL, with a decimal point, must be in accordance with the specifications indicated.

6.2 PLASTIC CONTAINERS

The aim with this section is to establish standards for materials and plastic components used for storing medicines and correlates. The standards and tests for the functional properties of containers and their components are supplied in Plastic Containers – *performance Tests* (6.2.3).

The articles of plastics are identified and characterized by infrared spectroscopy and differential scanning calorimetry. In this section are described the testing procedures and standards for the identification and characterization of different types of plastic. The degree of verification is based

The plastics may contain residues of the polymerization process, plasticizers, stabilizers, antioxidants, pigments and lubricants. The factors such as the composition of the plastics, processing and cleaning procedures, surface treatment, media contact, dyes, adhesives, absorption and permeability of wood preservatives and conditions of storage, also, may affect the appropriateness of a plastic for a specific use. The tests of extractables are planned to characterize the components extracted and identify the potential migrants. The degree or extent of tests to extract substances of a component depends on the purpose of use and the degree of risk of negatively impact on the effectiveness of the product. In this chapter are described the tests of extractables specific for polyethylene resins, polypropylene, poly (ethylene terephthalate) and poly (ethylene terephthalate glycol). All other plastics shall

be tested as described in *Physical Chemical Tests* of Test Methods (6.2.1.3). The test of *Buffering Capacity* must be tested for containers for packing a liquid product.

The plastic components used for high-risk products, such as those intended for inhalation; parenteral and ophthalmic preparations are tested using the Biological Testing of Test Methods (6.2.1.3).

The plastic containers intended for packaging of parenteral products shall comply with the requirements of the Biological Testing and *Physical Testing of Chemicals. Also, standards are provided for the receptacles in polyethylene used for packing dried oral pharmaceutical forms, not intended for constitution in solution.*

6.2.1 PLASTIC CONTAINERS AND CORRELATES

6.2.1.1 POLYETHYLENE CONTAINERS

Polyethylene of high and low density polymers are long-chain, synthesized under controlled conditions of heat and pressure, with the aid of catalysts and from, at the very least, 85.0% of ethylene and a total of 95.0% of olefins. Both the high-density polyethylene and low density has an infrared absorption spectrum of specific polyethylene and have thermal properties characteristics. The high-density polyethylene has a density between 0.941 and 0.965 g/cm3. The low density polyethylene has a density between 0.850 and 0.940 g/cm3. Other properties that may affect the adequacy of polyethylene include elasticity module, index of fluidity, resistance to breakage under environmental stress and degree of crystallinity after molding.

The standards and tests described in this section are characterised containers and components, produced from polyethylene of low or high-density homopolimeric or copolymeric resins.

All components of polyethylene are subject to testing of infrared spectroscopy and differential scanning calorimetry. When stability studies are carried out to determine the date of validity of a pharmaceutical form especially in a polyethylene container suitable, any other polyethylene container that complies with these requirements can also be used for packing the pharmaceutical form in question, provided that the appropriate stability programs are expanded to include the alternative container for ENSURE that the identity, strength, quality and purity of the pharmaceutical form are maintained during the period of validity.

TESTS

High Density Polyethylene

Infrared Spectroscopy. Use the accessory of attenuated total reflection, as described in item *medium Infrared* (2.5.14). The spectrum corrected sample must submit bands of higher absorption only ourselves wavelengths of the spectrum of the reference standard.

Differential Scanning Calorimetry. Proceed *as described in* Thermal Analysis *of Methods* of Testing

The thermograph of sample should be similar to the reference standard, determined in a similar manner, and the temperature endotermica (melting) in thermograph of sample should not differ by more than 6.0 ºC the reference standards.

Heavy Metals. containers shall meet the requirements for *Heavy Metals* in *Chemical Physical Tests, Methods of Testing* (6.2.1.3).

Non-Volatile Residue. Proceed as described in *Non-Volatile Residue* in *Physical Chemical Tests* in *Test Methods* (6.2.1.3), being that the *White should* be the same solvent used in each one of the conditions of test. The difference between the quantities obtained from the *Preparation of the Sample and the Blank* should not exceed 12.0 mg when the water maintained at 70ºC is used as a Means *of Extracting;* do not exceed 75.0 mg when the alcohol kept at 70ºC is used as a Means *of Extraction; and not exceed 100.0 mg when the hexane maintained at 50 ºC is used as a Means of Extracting.*

Substances Used in Contact with Oral Liquids.

Proceed as described in *Buffering Capacity* of *Physical Chemical Tests, Test Methods* (6.2.1.3).

Low Density Polyethylene

Infrared Spectroscopy. Use the accessory of attenuated total reflection, as described in item *medium Infrared* (2.5.14). The spectrum corrected sample must submit bands of higher absorption only ourselves wavelengths of the spectrum of the reference standard.

Differential Scanning Calorimetry. Proceed As described in *Thermal Analysis, Methods for Testing.* The thermograph of sample should be similar to the reference standard, determined in a similar manner, and the temperature endothermic (melting) in The thermograph sample must not differ by more than 8.0 ºC the reference standards.

Heavy Metals, and Non-Volatile Residue. **Prepare statements of the sample as described in** *the Sample Preparation* **in** *Tests of Physical Chemical Methods of Testing, with portion of 60 cm2, without considering the thickness for each Ml of Means of Extraction.*

Heavy Metals. **The containers must meet the requirements for** *Heavy Metals* **of** *Physical Chemical Tests, Methods of Testing* (6.2.1.3).

Non-Volatile Residue. Proceed as described in

Non-Volatile Residue Tests, Physical Chemical Methods of Testing (6.2.1.3), *being that the White should be the same solvent used in each one of the conditions of test. The difference between the quantities obtained from the Preparation of the Sample and the Blank should not exceed 12.0 mg when the water maintained at 70ºC is used as a Means of Extracting; do not exceed 75.0 mg when the alcohol kept at 70ºC is used as a Means of Extraction; and not exceed 350.0 mg when the hexane maintained at 50 ºC is used as a Means of Extracting.*

Substances Used in Contact with Oral Liquids. Proceed as described in *Buffering Capacity* **of** *Physical Chemical Tests, Methods for Testing* **(6.2.1.3).**

6.2.1.2 POLYPROPYLENE CONTAINERS

The polymers of polypropylene polymers are long-chain, synthesized with the aid of catalysts under controlled conditions of heat and pressure. Factors such as composition of the plastic material, processing and cleaning procedures, media contact, dyes, adhesives, absorption, adsorption, permeability of wood preservatives and storage conditions can affect the suitability of a plastic for a specific use. The adequacy of a polypropylene characteristic must be established by means of appropriate tests.

The polypropylene has a spectrum infrared and thermal properties distinctive characteristics. It has a density of 0.880 to 0.913 g/cm3. The properties of permeability of receptacles in polypropylene molded can be changed when the resprayed polymer is incorporated, depending on their proportion in the final product. Other properties that may affect the adequacy of polypropylene used in containers for packaging of medicinal products include permeability to oxygen and moisture, elasticity module, index of fluidity, resistance to breakage under environmental stress and degree of crystallinity after molding.

The standards and tests provided characterize containers in polypropylene, produced from homopolymers or copolymers, which are suitable for packaging of oral pharmaceutical forms dried solids and liquids. Considering that appropriate studies of stability have been carried out to determine the date of validity

A pharmaceutical form specifies in an appropriate container in polypropylene, any other container in polypropylene that meets these requirements can be used to package the same pharmaceutical form, provided that the appropriate stability programs are expanded to include this alternative container, in order to ensure that the identity, strength, quality and purity of the pharmaceutical form are maintained during the period of validity.

TESTS

Infrared Spectroscopy. **Use accessory attenuated total reflection, as described in item** *Infrared absorption Spectrophotometry* (2.5.14). **The spectrum corrected sample must submit bands of higher absorption only in the same wavelengths of the spectrum of the respective reference standard (homopolymer or copolymer of polypropylene) determined in a similar manner.**

Differential Scanning Calorimetry. **Proceed As described in** *Thermal Analysis of Methods of Testing* (6.2.1.3). **The temperature endotermica (melting) in thermograph must not differ by more than 6.0 ºC of reference standards for homopolymers. The temperature endotermica obtained of the thermograph of sample of polypropylene copolymer must not differ by more than ºC of the patterns of that substance.**

Heavy Metals and Non-Volatile Residue. **Prepare extracts of samples as described in** *the Sample Preparation, Testing, Physical Chemical Methods of Testing* (6.2.1.3), **with portion of 60 cm2, without considering the thickness for each 20.0 mL of Means of Extraction.**

Heavy Metals. **The containers must meet the requirements for** *Heavy Metals* **of** *Physical Chemical Tests, Methods of Testing* (6.2.1.3).

Non-Volatile Residue. Proceed as described in Non-Volatile Residue Tests, Physical Chemical Methods of Testing (6.2.1.3), *being that the White should be the same solvent used in each one of the conditions of test. The difference between the quantities obtained from the Preparation of the Sample and the Blank should not exceed 10.0 mg when the water maintained at 70ºC is used as a Means of Extracting; do not exceed 60.0 mg when the alcohol kept at 70ºC is used as the Means of Extraction; and not exceed 225.0 mg when the hexane maintained at 50 ºC is used as a Means of Extracting. The containers must meet the requirements for Non-Volatile Residue for all means of extraction.*

Note: hexane and alcohol are flammable. To evaporate these solvents, use a current of air with water bath; the dry residue, use oven explosion proof.

Substances Used in Contact with Oral Liquids.

Proceed as described in *Buffering Capacity* **of** *Physical Chemical Tests, Methods for Testing* (6.2.1.3).

6.2.1.3 CONTAINERS OF POLY (ETHYLENE TEREPHTHALATE) AND POLY (ETHYLENE TEREPHTHALATE GLYCOL)

Resins of poly (ethylene terephthalate) (PET) are long-chain crystalline polymers prepared by condensation of ethylene glycol with dimethyl terephthalate or terephthalic

acid. The PET copolymer resins are prepared in a similar way, except that, also, may contain a small amount of isophthalic acid (less than 3% of mol of resin) or 1,4-cyclohexane-dimethanol (less than 5% of mol of resin). The polymerization is conducted under controlled conditions of heat and vacuum; with the aid of catalysts and stabilizers.

The copolymer resins PET has physical properties and spectral similar to PET and, for practical purposes, they are treated as PET. The tests and the specifications provided in this section to characterize resins and containers of PET, apply also to the copolymer resins and to containers manufactured from them.

Generally, the PET and its copolymer resins exhibit a high degree of order in its molecular structure. As a result, exhibit a thermal behavior characteristic dependent on composition, including a glass transition temperature of approximately 76 ºC and a melting temperature of approximately 250 ºC. These resins have a spectrum of infrared absorption especially that allows the differentiation of other plastic materials, such as polycarbonate; polystyrene; polyethylene resins and poly (ethylene terephthalate glycol (PETG). The PET and its copolymer resins have a density between 1.3 and 1.4 g/cm3 and a minimum intrinsic viscosity of 0.7 dL/g, which corresponds to an average molecular weight of approximately 23,000 Da.

The PETG resins are polymers of high molecular weight prepared by condensation of ethylene glycol with dimethyl terephthalate or terephthalic acid and with 15 to 34% of 1,4-cyclohexane-dimethanol molar. PETG polymer resins are transparent, amorphous granules, with a glass transition temperature of approximately 81 ºC and without a crystalline melting point, as determined by differential scanning calorimetry. The resins PETG has an infrared absorption spectrum of particular which makes it possible to distinguish between other plastic materials, including the PET. The resins PETG has a density of approximately 1.27 g/cm3 and a minimum intrinsic viscosity of 0.65 dL/g, which corresponds to an average molecular weight of approximately 16,000 Da.

The PET resins and PETG contains no plasticizer, support of processing or antioxidants. When dyes are used in the manufacture of containers of PET and PETG, these should not migrate to the net.

The standards and tests provided in this section characterize containers of polyethylene terephthalate (PET) and polyethylene terephthalate glycol (PETG) that are used for packaging liquid oral pharmaceutical forms. Whereas

That appropriate studies of stability have been carried out to determine the validity of a particular liquid pharmaceutical form in a container that meets the requirements for containers of PET or PETG, any other container of these substances that meet these requirements can be used for packing the same pharmaceutical form, provided that appropriate stability programs are expanded to include this alternative container, to ensure that the identity, strength, quality and purity of the pharmaceutical form are maintained throughout the validity. The adequacy of a container of PET or PETG specific to be used in dispensing a liquid oral pharmaceutical form specifies must be established by means of appropriate tests.

TESTS

Infrared Spectroscopy. Using attenuated total reflection accessory, proceed as described

In *infrared absorption Spectrophotometry* (2.5.14). The spectrum corrected for sample shows bands of higher absorption only in the same wavelengths of the spectrum of reference standards, certain 47 similarly.

Differential Scanning Calorimetry. Proceed as described in item *Thermal Analysis in Testing Methods*. *For the polyethylene terephthalate, the thermograph of sample should be similar to the reference standard, determined in a similar manner; the melting point of the sample (Tm) should not differ from standards of reference in more than 9 ºC and the temperature of vitreous transition into more than 4 ºC. For the polyethylene terephthalate glycol,*

The Thermograph of sample should be similar to the reference standard, determined in a similar manner; the glass transition temperature of the sample (Tg) must not differ by more than 6ºC for the reference standards.

Extraction of Dyes. Select three containers to The Test. Cut a relatively flat portion of the side wall of a container and trimming it in so far as necessary to adjust the sample to the support of the spectrophotometer. Perform sweep (2.5.14) to obtain the visible spectrum of 350-700 nm of side wall. With approximation of 2 nm, determine the wavelength of maximum absorbance. Fill out the two remaining containers with 50% ethanol for containers PET and 25% ethanol for PETG. Prepare the containers with seals raincoats, as a sheet of aluminum, and close with the caps. Fill with solvent corresponding a glass container of the same capacity that the containers in test, prepare it with watertight seal, such as an aluminum foil, and close with a cover. Incubate the containers under test and the glass container to 49 ºC for 10 days. Remove the containers and wait until they reach the ambient temperature. Concomitantly, determine the absorbance (2.5.14) of solutions in test in cells of 5 cm at the wavelength of maximum absorbance, using the corresponding solvent container glass as white. For both solutions in test, the absorbance values, thus, obtained must not be less than 0.01.

Heavy Metals; Total Tereftaloila and Ethylene Glycol.

Means of extraction.

Purified Water

Ethanol 50 %. Dilute 125 mL of ethanol in water to 238 mL of solution and mix thoroughly.

Ethanol 25 %. Dilute 125 mL of Ethanol at *50%* in water to 250 mL of solution and mix thoroughly.

N-Heptane.

General Procedure. Use a means of extraction of *Ethanol at 50%* for containers of PET and *Ethanol at 25%* for PETG. For each means of extraction, fill a sufficient number of containers tests with 90% of its nominal capacity to obtain at least 30 mL. Fill a corresponding number of glass containers with *purified Water,* the same quantity of containers with *Ethanol at 50 %,* or *Ethanol at 25%* and the same number of glass containers with *n-Heptane* to be used as white means of extraction. Put in containers waterproof seals, such as aluminum foil, and cover them. Incubate the containers tests and the glass containers to 49 ºC for 10 days. Remove the containers tests with the samples and the whites of the means of extraction and stores- los at ambient temperature. Do not transfer the samples from the means of extraction for containers of alternate storage.

Heavy Metals. Pipette 20 mL of *purified Water* extracted from the containers tests, filtered as needed, put in one, or two tubes of 50 mL for comparison of color and save the *purified Water* remaining for use in test of Ethylene Glycol. Adjust the pH of the extract between 3.0 and 3.97 *M with acetic acid* or ammonium hydroxide 6 M, using an indicator paper short interval of pH. Dilute with water to about 35 mL and mix.

Pipette 2 mL of *lead standard Solution (10 ppm Pb),* Prepared on the day of use; transfer to a second tube of comparison of color and add 20 mL of *purified Water.* Adjust the pH between 3.0 and 4.0 *M with acetic acid* or ammonium hydroxide 6 M, using an indicator paper short interval of pH. Dilute with water to about 35 mL and mix.

In each tube, add 1.2 mL of thioacetamide SR and 2 mL of acetate Buffer *pH 3.5* (5.3.2.3) dilute with water to 165 mL of solution and mix thoroughly. Any color produced within 10 minutes in tube that contains the *purified Water* removed from containers tests, should not be more intense than that of the tube containing the *standard Solution of lead (10 ppm Pb), both* displayed on a white surface (limit 1 ppm).

Total tereftaloila. Determine the absorbance of the extract of *Ethanol at 50%* or *25% Ethanol* in a cell of CM, at the wavelength of maximum absorbance at about 244 nm (2.5.14), using as white one corresponding to the means of extraction. The absorbance of the extract must not exceed 0.150, which corresponds, at most, 1 ppm of total tereftaloila of means.

Dilute each solution with dilute sulfuric acid to fill the volume and mix. Concomitantly, determine the absorbance (2.5.14) of solutions from the *standard Solution* and the *test Solution* in cells of 1 cm, at a wavelength of absorbance maximum at about 575 nm, using white as the solution removed from the midst of extraction in *purified Water. The absorbance of the solution obtained from the test Solution* is not determine the absorbance of the extract of *n-Heptane* in a 1 cm cell at the wavelength of maximum absorbance at about 240 nm (2.5.14), using white as the means of extraction of *n-Heptane. The absorbance of the extract must not exceed 0.150, which corresponds to a maximum 1* ppm of tereftaloila of means.

Ethylene Glycol.

Periodic acid Solution. Dissolve 125 mg of periodic acid in 10 mL of water.

Dilute sulfuric acid. 50 mL of water, add slowly and with constant stirring, 50 mL of sulfuric acid and wait for that to reach ambient temperature.

Sodium bisulphite Solution Dissolve 71 g of sodium bisulphite in 10 mL of water. Use this solution within 7 days.

Solution of disodium cromotropato. Dissolve 100 mg of disodium cromotropato in 100 mL of sulfuric acid. Protect the solution from light and use it within 7 days.

Standard Solution. Dissolve an accurately weighed quantity of ethylene glycol in water and dilute, quantitatively, step by step, if necessary, to obtain a solution with a concentration of about 1.0 µg/mL.

Test Solution. Use the extract in purified Water.

Procedure. Transfer 1 mL of the standard Solution to a 10-mL volumetric flask. Transfer 1 mL of the *test Solution* for a second 10-mL volumetric flask. Transfer 1 mL of the means of extraction in *purified Water* for a third 10-mL volumetric flask. For each of the three flasks, add 100 µL of the Solution of periodic acid, stir to mix well and leave to stand for 60 minutes. Add to each flask 1 mL of sodium bisulphite Solution and mix thoroughly. Add 100 µL of the Solution of disodium cromotropato to each flask and mix well. All the solutions must be analyzed until

Time after adding the *Solution of disodium cromotropato. Add, carefully, 6* mL of sulfuric acid to each flask, mix and expect that the solutions to reach ambient temperature.

Note: the dilution of sulfuric acid produces considerable heat and may cause the boiling point of the solution. Perform this addition carefully. The gas of sulfur dioxide will be released. The use of a camera of exhaust ventilation is recommended.

Dilute each solution with dilute sulfuric acid to fill the volume and mix. Concomitantly, determine the absorbance (2.5.14) of solutions from the *standard Solution* and the *test Solution* in cells of 1 cm, at a wavelength of absorbance maximum at about 575 nm, using white as the solution removed from the midst of extraction in *purified Water. The absorbance of the solution obtained from the test Solution* is not the top of the solution obtained from the *standard Solution, corresponding, at most, 1 ppm of ethylene glycol.*

METHODS OF TESTING

Multiple Internal Reflectance

Equipment. Use infrared spectrophotometer capable of correcting for the spectrum of white and equipped with an accessory of attenuated total reflection and a board KRS-5 of internal reflection. The cristal KRS-5 of 2 mm thick, with an incidence angle of 45º provides a sufficient number of reflections.

Preparation of the sample. Cut two flat portions representing the average thickness of the container wall, and trimming them as needed, to obtain segments suitable for mounting on the attachment of multiple internal reflectance. To avoid scratching the surface, clean the samples with dry paper or, if necessary, with a soft cloth dampened with methanol and wait for drying. Firmly Engage the samples in both sides of the plate of internal reflection KRS-5, ensuring a suitable contact surface. Before placing the samples on the plate, compress them obtaining uniform thin films to be exposed to temperatures of approximately 177 ºC, under high pressure (15,000 psi or more).

General Procedure. Place the parties clipped from sample in multiple internal reflectance accessory and place the assembly in the light beam of infrared spectrophotometer. Adjust the position of the sample and the mirrors of equipment to enable the maximum transmission of light by reference beam non-attenuated. Complete the adjustments of the accessory, mitigate the reference beam to allow full scale deflection, during the scanning of the sample. Determine the infrared spectrum from 3500 to 600 cm^{-1} for polyethylene and polypropylene and from 4000 to 1300 cm^{-1} for PET and PETG.

Thermal Analysis

General Procedure. Cut a section with approximate weight of 12 mg and puts it into the slot for the sample. The close contact between the cage and the heating element is essential for reproducibility of results. Determine the thermogram done under nitrogen, using the conditions of heating and cooling as specified for the type of resin and use an equipment capable of performing the determinations.

For Polyethylene. Determine the thermogram done under nitrogen at temperatures between 40ºC and 200ºC, at a heating rate between 2ºC and 10 ºC per minute, followed by cooling to 40 ºC, at a rate between 2 ºC and 10 ºC per minute.

For Polypropylene. Determine the thermogram done under nitrogen at temperatures that vary between the ambient temperature and 30 ºC above the melting point. Maintain the temperature for 10 minutes, then cool to 50 ºC below the maximum temperature of crystallization at a rate of 10 ºC to 20 ºC per minute.

For poly (ethylene terephthalate). Heat the sample from ambient temperature up to 280ºC at a heating rate of approximately 20 ºC per minute. Keep the sample at 280 ºC for 1 minute. Rapidly Cool the sample to ambient temperature and reheats it to 280 ºC at a heating rate of approximately 5 °C per minute.

For poly (ethylene terephthalate) glycol. Heat the sample from ambient temperature up to 120ºC at a heating rate of approximately 20 ºC per minute. Keep the sample to 120 ºC for 1 minute. Rapidly Cool the sample to ambient temperature and reheats it to 120 ºC to a heating rate of approximately ºC per minute.

Biological Tests

The *in vitro biological tests are carried out in accordance with the procedures established in biological reactivity Tests in vitro* (6.2.5). The components that meet the requirements of in vitro tests *need not be subjected to additional tests. Any descriptive name of class of plastic is attributed to these materials. The materials that do not meet the requirements of in vitro tests are not suitable for use as containers for medicines.*

If the designation of class is needed for plastics and other polymers that meet the requirements laid down in

Biological reactivity Tests in vitro (6.2.5), perform the in vivo test specified for proper *Classification of Plastic in biological reactivity Tests in vivo* (6.2.6).

Physical Chemical Tests

The following tests to determine the physical and chemical properties of plastics and their extracts, are based on the extraction of plastic material, it is essential that the quantity designated plastic is used. In addition, the surface area specified must be available for extraction in determined temperature.

Test Parameters:

Means of Extraction. Unless directed otherwise in a specific test to follow, use *Purified Water* as a means of extracting, keeping the temperature at 70 ºC, during the extraction of sample Preparation.

White. Use *Purified Water where the white is specified in the tests that follow.*

Equipment. Use water-bath and Containers *of extraction, as described in biological reactivity Tests in vivo* (6.2.6). Proceed as described in the Preparation *of equipment in biological reactivity Tests in vivo* (6.2.6). The containers and equipment need not be sterile.

Preparation of the Sample. From a homogeneous sample of plastic, use an aliquot for each 20 Ml of means of extraction, equivalent to 120 cm2 of surface area total (uniting both sides), and subdivided into bands of approximately 3 mm in width and next to 5 cm in length. Transfer the sample subdivided for a measuring cylinder of glass type I, graduated, 250 mL with lid and add about 150 mL of

purified Water. Shake for approximately 30 seconds, drain, discard the liquid and repeat a second wash.

Extraction for Sample Preparation. Transfer the sample Preparation ready for a flagon of appropriate extraction and add the required quantity of means of extraction. Remove per 24 hours by heating in a water bath at the temperature specified for the means of extraction. Cool to temperatures not below 20 ºC. Pipette 20 mL of the extract prepared to a suitable container. Use this part in the test for *Buffering Capacity*. Decant, immediately, the residual extract in a suitable clean container and close it.

Non-Volatile Residue. Transfer, in appropriate, 50 mL aliquots of the Extract of *the sample Preparation* for a suitable tared crucible (preferably a crucible of fused silica which has been cleaned with acid) and evaporate the volatile part in a steam bath. Evaporate to form similar 50 mL of *White* in another crucible. If you expected an oily residue, examine repeatedly the crucible during evaporation and drying process and reduce the amount of heat, if the oil tend to slide down the wall of the crucible. Dry at 105 ºC for 1 hour. The difference between the quantities obtained from the *Extract for the sample Preparation* and the *White* should not be higher than 15 mg.

Waste by incineration (2.5.10). It is not necessary to perform this test when the test result of *Nonvolatile Residue* does not exceed 5 mg. Proceed with the retrieval of waste, from the *Extract for the Preparation of the sample* and *Blank* described in the test for *Non-Volatile Residue* above, using, if necessary, more sulfuric acid for the same amount in each crucible. The difference between the quantities obtained residue of ignition from the *Extract to the Preparation of the sample and the Blank* should not be higher than 5 mg.

Heavy Metals. Pipette 20 mL of the Extract *of the sample Preparation, filtered,* if necessary, for one of the two tubes of 50 mL for comparison of color. Adjust the pH to between 3.0 and 4.0 *M with acetic acid* or ammonium hydroxide 6 M, using an indicator paper short interval of pH. Dilute with water to about 35 mL and mix.

Pipette 2 mL of *lead standard Solution (10 ppm Pb),* Transfer to the second tube for comparison of color and add 20 mL of the Blank. *Adjust the pH to between* and with 4.0 *M acetic acid* or ammonium hydroxide 6 *M, using an indicator paper short interval of pH. Dilute with water to about 35 mL and mix. In each tube, add 1.2 mL of thioacetamide SR and 2 mL of acetate Buffer pH 3.5* (5.3.2.3), dilute with water to 165 mL of solution and mix thoroughly. Any color produced within 10 minutes in preparation that contains the Extract of the *Preparation of the extracted sample* containers of tests, do not should be more intense than in *standard Preparation, both displayed on a white surface (1* ppm in statement).

Buffering Capacity. Titrate potentiometrically, aliquots of 20 mL, previously collected, the *Extract of the Preparation of the sample* to a pH 7.0, using 0.010 *M hydrochloric acid* or sodium hydroxide 0.010 M, as needed. Deal, similarly, a 20 mL aliquot of the *White. If the same titrant is necessary for both certificated, the difference between the two volumes should not be greater than 10 mL; and if acid is necessary or to the Extract of the sample Preparation,* or for the *White, and the alkali for the other, the total of the two volumes requested should not be greater than 10 mL.*

6.2.2 LIDS OF ELASTOMER

Elastomer Covers are manufactured in materials obtained from the polymerization, poly addition or poly condensation of organic substances. The polymers obtained are, generally vulcanized. The formulations of elastomeric caps contains natural or synthetic organic and inorganic additives and to assist or control the vulcanization, provide physical and chemical properties, coloring, or stabilize the formulation of cover.

For caps formulated with substances of natural or synthetic elastomer, used for storage of long term. Does not apply to caps manufactured in silicone elastomer, but applies to the caps treated with silicone, such as dimethicone, and covers coated with other materials, such as lubricating materials related chemically, mechanically or the cover.

The following comments relate only covers laminated or coated with materials intended to provide or operate as a barrier to the base of the elastomer, for example poly (tetrafluoretileno (PTFE) or coatings varnished. It is not permissible to use a material with the aim of transforming a cover that is not within the specific requirements for a that is in compliance. However, all the physico-chemical tests apply the formula based on these covers, as well as the covers laminated or coated. The functionality tests should be performed using caps of elastomer laminated or coated. The biological tests apply to material coated or laminated, as well as the formula basis. The biological tests can be carried out in caps or materials coated or laminated and non-laminated in covers and not coated, being that the results should be reported separately. The basic formula used in tests- physical, chemical or biological must meet the specifications of a cover with barrier coating that should be similar to the coating of the cover in configuration and size.

The tests in this section are limited to the caps of elastomer Types I and II, being that the Type I are used for aqueous preparations and Type II are usually intended for the non-aqueous preparations. If a cover does not meet all the requirements of the test Type I, but meet The requirements for the testing of Type II, the cover receives the final classification of Type II.

In this section proposes to conduct an initial screening to identify elastomer plugs that may be appropriate for use with injectable preparations, based on their biological compatibility; in physico-chemical properties of their aqueous extracts and its features. All of the covers of elastomer suitable for use in injectable preparations comply both with the limits of the test of the Type I and the Type II. However, with this specification does not have

the intention to serve as a single evaluation criterion for the selection of such caps.

Among the requirements for evaluation of caps that are beyond the scope of this section is the establishment of identification tests and specifications of the cover, the cover check compatibility, physical chemistry of the product, the identification and determination of safety caps filterable found on the packaging of the product, to check the functionality of the product packaging under real conditions of storage and use conditions.

The user of the lids should obtain from the supplier guarantees that the composition of the cover does not vary and that is the same used in compatibility testing. When the supplier shall inform the end user about changes in composition,

The Compatibility test should be repeated, fully or partially, depending on the nature of the changes.

CHARACTERISTICS

The caps of elastomer are translucent, or opaque and has no coloring characteristic, depending on the additives used. Are homogeneous and practically free of materials bright and accidental, such as fibers, foreign particles, and rubber waste.

IDENTIFICATION

The covers are manufactured from a wide variety of polymeric coatings and elastomeric materials optional. Therefore, this section is not specific identification tests involving all possible presentations of covers. It is the responsibility of the supplier of the cover and the manufacturer of the finished product check the formulation of cover and any coated materials, or laminates used in accordance with the tests of appropriate identification. Some Examples of analytical tests that can be employed include specific density, analysis of ash, determination of sulfur content, thin-layer chromatography of the extract, ultraviolet absorption spectrophotometry of extract, or absorption spectrophotometry.

TESTING PROCEDURES

The caps of elastomer must be in accordance with the biological requirements; physico-chemical and functional.

Como as tampas de elastomero sao processadas pelo fornecedor before distribution to the end user, the supplier must demonstrate compliance of covers exposed to processing steps or sterilisation. In a similar manner, if the covers of elastomer received by end user are processed, or sterile, subsequently, the end user is responsible for proving the continued compliance of subsequent covers the processing conditions or sterilisation. This is important if the covers are exposed to processes or conditions that may have a significant impact on biological characteristics, physical-chemical or functional cover, such as the gamma radiation.

For plugs that are normally lubricated with silicone before use, is allowed to carry out the test in physico-chemical covers not lubricated to avoid potential interference of method and/or difficulties in the interpretation of test results. For caps supplied with other lubricants not occlusive, all tests should be performed using the cover coated.

For caps, coated or laminated with coatings intended to confer a barrier function, such as PTFE, or coatings varnished, the physico-chemical tests will be applied to the elastomer based not coated, as well as the caps coated. The lid does not coated subjected to physico-chemical tests should be similar to cover coated in size and configuration. The end users of caps coated, also, are responsible to prove compliance with the specifications of these covers physical- chemical, processed or treated in a way that simulates the conditions normally employed by the end user before use.

In all cases it is appropriate documenting the conditions of processing, pre-treatment, sterilisation or cap lubrication when reporting the results.

In Table 1 are summarized the requirements of the tests of the plugs and the responsibilities of the supplier and the end user.

Table 1 – Requirements of the tests of the plugs and the responsibilities of the supplier.

Types of caps (as supplied or Used)	*Physico- chemical Tests*	*Functionality Tests*	*Biological Tests*
Covers with or without silicone coating	The tests should be performed The use of silicone is optional Responsibility: supplier and end user	The tests should be performed The use of silicone is optional Responsibility: supplier and end user	The tests should be performed The use of silicone is optional Responsibility: supplier and end user
Covers with coating lubricants (Materials not occlusive, non-silicone)	The tests should be performed in covers coated Responsibility: supplier and end user	The tests should be performed in covers coated Responsibility: supplier and end user	The tests should be performed in covers coated Responsibility: supplier and end user
Caps with occlusive coatings	The tests should be performed in covers coated Responsibility: supplier and end user AND: The tests should be carried out in caps not coated (formula basis) Responsibility: supplier	The tests should be performed in covers coated Responsibility: supplier and end user	The tests should be performed in covers coated OR: The tests should be performed in caps not coated (formula basis) and material laminated/ coated (report the results separately) Responsibility: supplier and end user.

6

BIOLOGICAL TESTS

Are indicated two stages of testing. The first stage is the completion of the in vitro test. *The materials that do not meet the requirements of the in vitro test are submitted to the second stage of in vivo tests, as described in biological reactivity Tests in vivo* (6.2.6). The materials that meet the requirements for the in vitro tests *need not be subjected to in vivo test.*

The caps Type I and Type II must be in accordance with the tests of biological reactivity *in vitro and in vivo.*

PHYSICO-CHEMICAL TESTS

Development of Preparation S

Put the caps whole, not cut, corresponding to a surface area of (100 ±10) cm2 in a suitable glass container. Cover caps with 200 mL of purified water or water for injectables. If it is not possible to obtain a cover with the surface area of prescribed using caps do not cut, select a number of caps that

Iran is approaching 100 cm2, and adjust the volume of water used for the equivalent of 2 mL for each 1 cm2 of the surface area of the actual cover used. Boil for 5 minutes and rinsed five times with purified water or water for cold injectate.

Put the caps washed in a glass flask wide-neck Type I, add the same amount of purified water or water for injectables, initially added the caps and weigh. Cover the mouth of the bottle with a becher glass fibers of Type I. Sterilize in an autoclave, so that the temperature of 121 ºC ±2ºC is reached within 20 to 30 minutes and maintain this temperature for 30 minutes. Let cool down to ambient temperature for a period of approximately 30 minutes. Add purified water or water for injectables to return to the original weight. Shake, decant immediately and collect the fluid. This liquid should be shaken before being used in each of the tests.

Preparation of White

The preparation of the white should be performed similarly, using 200 mL of purified water, or water for injectables, omitting the caps.

Appearance of Preparation (Turbidity and Color)

Turbidity Determination

The turbidity determination can be performed by means of visual comparison (Procedure), or instrumentally using a turbidity meter from suitable (Procedure B). The instrumental evaluation of turbidity provides a test that does not depend on the visual acuity of the analyst.

Hydrazine sulphate Solution Dissolve 1.0 g of hydrazine sulfate in water and dilute with water to 100.0 mL. Leave to rest for 4 to 6 hours.

Solution of Hexamethylenetetramine. Dissolve 2,5 g of hexamethylenetetramine in 25.0 mL of water in glass bottle, with stopper, 100 mL.

Suspension Stock of Opalescence. Add 25.0 mL of the solution of ammonium sulfate solution of hydrazine to Hexamethylenetetramine in bottle, mix and leave to rest for 24 hours. This suspension is stable for 2 months, if stored in a glass container free of surface defects. The suspension should not adhere to the glass and must be mixed before use.

Preparation of Standard Opalescence. Prepare a suspension through dilution of 15.0 mL of suspension stock of opalescence with water for 1000,0 mL. The standard preparation of opalescence is stable, for approximately 24 hours after preparation.

Suspensions of Reference. Prepare according to **Table 2. Mix and shake before use. Stable Suspensions of freshly prepared formazine that can be used to prepare stable patterns are commercially available and can be used after the comparison with standards prepared as described.**

Table 2 – Preparation of suspensions of reference.

	Reference Suspension To	***Reference Suspension B***	***Reference Suspension C***	***Reference Suspension D***
Pattern of Opalescence	5.0 Ml	10.0 Ml	30.0 Ml	50.0 Ml
Water	95.0 Ml	90.0 Ml	70.0 Ml	50.0 Ml
Nephelometric Turbidity Unit	3 UTN	6 UTN	18 UTN	30 UTN

Procedure A. Visual Comparison – Use test tubes identical, glass colorless; transparent and neutral; with a flat base and an internal diameter of 15 to 25 mm. Fill tube with a length of 40 mm with the Preparation *S,* a tube of the same length with water and four other tubes of the same length with the Suspensions of Reference A, B, C and D. Compare the preparations in daytime running light diffuse 5 minutes after the preparation of Suspensions *of Reference, viewing,* vertically, against a black background. The conditions of light must be such that the Suspension of The Reference can be readily distinguished from the water and that the Suspension of Reference B can be readily distinguished from Suspension of Reference A.

Limit. The Preparation *S* should not be more opalescent glass from which the Suspension of Reference B for the covers of the Type I, and not more opalescent glass than the Suspension of Reference

C for the covers of Type II. The Preparation *S* is considered clear if the clarity is the same as that of the water when examined as described above, or if your opalescence is not more pronounced than the Suspension of The Reference (see **Table 3).**

Table 3 – Method of comparison of turbidity developed in preparations.

Requirements of Opalescence	***Procedure A (visual)***	***Procedure B (Instrumental)***
Caps Caps of Type I	Not more opalescent glass than the Suspension B	Not more than 6 UTN
Caps Caps of Type II	No more opalescent glass from which the Suspension C	Not more than 18 UTN

Procedure B. Comparison Instrumental: Measure the turbidity of Suspensions of Reference in a turbidity meter from calibrated properly. The white should be tested and corrected results for the white. The Suspensions of Reference A, B, C and D represent 3.6.18 and 30 Units of Turbidity Nephelometric (UTN) respectively. Measure the turbidity of the Solution S using the turbidity meter from calibrated.

Limit. The turbidity of the Solution S must not be greater than that for the Suspension of Reference B (6 UTN) for the covers of Type I, and is not greater than the Suspension of Reference C (18 UTN) for the covers of Type II (Table 3).

Determination of Color

Standard Color. Prepare a dilution 3.0 mL of Fluid of Correspondence with 97.0 mL of dilute hydrochloric acid.

Procedure. Use identical neutral glass tubes, colorless, traraparerte with a flat bottom and Internal 15 to 25 mm. Put in a pipe, the *Preparation S,* forming a liquid column of 40 mm in length and a second Color Standard forming the same liquid column. Compare the liquids in diffuse daytime running light, viewing, vertically, against a white background.

Limit. The Preparation *S* should not be more intensely colorless, than the Default Color.

Acidity or Alkalinity

Solution of Bromothymol Blue. Dissolve 50 mg of bromothymol blue in a mixture of 4 mL of sodium hydroxide to 0.02 M and 20 mL of alcohol. Dilute with water to 100 mL.

Procedure. Add 0.1 mL of bromothymol blue solution to 20 mL of the Preparation S.

If the preparation be yellow, titrate with sodium hydroxide to 0 701 *M until the blue end point is reached.*

If the preparation becomes blue, titrate with 0.01 *M hydrochloric acid until the end point yellow is reached.*

If the preparation turns green, it is neutral and is not required for titration.

Correction of White. Test 20 mL of white in a similar way. Correct the results obtained for the Preparation S through the subtraction or addition of the volume of titrant required to white, as appropriate.

Limit. Not more than 0.3 mL of sodium hydroxide to 0.01 M produces a blue color, or not more than 0.8 mL of 0.01 M hydrochloric acid produces a yellow color, or titration is not required.

Absorbance

Procedure. Perform this test in the space of time of 5 hours after developing the Preparation S. Filter Preparation S through a filter with pore 0.45 µm, discarding the first 70 mL of the filtrate. Measure the absorbance of the filtrate at wavelengths between 220 and 360 nm in a 1 cm cell using the white in a cell of correspondence in a reference beam. If the dilution of the filtrate is required before the measurement of the absorbance, correct the test results for dilution.

Limit. The absorbances at all these wavelengths should not exceed 0.2 for the covers of the Type I or 4.0 for the covers of Type II.

Reducing Substances

Procedure. Perform this test in the space of time of

Hours after developing the Preparation *S. A 20.0 mL of Preparation S add 1 mL of dilute sulfuric acid and*

Ml of potassium permanganate to 0.002 *M. Boil for 3 minutes. Cool, add 1 g of potassium iodide, and titrate immediately with sodium thiosulphate 0.01* M, using 25.0 mL of starch solution TS as indicator. Carry out the titration using 20.0 mL of white and noted the difference in the volume of sodium thiosulphate to 0.01 *M* needed.

Limit. The difference between the volumes of titration should not be greater than 3.0 mL for the covers of Type I and must not be greater than 7.0 mL for the covers of Type II.

Heavy Metals

Procedure. Proceed as directed for the Method

In Heavy Metals. Using 10.0 mL of Preparation S, in preparation problem.

Limit. 2 PPM of heavy metals such as lead.

Extractable Zinc.

Test Solution. Prepare a Test Solution by means of a dilution of 10.0 mL of Preparation S to 100 mL with hydrochloric acid to 0.1 M. Prepare the white test similarly, using white for the Preparation S.

Standard Solution of Zinc. Prepare a solution (10 ppm Zn) by dissolving zinc sulphate in hydrochloric acid 0.1 M.

Reference Solutions. Prepare at least 3 Reference Solutions by means of a dilution of the Standard Solution of Zinc with hydrochloric acid 0.1 M. The concentrations of zinc in these Reference Solutions are the extension of the expected limit of the Test Solution.

Procedure. Using an atomic absorption spectrometer; adequate and equipped with a source of electromagnetic radiation, and an appropriate air acetylene flame. An alternative procedure as an analysis by mass spectrometry or optical emission spectrometry with inductively coupled plasma, properly validated can be used.

Test each of the Reference Solutions in wavelength for Zinc selected at 213.9 nm, at least 3 times. Register stable readings. Rinse the equipment with the solution white, every time to ensure that the reading returns to the initial value of the white. Prepare a calibration curve from the average of the readings obtained for each Reference Solution. Record the absorbance of the Test Solution. Determine the concentration of zinc in ppm of the Test Solution using the calibration curve.

Limit. The Preparation S contains, at most, 5 ppm of zinc extracting.

Ammonium

Solution of Tetraiodomercurate (II) Potassium Alkaline. Prepare a 100 mL solution containing 11 g of potassium iodide and 15 g of mercury iodide in water. Immediately before use, mix 1 volume of this solution with an equal volume of a solution to 250 g per liter of sodium hydroxide.

Test Solution. Dilute 5 mL of the Preparation S in 14 mL of water. Make alkaline, if necessary, by the addition of 1 M sodium hydroxide, and dilute with water to 15 mL. Add 0.3 mL of the solution of tetraiodomercurate (II) potassium alkaline, and close the container.

Standard Solution of Ammonium. Prepare a solution of ammonium chloride in water (1 ppm of NH4). Mix 10 mL of the solution of 1 ppm of ammonium chloride with 5 mL

of water and 0.3 mL of a solution of tetraidomercurate (II) potassium alkaline. Close the container.

Limit. After 5 minutes, any yellow color in Solution Test should not be darker than the Standard Solution of Ammonium (at most, 2 ppm of NH4 in *Preparation* S).

Volatile Sulfide

Procedure. Put the caps, cut if necessary, with a total area of (20 ±2) cm2 in a 100 mL bottle, and add 50 mL of a solution of citric acid (20 g per L. In The same way and at the same time, prepare a control solution in a 100 mL bottle separately through the dissolution of 0.154 mg of sodium sulfide in 50 mL of a solution of citric acid (20 g per L. Put a piece of lead acetate paper over the mouth of each bottle, and hold the paper in position, placing on it a weighing bottle inverted. Heat the bottles in an autoclave at 121 ºC ±2ºC for 30 minutes.

Limit. Any black coloring on paper produced by *Preparing S is not* more intense than that produced by control solution.

FUNCTIONAL TESTS

The samples treated as described for the Preparation *S* and the air-dried should be used for the Tests of Functionality; of Penetrability; Fragmentation and Self-sealing Ability. The Functionality Tests are performed in caps intended to be penetrated by a hypodermic needle. The test of Capacity Self- Sealant is required only for caps designed for multiple-dose containers. The needle specified for each test is a hypodermic needle lubricated with bisel long (angle of the bevel 12 ±2º) [2].

Penetrability

Procedure. Fill 10 bottles suitable for nominal volume, with water, adjust the caps to be examined and close the bottles with their cover. Using a new hypodermic needle, as already described, for each cover, drill the cover with the needle perpendicular to the surface.

Limit. The strength for drilling of each cover must not be greater than 10 N (1 kgf), determined with an accuracy of ±0.25 N (25 gf).

Fragmentation

Caps for Liquid Preparations. Fill 12 bottles cleaned with water up to 4 mL less than the rated capacity. Adjust the covers that will be examined, close with a lid and leave to rest for 16 hours.

Lids for Dried Preparations. Adjust the covers to be examined in 12 bottles clean and close each one with a cover.

Procedure. Using a hypodermic needle as described previously, adjusted to a clean syringe, inject within each bottle 1 mL of water, while you remove 1 mL of air. Repeat this procedure 4 times for each cover, drill each time in a different location. Use a new needle for each lid, making sure it is not inch during the test. Filter the total volume of liquid in all flasks through a simple filter with nominal pore size not greater than 0.5 µm. From the fragments of rubber on the surface of the filter visible to the naked eye.

Limit. no more than 5 fragments visible. This limit is based on the assumption that the fragments with a diameter greater than 50 µm are visible to the naked eye. In case of doubt or dispute, the particles are examined microscopically to check their natures and sizes.

Self-sealing Ability

Procedure. Fill 10 bottles suitable with water up to the nominal volume. Adjust the covers to be examined and capping. Using a new hypodermic needle as previously for each cover, pierce each cover 10 times, each time in a different location. Immerse 10 bottles in a solution of methylene blue in 0.1% (1 g per L), and reduce the external pressure of 27 kPa for 10 minutes. Restore the atmospheric pressure, and leave the vials immersed for 30 minutes. Rinse the outside of the bottles.

Limit. None of the bottles should contain any trace of blue solution.

6.2.3 PLASTIC CONTAINERS – PERFORMANCE TESTS

In this section are proposed standards for the functional properties of plastic containers and their components used for storing medicines. The following tests are established to determine the permeability to moisture and light transmission of plastic containers applicable to each type of packaging.

A container intended to provide protection to light, or presented as container resistant to light must satisfy the requirement of *Test of light Transmission* (6.2.3.5), where protection, or the resistance is due to the properties specific to the material from which the container is composed, including any coating applied to it. A container clear and colorless, or translucent, manufactured as *resistant to light* by means of inclusion of composite opaque is exempt from the requirements of item *Tests of light Transmission* (6.2.3.5). As used in this chapter, the term container refers to the complete system covering the container itself, the coating when used, closing in the case of containers of multiple units and the caps and *blister* in cases of containers of single dose.

2 Refers to ISO 7864, hypodermic needles sterile, single-use with an external diameter of 0.8 mm (size 21).

6.2.3.1 CONTAINERS OF MULTIPLE UNITS FOR BOMBS AND TABLETS

Desiccant. Place a quantity of anhydrous calcium chloride (1.4 to 8 mesh in a shallow container, taking care to exclude any fine dust, dry to 110 ºC during one hour and cool in a desiccator.

Procedure. Select 12 containers of sizes and type uniforms, clean the surfaces of closing with a cloth free of fibers, close and open each container 30 times. Cap tightly and evenly every time that the container is closed. Capping the containers with screw cap with movement of torque which is within the range specified in Table 1. Add desiccants 10 containers, *containers designated tests,* fill each one up to 13 mm of closing if the volume is 20 mL or higher, or fill each one up to two-thirds of capacity if the volume of the container is less than 20 mL. If the internal part of the container has more than 63 mm of depth, a funnel or inert spacer must be placed on the bottom to minimize the total weight of the container and the desiccant; layer of desiccant in such container must not be less than 5 cm in depth. Close each one, immediately after the addition of the desiccant, applying the torque designated in Table 1 in the case of containers with screw cap. For each of the

Containers remnants, designated as controls, add a sufficient number of glass beads to achieve a weight approximately equal to those of *containers tests and* close by applying the torque designated in Table 1 in the case of containers with screw cap. Record the weight of the containers, individually, therefore, prepared to the nearest 0.1 mg if the volume of the container is less than 20 mL, or until the approximation in mg more next if the volume of the container is 20 to 200 mL, or until the approximation in 0,1 (10 mg) if the volume is 200 mL or higher. Store the relative humidity of (75 ±3) % and at a temperature of 23 ºC ±2 ºC. A system saturated with 35 g of sodium chloride for each 100 mL of water placed on the background of the desiccator maintains the humidity specified, or other methods may be employed to maintain these conditions. After 336 h ± 1 h (14 days), record the weight of the containers individually in the same way. Fill out completely, 5 empty containers of the same size and type of containers *tests with* water or a solid not compressible, free flow such as glass beads small well accommodated up to level indicated by the surface of the clamp. Transfer the contents of

Each container to a graduated cylinder, and determine the average volume of container in mL. Calculate the rate of permeability to moisture, in mg per day per L, By means of the formula:

$$(1000/14V)[(T_F\text{-}T_I)\text{-}(C_F\text{-}C_I)]$$

Whereas

V is the volume in mL of the container, *(TF – T)* is the difference in mg between the initial and final weight of each *container test; (CF – C)* is the difference in mg between the final average and the initial mean weights of 2 controls.

For containers used in medicines dispensed under prescription, the containers so tested are of type sealed containers, if not more than one of 10 *containers tests* exceed 330 mg per day per L in permeability to moisture, and none exceed 655 mg per day for L. To containers used for medicines dispensed under prescription, the containers are well closed if not more than one of 10 containers tests exceed 2000 mg per day per L in permeability to moisture and none exceed 3000 mg per day per L.

Table 1 – Torque applicable to container with cover type thread.

The Closing Diameter[a] (mm)	*Clamping Range suggested with torque manually applied[b] (inches/pounds)*
8	5
10	6
13	8
15	5-9
18	7-10
20	8-12
22	9-14
24	10-18
28	12-21
30	13-23
33	15-25
38	17-26
43	Detection 17-27
48	19-30-
53	21-36
58	23-40
63	25-43
66	Between 26 and 45
70	28-50
83	32-65
86	40-65
89	40-70
100	45-70
110	45-70
120	95
132	60-95

a – torque designated for the next largest diameter clamp must be applied in *containers tests that* have a diameter of close to intermediate diameters listed.

b – use appropriate equipment for measuring torque.

CONTAINERS OF MULTIPLE UNITS FOR BOMBS AND PILLS (without closing)

Polyethylene Container. Close the containers, with stamps inpenetrable obtained by means of heat sealing with an aluminum foil laminated with polyethylene or other suitable sealant. Test containers as described above. The containers of high-density polyethylene, tested meet the requirements if the permeability to moisture exceed 10 mg per day per L, at most, in 1 of 10 *containers tests and* do not exceed 25 mg per day per L in none of them. The

containers of low density polyethylene, thus, tested meet the requirements if the permeability to moisture exceed 20 mg per day per L, at most, in 1 of the 10 containers tests and do not exceed 30 mg per day per L in none of them.

Containers of Polypropylene. Close the containers, with stamps inpenetrable obtained by means of heat sealing with an aluminum foil laminated with polyethylene or other suitable closing. Test containers as described above. The containers meet the requirements if the permeability to moisture exceed 15 mg per day per L, at most, in 1 of the 10 *containers tests and do not exceed 25 mg per day per L in none of them.*

6.2.3.2 CONTAINERS OF UNIT DOSE AND SINGLE DISPENSATION FOR BOMBS AND TABLETS

To allow a reasoned assessment in relation to the suitability of the packaging for a specific type of product, the procedures and classification schemes below are presented to evaluate the characteristics of permeability to moisture of containers for single unit and for unit dose. Since the performance of the equipment and the operator can affect the penetration of moisture into a container formed or closed, the characteristics of moisture penetration of the packaging system used should be determined.

Desiccant. Dry the pads desiccants appropriate to 110 ºC for 1 hour before use. Use pads with approximate weight of 400 mg each and with a diameter of approximately 8 mm. If necessary, due to the limited size of the container of unit dose, can be used pads weighing less than 400 mg each and with a diameter less than 8 mm.

PROCEDURE

Method I. Seal not less than 10 containers of unit dose with a pad each one, and seal 10 additional units of unit dose containers empty for control, using fingers of gloves or tweezers padded to manipulate the sealed containers. Renumber the containers and record the weights, individually, with the approximation in mg more next. Weigh the controls as a unit and divide the total weight by the number of controls to obtain the average.

Store all containers the relative humidity of (75 ±3) % and at a temperature of 23 ºC ±2 ºC. A system saturated with 35 g of sodium chloride for each 100 mL of water placed on the bottom of a desiccator maintains the humidity specified, or other methods may be employed to maintain these conditions. After an interval of 24 hours, and in each one of its multiple, remove the containers from the camera, and leave balance during 15 to 60 minutes in the weighing area. Again record the weight of the containers individually and the controls combined in the same way. If no indicator tablet become pink during the procedures, or if the increase in weight of the pad exceeds 10 %, finish the test and consider only valid the first determinations. Return the containers to the camera from moisture. Calculate the rate of penetration of moisture in mg per day of each container using the formula:

$$(1/N)[(W_F - W_I) - (C_F - C_I)]$$

Whereas

N is the number of days in the period expired test (starting after the 24 hours of initial balance period);

$(W_F - W_I)$ is the difference in mg between the final and initial weights of each receptacle test;

$(C_F - C_I)$ is the difference in mg between the final and initial average weights of controls, with the calculated data with respect to two significant figures. When the penetration measured is less than 5 mg per day, and when it is observed that the controls they achieve balance in a period of 7 days, the individual penetration can be determined more precisely, using the container test of 7º day and the container control as W{ e C, respectively, in the calculations. In this case, an adequate range of test for Class A should not be less than 28 days from the time of balance of 7º day (a total of 35 days).

Method II. Use this procedure for packaging, such as cardboards that can be perforated, which incorporate a number of blister packs or containers sealed unit dose, separately. Seal a sufficient number of packages, at the very least, 4 and a total of at least 10 containers of unit dose or blisters filled with an orifice in each unit to be tested. Seal a corresponding number of empty packages, each containing the same number of containers of unit dose blister packs or equal to those used in packaging testing, as controls. Store all containers at a relative humidity of (75 ±3) % and at a temperature of 23 ºC ±2 ºC. A system saturated with 35 g of sodium chloride for each 100 mL of water, placed at the bottom of the desiccator keeps the moisture required, or other methods may be employed to maintain these conditions. After 24 hours and every 24 hours thereafter, remove the packaging from the camera and leave that to counterbalance ambient temperature for approximately 45 minutes. Record the weights of individual packages and returns them to the camera. Weigh the packages as a control unit and divide the total weight by the number of packages control to obtain the average weight of the empty packaging.

Indicator tablet turn to pink during the procedure or if the average weight of the pad exceeds 10% in any one of the packagings, finish the test and consider only valid the first determinations. Calculate the average rate of moisture penetration, in mg per day for each container of unit dose or *blister,* in each package in accordance with the formula:

$$(1/NX)\ [(W_F - W_I) - (C_F - C_I)\ ]$$

Whereas

N is the number of elapsed days within the period of the test (starting after the 24 hours of initial balance period);
X is the number of sealed units separately per package;
(WF – W) is the difference in mg between the initial and final weights of each packaging test;

(CF – C) is the difference in mg between the final and initial average weight of packaging control, being these calculated rates up to two significant figures.

Limits. The containers of individual unit dose, as tested in Method I, are classified as Class A is, at most, 1 of 10 containers tested exceed 0.5 mg per day in penetration rate of moisture and no more than 1 mg per day; are classified as Class B if, at most, 1 of 10 containers tested exceed 5 mg per day and no more than 10 mg per day; are classified as Class C if, at most, 1 of 10 containers tested exceed 20 mg per day and no more than 40 mg per day and are classified as Class D if the receptacles tested not satisfying any one of these requirements of penetration rate of moisture.

The packagings, how they are tested in Method II, are classified as Class A if no packaging tested exceed 0.5 mg per day of penetration rate of average moisture per blister; are classified as Class B if no packaging tested exceed 5 mg per day of penetration rate of moisture on average per blister pack; they are classified as Class C if no package exceed 20 mg of humidity rate on average per blister pack and are classified as Class D if no packaging tested meet the requirements of penetration rate of moisture on average per blister pack mentioned above.

With the use of desiccant described in Method I and Method

, After every 24 hours, the receptacles tests and controls are heavy; the testing intervals appropriate for the final weighings, WF and CF, shall be the following: 24 hours for Class D; 48 hours for Class C; 7 days for Class B, and at least 28 days for Class A.

6

6.2.3.3 MULTI REMOTE ENTRY CONTAINERS OF DOSE AND DOSE DISPENSATION FOR LIQUIDS

The standards and the tests presented in this section are used to measure the functional characteristics and performance of plastic containers used for packaging products

Aqueous through measurement of weight loss of liquid water as a percentage of its contents. This test, also, can be used to demonstrate a functional comparison and performance. During the entire procedure, determine the weights of the individual systems of closing of containers (container, sealing internal if used, and closing) both as weights of tara and weights of bottling, to an approximation of 0.1 mg if the maximum capacity is less than 200 mL; an approximation in mg if the maximum capacity is between 200 and 1000 mL or an approximation in centigrams (10 mg) if the maximum capacity is 1000 mL or higher.

Procedures for Testing of Closed Containers marketed (bunghole if applicable, internal seal and cover). Select 10 containers of type and size uniform and clean the sealing surfaces with a cloth free of fibers. Assemble each container with the bunghole, if applicable, and closing system. Renumber each clamp system and record the weight tared. Remove the closings and with the aid of a pipette, fill the containers with water up to the maximum capacity. Assemble the containers with the seals and apply the closings. If they are used screw caps, apply the torque specified in Table 1 in Containers *of multiple units for capsules and tablets* (6.2.3.1) and store the closed containers at a temperature of 25 ºC ±2 ºC and relative humidity of (50 ±2 %. After 168 h ± 1 h (7 days), record the weight of the containers individually. Return the containers to the place of storage for over 168 h ±1 h. after the second period of 168 h ± 1 h, remove the containers, record the weights of each system of container, individually, and calculate the rate of penetration of water vapor, in percentage of weight loss of water, for each container by means of the formula:

$$(W_7 - W_{14})\ 365 \times 100/(W_7 - W_T)7 = \text{Percentage per year}$$

Whereas

W_7 is the weight, in mg of container to 7 days;

W_{14} 4 is the weight in mg of container to 14 days;

W_T is the tare weight in g;

7 Is the time to test in days, after 7 days of period of balance. The containers so tested meet the requirements and are considered as containers tightly sealed if the percentage of weight loss of water exceeds 2.5% per year, at most, in 1 of the 10 containers tested and does not exceed 5.0 %, per year, in none of them.

The containers of unit dose liquids for complying with the requirements of a container tightly sealed if the average weight in weight loss of water is less than or equal to 2.5% (p/p) per year and 5% at the end of 2 years.

Procedure for Testing of Containers of Multiple Doses in Conditions of Use. Select 10 containers of type and size uniform. If you used an internal sealing, open containers, carefully, and remove the seals inside of each one. Assemble each container with spigot, if applicable, and your closing system. Renumber each clamp system of container and record the tare weight.

Open and close the containers 30 often, taking care not to lose liquid during this procedure. Close the containers with screw cap inside the torque range presented in Table **1** in Containers **of multiple units for capsules and tablets (6.2.3.1)** and store the sealed containers at a temperature of 25 ºC ±2 ºC and relative humidity of (50 ±2 %. After 168 h ± 1 h (7 days), record the weight of the containers, individually. Return to the place of storage during more 168 h ±1 h. After the second period of 168 h ± 1 h, remove the containers, record the weights of each system of container, individually, and calculate the rate of penetration of water vapor, in percentage of weight loss of water to each container by means of the formula:

$$(W_7 - W_{14})\ 365 \times 100/(W_7 - W_T)7 = \text{Percent per year}$$

Whereas

W_7 is the weight, in mg of container to 7 days;

W_{14} is the weight in mg of container to 14 days;

W_T is the weight tared in g and 7 is the test time in days, after 7 days of period of balance.

The containers so tested meet the requirements and are considered as containers tightly sealed if the percentage of weight loss of water exceeds 2.5% per year, at most, in 1 of the 10 containers tested and does not exceed 5.0% in any of them.

6.2.3.4 TEST OF LIGHT TRANSMISSION

Equipment. Using a spectrophotometer for sensitivity and accuracy, appropriate, adapted, to measure the amount of light transmitted by plastic materials, glass or translucent or transparent, used as pharmaceutical containers. Additionally, the spectrophotometer must measure and record the transmitted light diffuse as well as parallel rays.

Procedure. Select sections to represent the average thickness of the wall of the container. Circular Cut sections of two or more areas of container and trimming the necessary to provide segments of convenient sizes for their insertion in a spectrophotometer. Cut, wash and dry each sample, taking care to avoid scratches on the surface. If the sample is too small to cover the opening in support of sample, cover the discovery portion of opening with an opaque paper or adhesive tape, causing the length of the sample is larger than the opening in spectrophotometer. Immediately before assembling the sample holder, clean the sample with a fabric suitable for cleaning lenses. Assemble the sample with the aid of a viscous wax, or by means of other convenient means, taking care not to leave fingerprints or other marks on the surfaces through which the light must pass. Place the section in spectrophotometer with its cylindrical axis parallel to the plane of the opening and approximately centered in relation to the opening. When properly placed, the light beam is normal to the surface of the section and the losses by reflection are minimal.

Measure, continuously, the transmittance of the section with reference to air in wavelength of interest,

Limit. The transmission of light observed must not exceed the limits listed in Table **1** for containers intended for parenteral use. With a recording equipment or at intervals of approximately 20 nm with an equipment manual, in wave amplitude between 290 to 450 nm.

Table 1 – Limits for plastics classes I-VI.

Nominal Size (in mL)	*Maximum Percentage of light transmission at any wavelength between 290 and 450 nm*	
	Containers Termoselados	*Hermetically sealed Containers*
1	50	25
2	45	20
5	40	15
10	35	13
20	30	12
50	15	10

Any container, with an intermediate size of listed in Table **1,** presents a transmission not larger than the next largest size, listed in the table. For containers larger than 50 mL, apply the limits to 50 mL.

The transmission of light observed for plastic containers for products intended for oral administration or topical should not exceed 10% in any wavelength within the range of 290 to 450 nm.

6.2.4 BIOCOMPATIBILITY

In this section there are guidelines on procedures for the evaluation of the biocompatibility of plastic containers for medicines, lids of elastomer and correlates. The biocompatibility refers to the tendency of these products remain, biologically inert, when in contact with the body. In combination with the chemical tests, the biological processes can be used to detect and identify the inherent toxicity or acquired of correlates, before or during its manufacture and processing.

The procedures used for Evaluate the Biocompatibility of a correlative or its constituents were classified in a panel of biological effects or procedures of toxicity such as cytotoxicity, sensitization, irritation or reactivity Intracutnea, Acute systemic toxicity, subchronic toxicity (acute toxicity), genotoxicity, Application, Hemocompatibility, chronic toxicity (Prolongs in 10% the life expectancy of animal testing, or for more than 90 days), carcinogenicity, reproductive toxicity or development and biodegradation.

The pyrogenicity, in an area of special toxicity, is evaluated by the Test for Bacterial Endotoxins (5.5.2.2) and Test of Pyrogens (5.5.2.1). There is Currently no chapters that detail about awareness, subchronic toxicity, genotoxicity, chronic toxicity, carcinogenicity, Hemotoxicidade, reproductive toxicity or requirements of biodegradation test.

6.2.4.1 PLASTIC CONTAINERS AND LIDS OF ELASTOMERS

The plastic containers may be composed of polymers, which do not feature extraction, toxicity or does not alter the stability of the packaged product. The test requirements of biocompatibility of containers for medicines are related to Plastic Containers. The plastic, polymer or other portions of these products are tested in accordance with the procedures established in *biological reactivity Tests in vitro* (6.2.5), and those who do not meet the requirements of these tests are not suitable for a container of medicinal products. The materials that meet the requirements *in vitro* qualify- as biocompatible materials, without the need for other tests, and can be used in the manufacture of a container for medicines. If prompted for a description of class (classes I-VI) for plastics or other polymers, the appropriate procedures for test are performed as shown in Tests *of biological reactivity in vivo* (6.2.6) and Designation of Class.

The biocompatibility of an elastomeric material is assessed in two stages, as described in Procedures of *Biological Test in Covers of Elastomer (6.2.2)*.

Unlike plastic or other elastomeric polymers, a material that does not meet the requirements of the first phase of in vitro test, can be considered a biocompatible material, if approved in the second phase – *in vivo,* which consists in *Systemic Injection* and the *Intracutaneous Test* in Tests of *biological reactivity in vitro*

. No distinction of class or type is held between the elastomeric materials that meet the requirements of the first phase of testing and those that meet the second stage, qualifying it as biocompatible materials. The elastomeric materials are not classified in classes I-VI.

6.2.4.2 CORRELATES

The biocompatibility of the plastics, other parties elastomeric polymers and those products is tested according to the procedures described in *biological reactivity Tests in vitro* (6.2.5). If, also, is required a grade designation for a plastic or other polymer, are achieved adequate testing procedures described in *biological reactivity Tests in vivo* (6.2.6).

6.2.4.3 TESTS IN VITRO, IN VIVO TESTS AND DESIGNATION OF CLASS FOR PLASTICS AND OTHER POLYMERS

The requirements of tests in vitro and *in vivo* are developed to determine the reactivity of biological cultures of mammalian cells and the biological response of animals

The elastomeric materials, plastics and other polymers, when in direct or indirect contact with the patient. The reactivity of these biological materials may depend both on its surface characteristics, as of their chemical components extractables. The test procedures can be performed on the material, or an extract of the material under test, unless otherwise indicated.

Preparation of Extracts

Usually the evaluation of the biocompatibility of a correlative integer is not realistic and the use of portions representative, or extracts of selected materials can be a practical alternative for the conduct of tests. When portions or extracts are used, it is important to consider that the raw material may undergo chemical changes during manufacture; the processing and sterilisation of a correlative. Tests, in vitro, of raw material can serve as an important screening process, but the final evaluation of the biocompatibility of the correlative should be performed with parts of the finished product and sterilized.

The extractions can be performed at various temperatures (121.70.50, or 37 ºC), at various time intervals (1.24, or 72 hours) and in means of extracting different. The choice of the means of extraction for in vitro tests includes sodium chloride solution of injection at 0.9 %, or tissue culture medium with or without serum. When the serum-containing medium is used, the temperature of extraction may not exceed 37 ºC. To choose the conditions of extraction, select the temperature, the solvent and the time variables that best simulate the conditions of use of the product. The performance of several tests in various conditions can be used to simulate the variations of conditions “in use”. An assessment of biocompatibility is performed with the finished product and sterilized, although, a careful selection of extraction conditions allow the simulation of the conditions for the production and testing of raw materials.

In vitro Test

When in vitro tests are performed, the sample is biocompatible, if the cultures of cells did not show reactivity greater than the mild (grade 2), as described in biological reactivity Tests in vitro (6.2.5).

In vivo Test and designation of class

According to the definition of injection and deployment described in *biological reactivity Tests in vivo*, Plastics and other polymers are classified in classes I to VI. For description of plastics, or other polymers, the extracts of the test substance are produced in accordance with the procedures described in various ways. To evaluate the biocompatibility, the extracts are inoculated, systemic and intracutaneous, in mice and rabbits. In accordance with the requirements for injection, a plastic or other polymer can be initially classified as I, II, III, or V If, in addition to the injection test is performed the test of deployment with the same material, the plastic or the polymer can be classified as class IV or VI.

6.2.4.4 BIOCOMPATIBILDADE CORRELATES OF

In addition to assessing the correlates for sterility Tests, *in vitro* and *in vivo,* the correlates are evaluated for sensitization, subchronic toxicity, genotoxicity, hemocompatibility, Toxicity Chronic, Carcinogenicity, reproductive toxicity or development and biodegradation.

In international guidelines there is no indication that the extension of the tests performed for a correlative depends on the following factors: the similarity and the exclusivity of the product in relation to products previously marketed, as considered in Decision Flow chart; the extent and duration of the contact between the product and the patient, as described in the Categorization of correlates and the composition of the material of the product, as considered in sections Decision Flow chart, in vivo Tests and Designation of Classes.

FLOWCHART OF DECISION

The guidelines for the comparison of a correlative with marketed products previously are provided by Decision Flow chart of Biocompatibility (Figure 1).

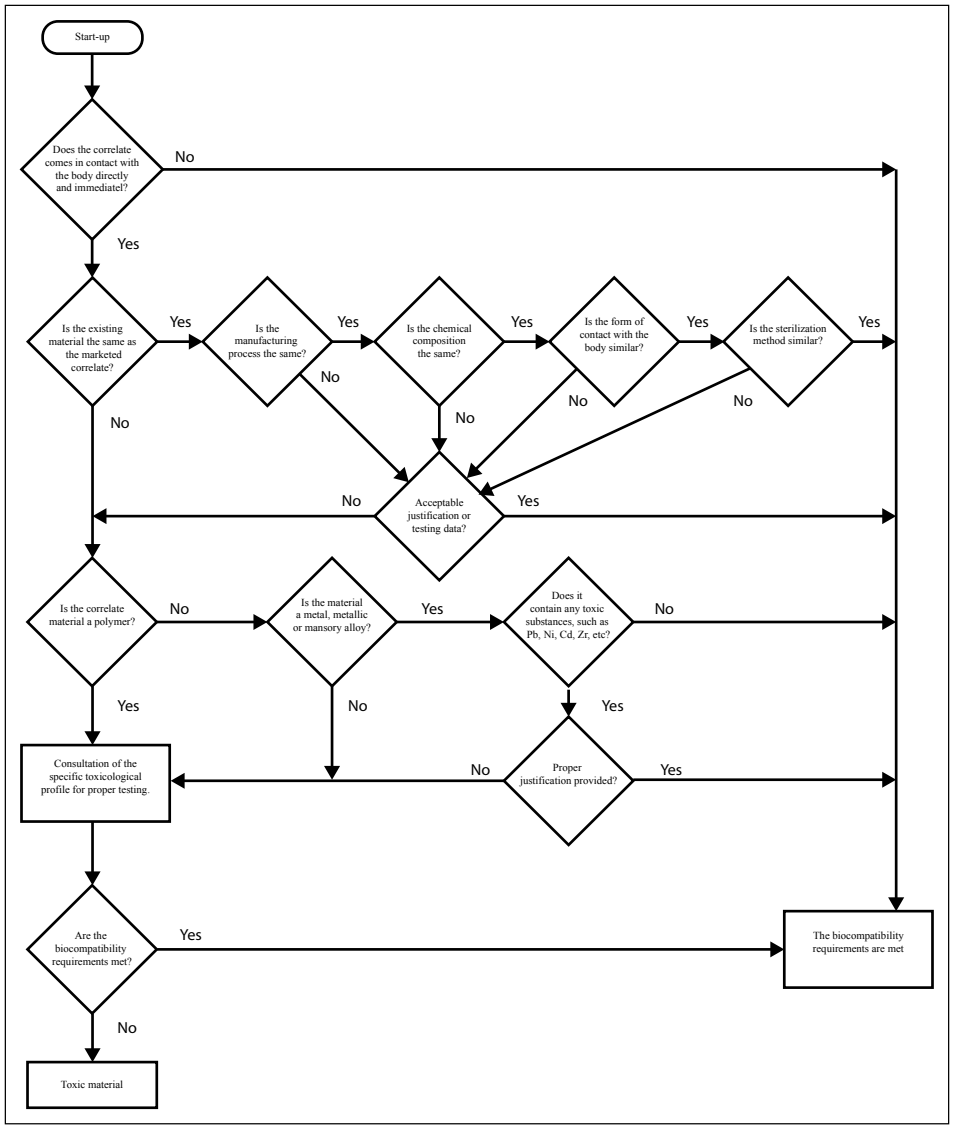

Figure 1 – Flowchart of biocompatibility adapted from the FDA *Blue Book Memorandum # G95-1.*

The goal with the flowchart is to determine if the available data related to previously marketed are sufficient to ensure the safety of correlative in question. As indicated in the flow chart, the composition of the material and the techniques of manufacture of a product are compared with the correlates already marketed, that come in direct contact with the body. In addition, Flow chart there is no requirement for an assessment of the toxicity of an exclusive material that has not been previously used in related products. The responses to the questions raised in the flow diagram leads to the conclusion that the available data are sufficient, or that additional tests are necessary to ensure the safety the product. Guidelines for the identification of appropriate procedures for additional tests are provided in section Array of Selection Test.

CATEGORIZAÇÃO CORRELATES OF

To facilitate the identification of adequate testing procedures, correlates are divided and subdivided, as is recorded in Table 1 according to the nature and extent of your contact with the body. The main categories are related to surface, communication cardiopulmonary bypass and implantable devices. Then, these ratings are subcategorized and examples of correlates belonging to each one of the subcategories (Table 1).

Table 1 – Classification and examples of correlates.

Category of Correlative	*Subcategory of Correlative*	*Nature or Extent of Contact*	*Examples*
Surface	Skin	Correlates that come into contact only with the intact surface of the skin.	External Electrodes, prostheses, retaining straps, compression bandage and monitors of various types.
	Mucosa	Correlates that communicate with intact membranes of the mucosa.	Contact Lenses, urinary catheters, intravaginal devices and intra intestinal (tubes of stomach, sigmoidoscopios, colonoscopios, gastroscopios), endotracheal tubes, broncoscopios, dentures, braces and intrauterine devices.
	Surfaces Compromised or not	Related Conditions that come into Contact with surfaces Corporal compromised or non-undamaged.	Dressings, devices for healing And occlusive bandage for ulcer, Burn and granulated tissue.
	Blood Vessel, Indirect	Correlates that are entering In contact with the blood vessel at a point and serve as input channel to the vascular system.	Set of administration of Solution, transfer and administration of blood, extenders.
Communication Cardiopulmonary bypass	Communication With Tissue, Bone or Dentin	Correlates and materials That communicate With tissue, bone or dentin/pulp system.	Laparoscopes, arthroscopies, systems of Drainage, cement, dental material Dental filling and staples of skin.
	Blood Circulation	Correlates that come into contact with the blood circulation.	Intravascular Catheters, pacemaker electrodes, oxygenators, temporary tube oxygenator extracorporeal and accessories, dialyzers, tube of dialysis and accessories, hemoadsorventes and imunoadsorventes.
Implantable	Tissue or Bone	Correlates that come in contact primarily with the bone, tissue or with the fluid of tissue.	Examples of mold as orthopaedic pins, plates, replacement gaskets, prosthetic devices and bone cements, intra-osseous. Examples of the latter are brand-steps, devices for supply of medicines, sensors and neuromuscular stimulators, tendons of replacement, breast implants, artificial larynxes, subperiosteal implants and staples of connection.
	Blood	Related mainly in contact with blood.	Electrodes of pacemaker, arteriovenous fistula, artificial heart valves, valve graft, catheters of internal administration of medicines and ventricular assist devices.

ARRAY OF SELECTION OF TEST

In the matrix there are guidelines for the identification of appropriate procedures for biological tests for the three categories of correlates: Tests for surface Devices (Table 1 in *Guide to the selection of plastics and other polymers* (as described in section 6.2.4.5)), Tests for Communication Devices Cardiopulmonary Bypass (Table 2 in *Guide to the selection of plastics and other polymers* (as described in section 6.2.4.5)), and Tests for Implantable Devices (Table 3 in *Guide to the selection of plastics and other polymers* (as described in section 6.2.4.5)). Each category is related subcategorized and subdivided according to the duration of contact between the device and the body. The contact duration is defined as limited (less than 24 hours); prolonged (24 hours to 30 days) or permanent (more than 30 days). The biological effects that are included in the matrix are: cytotoxicity, sensitization, intracutaneous irritation or reactivity, systemic toxicity, subchronic toxicity, genotoxicity, deployment, hemocompatibility, chronic toxicity, carcinogenicity, reproductive toxicity or development and biodegradation.

In the matrix, for each subcategory there is an instrument associated with the requirements of test and, generally, the number of tests

Increases as the duration of the contact between the device and the body is extended and in accordance with the proximity of contact between the device and the circulatory system. Within the subcategories, the option to perform additional tests should be considered case by case. The specific situations, such as the use of implantable devices or permanent communication with cardiopulmonary bypass in pregnant women, should be considered by the manufacturer who shall decide as to the inclusion of test of reproduction or development. Guidance on the identification of any additional procedures for testing are provided in the matrix of each subcategory of correlates.

6.2.4.5 GUIDE FOR THE SELECTION OF PLASTICS AND OTHER POLYMERS

Description of Class to Correlate

In Figure 1 there is no guidance for the choice of the designation of the appropriate grade of plastic or other polymer for a correlative and each subcategory of Devices of Surface and in Figure 2 for Communication Devices. The descriptions of class can be found in *biological reactivity Tests in vivo* (6.2.6).

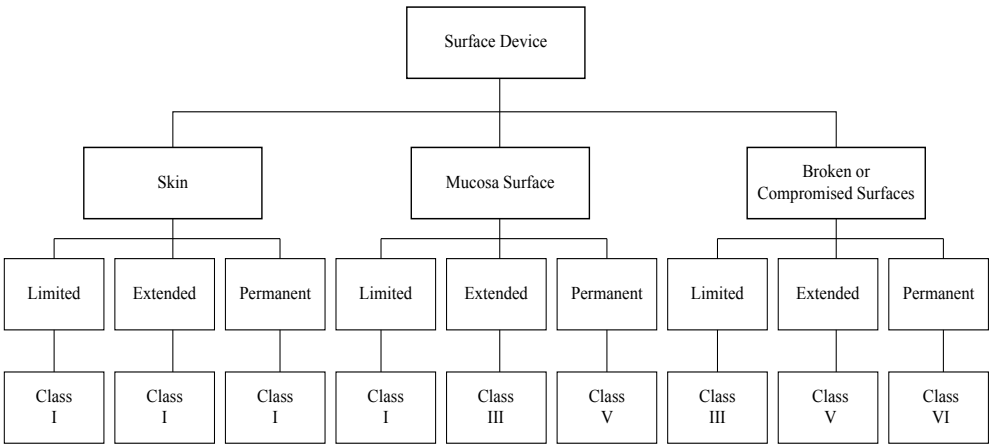

Figure 1 – Requirements of Class of plastics and other polymers for surface devices.

* Categorization based on the duration of contact. Limited: less than 24 hours; prolonged: from 24 hours to 30 days; permanent: more than 30 days. Description Class of Plastics.

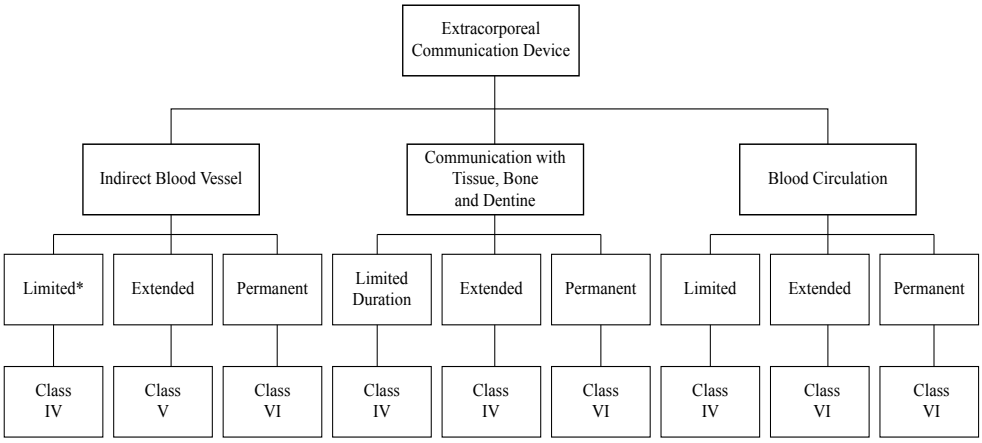

Figure 2– Requirements of Class of plastics and other polymers paradispositivos communication cardiopulmonary bypass.

* Categorization based on the duration of contact. Limited: less than 24 hours; prolonged: from 24 hours to 30 days; permanent: more than 30 days. Description Class of Plastics.

The number of class indicated increases as the duration of contact between the device and the body (risk). In the category of Implantable Devices, the exclusive use of class VI is mandatory. The designation of classes of plastic is based on selection matrices tests illustrated in Tables 1.2 and 3.

The assignment of class of a plastic or other polymer to a subcategory is not intended to restrict the use of higher categories of plastics or other polymers.

Although the assigned name set the lowest numerical class of plastic or other polymer that can be used in corresponding correlative, the use of a class of plastic numerically greater is optional. When a correlative belong to more than one category, plastic, or other polymers must satisfy the requirements of the class numerically higher.

Table 1 – Array of selection tests for surface devices. *

Contact with the Body		***Biological Effect*** [b]											
Contact with the Body	*Duration of Contact* [a]	*Cytotoxicity*	*Awareness*	*Irritation or Reactivity Intracutnea £*	*Systemic Toxicity (Acute)*	*Subchronic Toxicity (acute)*	*Genotoxicity*	*Deployment*	*Hemocompatibility*	*Chronic Toxicity*	*Carcinogenicity*	*Reproductive Toxicity in the development*	*Biodegradation*
Surface Devices													
Skin	A	X	X	X	—	—	—	—	—	—	—	—	—
	B	X	X	X	—	—	—	—	—	—	—	—	—
	C	X	X	X	—	—	—	—	—	—	—	—	—
Mucosa	A	X	X	X	—	—	—	—	—	—	—	—	—
	B	X	X	X	O	O	—	O	—	—	—	—	—
	C	X	X	X	O	X	X	O	—	O	—	—	—
Compromised Surfaces or undamaged	A	X	X	X	O	—	—	—	—	—	—	—	—
	B	X	X	X	O	O	—	O	—	—	—	—	—
	C	X	X	X	O	X	X	O	—	O	—	—	—

a Legenda A: limitada (menos de 24 horas); B: prolongada (de 24 horas a 30 dias); C: permanente (mais de 30 dias).

b Legenda X: Testes de avaliação ISO para consideração; O: testes adicionais que podem ser aplicados.

***** Adaptado do FDA's *Blue Book Memorandum #G95-1*(**Tabela 1**. Testes de Avaliação Inicial para Consideração e **Tabela 2**. Testes de Avaliação Complementar para Consideração).

Table 2 – Array of Selection Tests for Communication Devices Extracorporea. *

Related Categories			*Effect Biologico*[b]											
Contact with the Body		*Duration of Contact* [a]	*Cytotoxicity*	*Awareness*	*Intracutaneous Irritation or Reactivity*	*Systemic Toxicity (Acute)*	*Subchronic Toxicity (acute)*	*Genotoxicity*	*Deployment*	*Hemocompatibility*	*Chronic Toxicity*	*Carcinogenicity*	*Reproductive Toxicity or Development*	*Biodegradation*
Communication Devices with Fabric,	Blood Vessel, Indirect	A	X	X	X	X	—	—	—	X	—	—	—	—
		B	X	X	X	X	O	—	—	X	—	—	—	—
		C	X	X	O	X	X	X	O	X	X	X	—	—
	Communication	A	X	X	X	O	—	—	—	—	—	—	—	—
	Bone or Dentin	B	X	X	O	O	O	X	X	—	—	—	—	—
	Cardiopulmonary Bypass	C	X	X	O	O	O	X	X	—	X	X	—	—
	Blood Circulation	A	X	X	X	X	—	O	—	X	—	—	—	—
		B	X	X	X	X	O	X	O	X	—	—	—	—
		C	X	X	X	X	X	X	O	X	X	X	—	—

a Legend: limited (less than 24 hours); B: prolonged (24 hours to 30 days); C: permanent (more than 30 days),

b Legend X: evaluation Tests ISO for consideration; A: additional tests that can be applied.

* Adapted from the FDA's *Blue BookMemorandum #G95-1* (Table 1. Tests of Initial Evaluation for Consideration and Table 2. Tests of Supplementary Assessment for Consideration).

Table 3 – Array of Selection of Tests for Devices Implantaveis. *

Related Categories			*Effect Biologico*[b]										
Contact with the Body		*Duration of Contact* [a]	*Cytotoxicity*	*Awareness*	*Intracutaneous Irritation or Reactivity*	*Systemic Toxicity (Acute)*	*Subchronic Toxicity (acute)*	*Genotoxicity*	*Deployment*	*Hemocompatibility*	*Chronic Toxicity*	*Carcinogenicity*	*Reproductive Toxicity or Development*	*Biodegradation*
Devices Implantable	Tissue or Bone	A	X	X	X	O	—	—	—	—	—	—	—	—
		B	X	X	O	O	O	X	X	—	—	—	—	—
		C	X	X	O	O	O	X	X	—	X	X	—	—
	Blood	A	X	X	X	X	—	—	X	X	—	—	—	—
		B	X	X	X	X	O	X	X	X	—	—	—	—
		C	X	X	X	X	X	X	X	X	X	X	—	—

a Legenda A: limitada (menos de 24 horas); B: prolongada (de 24 horas a 30 dias); C: permanente (mais de 30 dias).

a Legend: limited (less than 24 hours); B: prolonged (24 hours to 30 days); C: permanent (more than 30 days),

b Legend X: evaluation Tests ISO for consideration; The: additional tests that can be applied.

Adapted from FDA's *Blue BookMemorandum #G95-1 (Table 1. Tests of Initial Evaluation for Consideration and Table 2. Tests of Supplementary Assessment for Consideration).*

1 Document of ISO 10993-1:1997 entitled *Biological Evaulation ofMedicai Devices-Part 1: Evaluation and Testing (*Biological Evaluation of Devices Medicos-Parte 1: Evaluation and Testing).

Adapted from the FDA *'s Blue Book Memorandum #G95-1 ("Use of International Standard ISO-10993. 'Biological Evaluation of Medicai Devices-Part 1: Evaluation and Testing. ' ") ("Use of the International Standards of ISO-10993. 'Biological Evaluation of Devices Medicos-Parte 1: Evaluation and Testing. ' ").*

6.2.5 BIOLOGICAL REACTIVITY TESTS *IN VITRO*

The following tests are designed to determine the reactivity of biological cultures of mammalian cells, after contact with plastics elastomers and other polymeric materials, which come into direct contact, or indirect with the patient, or after contact with specific extracts prepared from the material under test. It is essential that the tests are carried out on the surface area specified. When the surface of the sample cannot be determined, use 0.1 g of elastomer or 0.2 g of plastic, or other material, for each mL of fluid extraction.

Three tests are described: Test of Diffusion in Agar, Direct Contact Test and Test of Elution. The decision of which type or the number of tests to be carried out to assess the potential of the biological response of a specific sample or of an extract, depends on the material, the final product and its intentions to use. Other factors that can also affect the adequacy of the sample for a particular use are: polymeric composition; processing procedures and cleaning; means of contact; dyes; adhesives; absorption, adsorption and permeability of wood preservatives and the storage conditions. The assessment of such factors should be performed by appropriate additional specific tests, before determining that a product produced by means of a specific material, it is suitable for its intended use.

Preparation of Cell Culture. At a minimum essential medium supplemented with serum of seeding density of approximately 105 cells per mL, prepare multiple cultures of fibroblastic cells L-929 cell line (ATCC CCL 1, NCTC clone 929). Incubate the cultures at 37 ºC ±1 ºC in a humidified incubator with an atmosphere of (5 ±1) % of carbon dioxide, for at least 24 hours until the obtaining of monolayer, with confluence exceeding 80 %. Examine the cultures prepared with a microscope to ensure a uniform level of almost confluent monolayers.

Extraction Solvents. Solution of sodium chloride injectable (see relevant monograph). Alternatively may be used means free or supplemented with serum for culture of mammalian cells. The supplementation of whey is used when the extraction is performed at 37 ºC for 24 hours.

Equipment

Autoclave. Employ an autoclave capable of maintaining a temperature of 121 ºC ±2ºC and able to cool the test vessels at around 20 ºC.

Greenhouse Gases. preferably Use a model of mechanical convection, capable of maintaining the operating temperatures in the range of 50ºC to 70 ºC ±2 ºC.

Incubator. Use incubator capable of maintaining a temperature of 37 ºC ±1 ºC and a wet atmosphere with (5 ±1 %) of carbon dioxide in the air.

Containers of Extraction. only Use glass containers Type I, such as test-tube culture with screw cap, or equivalent. The screw cap must have appropriate elastomeric coating. The exposed surface of this coating must be fully protected with an inert solid disc of 50-75 ^m of thickness.

Preparation of Equipment. Clean, completely, the entire glassmaker with cleaning solution of chromic acid and, if necessary, with hot nitric acid, followed by prolonged rinsing with sterile water for injectables. Sterilize and dry the containers and equipment used for extraction, transfer or administration of the test material, by means of appropriate process. If the ethylene oxide is used as sterilizing agent, wait at least 48 hours to complete degassing.

Procedure

Preparation of the Sample for Extract. Prepare as described in Procedure for *biological reactivity Tests in vivo* (6.2.6).

Preparation of Extracts. Prepare as described in Procedure for *biological reactivity Tests in vivo,* using sodium chloride solution injectable (0.9% NaCl) or medium free of serum for culture of mammalian cells as described in Solvent Extraction. If the extraction is performed at 37 ºC for 24 hours in the incubator, use cell culture media supplemented with serum. In any case, the conditions of extraction must cause physical changes, such as fusion or melting of the portions of the material, except a mild adherence.

TEST OF DIFFUSION IN AGAR

This test was developed for elastomeric materials of various models. The agar layer acts as a support to protect the cells from mechanical damage, allowing the diffusion of chemicals leachables of polymeric samples. On a piece of filter paper, are applied the extracts of materials to be tested.

Preparation of the Sample. Use extracts prepared as described or portions of samples with flat surfaces and not less than 100 mm^2.

Preparation of the Positive Control. Proceed as DESCRIBED in *the Sample Preparation.*

Preparation of the Negative Control. Proceed as DESCRIBED in *the Sample Preparation.*

Procedure. Use 7 mL of the cell suspension prepared as described in Preparation of Cell Culture and prepare the layers in plates of 60 mm in diameter. After the incubation, aspirate the culture medium from layers and replaces it with medium supplemented with serum containing quantities of up to 2% of agar. The quality of agar should be adequate to sustain the cell growth.

The agar layer should be thin enough to allow the diffusion of chemicals leachables. Place the flat surfaces of the sample, negative control and a positive control, or its extracts, in contact with the surface of agar solidified, in duplicate. Do not use more than three samples in each card

ready. Incubate all cultures at 37 ºC ±1 ºC for at least 24 hours, in appropriate incubator. Examine, visually, or with a microscope every culture around the sample; negative control and positive control, using appropriate coloring, if necessary.

Interpretation of Results. The biological reactivity, i. e. ma-formation and cellular degeneration, is described

And rated on a scale of 0 to 4 (Table 1). Measure the responses of cell cultures of sample, negative control and positive control. The test system of cell culture is appropriate if the responses observed are classified as 0 (without reactivity) for the negative control and at least 3 (moderate) for the positive control. The sample meets the requirements of the test if the response is not higher than the rating 2 (gently reactive). Repeat the procedure, if the adequacy of the system is not confirmed.

Table 1 – Classification of reactivity for Test of Diffusion in Agar and Direct Contact Test.

Classification	*Reactivity*	*Description of the Area of Reactivity*
0	None	Any detectable around or under the sample.
1	Lightweight	Some cells poorly formed or degenerated under the sample.
2	Gentle	Limited Area the area under the sample.
3	Moderate	Zone extends from 0.5 to 1.0 cm beyond the sample.
4	Strong	Zone extends more than 1.0 cm beyond the sample.

DIRECT CONTACT TEST

This test is set for materials in various formats. The procedure enables simultaneous extractions and testing of chemicals leachables from sample in a medium supplemented with serum. The procedure is not suitable for materials with density too high or too low, because it can cause mechanical damage to cells.

Preparation of the Sample. Use portion of the sample with flat surface not less than 100 mm2.

Preparation of the Positive Control. Proceed as described in the Sample Preparation.

Preparation of the Negative Control. Proceed as described in the Sample Preparation.

Procedure. Use 2 mL of the cell suspension prepared as described in Preparation of Cell Culture, prepare the layers in plates of 35 mm in diameter. After the incubation, aspirate the medium of cultures and replace it with 0.8 mL of fresh culture medium. Put a single sample, negative control and positive control in each one of the duplicates of the culture medium. Incubate all cultures at 37 ºC ±1 ºC for at least 24 hours, in appropriate incubator. Examine, visually, or with a microscope every culture around the sample; the negative control and positive control, using appropriate coloring, if necessary.

Interpretation of Results. Proceed according to the interpretation of Test results of Diffusion in Agar. The sample meets the requirements of the test, if the response of the sample is not greater than the classification 2 (gently reactive). Repeat the procedure, if the adequacy of the system is not confirmed.

ELUTION TEST

This assay is defined for the evaluation of extracts of polymeric materials. The procedure allows the

extraction of samples per time intervals varied and in physiological and non-physiological temperatures. IS It appropriate for materials of high density and assessments of dose response.

Preparation of the Sample. Prepare as described in Preparation of Extracts, using sodium chloride solution injectable (0.9% NaCl) or medium free of serum for culture of mammalian cells as Extraction Solvents. If the sample size cannot be readily measured, can be used a mass of at least 71 g of elastomeric material or 0.2 g of plastic or polymeric material, per mL of medium of extraction. Alternatively, to simulate conditions closer to the physiological, use for the extraction, a culture medium of mammalian cells, supplemented with serum. Prepare the extracts by means of heating at 37 ºC ±1 ºC for 24 hours in an incubator appropriately. Higher Temperatures can cause denaturation of whey proteins.

Preparation of the Positive Control. Proceed as described in the Sample Preparation.

Preparation of the Negative Control. Proceed as described in the Sample Preparation.

Procedure. Use 2 mL of the cell suspension prepared as described in Preparation of Cell Culture, prepare the monolayers in plates of 35 mm in diameter. After the incubation, suck up the middle of the layers and replaces it with extract of the sample; the negative control and positive control. The extracts of media supplemented or not with sera are tested in duplicate, without dilution (100 %). The extract of the sodium chloride solution of injectate is diluted with cell culture medium supplemented with serum and tested, in duplicate, at a concentration of 25 %.

Incubate all cultures at 37 ºC ±1 ºC for 48 hours in an incubator appropriately. Examine with a microscope each culture after 48 hours, using appropriate coloring, if necessary.

Interpretation of Results. Proceed as interpretation of Test results *of Diffusion in Agar,* but using the Table 2. The sample meets the requirements of the test, if the response of the sample is not greater than the classification gently (reactive). Repeat the procedure if the adequacy of the system is not confirmed. For assessments of dose- response, repeat the procedure, using quantitative dilutions of the sample extract.

Table 2 – Classification of reactivity for test of elution.

Classification	*Reactivity*	*Crops Conditions*
0	None	Intracytoplasmatic Granules discontinuous; without cell lysis.
1	Lightweight	Up to 20 % of the cells are round, vaguely united, without granules intracytoplasmatic; lysed cells are occasionally present.
2	Gentle	Up to 50% of the cells are round and devoid of cytoplasmic granules; without extensive cell lysis and empty areas between cells.
3	Moderate	Up to 70% of the layers contain rounded or lysed cells.
4	Strong	Destruction almost full of layers of cells.

6.2.6 BIOLOGICAL REACTIVITY TESTS *IN VIVO*

The following tests are designed to determine the biological response of animals to elastomeric materials, plastics and other polymeric materials, which come into direct contact, or indirect with the patient, or the response to inoculation of specific extracts prepared from the material under test. It is essential to make the specific surface area for extraction. When the surface area of the sample cannot be determined, use 0.1 g of elastomer or 0.2 g of plastic, or other material, for each mL of fluid extraction.

Three tests are described to sort plastics and other polymers, which are applicable to the materials and correlates, based on tests of biological reactivity in vivo. Test of Systemic Injection and Intracutaneous Test are used for elastomeric materials, especially for materials in which the biological reactivity Test in vitro (6.2.5) suitable indicated significant biological reactivity. The Test of Implant is used to check the adequacy of plastics and other polymers, used in the manufacture of containers and accessories; in parenteral preparations, correlates, implants and other systems.

In this chapter the following definitions apply: sample is the material under test, or the extract prepared from a given material. The white consists of the same quantity of the medium that is used for the extraction of the sample, being treated in the same way that the medium that contains the sample analyzed. The negative control is a sample that shows no reaction in the test conditions.

Classification of Plastics. Six classes of plastic are defined (Table 1), based on the responses to a series of in vivo experiments in which the extracts, materials and routes of administration are specified. These tests are, directly related, with the final use of articles of plastic. In preparations in which the plastics are likely to come into contact with the vehicles, the choice of the extraction solution is representative. The classification recorded in Table 1 summarizes the tests to be performed in containers for injectables and medical devices, where there is a need for classification.

Table 1 – Classification of plastics and tests to be performed.

Classes of Plastics						*Tests to be Performed*			
I	***II***	***III***	***IV***	***V***	***VI***	***Test Material***	***Animal***	***Dose***	***Procedures b***
X	X	X	X	X	X	Extract of Sample	Mice	50 Ml/kg	(IV)
X	X	X	X	X	X	In Sodium chloride Solution of injectate	Rabbit	0.2 Ml/ animal in each of the 10 sites	B
	X	X	X	X	X	Extract of Sample of Solution of Alcohol	Mice	50 Ml/kg	(IV)
	X	X	X	X	X	1:20 Solution of Sodium chloride injectable	Rabbit	0.2 Ml/animal in each of the 10 sites	B
		X		X	X	Extract of Sample in Polyethylene glycol 1300	Mice	10 G/kg	The (IP) B
				X	X		Rabbit	0.2 Ml/ animal in each of the 10 sites	
		X	X	X	X	Sample Extract in Vegetable Oil	Mice	50 Ml/kg	The (IP) B
			X	X	X		Rabbit	0.2 Ml/ animal in each of the 10 sites	
			X		X	Strips of implantation of Sample	Rabbit	4 Strips/animal	C

a Tests required for each class indicated with an "x" in the appropriate column.

b Legend: The (IP) *Test of Systemic Injection* (intraperitoneally); (IV) *Test of Systemic Injection* (intravenously); B *Test Intracutaneous* (intracutnea); C *Test* deployment Deployment (intramuscular).

With the exception of Testing of Implant, the procedures are based on the use of extracts that, depending on the thermal resistance of the material, are prepared in one of three standard temperatures: 50.70 and 121 ºC. For this reason, the description of the class of a plastic material must be accompanied by an indication of the temperature of extraction (for example IV-121 °C, is the designation of class IV, of a plastic extracted at 121 ºC; I-50 °C, is the designation of class I, of a plastic extracted to 50 ºC). The plastic can be classified in classes I to VI, based on the criteria of response recorded in Table 1.

This classification does not apply to plastics that are intended to be used as containers for topical products or oral, or which may be used as an integral part of a formulation of the medication. The information recorded in Table 1 does not apply to natural elastomers, which are only tested by means of sodium chloride solution injectable and vegetable oils.

The Test of Systemic Injection and the Intracutaneous Test are designed to determine, respectively, the biological responses to systemic and local; in animals exposed to plastics and other polymers, by inoculation of single dose of specific extracts from the sample. The Test of Implant is designed to assess the reaction of the living tissue to plastics and other polymers, through the deployment of their own sample in animal tissue. The proper preparation and placement of the samples under aseptic conditions are important in performing the Test of Implant.

These tests are designed for application in materials in the conditions in which they are used. If, before his End use, the material should be exposed to any process of cleaning or sterilisation, the tests should be performed on a sample submitted to such processes.

Means of Extraction

Solution of sodium chloride injectable. See relevant monograph.

Solution of Alcohol 1:20 in a solution *of sodium chloride injectable).*

Polyethylene glycol 1300. See relevant monograph.

Vegetable Oil. Use sesame oil, cotton seed oil or other vegetable oils appropriate (see monograph). If possible, obtain newly refined oils. Using three animals duly prepared and inoculate intracutaneamente in each animal a dose of 0.2 mL of oil, in each of the 10 sites, and observe the animals by 24.48 and 72 hours after inoculation. Classify the observations of each site, as the numerical scale indicated in Table 2. In any time of observation, the mean response in 3 rabbits (30 sites of inoculation) must not be greater than 0.5 for erythema, must be less than 1.0 for the edema, and in none of those places can occur a tissue reaction greater than 10 mm in diameter total. The oil residue in the inoculation site should not be interpreted as edema. When pressed gently, tissue edema is whitish.

Water for injectables. See relevant monograph.

Table 2 – Evaluation of the reactions of the skin.

Erythema and Eschar Formation	*Score*
Without erythema	0
Mild Erythema (very little perceptible)	1
Well-defined Erythema	2
Moderate to severe Erythema	3
Severe Erythema (red beet) the lightweight training of bedsore injuries (deep)	4
Edema Formation	*Score*
Without edema	0
Edema very smooth (very little perceptible)	1
Edema (smooth edges with well defined area by increasing accurate)	2
Moderate Edema (approximately 1 mm protrusion)	3
Severe Edema (with more than 1 mm of protrusion and beyond the area of exposure)	4

* Excludes the non-inflammatory edema (mechanical) from white or fluid extraction.

Equipment

Autoclave. Employ an autoclave capable of maintaining a temperature of 121 ºC ±2ºC and able to cool the test vessels at around 20 ºC.

Greenhouse Gases. preferably Use a model of mechanical convection, capable of maintaining the operating temperatures in the range of 50 to 70 ºC ±2 ºC.

Containers of Extraction. only Use glass containers Type I, such as test-tube culture with screw cap, or equivalent. The screw cap must have appropriate elastomeric coating. The exposed surface of this coating must be fully protected with an inert solid disc of 50-75 µm of thickness.

Preparation of Equipment. Thoroughly Clean all glassware with cleaning solution of chromic acid and, if necessary, with hot nitric acid, followed by prolonged rinsing with water. Before use in subdivision of the sample, clean the cutting equipment by means of a suitable method, such as successive cleanings with acetone and methylene chloride. Clean all other equipment by means of a complete flush with Suitable detergent and rinse extended with water. Sterilize and dry the containers and equipment used for extraction, transfer or administration of the test material, by means of appropriate process. If the ethylene oxide is used as sterilizing agent, allow adequate time for complete degassing.

Procedure.

Preparation of the sample. The Test *of Systemic Injection* and the *Intracutaneous Test* can be performed with the same extract or extracts with distinct. Select and subdivide into parts the sample size indicated in Table. Remove the particulate material of each sample subdivided, or the negative control, by placing the sample in a 100 mL measuring cylinder, glass of type I, clean and with lid, and add about 70 mL of water for injectables. Stir for about 30 seconds and drain the water, repeat this step and dry the parts prepared for extraction with oil in an oven up to 165 ºC. Do not clean the sample with dry cloth or wet or wash and rinse with organic solvent, tenside, etc.

Preparation of extracts. Put a sample, duly prepared, to be tested in a container of extraction and add 20 mL of

Table 3 - surface Area of the sample to be used.

Form of Material	*Thickness*	*Quantity of the Sample for each 20 mL of Means of Extraction*	*Subdivided into 1*
Film or foil	<0,5 mm	Equivalent to 120 cm^2 of total surface area (both sides combined)	Parties with approximately 5 × 0,3 cm
	0,5 a 1 mm	Equivalent to 60 cm^2 of total surface area (both sides combined)	
Tube	<0,5 mm (wall)	Length (in cm) = 120 cm^2/ (sum of the circumferences of internal and external diameter)	Parties with approximately 5 × 0,3 cm
	0,5 a 1 mm (wall)	Length (in cm) = 60 cm^2/ (sum of the circumferences of diameter internal and external)	
Strips, tube and molded items	>1 mm	Equivalent to 60 cm^2 of total surface area (both sides combined)	Parties with approximately 5 × 0,3 cm
Elastomers	>1 mm	Equivalent to 25 cm^2 of total surface area (both sides combined)	Without subdivision[2]

the appropriate medium. Repeat these steps for each means of extracting necessary for the test. Prepare, also, a white 20 mL of each medium for injections and parallel comparisons. Remove by heating in an autoclave at 121 ºC for 60 minutes, and in the case of a furnace at 70 ºC for 24 hours, or 165 ºC for 72 hours. Allow sufficient time for the liquid from the container reaches the temperature of extraction. At any time the conditions of extraction must cause physical changes, such as fusion or melting of the parts of the sample, not to result in a decrease of the surface area available. A lightweight grip of the parties can be tolerated. Always Add, individually, the cleaned parts to means of extraction. If the culture tubes are used for the extraction of vegetable oil with autoclave, reseal properly screw caps with adhesive tape pressure sensitive. Cool to room temperature, however, not less than 20 ºC, shake vigorously for several minutes, and immediately decant each extract of aseptic manner, in a sterile container and dry. Store the extracts at a temperature between 20ºC and 30 ºC and do not use for tests after 24 hours.

SYSTEMIC INJECTION TEST

This test is designed to assess the systemic responses to the extracts of materials tested by means of inoculation in mice.

Animal testing. Use albino mice healthy, not previously used, weighing between 17 and 23 g. For each test group, only use the mice of the same origin. Water and food of known composition, commonly used in laboratory animals, are allowed to desire.

Procedure. Before removing the dose of inoculation, shake vigorously, each extract to ensure uniform distribution of the extracted matter. The visible particles should not be administered intravenously. In a test group, inoculate in each one of the five mice to sample or the white, as described in Table **4, diluting each g of the extract of the sample prepared with polyethylene glycol 400 and the corresponding white, with** volumes of sodium chloride solution injectable, to obtain a solution with a concentration of approximately 200 mg of polyethylene glycol per mL.

Table 4 – Procedure for inoculation – Test of Systemic Injection

Extract or White	*Dose per kg*	*Route of Administration* *	*Speed of Inoculation, pL per second*
Alcohol Solution 1:20 solution of sodium chloride injectable	50 Ml	IV	100
Polyethylene glycol 1300	10 G	IP	-
Vehicle of medicines (where applicable)	50 Ml	IV	100
	50 Ml	IP	-
Vegetable Oil	50 Ml	IP	-

* IV = intravenous (aqueous sample and blank); IP = intraperitoneally (sample oily and white).

Observe the animals at the following times: immediately after the inoculation, after 4 hours and, at least after 24.48 and 72 hours. If during the period of observation, none of the animals treated with the extract of the sample presents a biological reactivity significantly higher than those treated with the white, the sample satisfies the requirements of this test. If two or more mice die or submit an abnormal behavior, such as convulsions or prostration, or if you experience loss of body weight exceeding the

G in three or more mice, the sample does not meet the requirements of the test. If any animal treated with the sample show only mild signs of biological reactivity, and if only one animal present severe symptoms of organic reactivity or die, repeat the test using groups of 10 mice. In repetition, during the observation period, all 10 animals treated with the sample must not show any significant biological reactivity to more than those treated with white.

INTRACUTANEOUS TEST

This test was developed to assess local responses to the extracts of materials tested, after inoculation intracutnea in rabbits.

Animal testing. Select healthy albino rabbits, whose by makes it possible be trapped close to the skin, this being, fine and free of irritation or trauma. When dealing with the animals during the observation periods, avoid touching the places of inoculation, except to differentiate a edema and an oil residue. The rabbits previously used in independent tests, such as the test of pyrogen **(5.5.2.1), and that rested the planned period, may be used for this test, since that have skin clean, without stains.**

Procedure. Before removing the dose of inoculation, shake vigorously, each extract to ensure uniform distribution of the extracted matter. On the day of the test, secure, carefully, by the coasts of the animal, on both sides of the vertebral column, on top of a test area large enough. Avoid the irritation and trauma. Remove the loose through vacuum. If necessary, before the inoculation, clean the skin with diluted alcohol and dry. More than a statement of a given material can be used per rabbit, if it is determined that the results are not affected. For each sample, using two animals and inoculate track intracutnea, using one side of the animal for the sample and the other for the white, as described in Table **5.** Dilute each g of the extract of the sample prepared with polyethylene glycol 400, and the corresponding white with 7.4 volumes of sodium chloride solution injectable to obtain a solution with a concentration of approximately 120 mg of polyethylene glycol per mL.

Table 5 – Intracutaneous Test.

Extract or White	*Number of Sites (per animal)*	*Dose, µL per site*
Sample	5	200
White	5	200

Examine the sites of inoculation to highlight any tissue reaction, such as erythema, edema and necrosis. If necessary, gently clean the skin with alcohol diluted to facilitate reading of places of inoculation. Observe all animals 24.48 and 72 h after inoculation. Classify the observations into a numerical scale for the extract of the sample and for the white, using the **Table 2.** If necessary, re-secure the hair during the observation period. The average score of erythema and edema to the locations of the sample and the blank are determined for each rabbit and each score range after 24.48 and 72 hours of inoculation. After the score for 72 hours, all the scores of erythema, edema of the most are summed separately, for each sample and blank. Divide each total by 12 (2 animals x 3 periods of score x 2 categories of score) to determine the average total for each sample versus each corresponding white. The test requirements are met if the difference between the average score of the sample and the blank is less than or equal to 1.0. If in any period of observation, the average for the reaction of the sample is questionable to be greater than the average for the reaction of the white, repeat the test using three additional rabbits. The test requirements are met if the difference between the average score of the sample and the blank is less than or equal to 1.0.

TEST OF IMPLANT

The test of implant is prepared for evaluation of plastics and other polymers when they come into direct contact with living tissue. The proper preparation of strips of implant and its deployment should be performed under aseptic conditions. Prepare for deployment 8 strips of the sample and 4 strips of pattern. Each strip should measure at least 10 x 1 mm. The edges of the strips should be as smooth as possible, to avoid additional mechanical traumas in deployment. The strips of minimum size specified are deployed through a hypodermic needle (size 15 to 19) with intravenous and a trocar tip sterile. Use a needle or other pre-sterilized Whereas sterile plastic strips are inserted, aseptically, or insert each strip clean in a needle whose cannula and the center hole are protected with an appropriate cover, and then submitted to the appropriate procedure for sterilisation.

Animal testing. Select rabbits healthy adults with minimum weight of 2.5 kg, and that possess paravertebral muscles sufficiently large to enable the deployment of test strips. Do not use any muscle tissue than those situated in paravertebral area. The animals must be anesthetized with an anesthetic agent commonly used to a degree of sufficient depth to prevent muscle movements, such as infantile spasms.

Procedure. Perform the test in a clean area. On the test day or up to 20 hours before, attach the hair of animals on both sides of the vertebral column. Remove loose hairs through vacuum. Before inoculation, clean the skin with diluted alcohol and dry it. Deploy Four strips of the sample in paravertebral muscles, distant approximately 2.5 cm of one another, in a side of the column of each of the two rabbits, 2.5 to 5.0 cm from the median line and parallel to

the vertebral column. Similarly, deploy two strips pattern in the muscle opposite of each animal. Insert a catheter in sterile needle to hold the strip implantation in tissue with withdrawal of the needle. After the deployment of a comic strip, if there is an excessive bleeding, put another piece in duplicate in another location.

Keep the animals for a minimum period of 120 hours, and sacrifice unto them at the end of the period of observation with an overdose of an anesthetic agent or other appropriate players. Allow spend enough time to cut the fabric, without bleeding. Examine macroscopically the tissue area around the central part of each strip of implant. Use a magnifying glass and a source of auxiliary light.

Observe if there is hemorrhage, necrosis, discolorations and infections in implantation sites of the sample and the control and record the observations. If there is no encapsulation, measure and record the width of the capsule, rounding to the nearest 0.1 mm, from the periphery of the space occupied by implantation of the control or the sample until the periphery of the dish. Punctuating the package, as shown in Table **6.**

Table 6 – Evaluation of encapsulation in Implant.

Width of Capsule	*Score*
None	0
Up to 0.5 mm	1
0.6-1.0 MM	2
1.1-2.0 MM	3
Exceeding 2.0 mm	4

Calculate the differences between the average score for the sites of sample and control. The test requirements are met if the difference is not more than 1, or if the difference for more than one of the four places of implant, does not exceed 1 in any one of the animals.

7 PREPARATION OF STERILE CLEANROOM COVERALL PRODUCTS

7.1 ESTERILIZAÇÃO AND STERILITY ASSURANCE

Sterility is the absence of viable micro-organisms. As obtaining the sterility of any item isolated from a population subjected to sterilisation process can not be guaranteed and demonstrated, the sterility of a batch is defined in accordance with probabilistic by means of a production process properly validated.

The inactivation of micro-organisms by physical or chemical means follows an exponential law and, therefore, there is a statistical probability that micro-organisms can survive the sterilisation process. For a given process, the probability of survival is determined by the number, type and resistance of micro-organisms present and by environment during sterilisation. The sterility assurance level of a sterilisation process translates the security with which the proceedings in question sterilizes a set of items, and is expressed as the probability of an item not effete Whereas population. The sterility assurance level of 10^{-6}, for example, indicates the probability of not more than a micro-organism in 1 x 106 viable items sterilized by the final product. The sterility assurance level of a process for a given product is established by means of validation studies is appropriate and generally accepted that injectable products or critical devices sterile undergoing terminal sterilisation reach a probability of microbial survival of $10^{-6.}$ With thermos table products, the approach is often exceed the critical time needed to achieve the likelihood of microbial survival of 10^{-6} (death). However, for products, the latexes death approach can not be used and the development of the sterilisation cycle depends on the knowledge of the microbial load of the product.

The D-value, decimal reduction time, is the time in minutes required to reduce the microbial population by 90 %, or 1 logarithmic cycle, to a specific condition, that is, for a fraction survivor of 1/10. Therefore, where the D-value of a preparation of biological indicator of, for example, spores of Geobacillus *stearothermophilus* is Minutes under the parameters of total process, that is, to 121 ºC, if treaty for 12 minutes under the same conditions, you can declare that the increment of lethality is 8 D. The application of this increment in sterilisation of the product depends on the initial microbial load. Assuming that the microbial load of the product presents resistance to sterilisation process equal to the resistance of the biological indicator and that the initial load of the product is 102 micro-organisms, the increment of lethality of D would reduce the microbial load to 1 (theoretically 100) and, consequently, 6 D would result in an additional probability of microbial survival calculated from 10^{-6}. Under the same conditions, an increment of lethality of 12 D can be used as typical approach for obtaining death. Generally, the probability of survival of microbial load in the material, whose process of validation of sterilisation is being carried out, is not the same as the biological indicator.

For use valid, therefore, it is essential that the resistance of the Biological Indicator is greater than that of the bioburden of the material to be sterilized, being necessary take the situation of worst case during the validation. The D value of biological indicator to be employed should be determined or verified for each program of validation and, also, in the event of amendment of this program.

The determination of survival curves, or lifecycle approach fractionated, can be used to determine the D value of biological indicator chosen for the sterilisation process specific. This approach, also, can be used to evaluate the resistance of the bioburden of the product. Dispensing Cycles are used to assess the reduction of the microbial count or to achieve fraction negative. These numbers can be used to determine both the lethality of the process under conditions of production as to establish appropriate sterilisation cycles. A biological indicator suitable, such as the preparation of Geobacillus *stearothermophilus,* also, must be used during the sterilisation of routine. Any method of microbial load, used to guarantee sterility requires adequate surveillance of microbial resistance of item to detect any changes.

7.1.1 STERILISATION METHODS

With a method of sterilisation have-whether by purpose remove, or destroy all forms of life, animal or vegetable, macroscopic or microscopic, saprophytes or not, the product under consideration, without guaranteeing the inactivation of toxins and cellular enzymes. The selected procedure to achieve the level of sterility assurance depends on the knowledge of the nature of the material to be sterilized; the sterilisation process to be employed and the changes that may occur in the material, as a function of sterilisation. The knowledge of the type, quantity and source of contaminants in products, before sterilisation, and the application of methods to minimize such contamination and prevent it post-processing contribute to ensuring the success of sterilisation.

In this chapter are registered concepts and principles involved in quality control of products that must comply with the requirement of sterility and includes description of methods of sterilisation and instructions for aseptic process.

7.1.1.1 PHYSICAL METHODS

7.1.1.1.1 Sterilisation by heat

The heat is the sterilizing agent more simple, economic and safe that if offers, however the sensitivity of different micro-organisms the action of heat is quite varied, being forms sporulating bacteria the most resistant. The efficiency in inactivation of micro-organisms is dependent on the temperature, time of exposure and the presence of water, because in the presence of this are required lower exposure times and temperatures. The sterilisation by moist heat causes the coagulation of proteins of cell micro-organisms, while the sterilisation by dry heat in function of oxidative processes, which require high temperatures and long exposure time.

WET HEAT

The sterilisation process employing saturated steam under pressure is conducted in camera called an autoclave. The basic principle of operation is the replacement of the air of the chamber by saturated steam. To move air more efficiently and camera inside of the products, the sterilisation cycle can include stages of evacuation of air and steam. For this method of sterilisation, the reference condition for sterilisation of aqueous preparations is for heating, at a minimum, 121 ºC for at least 15 minutes. Different Combinations of temperature and time can be used, provided that validated and that demonstrate the effectiveness of the chosen procedure, providing an appropriate level of lethality and reproducible when operated, routinely, within the tolerances established. Are applied procedures and precautions in order to achieve a level of security of sterility of 10^{-6} or better. Time and temperature Combinations should be established based on factors such as nature of the material and its thermolability and alter, penetrability of steam in product to be sterilized and other parameters defined in the process of validation. When used different sterilisation temperature of 121 ºC, the concept of F0 must be employed. The F0 in a particular different temperature of 121 ºC, is the time in minutes required to deliver the lethality equivalent to that provided to 121 ºC during a specified time. F0 is a measure of the effectiveness sterilizing agent, that is, it is the number of minutes of thermal sterilisation by steam at certain temperature supplied to a container or unit of product within a given value Z.

To ensure the efficiency of the sterilisation process, the load distribution in the chamber must be carried out in such a way as to facilitate the contact of steam with the regions of more difficult access. For materials sterilized by moist heat, it is acceptable that reach a probability of microbial survival of the order of 10^{-6}. For thermostable products, the time required to reach the previous condition can be exceeded, resulting in death, which does not apply to products which may change as a function of excessive exposure to Heat. In this situation, the development of the sterilisation cycle depends, in particular, the knowledge of microbial load in the product, which must be determined in substantial quantity of lots of product, previously the sterilisation. The D value of the appropriate biological indicator used, as Geobacillus stearothermophilus, should be assessed in validation program and on the occurrence of any amendment to this program.

DRY HEAT

The thermal sterilisation by dry heat is carried out in a greenhouse with homogeneous distribution of heat, which can be obtained by forced circulation of air. Can be sterilized articles such as glass, metals, powders, vaselines, fats, waxes, solutions and oily suspensions, and special fabrics. This process is applied, especially for sensitive materials sterilisation by moist heat. For this method of sterilisation, the reference condition is a minimum temperature of 160 ºC for at least 2 hours. Different Combinations of temperature and time can be used, provided that validated and that demonstrate the effectiveness of the chosen procedure, providing an appropriate level of lethality and reproducible when operated routinely within the established tolerances.

A sterility assurance level of 10-12 is considered acceptable for thermostable products. An example of biological indicator to validate and monitor the dry heat sterilisation is the preparation of Bacillus atrophaeus spores.

The process using the dry heat, also, can be used for sterilisation and depyrogenation as an integral part of the process of aseptic filling, which requires very high temperatures due to less time of exposure to heat. In continuous processes, usually, there is a need for a cooling stage preceding the process of bottling. On the basis of the shorter time of exposure of the material. With the validation program should cover parameters such as the uniformity of temperature and residence time.

The dry heat at temperatures greater than 220ºC can be used for sterilisation and depyrogenation of glassware. In this case, a challenge with bacterial endotoxin should be part of the validation program, showing a reduction of at least 3 log cycles of endotoxin resistant to heat, i. e. test materials inoculated with at least 1,000 units of bacterial endotoxin. The test, with lysate *Limulus amoebocyte lysate,* can be used to demonstrate that the endotoxin was inactivated at no more than 1/1000 of the original amount, being that the remainder of endotoxin is measured to ensure the reduction of 3 log cycles.

7.1.1.1.2 Sterilisation by ionizing radiation

The ionizing radiation emissions are high-energy, in the form of electromagnetic waves or particles, that when they go plummeting with the atoms of the irradiated material alter its electrical charge by displacement of electrons, transforming the atoms irradiated in positive ions or negative. When these radiation crossing the cells create

hydrogen free hydroxyl radicals; and some peroxides, which in turn can cause different intracellular lesions.

The main sources of radiation are: alpha emitter; beta gamma and X-rays; The two types of ionising radiation in use are decay radioisotope (gamma radiation) and radiation by electron beam. The products are exposed to ionizing radiation in the form of gamma radiation from a source radionuclide ventriculographic adequate (for example, cobalt 60) or an electron beam powered by means of an accelerator of electrons.

Besides the possibility of processing at low temperatures, which enables the sterilisation of products latexes, sterilisation by ionizing radiation has advantages such as low chemical reactivity and the fact that there are few parameters to be controlled, being essential control of radiation dose absorbed. The established radiation dose for sterilisation must ensure the non-impairment of the materials to be sterilized. For the gamma radiation, the validation of the process includes the establishment of the compatibility of the material, the establishment of the model of loading of the product and the mapping of dose in container for sterilisation, by identifying the areas of maximum and minimum dose of radiation, the definition of the exposure time and the attesting the application of sterilisation dose required. For irradiation by electron beam, must be controlled, still, voltage, current, conveyor speed and size of sweep of the electron beam. For this sterilisation process, the absorbed dose of reference is 25 kGy, however in some situations there is a need to select a higher or lower dose. The dose chosen must offer a level of lethality adequate and reproducible when the process is operated routinely within the established tolerances. Procedures and precautions should be applied to achieve a sterility assurance level of 10^{-6} or better.

To validate the effectiveness of sterilisation, especially when using lower doses, it is necessary to determine the resistance to radiation of the microbial load of the product. Patterns of loading of specific product and the distribution of minimum and maximum doses absorbed should be established. The absorbed doses are normally measures by means of specific dosimeters, as plastic support standardized that shows intensification of color proportional to the quantity of radiation absorbed. The approach of discontinuous cycle provides the data used to determine the D10 value of biological indicator, information applied to extrapolate the amount of radiation absorbed, to establish a probability of adequate microbial survival. Currently, the dose is based on resistance to radiation from natural heterogeneous microbial load contained in the product to be sterilized. The validation procedures can use the exposure of product inoculated, using resistant organisms such as *Bacillus pumilus, or exposure of samples of finished product production line to sublethal dose of process.*

The procedure for selection of the radiation dose, based on assessment of resistance of microSterilisation of health care products *Radiation – Part 1: Requirements for development, validation and routine control of a sterilisation process for medical devices*).

The efficiency of sterilisation cycle should be evaluated periodically, by determination of the microbial load of the product, or by employment of biological indicator and by the use of dosimeters calibrated.

7.1.1.1.3 Sterilisation by filtration

The filtration is used for sterilisation of solutions latexes by physical removal of contaminating micro-organisms. The filter material may not release fibers or materials extractables undesirable for the filtered solution, which restricts the nature of the filter element to glass, metal, polymers synthesized and polymeric membranes. The fitting of a filter consists of a porous matrix inserted in a waterproof shelter. The efficiency of an environment, or substrate filter depends on the pore size of the material, the adsorption of micro-organisms on or within the matrix of the filter and the mechanism of sieve or exclusion. The effect of exclusion by size is a function of aperture (diameter) of the pores, and the adsorption depends on the composition, thickness of the filter element and fluid that is being filtered.

The pore size membrane filters is estimated by nominal value which reflects the capacity of the filter membrane to retain micro-organisms represented by specific strains. The filtration for purposes of sterilisation is normally carried out with membranes of graduation of nominal pore size of 0.2 µm, or less. These membranes of sterilizing filtration, classified as 0.22 or 0.2 µam, depending on the manufacturer, are able to retain 100% of a culture containing 107 micro Brevundimonas diminuta ATCC 19146, per cm2 of surface area of membrane filter, under a minimum pressure of 30 psi (2.0 bar).

The user is responsible for choosing the filter as a function of the nature of the material to be filtered, that meets the need of the sterilisation process, and should, also, determine if the parameters employed in production will influence the efficiency of microbial retention. Since the efficiency of the filtration process, is also influenced by the bioburden solution to be filtered, it is important to the determination of the microbial quality of the solutions before the filtration, as well as the establishment of parameters such as pressure, flow rate and characteristics of the filter unit.

The value of logarithmic reduction, also, can be used to evaluate the retention capacity of the membrane filter. For example, a filter of 0.2 |am, which can hold 107 micro-organisms of a strain specific, will have a value of logarithmic reduction of, at least, 7, under conditions declared.

The membrane filters commercially available include cellulose acetate, cellulose nitrate, fluorcarbonate, acrylic polymers, polyester, polycarbonate, polyvinyl chloride, vinyl, nylon, polytef and still, metal membranes. The membrane filters, by being polymeric films, offer many

advantages and some disadvantages when compared to the filters of depth as porcelain or sintered material. As a good part of the surface of the membrane is an empty space or open, the correct assembly and sterilisation filter provide the advantage of a high flow rate. A disadvantage is that, due to the fragility of the membrane, you must ensure the absence of rupture during assembly, sterilisation, or use.

The filtration system must be tested before and after the filtration process to ensure the maintenance of its integrity during the filtration process. Typical Tests of use include the test of point of bubble, the air flow test, the test of diffusive retention under pressure and flow test progressive. The bubble point test consists in non-destructive test, whose name derives from the visualization of bubbles after the application of a predetermined pressure on the filter. As an example, after filtration of approximately two liters of sterile distilled water, applies constant pressure of nitrogen, during 5 minutes for membranes of ester of cellulose 0.2 |am. For each type of filter there is a pressure limit value to be supported, without which presents the formation of bubbles, indicating the resistance of the filter material. The tests must be correlated with the retention of micro-organisms. Additional Tests performed by the manufacturer of the filter, such as the 30-42 challenge, are normally not repeated by the user.

7.1.1.2 CHEMICAL METHOD

7.1.1.2.1 Ethylene oxide Gas

The sterilisation by gas may be the method of choice for materials that do not withstand high temperatures such as in processing by dry heat or damp heat. The active agent usually employed in sterilisation by gas is The ethylene oxide. Among the disadvantages of this sterilizing agent are its mutagenic properties; the possibility of toxic residues in treated material and its nature highly flammable, except when in certain mixtures with inert gases. The sterilisation process is generally carried out in a pressurized chamber designed in a manner similar to the autoclave, but with specific characteristics such as system for degassing after sterilisation and minimisation of exposure of operators to gas.

The program qualification process of sterilisation with ethylene oxide is broader than that of other sterilisation processes, since besides the temperature, must be controlled humidity; positive pressure / vacuum and the concentration of ethylene oxide. It is important to determine and demonstrate that all the critical parameters of the process are suitable within the sterilisation chamber during the entire cycle. As the parameters of sterilisation applied to products to be processed are critical, it is recommended that the pre-conditioning of load to minimize the time of exposure to the temperature required. The validation program is usually carried out by employing the product inoculated, or product simulated inoculated with appropriate preparations as Bacillus atrophaeus spores. biological indicators, typically, are employed to establish the final probability of microbial survival, using the concept of fractional cycle, to design a cycle of sterilisation with ethylene oxide, and should be used in loads of product, or product, with simulated filled chamber.

The biological indicator should be employed in the monitoring of cycles of routine, in addition to the planning of the cycle of sterilisation by ethylene oxide. Another important aspect of the planning of the sterilisation process is defining the type of packaging material to be processed and their distribution in sterilizing chamber, due to the limited capacity of diffusion of ethylene oxide in areas most internal product.

7.1.2 THE VALIDATION PROCESS OF ESTERILIZAÇÃO

The validation must demonstrate documented that the sterilisation process established will consistently provide products that meet the level of sterility assurance required. Sterile products in accordance with the validated process must meet the specifications pre-determined and the characteristics of quality related to functionality and safety.

Once the process validated, he should be revalidated periodically, and after changes to the product; equipment and process, which may compromise the sterility assurance level specified.

The main elements of validation are: Qualification of Installation; Operation Qualification and Performance Qualification

7.1.2.1 QUALIFICATION OF INSTALLATION

The implementation of the plan of qualifying facility shall provide documented evidence that the equipment and all auxiliary items were supplied, installed and operate in accordance with the specifications. It must be demonstrated that the sterilisation equipment, its components, auxiliary items and supplies, such as steam, water and air, were properly designed, installed and calibrated.

In order to meet the parameters and limits recommended for sterilisation, is required the employment of appropriate instrumentation to monitor and control the critical parameters such as temperature, time, humidity, gas concentration or sterilizing radiation absorbed. These instruments should be evaluated in qualifying for installation.

The qualification of installation comprises the following elements: equipment, installation and function.

With regard to equipment and installation, the specifications of the sterilizer; auxiliary items and services; operating procedures; the installation location and the documentation must be previously defined and verified in qualifying installation, guaranteed their conformity. To ensure the function, it must be verified that the equipment and systems of operational security work in accordance with their specifications; the cycles of operation are in accordance with the defined and that there is no evidence of leakage of utilities or steriliser, when applicable.

In documented procedures for the qualification of installation must be specified as each element of qualification is planned, executed and reviewed. The documentation that provides support to the qualification of installation includes description of the physical and operational characteristics of the equipment; its components and services. Drawings and process and instrumentation diagrams should be checked against the proposed configuration and updated when necessary. Security Systems applicable should be evaluated to ensure performance; quality and safety of equipment and operators.

The qualification of the installation is required for new equipment, or when the existing sterilizer is replaced or relocated. The qualification should be redone to defined time intervals, and at least partially when changes occur that may alter the effectiveness of the sterilisation process, such as replacement or retirement of equipment, or parts thereof, changes in supplies of process and change in radioactive source

7.1.2.2 OPERATION QUALIFICATION

In operation qualification must demonstrate that the installed equipment is capable of performing the sterilisation process specified within the defined intervals. The range of parameters and limits of operation should be laid down in the definition of the process. Before operation qualification, calibration status of all instrumentation used to monitor, control, indicate and record should be confirmed.

For autoclaves and other sterilizers that employ thermal process, studies should be conducted of heat distribution in different positions considering the size of the camera and the load. It must be confirmed that the camera (empty and full) operates within the critical parameters in all its major locations. The number and position of thermocouples are determined by the type and configuration of the load; equipment size; type of instrument and cycle used. An acceptable range of temperature in the chamber is empty ± 1 °C when the temperature of the chamber is 121 ºC. For sterilizers the ethylene oxide, the relative humidity; the gas concentration and temperature must be monitored by sensors distributed in suitable positions. Security Systems applicable should be tested. *Control Software* must be validated and challenged under fault conditions. The penetration and distribution of ionizing radiation in charge must be held and monitored by dosimeters. The operation of qualification of sterilizing filters is made by means of the test of integrity of filters; measures of differential pressure and flow velocity. As the fluids sterilized by membrane filters can be exposed to the environment during the next processing, environmental control and the qualification and/or validation of aseptic handling area should be an integral part of the process of sterilisation by filtration.

7.1.2.3 QUALIFICATION OF PERFORMANCE

In performance qualification must demonstrate that the sterilisation process is capable of achieving, repeatedly, the level of sterility assurance pre-determined for the loads defined products; that the equipment operates consistently in accordance with pre-determined criteria and that the product meets the specified requirements of safety, quality and performance.

The performance qualification comprises physical and microbiological evaluations that demonstrate the efficacy and reproducibility of the sterilisation process, while maintaining the specified characteristics of the product.

The studies should be considered: physical criteria as representative test load process packaging; identical to the product; pre-conditioning; profile of temperature and temperature at the point of reference; response of chemical indicators; integrity of packaging; documentation; among others. The load for sterilisation must be established and documented, taking into account parameters such as configuration, distribution, orientation, density, size, material composition, use and type of pallets. The product or material with similar characteristics to the product (product simulated) used for qualification must be identical to the product packaging and represent, at the very least, the worst case load of routine production, i. e. the configuration more difficult to sterilize. Criteria for reuse of load must

be defined, and that it should be balanced environmental conditions or aerated before reuse. With the data generated should demonstrate- if compliance with the physical and chemical parameters applicable. The relationship between the conditions of positions of monitoring during the qualification and routine should be established.

In the qualification of physical performance should demonstrate the reproducibility of the process with a minimum of three consecutive cycles to check the attendance of all the acceptance criteria.

In microbiological qualification you must follow specific requirements for each sterilizing agent. Different methods can be used in the validation of the sterilisation process and include three categories: process based on inactivation of microbial load (natural bioburden); process combined with base in inactivation of microoverkill). Indicates that the challenge 30-42 is run on minimum parameters of process and must meet the level of sterility assurance for all combinations of load, and can use the worst case of product representative of families. For each type of load to be sterilized, the reproducibility of the process must be demonstrated using at least three consecutive cycles. The biological indicators used should be positioned in and/or on the product at defined location.

The performance qualification should be repeated when significant changes are proposed, such as changes in the design and packaging of the product; setting or load density and equipment or sterilisation process. The effects of these changes in the stages of validation of the sterilisation process must be evaluated.

7.1.3 REVIEW AND APPROVAL OF VALIDATION

The documented review of validation data, generated in the qualifications of installation, operation and performance must be made to confirm the acceptability of the sterilisation process and define the specification of the process, including parameters and tolerance.

The final stage of the program of validation requires the documentation of data to support developed in the implementation of this program.

7.2 BIOLOGICAL INDICATORS

The biological indicator is defined as a preparation characterized micro-specific body which provides a resistance defined and stable at a given process of sterilisation. Spore-forming Bacteria are the micro-organisms recognized as appropriate for employment as biological indicators once that, with the exception of ionizing radiation, these microSpore forming bacteria are microorganisms acknowledged as proper for usage as biological indicators once, except for ionizing radiation, these microorganisms are significantly more resistant to the sterilization processes than microorganisms with natural microbial load of the product. A biological indicator may be used in the performance qualification of the sterilization equipment and development and establishment of the sterilization process for a certain piece of equipment. Biological indicators are used in processes for obtainment of sterile product is its final vessel and equipment, material and packaging component sterilization, deployed in the aseptic process. Biological indicators may also be used for monitoring sterilization cycles in periodical revalidations and for assessing the process capacity used in the isolator decontamination or clean rooms.

7.2.1 TYPES OF BIOLOGICAL INDICATORS

There are at least three types of biological indicators, being that each type incorporates a microbial species with known resistance to the sterilisation process.

A type of biological indicator includes the spores that are added to a support or carrier (disc, or strip of filter paper, glass, plastic, or other material) packaged in such a way as to maintain the integrity and viability of the material inoculated. The towpath and primary packaging must not contain any type of chemical contamination, physical or microbiological that can compromise the performance and stability of biological indicator and may not undergo a change in function of the sterilisation process submitted. The towpath and primary packaging should resist the transportation and handling up to the time of use and should prevent the loss of the original inoculum during the transportation, handling and storage until the expiration of the period of validity.

Another type of biological indicator consists of a suspension of spores inoculated into representative units of the product to be sterilized. When this is not possible the employment of real product, you can inoculate a simulated product, which differs from the actual product in some characteristics, but behaves in a similar manner when subjected to the test conditions, or sterilisation. A spore suspension of D-value known should be used for inoculation of the real product or simulated, ensuring that the product to be used simulated, this does not undermine the strength of the biological indicator. The physical configuration of the product to be inoculated (real or simulated) can affect the resistance of microbial suspension was inoculated. In the case of liquid products it is recommended that the determination of the value of D and Z value of biological indicator on liquid product specified. The population, D value, Z value where applicable and time of destruction of the micro-organism should be determined.

Value Z is the elevation of temperature in degrees, required to reduce the value D in 90 %, or produce the reduction of a cycle in logarithmic curve of thermal resistance. The third type is the biological indicator self contained, presented in such a way that the primary packaging intended for hatching after sterilisation contains the means of growth

required for recovery of the micro-organism. In this case, the system composed by biological indicator and the means of growth of micro

The self contained biological indicator, also, may consist of a suspension of spores in a culture medium containing pH indicator that allows viewing the presence or absence of growth after hatching. The resistance of the system self contained is dependent upon the penetration of the sterilizing agent in packaging, which should be controlled by the manufacturer by means of drawing and composition of the material that constitutes the packaging, ampoule or container. The self contained biological indicator in form of ampoule can be incubated directly after exposure to sterilisation process, under the specified conditions. The absence or presence of microbial growth is determined visually, from the change of color of an indicator built into the medium, or by turbidity resulting from the development of the micro-organism; or even, by microscopic examination of the inoculated medium. The self-contained biological indicator must withstand transport and handling during use without that occur breaks or loss of the original inoculum. During or after the sterilisation process, the material of which it is composed the system self contained should not hold or release any substance that can inhibit the growth of micro-organisms survivors. The growth promoting capacity of culture medium after exposure to sterilisation process must be proven.

7.2.2 PREPARATION OF THE BIOLOGICAL INDICATOR

All the operations involved in the preparation of biological indicators should be monitored by means of a system of quality documented that enables traceability of all materials and components built into the microbial suspension; the carrier inoculated, or the biological indicator. The preparation of stock suspensions of spores of selected micro-organisms as biological indicators requires the development of appropriate procedures including its cultivation, collection, purification and maintenance. The Suspensions inventory should contain, predominantly, dormant spores (not germinal) kept in liquid non-nutritive. The final product provided by manufacturers (suspension, acarrier inoculated microbial or biological indicator) should not contain micro-organism different from micro-organism test in sufficient number that may affect the product. The system to minimize the presence of micro-organisms of different micro-organism test must be validated, monitored and recorded.

7.2.3 INDICATOR SELECTION FOR THE BIOLOGICAL PROCESS OF STERILIZATION

The choice of biological indicator requires knowledge of their resistance to sterilisation process specific to ensure that the system of biological indicator provides greater challenge that the microbial load on the product.

The efficient use of biological indicators for the development of the cycle, process and validation, or for monitoring the process of sterilisation routine requires knowledge of the material to be sterilized including its components and packaging material. Just biological indicators recognized and specified in the monographs should be used in the development or validation of a sterilisation process to ensure that the biological indicator selected will provide a greater challenge to the sterilisation process of the microbial load on the product.

In cases of the use of biological indicators with different characteristics than those commercially available, can cultivate if micro-organisms described in scientific literature for preparation of biological indicators. The user must be able to determine the values of D and Z for the indicators household. When biological indicator non-commercial is used, the population, purity and validity should be confirmed to ensure the legitimacy of the tests to be performed using this indicator.

When the definition of the sterilisation process is based on microbial load of product, this should be quantified and the resistances of the biological indicator and microbial load should be compared. The sterilisation process must result in sterility assurance level of at least 10^{-6}.

The method of death *(overkill)* can be employed in the development of the sterilisation process and, in this case, consideration should be made regarding the alleged specific resistance used in the establishment of requirements of lethality of the process. In general, the processes of death are developed with the assumption that the microbial load is equal to 106 micro-organisms highly resistant. A process 12 D is defined as the process that can prove lethal enough to reduction of 12 logarithmic cycles, equivalent to 12 times the D value for micro

The micro-organisms present in the microbial load of the product. To assume a microbial load of 106, a process of death will result in a probability of sterility not less than 10^{-6}. The use of the process of death and their validation can minimize or avoid the need for quantification and identification of microbial load of product.

For the process of wet heat, spores of appropriate strains of Geobacillus stearothermophilus are available commercially as biological indicators. Other micro-organisms form spores resistant to moist heat such as Clostridium sporogenes, Bacillus atrophaeus and Bacillus coagulans, also, can be used in the development and validation of a process of sterilisation by moist heat.

Table 1 – Characteristics indicative of systems of biological indicators available commercially.

Sterilisation Process	*D Value (minutes)*	*Range of D value for the selection of biological indicator (minutes)*	*Limits for proper resistance (dependent on the D value in minutes)*	
			Survival Time	*Time of death*
Heat secoa	1.9	Min. 1.0	Min. 4.0	10.0
(160ºC)		Max. 3.0	Max. 14.0	32.0
Etilenob oxide	3.5	Min. 2.5	Min. 10.0	25.0
(600 MG/L, 54 ºC, 60% RH)		Max. 5.8	Max. 27.0	68.0
Heat umidoc	1.9	Min. 1.5	Min. 4.5	13.5
(121 ºC)		Max. 3.0	Max. 14.0	32.0

[a] – for 1.0 x 106 to 5.0 x 106 spores /acarrier

[b] to 1.0 x 106 to 5.0 x 107 spores /acarrier

[c] for 1.0 x 105 to 5.0 x 106 spores /acarrier

For the validation of the sterilisation process, via dry heat, can be employed Bacillus atrophaeus spores. During the validation studies could be performed for evaluation of depyrogenation in place of microbial inactivation, since the rate of inactivation of endotoxins bacterins is much slower than the inactivation of Bacillus atrophaeus spores. In practice, a reduction of the order of at least three cycles log level of endotoxin results in a probability of sterility not less than 10^{-6}.

To monitor the sterilisation processes employing ionizing radiation, spores of Bacillus pumilus has been used despite not be usual practice. The method of establishing the dose of radiation, more employee, does not use biological indicators. Some micro-organisms in the microbial load of the material to be sterilized, may provide greater resistance to the process of sterilisation by radiation in comparison with the spores of Bacillus pumilus.

For the process of sterilisation by ethylene oxide are commonly used spores of subspecies of Bacillus atrophaeus var. niger, when employs ethylene oxide 100 %, or different mixtures of gases.

7.2.4 EVALUATION OF PERFORMANCE

7.2.4.1 THE MANUFACTURER'S LIABILITY

Are the manufacturer's responsibilities: determination and delivery of the performance characteristics of the batch of biological indicator by means of certificate of analysis documenting that the validity of the performance stated on the product packaging; definition of the sterilisation process for which the biological indicator is recommended; characterization of each type of biological indicator, using standardized conditions and appropriate equipment; D-value and the method by which this value has been defined; microbial count; stability of resistance until the validity indicated on the label; storage conditions, including temperature and relative humidity; guidelines on the environment of culture to be employed and the conditions of recovery of micro-organisms after exposure to sterilisation process and its disposal.

7.2.4.2 USER RESPONSIBILITY

COMMERCIAL PRODUCTS

When a biological indicator is purchased commercially, its suitability for use in a sterilisation process must have been established in studies, to not be that data are available to confirm the employment indicator in a specific process. The user must establish within their institution, the acceptance criteria for the lots of biological indicator. To acquire a biological indicator, this must be accompanied by a certificate issued for each batch. If the biological indicator is employed in a manner different from that indicated by the manufacturer, the user must proceed with the registration of the conditions of use, the checks and the performance of the biological indicator.

After receiving a lot of biological indicator, the user must quantify the load of spores per unit and proceed to check the morphology and purity of micro-organisms, confirming, at least, the kind of micro-organism. The information regarding the D value; the conditions of storage; the term of validity and the stability of biological indicator should be observed and recorded. The user may consider the need to audit the value D before acceptance of the lot of biological indicator. For storage for a long period, it is important to

check the D-value and the stability of the count. In the case of storage of the spore suspension, for a period exceeding 12 months, under conditions documented, the confirmation of the count and the attesting the resistance of spores should be carried out, unless the performance of a previous crop has been validated after long period of storage. The results of tests of resistance and spore count must be within the range of acceptance established during the approval of the lot of the spore suspension.

NON-COMMERCIAL PRODUCTS

The user can decide cultivate micro-organisms for development of biological indicators to be employed in the development or validation of a sterilisation process. In the case of user become a producer, the performance requirements of the biological indicator must be met. If a system of biological indicator is employed for the development of a new process of sterilisation or validation of a process already exists, the same performance criteria for commercial products should be complied with.

7.2.4.3 PREPARATION OF THE SPORE SUSPENSION

The records of identification of spore suspensions must be kept by commercial or non-commercial producers and should include information about the source of the initial culture of micro-organisms; identification; the traceability of culture mother of spores, the frequency of subculture; the culture medium used for sporulation; the changes that have occurred in the preparation of the medium; the comments about contamination of suspension; the previous data and subsequent thermal shock; the records of the use of the spore suspension and resistance to sterilisation (particularly, values of D and Z, where applicable).

7.2.5 USE OF BIOLOGICAL INDICATOR VALIDATION

Independent of the sterilisation process to be employed, the initial population of micro-organisms, their resistance to sterilizing process and the site of inoculation of the product can influence the rate of inactivation of indicator

Biological. During the validation process, in several places of the product must be inoculated the biological indicator, ensuring the challenge of both packaging and the product contained within it, to ensure that a sterility assurance level of 10^{-6} for the product and for the packaging. It may be necessary, through laboratory studies, to determine if the product components are more difficult to sterilize than, for example, a solution, or medication in it. The phase of qualifying product performance should identify the most important parameters of the process for inactivation of micro-organisms in places more difficult to sterilize. The survival of biological indicator is a consequence of the strength and size of the microbial population. Therefore, it is not always a biological indicator with population of 106 is required to confirm a sterility assurance level of 10^{-6}. The appropriate use of biological indicators is to confirm that the parameters established in sterilisation process ensures the security level of sterility desired. In sterilisation by moist heat, the employment of biological indicator confirms the lethality rate determined by physical parameters. Biological Indicators with D value relevant populations and substantially smaller than 106 are suitable to validate many processes of sterilisation and decontamination. It is important that users are trained to justify scientifically, the choice of a biological indicator.

7.3 PROCESS ASSÉPTICO

Although the terminal sterilisation of a packaged product is the procedure that ensures minimal risk of microbial contamination in the production of a batch, there are classes of products that may not be sterile in its final packaging and that must be prepared using aseptic process. This process is designed in such a way as to prevent the contamination of sterile components per viable micro-organisms, or even in the intermediate phase of production, when some component must be provided free of micro-organisms. A product defined as processed aseptically consists of components that were sterilized by one of sterilisation processes such as, for example, filtration, in case it is a liquid. In the case of packaging material consisting of glass, can be employed the dry heat and, when it comes to packaging material, such as polymeric covers, can be used to autoclaving or ethylene oxide.

The aseptic process, the environment where the inputs are handled and the step of aseptic filling are considered critical points. The requirements for a project properly validated and that hold the necessary conditions for the aseptic process include an environment free of viable micro-organisms, where the air quality is guaranteed by appropriate equipment, by trained personnel and paramentado in accordance with the requirements of the environment and by operation to be performed. The desired environment can be obtained by means of the technology of air filtration that provides the air supply with the microbial quality required.

The planning of the plant should provide a system of cascade of air flow with positive pressure increased, the most critical areas (aseptic) to those of intermediate demand (preparation areas) and finally, those of lesser requirement of control; and yet, it must allow the frequent exchange of air, in addition to the employment of unidirectional flow of air in the immediate vicinity of the product or components exposed and the control of temperature and humidity (when applicable). The installations must include systems for primary isolation (near the product) and secondary (where the aseptic process is performed) by means of physical barriers. The surfaces, such as walls and ceiling should be smooth allowing frequent sanitization. Changing rooms must have adequate space for the staff and storage of sterile dressings. The training of staff regarding the

appropriate adornment, should cover the correct use of vestments as, overalls, gloves and other items that promote the coverage of the surface of the body. The whole process of sanitization should be documented. Certification and validation of aseptic process and installations are carried out by means of the confirmation of the efficiency of filtration systems; by procedures for microbiological monitoring of the environment and by the simulation of aseptic filling of the product, employing sterile culture medium. The monitoring of aseptic installation must include the periodic examination of environmental filter, the routine monitoring of particulate material and viable and fill simulated with sterile culture medium.

7.4 CLEANROOMS AND ASSOCIATED CONTROLLED ENVIRONMENTS

In this section are included some aspects related to aseptic processing of products with the establishment; maintenance and control of microbiological quality of rooms and clean zones. Includes the classification of these controlled environments based on limits of particle count; in microbiological assessment for controlled environments; training of staff; on the critical factors in the design and implementation of a program of microbiological assessment; development of a sampling plan; in the establishment of levels of microbiological alert and action; the methods and equipment used for microbiological sampling; in culture media and diluents used; in microbiological identification of isolates and in operational assessment by means of the filling of culture medium *(average fill)*.

Media fill is a test for simulation of aseptic operations in which the product is replaced by a culture medium and serves to ensure that the processes used are able to lead to sterile products.

There are alternative methods to assess and monitor the microbiological status of rooms and clean zones, with variety of Equipment and methods for microbiological sampling. The improper application of sampling and microbiological analyzes can cause significant variability and potential for inadvertent contamination. A large number of sterile products is manufactured by aseptic processing, which depends on the exclusion of micro-organisms of the processing line and, therefore, preventing the entry of micro-organisms in open containers during the bottling and the microbial load of the product and the manufacturing environment are important factors related to the level of assurance of sterility of these products.

7.4.1 CLASSIFICATION OF CLEANROOMS AND ASSOCIATED CONTROLLED ENVIRONMENTS

Clean Room is the room in which the concentration of particles suspended in air is controlled; it is constructed and used in such a way as to minimize the introduction, generation and retention of particles inside the room, in which other relevant parameters, such as, for example, temperature, humidity and pressure, are controlled as needed.

The classification of air cleanliness of rooms and clean zones, through the analysis of concentration of particles suspended in air, is regulated by ABNT NBR ISO 14644-1 – clean Rooms and associated controlled environments – Part 1: classification of air cleanliness. This document applies to particles suspended in air within a controlled environment, but don't want to characterize the nature of viable or non-viable particles.

The application of this standard has been used by manufacturers of rooms and clean zones to guide the construction, the preparation and maintenance of these facilities. However, it provides no relation between the number of particles is not viable and the concentration of viable micro-organisms.

The pharmaceutical industry is concerned with the particle count viable and, in the case of injectable products, there is additional concern with the particle count totals. The background of that, the smaller the number of particles present in a clean room, less likely that micro-organisms carried by air are present, it is acceptable and New difficulties are then appearing in design, construction and operation of rooms and clean zones.

In Table 1 are described the classes of air cleaner according to ABNT NBR ISO 14644-1, which is based on limits of particle sizes of 0.1 to 5 ^m. In Table 2 there is a relationship between the different classification systems for clean rooms.

IS It acceptable that, if a smaller number of particles are present in the clean room or controlled environment, the microbial count under operating conditions will be less, since there are no changes in the flow of air, the temperature and humidity. Clean Rooms are kept under a state of operational control based on dynamic data (operational).

Table 1 – Classes of cleaning the air for particles in suspension, selected for rooms and clean zones.

Number of Classification ISO	*Maximum concentration Limits (particles/m3 of air) for particles equal to or larger than the sizes considered*					
(N)	*0.1 PM*	*0.2 PM*	*0.3 PM*	*0.5 PM*	*1 PM*	*5 PM*
ISO Class 1	10	2				
ISO Class 2	100	24	10	4		
ISO Class 3	1 000	237	102	35	8	
ISO Class 4	1.000	2 370	1 020	352	83	
ISO Class 5	10.000	2.700	10,200	3 520	832	29
ISO Class 6	1 000000	23.000	10.000	3.200	8 320	293
ISO Class 7				35.000	8.200	2 930
ISO Class 8				3 52.000	83.000	2.300
ISO Class 9				3.20.000	8 32.000	293000

Table 2 – Comparison between the different systems of classification of air cleaner.

WHO and EECs (GMP)	*United States (usual)*	*ISO*
Class A	Class 100	ISO 5
Class B	Class 100	ISO 5
Class C	Class 10,000	ISO 7
Class D	Class 100,000	ISO 8

7.4.2 PROGRAM OF MICROBIOLOGICAL ASSESSMENT FOR CLEANROOMS AND ASSOCIATED CONTROLLED ENVIRONMENTS

The monitoring of total particles suspended in air in rooms and clean zones does not provide information on the microbiological content of the environment. The basic limitation of the counters of particles is that typically measure particles of 0.5 ^m or greater and the micro-organisms are not loaded by air cells that float freely, they are not alone and is often associated with particles of 10 to 20 ^m. Particle Counts, as well as microbial counts within rooms and clean zones, vary with the activities conducted during the sampling and its location. The monitoring of the environment for non-viable particles and micro-organisms is important because both are necessary to achieve the requirements relating to 0,220 and sterility laid down for the products.

Programs of microbiological monitoring for rooms and clean zones should assess the effectiveness of the cleaning and disinfection practices that may have an impact on the microbial load of the environment. The microbiological monitoring, normally, not identifies and quantifies all microbial contaminants in environments; however, the routine monitoring should provide enough information to make sure that the environment is operating within the state of proper control.

The environmental microbiological monitoring and analysis of data carried out by qualified persons allow control state is maintained in rooms and clean zones. The environment should be sampled during normal operations to enable the collection of meaningful data and microbial sampling must occur when materials are in the area, the processing activities are occurring and all employees are in operation on the site.

The microbiological monitoring of rooms and clean zones must include the quantification of microbial content of environmental air; the compressed air that enters the critical area; surfaces; equipment; the containers; the floors; the walls and the vestry of people. The objective desired with the program is to obtain representative estimates of microbial load of the environment and, once compiled and analyzed, any trends should be evaluated by trained persons. It is important to review environmental results based on specified frequency, as well as reviewing results for prolonged periods to determine if there are trends present. Trends can be visualized by means of tables of statistical control that included levels of alert and action. The microbiological control of controlled environments, also, can be assessed on the basis of trend data. Periodic Reports or summaries should be issued to alert the responsible for the area.

When the microbiological level specified for a controlled environment is exceeded, review of documentation and research should occur. The research should include the

review of maintenance documentation of the area; the documentation of disinfection; the physical parameters or inherent operational, such as, changes in temperature Environmental and relative humidity and the stage of training of the staff involved.

Then the research, the actions taken may include the strengthening in the training of people to emphasize the microbiological control of the environment; the additional sampling in frequency increased; the additional disinfection; additional tests of product; the identification of the contaminant 30-42 and its possible source and the reassessment and revalidation of current standardized operational procedures, if necessary. Based on a review of the research and the results of the tests, the significance microbiological level exceeded and the acceptability of operations or products processed under that condition can be defined. All research and justification of actions should be documented and made part of the system of management of quality.

Luggage and clean area are defined by certification in accordance with the applicable standard, being that the parameters evaluated include integrity of filters, pressure differentials and speed, patterns and changes in the air. An example of method for conducting the challenge test of particles to the system consists in increasing the concentration of particles in the environment through smoke in the vicinity of work areas and critical view the movements of the air. The presence of vortices and turbulent zones can be visualized and the pattern of air flow can be finely adjusted to eliminate or minimize undesirable effects. This evaluation is made under simulated production conditions; however, with equipment and staff on site.

The appropriate test and the optimization of the physical characteristics of the clean room or controlled environment are essential before completing the validation of microbiological monitoring program. The guarantee that the environment is operating properly and in accordance with their specifications will provide greater assurance that the microbial load of the environment will be appropriate for aseptic processing. These tests should be repeated during the certification of the routine room or clean area and whenever changes considered significant are made in operation, such as changes in the flow of people, processing, operation, material flow systems, air handling or layout of equipment.

7.4.3 ALTERNATIVE TECHNOLOGIES TO ASSEPTIC PROCESS

Due to the strong correlation between the involvement and human intervention and the potential risk for contamination of product in aseptic packaging, production systems where people are removed from critical areas have been implemented, employing, therefore, strategies advanced aseptic processing, with reduced requirements for environmental monitoring of viable and non-viable particles.

Here are some definitions of systems used to reduce the rate of contamination in aseptic process.

Barriers: device that restricts the contact between the operator and the aseptic field. Barriers may not be sterile and not always have transfer systems that allows the passage of materials for inside or outside the system without exposure to surrounding environment. There are different types of barriers, since plastic curtains in critical areas until rigid barriers in the equipment, which may incorporate elements such as support of gloves and transfer port.

Blow /Fill/Seal*:* this system combines the fitting of container with the bottling of product and sealing in a single product. From a microbiological point of view, the sequence of formation of the container, filling of sterile product and the formation and implementation of the seal are obtained, aseptically, in an uninterrupted operation with minimal exposure to the environment. These systems have existed for many years and contamination rates are lower than 0.1 %.

Isolators: technology used for dual motion, to protect the product from contamination of the environment and of people during filling and closing and to protect people from toxic products or deleterious during its production. This technology is based on the principle of putting materials previously sterilized, such as containers, products, and covers in a sterile environment, which remain sterile during the entire operation, once that persons or non-sterile components are not inside the insulator. The barrier of the isolator is an absolute barrier that does not allow trade between protected environments and not protected. Insulators can be physically sealed against the entry of external contaminants or can be effectively sealed by continuous application of overpressure. Material Handling by employees is performed by means of gloves or clothing complete or partial. The air that enters the isolator passes through a HEPA filter or/ ULPA and the air exhaust usually passes through a HEPA filter. Vapors of hydrogen peroxide or peracetic acid are typically used for sterilisation of surfaces or internal environment. The sterilisation of the interior of the insulators and all content are usually validated for a sterility assurance level of 10^{-6}.

The introduction of equipment; components and materials can be done in various ways, such as the use of autoclave dual port, introduction continues to components through a treadmill that passes through the tunnel of sterilisation or use of a system of dock. IS It necessary to monitor the integrity, calibration and maintenance of the insulator.

The requirements for controlled environments adjacent to these new technologies used in aseptic processing depend on the type of technology used.

Blowing Equipment /Enchimento/Selagem that limit the contact of the operator with the product can be installed

in a controlled environment, especially if any operator intervention is possible during production.

Barrier Systems require some form of controlled environment. On the basis of numerous types and applications, the requirements for the adjacent environment may vary.

The strategies of design and operation for the environment where circulate these systems should be developed by producers using a logical criterion and rational and the ability of the system to provide sterile products must be validated in accordance with pre-established criteria.

In isolators, air enters through HEPA filters integrals of quality, or better, and its interior is, typically, sterilized with a sterility assurance level of 10^{-6}. Therefore, isolators which contains sterile air do not exchange air with the surrounding environment and are free from human operators. However, when the insulator is in a controlled environment, the potential for product contamination is reduced in the event of a leak in the gloves or clothing.

The extent and scope of environmental microbiological monitoring depends on the system used. Producers must balance the frequency of environmental sampling that requires human intervention, with the benefit accrued by the results of the monitoring. Since that barriers are designed to reduce the human intervention, remote sampling systems should be used in replacing the intervention of people. In general, once the validation has established the effectiveness of the barrier, the frequency of sampling to monitor the microbiological status of the aseptic processing area can be reduced when compared to the frequency of a classical system of aseptic process.

Systems of isolators require lower frequency of microbiological monitoring. The continuous monitoring of total particles can provide assurance that the system of filtration of air inside the insulator is functioning properly. Traditional Methods for quantitative microbiological air sampling may not be sufficient to test the environment inside the insulator. Experiences with isolators indicate that, under normal conditions of operation, leakage or rupture in the gloves represent the greatest potential for microbiological contamination, which requires frequent testing of integrity of gloves and monitoring of their surfaces. The monitoring does not frequent surfaces inside the isolator should be assessed and can be beneficial.

7.4.4 TRAINING OF OFFICIALS

Aseptically processed Products require a lot of attention to detail, strict discipline and strict supervision of persons, in order to maintain the level of environmental quality appropriate for sterility assurance of the final product.

The training of all employees who work in rooms and cleaned areas is critical. This training, also, it is important for the people responsible for microbiological monitoring program, a time that the contamination of the work area may inadvertently occur during sampling microbiological, by use of improper techniques. In highly automated operations, monitoring can be accomplished by people who have direct contact with critical areas within the processing area. The monitoring of officials should be conducted before and after the work in the processing area.

The management of the facility must ensure that all persons involved in the operations in rooms and clean zones know relevant microbiological principles, including basic principles of aseptic processing and the relationship of manufacturing procedures and manipulation with potential sources of contamination of the product. Also, they must have knowledge of basic principles of microbiology; microbial physiology; cleaning, disinfection and sterilisation; selection and preparation of culture media; according to the involvement of employees in the process. The people involved in microbial identification require specialized training in laboratory methods apply. Additional Training in managing environmental data collected must be supplied. Knowledge and understanding of standard operating procedures applicable are critical, especially those related to corrective actions that are taken when the environmental conditions dictate. The understanding of the policies of adherence to regulatory requirements and the responsibility of each individual, relating to Good Manufacturing Practices should be an integral part of the training program, as well as training on how to conduct research and analyze data.

The control of microbial contamination associated with people is one of the most important elements of environmental control program. Contamination can occur from the spread of micro-organisms by individuals, especially those with active infections and, therefore, only healthy individuals should be authorized to access controlled environments. The good personal hygiene and careful attention to details of appropriate adornment aseptic procedures are important items. The staff appropriately wore plumb should be careful to maintain the integrity of their gloves and aprons during the entire period of stay in controlled environments.

As the environmental monitoring program is not able to detect all events of the aseptic processing that could compromise the microbiological quality of the environment, periodic studies of bottling of culture medium or simulation of process are needed to ensure that the operational controls and appropriate training are actually kept.

7.4.5 DESIGN AND DEPLOYMENT OF THE PROGRAM OF ENVIRONMENTAL MICROBIOLOGICAL CONTROL

It is the responsibility of the manufacturer develop, initiate, implement and document a 30-42 environmental monitoring program that is capable of detecting an adverse event in microbiological conditions in time to permit corrective actions meaningful and effective. It is imperative that the program is tailored to the conditions and specific installations.

A culture medium of general microbiological growth as the means of casein / soybeans, should be adequate in most cases. This means it can be supplemented with additives to circumvent or minimize the effects of disinfecting agents or antibiotics, if used or processed in these environments. The detection and quantification of yeasts and molds should be considered. Means, generally accepted, are such as Sabouraud agar and Sabouraud Dextrose agar modified. Other means validated to promote the growth of fungi may be used, such as casein Agar / soybeans. In general, the analysis of anaerobic micro-organisms mandatory is not performed in the routine, to not be that conditions or investigations require. The ability of the culture media selected to detect and quantify anaerobic micro-organisms or microaerophilics should be evaluated.

The sterilisation processes used to prepare culture media for the environmental program must be validated and should be examined for sterility and promotion of growth. The media should be able to maintain the growth when inoculated with less than 100 CFU. The selection of temperature and time of incubation is done once the appropriate means have been selected. Typically, temperatures of incubation in intervals of ºC ±2.5 ºC and 32.5 ºC ±2.5 °C has been used with incubation times of 72 and 48 h, respectively.

The environmental control program should include identification and evaluation of sampling locations and validation of methods for microbiological sampling of the environment.

7.4.6 PLAN AND SAMPLING LOCATIONS

During the initial phase of activities, as well as in the preparation of a clean room, or other controlled environment, specific locations for air sampling or surfaces must be determined. Should consider the proximity of the product, if the existing air and surfaces in the room are in contact with him or with internal surfaces of closing systems of containers.

The frequency of sampling will depend on the criticality of specified locations and subsequent treatment to the aseptic process.

As manual interventions during the operation and the potential for personal contact with the product increase, grows the importance of environmental monitoring program. This is more critical for aseptically processed products than for those undergoing terminal sterilisation. When the sterilisation cycle terminal is not based on concept of death, the program of microbial load prior to sterilisation is critical. The sampling plans must be dynamic with frequencies of monitoring locations and adjusted based on the performance of trend. It is appropriate to increase or decrease the sampling on the basis of this performance.

7.4.7 MICROBIOLOGICAL LIMITS ALERT AND ACTION IN ROOMS AND CLEAN ZONES

The principles and concepts of control of statistical processes are useful to establish levels of alert and action, as well as mechanisms for control of trends.

The alert level for monitoring environmental microbiological evidence contamination level significantly higher than the normal operating conditions. Exceed the alert level does not necessarily require corrective action must, however, at least lead to a research monitoring documented, which may include modifications to the sampling plan.

The indicative of action in environmental microbiological monitoring when exceeded, requires immediate follow-up and, if necessary, corrective action.

Alert Levels are based usually in historical information obtained from routine operations of the process in a controlled environment specific.

In a new installation, these levels generally are based on previous experience of installations and similar processes; and, on data obtained in the course of several weeks. These levels are usually re-examined for adequacy at a set frequency. Trends to a deterioration of environmental quality require attention to determine the cause and to establish a plan of corrective action, in order to bring the conditions back to previous levels. An investigation should be implemented, and the assessment of the potential impact on the product should be performed.

7.4.8 METHODS AND EQUIPMENT USED FOR ENVIRONMENTAL MONITORING

Viable Micro-organisms in the air can affect the microbiological quality of products manufactured in rooms and clean zones. The quantification of these micro-organisms may be influenced by instruments and procedures used in the tests. The employment of methods, or alternative equipment must be preceded by the verification as to the equivalence of the results. There are different ways of monitoring types and types of equipment available to quantify viable micro-organisms, including sediment samplers, impact and centrifugal. The selection and suitability of the method to be used is the responsibility of the user.

The method using settle plates is still the most widely disseminated due to its simplicity and low cost and provides qualitative information about the environment of prolonged exposure, however, the exposure of Petri plates open and containing agar medium is not for quantitative assessment of the levels of microbial contamination of critical environments.

One of the main limitations of mechanical air samplers is the sample size of air that is being tested, because the level of micro-organisms in the air of a controlled environment is usually reduced and a large volume of air must be tested to ensure that the result is precise and exact, which, many times, it is not practical. To demonstrate that the microbial counts in the environment do not are increasing after sampling, it can be extended to determine if the sampling time is a limiting factor to obtain a representative sample. There is No equipment able to be sampled high rates of volume of air, but it must be considered whether the disruption of the flow of air in critical areas or the creation of turbulence that may increase the likelihood of contamination.

Centrifugal Samplers demonstrate selectivity for larger particles and, therefore, the use of such equipment may result in larger counts of particles in the air. To use these samplers, should consider-if its effect on linearity of air flow in a controlled area where is positioned for sampling. The use of probes remote requires that it be determined if the extra vial used does not have adverse effect on particle count viable, because this effect should be eliminated, or a correction factor should be used for the results obtained.

7.4.9 METHODS AND EQUIPMENT USED FOR MONITORING PARTICULATE VIABLES IN CLEANER

The sampling equipment surfaces, of areas and officials is a component of the program of microbiological control of controlled environments. To minimize the disruption of critical operations, normally the sampling is performed at the end of operations. The sampling can be done using contact plates or swab.

The monitoring is carried out usually in areas that come into contact with the product and in adjacent areas. Contact Plates with nutrient agar are used to be sampled flat surfaces and are incubated at a temperature suitable for quantification of viable particles. Specific Agar can be used to quantify fungi, spores, etc. The swab is employed on uneven surfaces, especially in equipment. The swab is placed in a suitable thinner and the estimation of microbial count is made plaqueando an aliquot appropriate in specific nutrient agar. The area to be sampled using *swab* is defined using a mold of appropriate size sterile, in general between 24 to 30 cm2. The result is given by contact plate, or per swab.

7.4.10 CULTURE MEDIA AND DILUENTS FOR SAMPLING AND QUANTIFICATION OF PARTICULATE VIÁVEIS

The culture media and diluents used for sampling and quantification of micro-organisms in rooms and clean zones depend on procedures and equipment used. The agar casein / soybean is the solid medium normally used, but there are different media and diluents available for different proposals. Alternative Means must be validated for the proposed use. When using disinfectants or antibiotics in the controlled area, you must consider the employment of means with appropriate agents.

7.4.11 IDENTIFICATION OF MICROBIAL ISOLATES

The environmental control program includes an appropriate level of identification of flora obtained in sampling. The knowledge of the normal flora of the rooms and clean zones it is important to define the monitoring of the area, the effectiveness of the procedures for cleaning and disinfecting and methods microbial disinfection.

The information obtained using the identification program can be useful in the investigation of sources

of contamination, especially when the action limits are exceeded. The identification of micro

7.4.12 OPERATIONAL ASSESSMENT OF MICROBIOLOGICAL STATUS OF PRODUCTS ASEPTICALLY PACKAGED

Rooms and clean zones are monitored by an environmental monitoring program appropriate. To ensure microbial load minimum, additional information on the assessment of the microbiological status of the environment can be obtained by means of the test of aseptic filling of culture medium (media fill test). The average fill is employed to evaluate the aseptic processing using sterile culture medium instead of the product. Satisfactory Results of media fill demonstrate the appropriateness of the line for the manufacture of the product. However, other factors are important, such as construction of areas; environmental monitoring and training of people.

When an aseptic process is developed and installed, you must qualify the microbiological status of the process, performing at least three consecutive media fill. The problems in the development of the program of media fill to be considered shall include procedures of bottling means; selection of means; volume of bottling; incubation time and temperature; inspection of units potted; interpretation of results and possible corrective actions required.

Once the average fill is performed to simulate the aseptic processing of a product, it is important that it be carried out under normal production conditions. This includes maximum number of people and the use of all the steps and materials used in the process of normal production. During the conduct of the media fill, interventions pre-documented known should be planned during the races of normal production, such as exchange of nozzles for bottling, fixing components, etc. Alternatively, to add a margin of safety, a combination of possible conditions can be used, and examples include impairments frequently; repairs not expected; exchange of filters, etc.

The qualification of an aseptic process must be performed for all products and for each line. Since the geometry of the container (such as size and openness) and the line speed are factors that are variable. The appropriate combination of these factors, preferably in the extremes, must be used in the qualification. A rational analysis of the products used must be documented.

It is recommended that the average fill will be carried out to cover all shifts of production to line / product / combinations of containers for initial qualification and Regularly revalidated. The program of media fill must simulate production practices in prolonged and can be carried out at the end of the shift of production.

Culture Media rich can be used, such as casein / soy broth. After the aseptic processing of culture medium, these should be incubated at 22.5 ºC ±2.5 ºC or 32.5ºC ±2.5 ºC, for at least 14 days. If two temperatures are used for the incubation of samples of culture medium, these must be incubated overnight, at the very least, 7 days in each one of them. After incubation, the samples must be inspected for growth. Isolates should be identified to genus and, when possible, to species in order to facilitate the investigation of the sources of contamination.

Critical Points in the implementation of the media fill are number of containers to qualify the aseptic process; number of units filled to the media fill; interpretation of results and implementation of corrective actions. Normally three races of media fill are used for initial qualification, or beginning of an area to demonstrate consistency in aseptic filling line. The minimum number to demonstrate contamination rate of not more than 0.1 %, acceptance criterion for race of media fill, is, at the very least, 3000 units. Plants pilots that prepare small batches can use smaller number of units.

Once the employees are a critical source of contamination in cleanrooms, visual documentation can be useful to check the correlation of production activities with events of contamination.

7.5 PROCEDURES FOR RELEASE

A program should be established for quality assurance that describe in detail the steps and required documentation for the release of the load or batch. The release of sterilized products will depend on release which can be conventional or parametric.

RELEASE PARAMÉTRICA PRODUCTS WITH ESTERILIZAÇÃO TERMINAL

The release parametric Curve is defined as the release of loads or batches of products subjected to sterilisation terminal through the fulfilment of critical parameters of the sterilisation process without the need for completion of the sterility test. The release parametric Curve is a possibility when the sterilisation process is very well known, the important points of process control are well defined, predictable and measurable and the lethality of the sterilisation cycle has been validated with appropriate biological indicator, or, in the case of sterilisation by ionizing radiation, the achievement of microbiological tests and appropriate dosimetric. The use of parametric release for sterilisation processes requires prior approval by the regulatory agency, which must assess the scientific justification for the sterilisation process employed and the documented data validation.

It is important to consider the limitations of the sterility test in the evaluation of products subjected to terminal sterilisation, which has a sensitivity compromised and is statistically limited due to the low probability of the presence of contaminated units. Therefore, once the sterilisation process is fully validated and operating, consistently, the physical data of sterilisation combined with other methods, such as, for example, biological indicators, indicators thermochemical integrators and physico-chemical, can provide information more exact than the sterility test for the release of products submitted to terminal sterilisation.

Four sterilisation processes can be qualified for parametric release: moist heat, dry heat, ethylene oxide and ionizing radiation. The products subjected to terminal sterilisation represent the category of lower risk among the sterile pharmaceutical products. Unlike sterile products obtained by aseptic production in controlled environments, products submitted to terminal sterilisation present level of assurance of sterility measurable.

The sterile products obtained by terminal sterilisation must meet a sterility assurance level of 10^{-6}, i. e., no more than one unit contaminated in a million units produced. The appropriate application of the methods used to process development terminal requires extensive knowledge of the scientific method of sterilisation selected, within three categories, for use with specific product:

a) Process based on microbial load (bioburden);

b) Process combined: biological indicator and bioburden;

c) Process of death.

The process based on bioburden requires broad knowledge of microbial load of product. It should be noted that different procedures of establishment of dose in case of sterilisation by radiation use

The Knowledge of the microbial load of the product and its resistance to radiation. This method also requires a sterility assurance level of at least 10^{-6}. The method based on the determination of bioburden requires developed critical control points of the process regarding the microbial load of the product. Risk analysis Procedures, such as Hazard Analysis and Critical Control Points (HACCP), are useful to establish conditions for manufacturing control and appropriate parameters of control in the process.

For products that allow the survival of microbial load are necessary production environments more controlled and process controls more accurate. This process is more indicated for clean products, with low level of microbial load and low frequency of micro-organisms form spores. This process, also, may be useful for products which may undergo changes when subjected to sterilisation processes more drastic.

Combined process that uses biological indicator and bioburden is generally employed for products that can lose attributes when using process of death and when you want a sterilisation process that demonstrates the inactivation of high numbers of micro6 spores and D value greater than

Minute. Dispensing Cycles are used to determine the resistance (D value) on between product inoculated with the micro-organisms of biological indicator and with those, often found in the microbial load. This process is employed usually for development of sterilisation cycles of parenteral products employing terminal sterilisation and sterilisation of correlates with ethylene oxide.

The process of death is used when the product to be sterilized is not deleteriamente influenced by sterilizing agent or conditions of the sterilisation process. When employing this process, it is important to know the microbial load of the product and the prevalence of micro0 minimum of 12 minutes.

THE VALIDATION PROCESS OF ESTERILIZAÇÃO

The parametric release requires that the sterilisation process chosen is developed and consistently validated, for inactivation of microbial load and handling a sterility assurance level of 10-6. The validation of the majority of sterilisation processes includes the validation of physical parameters and the microbiological effectiveness through the use of biological indicators to demonstrate a reasonable correlation between the lethality obtained by means of physical measurements (F0) and the lethality biological determined with the use of biological indicators.

Once the effectiveness of the sterilisation process terminal defined in function of the bioburden is associated with the number and the resistance of micro-organisms in the product, one of the components of parametric release is the active program of microbiological control to monitor the counting and resistance of microbial load of product. The control of microbial load and its enumeration is not a crucial factor when it employs the method of death, because, in general, the method of death does not require extensive evaluation of microbial load in the course of the process and requires less control in the process of the production environment.

8 STATISTICAL PROCEDURES APPLICABLE TO BIOLOGICAL TESTS

8.1 GLOSSARY OF SYMBOLS

Symbol	*Definition*
$a_1...z_1$	Doses of preparations tested (samples) A... Z.
a	Statistical significance of a result or measure estimated the degree to which this result is "true".
b_0	Intersection of responses (y) on log doses (x) in the line of regression.
b, b_1	Estimate the slope of the regression line of the response (y) in relation to the logarithm of the dose (x).
bl	Number of blocks (animals) in a crossover clinical trial.
c'	Constant used in the evaluation of the confidence limits (Table 15).
d	Number of dose levels for each test preparation in a balanced.
f	Number of differences in the responses between Paired Standard and sample, in tests carried out By a randomized 5 x 1.
gl	Degrees of freedom.
h	Number of preparations in a trial, including the standard preparation.
h'	Number of samples tested.
k	Number of different treatments within a test *k* = dh.
k'	Number of logarithms of powers in the tests carried out by design 5 x 1, for the same sample.
n	Number of replicates for each treatment.
n'	Number of individual estimates of power.
n"	Degrees of freedom used to estimate the variance s^2_M in 5 x 1
p	Probability
$p_1 p_2 p_3$	Doses lower, medium and higher of the preparation standard P; in tests with only two levels of doses, p2 represents the highest dose.
r	Pearson's correlation coefficient
s^2	Variance estimation provided by mean squared error in the analysis of variance. Also used with a letter suffix, for example, s^2_M represents the variance of the log power M.
s	An estimate of the standard deviation, i. e., the square root of s^2.
t	Statistic Student (Table 3).
t'	Statistics of Dunnett's test (Table 12).
v	Variance for heterogeneity between trials.
w	Weighting coefficient.
x	Log dose – also used with index to indicate a special preparation.
$\bar{x}$	Average of log dose.
y	Individual response or individual response transformed.
y'	Response calculated to replace a lost value.
$\bar{y}_P \cdots \bar{y}_Z$	Average response for the preparations and standard sample.
A...*Z*	Samples tested.
A...Z	Sum of responses for the samples *A*... *Z*.
$A_1 A_2 A_3$	Sum of responses to the doses lower, medium and higher sample *A*. For a test with two dose levels, A2 represents the response to the highest dose. Similarly to other samples tested.
$B_1...B_{2n}$	Sum of responses for each subject (1 to 2n) in test double crossover.
B´	Total incomplete responses in queue or block that has a lost value.
C	Statistics used in the calculation of the confidence limits (Formula 14).
$C_1...C_n$	Sum of responses in each column (1 to n) in Latin square design.
C'	Sum of incomplete responses in a column of Latin square design with a lost value.
CV	Coefficient of variation.
χ^2	Constant statistic from Table 18.
χ^2_M	Constant statistic to test homogeneity of individual estimates of logarithm of power.

Symbol	*Definition*
E	Sum of squares for regression (Table 10).
F	Reason of two independent estimates of variances (Tables 4 and 5).
F_I, F_{II}	Sum of responses in phase I or phase II a crossover clinical trial.
F_1...F_n	Sum of responses in each of the queues 1 to n in a randomized Latin square, or in each block of a randomized complete block design.
G_1, G_2, G_3	Statistic used to test for outliers.
G’	Total incomplete responses in a test with the exception of lost value.
I	Log interval between adjacent doses, in straight, parallel.
K	END of correction used in analysis of variance K = (Sy) 2/N.
L	Confidence interval in logarithms.
L_C	Confidence interval in logarithms for average semiponderada.
L_P...L_Z	Linear contrasts for preparations standard and sample.
M	Estimate of the log of the power or the log of the ratio of power used with a letter suffix in a multiple test, to denote a special preparation (M = log R).
M_i, M_s	Confidence limits of the estimate of the log of power.
$\overline{M}$	Average of several independent estimators of M.
M´	Estimate of the log of the power of The sample or the log of the ratio of powers before correcting by power supposed (M’ = log R’).
M'_s, M'_i	Upper and lower limits of the estimate of the log of power, before correcting by power supposed.
N	Total number of responses of the test.
N_P, N_A	Total number of responses for the preparations *P* and A.
P	Standard preparation.
P	Sum of the responses to the standard preparation.
P_1, P_2, P_3	Sum of responses to the doses lower, middle and top of the preparation standard *P. For testing only two dose levels, P2* represents the responses to the highest dose.
Q	Sum of squares for linearity in the same direction (Table 10).
QM	Sum of squares due to a source of variation divided by its degree of freedom.
Q_P...Q_Z	Contrast quadratic for the preparations and standard sample (Table 9).
R	Estimation of the power of the sample.
R_i, R_s	Confidence limits upper and lower estimate of power.
R’	Estimate the ratio of powers before correction by power supposed.
R+	Constant specifies to test atypical values (Table 2).
SA	Power supposed to sample A, when preparing the doses.
SQ	Sum of squares due to a source of variation.
T’	Total incomplete responses for a treatment excluding the value lost.
V = 1/W	Variance of the logarithm of individual power.
X	Differences in responses between paired sample and standard, divided by the coefficient of regression (b1), in a 5 x l
W	Statistical weighting used in the combination of several independent estimates of log power.
W’	Semi-weighting of each logarithm of power in a series of tests.
χ^2	Chi-square statistics (Table 18).

Note: tables 1 to 20 are found in section 8.9 TABLES STATISTICS. The Tables of 21 to 47 are found in

Section 8.10 EXAMPLES OF STATISTICAL TESTS.

8.2 SUBMISSIONS

BIOLOGICAL ASSAYS

Procedures are intended to evaluate the power of active principles contained in raw materials and preparations for pharmacopoeial, using biological reagents such as micro-organisms, animals, fluids and organs isolated from animals. The characteristic feature of reactive biological is its variability. While the reactive chemical physical can be defined and standardized to provide identical results in all laboratories, it is impossible to fully define the biological reagents, despite the efforts of international organizations in this regard. This variability inherent to biological reactive becomes imperative: 1) The use of reference standards suitable to obtain relative potencies and 2) the use of statistical methods for the analysis of experimental designs and results.

EXPERIMENTAL DESIGNS

The delineation of a test shall comprise: a) selection of the set of standard doses of (P) and of the samples of the unknown (A) that will be tested; b) specification of experimental units (animals, micro-organisms, BTV, blood etc.); c) rules by which it will distribute the doses for the experimental units; (d) specification of measures or other records that need to be made in each experimental unit. The best experimental design is that which produces the desired information with greater efficiency. By practical difficulties, it may be impossible to achieve this goal. Therefore, for each test can employ different experimental designs, according to the availability of personnel, reagents and time. All the designs that provide valid tests and of adequate accuracy, as a final result, are scientifically acceptable. In addition, they must understand any system that ensures that the random distribution of experimental units for the different doses used.

CHANCE AND VÍCIO

Must be randomized distribution using apparatus employed in gambling or table of random numbers. It should be noted that this procedure does not eliminate all vices. For example, the effect of chance, the animals of greater weight may be intended for a particular dose and this difference of weights vitiates the results. Therefore, it should be created the balance, i. e. they must sort-if the animals by weight range and distribute, at random, those of same weight for all the doses and preparations (standard and sample).

STATISTICAL ANALYSIS

The mathematical procedure is applied to experimental results with that aims to estimate the power of the sample and evaluate the validity and accuracy of the test. The methods of analysis are related with the experimental designs used.

RESULTS

Express the results of biological evaluation as an estimate of the power supposed to a sample (R), which will be the expression of true relative potency of the sample in relation to the pattern (p). This last is impossible to be calculated with precision due to the variability of biological reactive. Such an estimate of power supposed (R) should be accompanied by confidence limits upper and lower (Ri, Rs), or range that covers the true relative potency of the sample (p). In the monographs are established specifications for acceptable amplitude of these intervals in relation to estimated power. These specifications take into account the difficulty of the methods and the practical need to estimate the true power with certain accuracy. To achieve the confidence limits specified must, at times, be more of a test. To obtain an estimate of the power with confidence interval reduced, should combine-If, statistically, the results of these independent assays.

The probability, which measures the degree of confidence that the power is outside of the confidence limits upper and lower, is given by statistical significance (α) of an outcome or measure estimated the degree to which this result is "true". The level of significance used most in biological assays is 5% (α = 0.05) or 1% (α = 0.01). In cases not explicitly specified understood to the level of significance used in the calculation of limits is α = 0.05.

The calculation procedures are planned for the test for a single sample. In the case of being tested several samples, simultaneously, employ the modifications described in this volume.

8.3 ATYPICAL VALUES

All the responses obtained without obey strictly the protocol pre – established must be eliminated. When, after the registration of the responses, if they observe values apparently atypical, the decision to keep or delete them must be based on statistical criteria, such as those described below:

Criterion based on the variation within a single group of responses supposedly equivalent

On average, for relatively few responses identical within the group, will be flouted valid points in 2 or 4% of the evidence. Starting with the value supposedly atypical, indicate the responses in order of magnitude of *y1 to yn, where n* represents the number of observations in group or replicas of the same treatment. Calculate

$$G_1 = (y_2 - y_1) / (y_n - y_1), \text{ quando } n = 3 \text{ a } 7$$

$$G_2 = (y_3 - y_1) / (y_{n-1} - y_1), \text{ quando } n = 8 \text{ a } 13 \text{ ou}$$

$$G_3 = (y_3 - y_1) / (y_{n-2} - y_1), \text{ quando } n = 14 \text{ a } 24$$

If G_1, G_2 or G_3 exceed the critical value recorded in Table 1 for the corresponding value of n, there is no statistical basis for the elimination of the value I suspect.

Criterion that contemplates the amplitude of a series K = 2 or more groups of equal size

The groups may receive different treatments, but all the n responses within each group are derived from the same treatment. In this test, it is studied the variation of the values for each treatment that is obtained by the difference between the largest and smallest value. The value obtained with greater difference should be divided by the sum of all the differences and must not exceed the value of tariff (R+) in Table 2 for *k* = number of doses and *n* = number of replicas. If the calculated value exceeds the value column tariff, the suspicion must be investigated to detect the value discrepant. If k is less than or equal to 10, using the values presented in Table 2; if greater, multiply R+ by (k + 2) and interpolate, if necessary, between the values presented in Table 2a. If R+ exceeds the value tariff or interpolated, the group with higher interval is suspect (a = 0.05) and the observation of their data allow us to identify the value that,

then, is considered atypical. The procedure can be repeated with the remaining intervals if there is suspicion of atypical value in a second group.

8.4 DIRECT ASSAYS

Measure-if, directly, the doses of each preparation (standard and sample) necessary to produce responses pre-determined in each experimental unit of two equivalent groups of animals or other reactive organic. Typical Example is the biological testing of digital. Prepare the standard solutions and sample so that contain approximately the same power, taking into consideration the activity declared of the sample or the estimated in previous trials (S_A). Transform each result (effective dose) in logarithms (x) and calculate the average values of logarithms of effective doses for the default ($\bar{x}_P$) and for the sample ($\bar{x}_A$). Calculate the relative potency of the sample (R'), before adjusting the power supposed, as the antilogarithm *M, whereas:*

$$M' = \bar{x}_p - \bar{x}_A \quad (1)$$

Calculate the variance of M' as the sum of the variances of the two averages, from the equation

$$S^2_{M'} = S^2_x \left(\frac{1}{N_P} + \frac{1}{N_A} \right) \quad (2)$$

Whereas

$$S^2_x = \frac{\left[\sum_P x^2_p - \left(\sum_P x_p \right)^2 \Big/ N_p \right] + \left[\sum_A x^2_A - \left(\sum_P x_A \right)^2 \Big/ N_A \right]}{N_{p+} N_A - 2} \quad (3)$$

N_P and N_A numbers of animals are treated as standard and sample; ^{2} and $^{2\ represent}$ sum of calculated results for the two preparations. Calculate the confidence limits as:

$$\frac{R'_s}{R'_i} = antilog \left(M' \pm t_{s_{M'}} \right) \quad (4)$$

Obtain the appropriate value of t in Table 3, according to the degrees of freedom (gl) data by the denominator of equation (3).

Calculate the relative potency of the sample and the confidence limits, taking into account the power supposed sample (S_A) used to prepare the dilutions:

$$R = anti \log M \quad (5)$$

Whereas

$$M = M + \log S_A \quad (6)$$

With confidence limits

$$\frac{R'_s}{R'_i} = antilog \left(M' \pm t_{s_{M'}} \right) \quad (7)$$

In this trial, s_M is equal to s_M'.

For a test to be valid, the variance of *xP should be* the same of xA, differing only by sampling errors. To test, calculate the variances and divide the greater by less. That way, you get a ratio of variances (F).

Calculate the variance of *xP* as follows:

$$S^2_{xp} = \frac{\sum_P x^2_p - \left(\sum_P x_p \right)^2 \Big/ N_p}{N_{p-1}} \quad (8)$$

Calculate similarly $S^2_{x\Delta}$.(8a)

The distribution of the ratio of variances (F) is shown in Tables 4 and 5, but for this test the values in Table 4 correspond to the levels of significance at = 0.05 and in Table 5 to a = 0.01. The F value of the test shall not exceed the value in the table, corresponding to the degrees of freedom of the numerator and denominator with what is obtained F. The degrees of freedom are those of denominators of variances of the equations (8) and (8a).

QUANTITATIVE TESTS INDIRECT

NATURE AND VALIDITY

In general it is not possible to measure directly the effective dose. For this reason, the power is determined indirectly, by comparing the responses produced in quantitative scale, for example, weight, per doses known the pattern with those produced by one or more doses of sample.

In a restricted range of doses, responses or their processing convenient (logarithm, probito, etc.), linear relation with the logarithm of corresponding doses. Use two or more dose levels of default or, preferably, of the standard and the sample to determine the position and inclination of the straight line. Proceed in each test this way because, depending on the sensitivity of biological reactive used, can vary both the position and the slope of the straight line.

Each treatment consists of a fixed dose of default (p_1, p_2, p_3, etc.) or sample (a_1, a_2, a_3, etc.) and is administered to a certain number (n) of experimental units (animals, organs, cultures, pipes etc.). Register *No* responses, i. e. one for each experimental unit. For the methods presented in this chapter are valid, you must meet the following conditions:

1) The experimental units corresponding to each treatment should be selected at random.

2) For each treatment, the responses or their transformations used in the calculation (y) constitute sample of normal distribution;

3) The standard deviation of the response or of its processing is independent of the level of response, i. e., is the same for all treatments, only differing by sampling errors;

4) The response, or its transformation used in the calculations (y), has re1action linear with the logarithm of the dose (x) in the range of doses used;

5) The straight line corresponding to one or more samples must be parallel to the pattern.

From preliminary studies of the test method, it is possible to suppose the fulfilment of conditions 2 and 3. In possession of the results of each test, you can test the conditions 4 and 5. The condition 4 (linearity) can only be verified in tests in which they apply at least three dilutions of each preparation. When performing test with only two dilutions, it is presumed that the linearity of the system was previously established. The condition 5 (parallelism) should be tested in each assay. In this, they should never be used less than two dilutions of each preparation.

If it is not complied with any of the conditions 1 to 5, the calculation methods described in this chapter may not be applied and studies are necessary to establish the conditions recommended.

It is desirable that the sample is tested with doses whose responses are approximately equal to those obtained with the corresponding doses of default. This increases the precision of the result. Renamed the power supposed to sample SA.

EXPRESSION OF POWER AND RESTRICTIONS

Tests of validity corresponding and being satisfactory results, you can express the relative potency of each sample in relation to the pattern with a ratio of powers or convert into appropriate units for each sample, for example, international units, national, units of weight etc. also, can calculate- if the confidence limits from the set of data obtained in the test.

To simplify the calculations of the statistical analysis presented in this chapter, it is necessary to impose the following restrictions on the design of the tests:

a) Test each preparation, pattern and sample, with the same number of dilutions. We present formulas for testing pharmacopoeial assays, using two and three dose levels for each preparation as well as a randomized 5 x 1;

b) Maintain constant in each test, the ratio of consecutive doses for all treatments and

c) Obtain the same number of responses for each treatment.

If any response is lost, this can be estimated by methods appropriate to each design presented in this chapter; if there is loss of a treatment, meet the *Tests specified in section of partially balanced.*

8.5.1 TYPES OF DESIGN

THE CHANCE

When the experimental units are, in their entirety, reasonably homogeneous and there is no indication that the variability of the response may be lower in certain subgroups, carry out the distribution of experimental units for the different treatments at random.

There is possibility of some subgroups such as, for example, layers, positions in bookshelves or days of the experiment, they are more homogeneous than the totality of the units, the accuracy of the test can be increased by introducing one or more restrictions on experimental design.

RANDOMIZED BLOCKS

Enables segregate one source of variation as the sensitivity of different breeds of animals or the variation between the petri plates on microbiological test by diffusion. This planning requires that each treatment is applied once in each block (brood, board, etc.) and can only be performed when the block is large enough to accommodate all the treatments.

CROSSOVER

Use this planning when the experiment can be adjusted into blocks. However, it is only possible to apply two treatments per block. for example, a block can be an animal can be tested in two different occasions. Its purpose is to increase the accuracy, eliminating the influence of the variation of the animals, at the same time that balance out the effects of any difference between the general levels of response, in the two stages of the test. Renamed *double crossed the* test with two doses of the standard and the sample, and triple *crossed* one of three doses of each preparation. carry out the test in two stages according to the period of time defined in the method. Distribute the animals in four or six groups and perform a treatment in each group in the first phase. In the second phase, the animals that received a preparation will receive another; the animals that received lower doses, this step will receive the largest. Follow the diagram Table 6.

LATIN SQUARE

When the Appropriate response can be affected by two sources of variation, each of which can have *k* different levels. For example, if you perform the experiment in *k* different days and by *k* experimenters, or if performs a test of antibiotics by diffusion on board, in which the treatments can be applied in a diagram of *k x k,* where each treatment occurs only once in each row and in each column. Use only when the number of columns, rows and treatments are equal.

The responses are recorded in the form of a square called Latin. There are many possibilities of Latin squares found

in the specialized literature. From a can prepare them if other, alternating at random queues and/or columns. In Table 7 there are example of Latin square with two doses of the standard and the sample.

For any design, the distribution of experimental units in blocks must be made per procedure to chance, being the units kept the more evenly as possible before and during the experiment.

8.5.2 ANALYSIS OF VARIANCE

To carry out this analysis has as objective to study the validity of the test and calculate the residual error. With the exception of the calculation of the residual error, the analysis of data from a test is identical for all randomized designs, randomized block and Latin square. The following will be described, the formulas for analysis of each type of test. See the glossary of symbols. The formulas are appropriate for the case that if you're comparing a single sample *(A)* against the reference standard *(P),* such as, also, for the case of multiple assays where are included h-l samples *(A... Z)*. The formulae for the crossover trials do not fall under the general scheme and will be presented separately.

If necessary, turn the responses (y) to comply with the conditions of validity described. Add together all the y values for each treatment and for each preparation, as can be observed in Tables 8 and 9. From these data, obtain the related linear contrasts with the inclinations of dose-response lines.

When are tested three doses of each preparation, if you get, also, contrasts quadratics that represent the curvature of the lines. See formulas in Tables 8 and 9.

The total variation of responses arising from different treatments can be as shown in Table 10. The sums of squares are obtained from the values in Tables 8 or 9. K represents the square of the sum of all the responses obtained in the test divided by the total number of them:

$$k = \{\sum y\ )^2/N\}$$

Calculate the residual error of the assay by subtracting the variations controlled the total variation in responses (Table11). In this table, Sy2 represents the sum of the squares of all the responses recorded in the test. It should be noted that the sum of squares, reduced, corresponding to item treatments is equal to the sum of the sums of squares reduced (Table10) and that, for the Latin square, the number of responses replicated *(n)* is equal to the number of rows, columns or treatments (*k)*.

8.5.3 TESTS OF VALIDITY

To test the significance of the sources of variation related to Table 10, each sum of squared reduced obtained in the table must be divided by the corresponding degree of freedom to obtain the mean squared. The mean squared error of the residual (s^2) is similar quotient, obtained the appropriate line in Table 11.

To obtain the reason known as F, divide the Mean Square of each source of variation to be tested by variance (s^2). Calculate the significance of each source and compare with the tabulated values (Tables4 and 5) at a significance level of 5% (a = 0.05) and 1% (a = 0.01). The F values are obtained in the column corresponding to the number of degrees of freedom associated with the mean squared the source tested *(gl_1)* and in the queue of the table corresponding to the number of degrees of freedom associated with s2 ($g1_2$). If the value of F calculated is greater than the value tariff, the source of variation tested is considered "significant" for the level of probability used.

Consider the tests "statistically valid" if the tests show the following results:

Delineation of straight, parallel

1) Significant Regression, i. e. F calculated is greater than the tariff at a significance level of 1% (a = 0.01). Indicates that the slope of the dose-response line is satisfactory;

2) Terms quadratics not significant, i. e., the values of F calculated should be smaller than those tabulated at a significance level of 5% (a = 0.05). Equates to satisfy the condition of linearity of the relationship between the transformation of response used and the logarithm of the dose;

3) Parallelism is not significant, i. e., F calculated must be less than the value tariff at a significance level of 5% (a = 0.05) indicating that the straight lines of the standard and sample are parallel. If they are rehearsing-if several samples, simultaneously, and get it if a significant deviation from parallelism, this may be due to the use of some preparation which supplied line dose-response with a different tilt in relation to the other samples. In this case, calculate the value of t' for each preparation A... Z, using the equation

$$t' = \frac{L_P - L_A}{2s\sqrt{n}} \qquad (9)$$

Each t' calculated should be compared with the value from the Table 12, where *g11 = h* -1 and *g12* is equal to the number of degrees of freedom associated with s2. If you find the value of t "significant" for some sample, all the data relating to this preparation should be eliminated from the test and the analysis repeated from the beginning.

In assays with residual error too large, a reason F "significant" for the term preparations may indicate that the assumption of power that served as the basis for the preparation of dilutions was not correct. This is not a

condition of nullity. Coming to this conclusion, the power estimated test can be used as power supposed in later trials.

In tests of parallelism and quadratics can occur by chance F values very low, less than 1. If this happens, repeatedly, can be an indication of that is not fulfilled the conditions supposed, which should be investigated more deeply.

A Randomized 5 x 1

The validity is established when:

1) Significant regression, i. e. F calculated must be greater than the value tariff for significance level of a = 0.01. Indicates that the slope of the dose-response line is satisfactory;

2) No significant deviation from linearity. The relationship between both variables (logarithm of the dose and inhibition halo) should be linear. The value of F calculated must be shorter than the tariff for significance level of a = 0.05. Another measure to be carried out is the Pearson's correlation coefficient (r) which must be greater than 0.98;

3) Coefficient of variation (CV) less than 5% is appropriate. The variability of the response from the calibration curve must be constant.

In the case of *crossover trials, with diagram of special calculation, the formulas to be used are shown in Tables 13 and 14.*

There are three terms of interactions due the replicas within each group: Phases X Preparations, Phases and Stages X X Regression Parallelism.

As we previously discussed designs, each sum of squares reduced must be divided by the corresponding number of degrees of freedom to get the squares dipped headlights.

In the case of randomized double crossover, you get two squares average corresponding to errors I and II, which are called s^2_I and s^2_{II}. Divide the Mean Square of each source of variation by s^2 appropriate to obtain the reason F.

For the sources Parallelism, Phases Phases X X Preparations, Regression, uses-if s^2_I. For the other sources, uses-if s^2_{II}.

Calculate the significance of the source using the Tables 4 and 5. If F calculated is greater than the value tariff, for the degrees of freedom of the source tested ($g1_1$) and the corresponding s^2 ($g1_2$), the source of variation is considered "significant" for the level of significance (a = 0.05, or = 0.01).

For a test to be valid, the regression *should be significant and the parallelism and the three interactions should not be significant.*

In crossover, the parallelism test is not very sensitive, because it depends on the variation between blocks (animals).

Established the statistical validity of tests made with any delineation, calculate the power and the limits of confidence by the methods described below.

8.5.4 ESTIMATION OF THE POWER AND LIMITS OF TRUST

Calculations for the design of straight, parallel (3 x 3 or X 2)

First, calculate the average response for each preparation

$(\bar{y}_P, \bar{y}_A, ..., \bar{y}_Z)$

$$\bar{y}_P = \frac{P}{N_P} \qquad (10)$$

And, similarly, for other preparations.

Calling them if I the interval in logarithm of the concentrations, for each preparation, in tests with two doses you get the common slope (b), from the equation

$$b = \frac{L_P + L_A + ... L_z}{Inh} \qquad (11)$$

For tests with three doses each preparation, the denominator Inh should be replaced by 2 Inh.

The logarithm of the power ratio of The sample *(M'_A), before correcting by value of S_A, it is*

$$M'_A = \frac{\bar{y}_A - \bar{y}_P}{b} \qquad (12)$$

The power calculated is the estimate of the true power of each sample. The confidence limits (with 5% probability of delete the real power or = 0.05) can be calculated as the antilogarithm of formula

$$\begin{matrix} M'_{As} \\ M'_{AI} \end{matrix} = CM'_A \pm \frac{ts\sqrt{C}}{b}\sqrt{\frac{1}{N_P} + \frac{1}{N_A} + \frac{(\bar{y}_A - \bar{y}_P)^2}{E\text{-}s^2t^2}} \qquad (13)$$

Whereas

$$C = E/(E - s^2t^2) \qquad (14)$$

Obtain AND **Table 10. The s2 is the** residual error from Table Divided by its degrees of freedom and t is shown in Table **3** in accordance with the degrees of freedom of s2.

For tests 2 and 3 balanced doses per preparation, the formula for the limits of equation 13 can be simplified:

$$\begin{matrix} M'_{As} \\ M'_{AI} \end{matrix} = CM'_A \pm \sqrt{(C\text{-}1)\,(CM'^2_A + c'I^2)} \qquad (15)$$

Whereas c' is the coefficient obtained in Table **15** and C is the measure of significance of the regression. In test with Tilt well defined the value of C will be very close to the unit.

Calculation for a randomized 5 x 1

Procedure for construction of the dose-response curve – In turbidimetric method, measure the turbidity in tubes with liquid medium.

In the method by diffusion in agar, measure the inhibition halos for each of the concentrations of standard ((P_1, P_2, P_3, P_4, e P_5) in 4 sets of plates. The average of 36 readings of the intermediate concentration of default (P3) is used to correct the averages of each of the other concentrations of standard P_1, P_2, P_4, P_5.

The correction if logs as follows: measure the 36 readings of P_3 in all the plates and calculate the average. Measure the 9 readings of P3 in the set of cards (3) for other concentrations (P_1, P_2, P_4 and P_5) and calculate the average. Calculate the difference between the average total and the average in the 3 plates for each concentration, to which must be added the measures of the other concentrations.

Example:

Average value of P_3 in 36 readings: 18.2 mm average value of P_3 on the boards with P_1.18.0 mm

Value of P_1 in the first reading of 9 plates: 17.3 mm corrected value at the first point En: (18.2 – 18.0) + 17.3 = 17.5 mm

Value of P_t in second reading of 9 plates: 16.9 mm corrected value in point P_t: (18.2 – 18.0) + 16.9 = 17.1 mm

Build a table with the corrected response for their concentrations (P_1 to P_5) according to Table 19 and perform the analysis of variance. Confirmed the validity of the results, calculate the difference in responses between paired sample and standard on the central point of the curve by the equation

$$X = (y_A - y_p)/b_1 \qquad (16)$$

Whereas *yA* is one of the responses of the sample among the f replays, *yp* is the response pattern of paired and bt is the regression coefficient given by Table 20.

The logarithm of the power ratio is

$$M'_A = \sum X/f \qquad (17)$$

Where f is the number of paired differences in responses between the sample and the standard

When a number of tests on the same sample is obtained through the same curve, calculate the coefficient of variation (CV) for the results of the samples.

$$CV = \frac{\text{standard deviation(s)}}{\text{mean (y) of each sample}} \times 100$$

Where $S = \sqrt{\frac{\sum y^2 - \sum(y^2)/n}{N-1}}$ and y is response from 1 to N for a same sample. (18A)

The variance is calculated over the f values of X for the total samples tested as

$$S_M^2 = \frac{\sum X^2 - \sum(Tx^2/f)}{n''} \qquad (19)$$

Where Tx = ΣX for a single sample and $n'' = \sum f - h'$ e *h and h'* is the number of samples tested.

The logarithm of the confidence interval for each sample is

$$L = \frac{2s_M t}{\sqrt{k'}} \qquad (20)$$

Where s is the standard deviation for the total of differences X, t is shown in Table 3 with the degrees of freedom of s2M and *k' is the number of paired differences per sample tested.*

The confidence limits (with 5% probability of delete the real power) can be calculated with the antilogarithm of formula

$$\begin{matrix} M'_{As} \\ M'_{AI} \end{matrix} = M'_A \pm 1/2L \qquad (21)$$

Get the power ratio (RA) and the confidence limits (Rs, Ri) taking the antilogarithms of the values obtained from the formulas 12 and 15 (straight, parallel design 3 x 3 or 2 x 2) and 17 and 21 (a randomized 5 x 1), after adding log SA to both:

$$M_A = M'_A + \log S_A \qquad (22)$$

$$R_A = anti\log M_A \qquad (23)$$

$$M_{As} = M'_{As} + \log S_A \qquad (24)$$

$$M_{Ai} = M'_{Ai} + \log S_A \qquad (25)$$

$$R_{As} = anti\log M_{As} \qquad R_{Ai} = anti\log M_{Ai}$$

Lost Values

In trials balanced requires the same number of observations for each concentration. If any response is lost by cause not related to the treatments applied, such as the death of an animal or a breach of any test tube, the statistical analysis becomes much more complex. You can restore the balance of two modes:

1) Reduce the number of observations in larger groups until the number of responses is the same for each treatment. If the design is totally at random, you can subtract the mean of each larger group, as many times as needed, or eliminate one or more responses to each larger group by selecting them at random. For testing in randomized blocks, keep only the full blocks;

Alternatively, a smaller group, incidentally, can be restored to the original size when the number of missed replies is not greater than one in any treatment or 5% in the total test. In this case, calculate the replacement of lost value. *You Lose a degree of freedom in the error variance s^2 for each substituted value:*

a) If the design is totally at random, replace the lost value by the average of the remaining responses group incomplete;

b) If the design is in randomized blocks, replace the lost value by applying the formula

$$y' = \frac{n\mathrm{B}' + k\mathrm{T}' - \mathrm{G}}{(n-1)(k-1)} \qquad (26)$$

Whereas B' is the total of incomplete responses in the block that contains the lost value, T' is the total number of incomplete responses in treatment that contains the lost value, G' is the sum total of the responses obtained in the test. As previously defined, *n is the number of blocks and k is the number of treatments or doses;*

c) If the test is based on a randomized Latin square, the lost value (y') is obtained by the equation

$$y' = \frac{k(\mathrm{B}' + \mathrm{C}' + \mathrm{T}') - 2\mathrm{G}}{(k-1)(k-2)} \qquad (27)$$

Whereas (B' and C' are the sums of the responses in the rows and columns, respectively, that contain the value lost. In this case, $k = n$.

If there is loss of more than one value, replace, temporarily, by the average of the respective treatment, all the empty seats, except one. Replace this place with the value y', calculated by equation 27. Replace one by one the values that had been placed, temporarily, by the average up to obtain stable set of values for all the responses lost.

If the number of substituted values is small in relation to the total number of observations in the test (less than 5 %), the approximation resulting from substitutions described and reduction of degrees of freedom, equivalent to the number of substituted values, is generally satisfactory. However, the analysis should be interpreted with caution, especially if there is a predominance of lost values in a particular treatment or block. The same is valid for the case of lost values in planning crusaders.

Tests partially balanced

If the presumed power of the samples (used to calculate the doses of the test) is very different from the real power, it is possible that the higher dose provide maximum response or that the lower dose response provide very low or zero. These responses will be outside the zone curve linear log doserresposta and the validity tests indicate curvature and/ or diversion of parallelism "significant".

In this case, the responses to the higher dose or less of the sample can be neglected, by calculating a value

Power on from the remaining data. This power may be taken as power supposed to select doses of sample for another test, with the objective of obtaining similar responses to standard and, thus, increasing the precision of the result. The equation that employs to calculate the power is:

$$\mathrm{M'}_{\mathrm{A}} = \frac{\bar{y}_A - \bar{y}_P}{\mathrm{b}} \pm \frac{I}{2} \qquad (28)$$

This formula is similar to the formula 12, however, subtracts to half the log interval dose when it omits the responses of the lower dose and adds them if the same interval when you despise the highest dose.

The responses medium y_A and y_P are obtained from the same formula that we tests fully balanced formula (10), however, it should be introduce modification in the calculation of the slope (b) in accordance with the design of the test.

For *multiple assays, which, necessarily, would have two doses of each preparation, the linear contrasts (Lp... Lz)* must form excluding L_A (as responses to a1 or a2 were eliminated, it is not possible to form a contrast L_A). Calculating the slope from the average of the values of L divided by In:

$$\mathrm{b} = \frac{\mathrm{L_P} + \ldots + \mathrm{L_z}}{\mathrm{I}n(h+1)} \qquad (29)$$

For simple test with a sample:

$$\mathrm{b} = \frac{\mathrm{L_P}}{\mathrm{I}n} \qquad (30)$$

For multiple assays with three doses of each preparation and the other contrasts, see Table 9. The equation for the slope is:

$$\mathrm{b} = \frac{2(\mathrm{L_P} + \ldots + \mathrm{L_z}) + \mathrm{L_A}}{\mathrm{I}n(4h-3)} \qquad (31)$$

If there is a *single sample, the equation reduces to:*

$$\mathrm{b} = \frac{2\mathrm{L_P} + \mathrm{L_A}}{5\mathrm{I}n} \qquad (32)$$

8.6 MOVABLE MEDIUM

In the particular case of biological assay of heparin, the interval between the dose that makes the coagulation and one that inhibits is so small that the dose-response curve can not be determined explicitly. To interpolate the logarithm of the dose corresponding to 50% of coagulation, both for the standard as for the sample, using the moving averages.

Calculation of power

LOGARITHM Transform in the volumes of standard preparation used in 5 or 6 tubes that form the series, so that 2 or 3 tubes present degrees of coagulation equal to or smaller than 0.5 and 2 or 3 tubes have degrees equal to or larger than 0.5.

Venovenous table correlating the pipes, numbered consecutively, with the degree of coagulation was observed.

Renamed *x* the logarithms of volumes used and *y* the corresponding degrees of coagulation. Calculate the averages 511 x_i and y_i of tubes 1, 2 and 3; the pipes 2, 3 and 4 and the pipes 3, 4 and 5, and when the series consist of 6 tubes, pipes 4, 5 and 6, respectively. If for one of these pairs of means the degree of coagulation medium y_i is exactly 0.50, the corresponding x_i is the median of the logarithm of the volume of preparation standard x_p. If this does not occur, interpolate the x_p from the paired values y_i, x_i and y_i+1, x_i+1 that occur, immediately below and above the grade 0.50, such as:

$$x_p = x_i + (y_i - 0{,}5)\,(x_{i+1} - x_i) / (y_i - y_{i+1}) \qquad (33)$$

From the paired data obtained in tubes of sample, calculate the same way the median of the logarithm of the volume xA. The logarithm of the power of the sample is:

$$M_A = x_p - x_A + \log S_A \qquad (34)$$

Whereas S_A is the assumption of the power of the sample made in the preparation of the corresponding solution of the sample tubes.

Repeat the test, regardless, and calculate the mean of two or more values of M for obter $\overline{M}$. If the second determination of M differs from the first more than 0.05, continue performing tests until the logarithm of the confidence interval, calculated as end of section Combination of estimates of power, does not exceed 0.20.

The power of heparin sodium is:

$$R = anti \log M$$

8.7 TESTS INDIRECT "ALL OR NOTHING"

In some trials is not appropriate or possible measure the effect in each experimental unit (for example, animal) in quantitative scale. In this case, it may measure effects of everything or nothing, such as death or occurrence of symptom pre- established. The proportion of experimental units that exhibit the symptom is the result. These tests are called quantais. In this chapter will be presented an approximate calculation. In the case of disposal of computing facilities, you can use the theoretical calculation exact. You must register for each dose, the percentage of animals with positive effect. Example: percentage of mice in convulsion. Transform the percentages in probit, using Table 16. Each probito will be regarded as the value of the response transformed (y). The following method is used when not occur responses equivalent to percentages zero or 100. In this case, to employ statistical methods to complete maximum Probability (log or probito). For each value of *y, you should get* a value of weighting (w) in Table 17.

The formulas of the sums of squares for the validity tests are the same used in quantitative indirect tests (Table 10), by taking n = 1, with the exception of the term error (s^2), which has degrees of freedom equal to infinity, and it is estimated as:

$$s^2 = \frac{k}{n\sum w} \qquad (35)$$

Whereas *k* = number of treatments, *n* = number of animals used in each treatment.

Calculate the power and the confidence limits using the formulas 12 and 25. This method is useful when the approximate test is outlined so that the responses in percentage corresponding to the smaller and larger doses are evenly spaced around 50 %. If one of the doses tested provide responses zero or 100 %, these can be neglected. In this case, to obtain the estimate of power by the methods described in section *Tests partially balanced.*

8.8 COMBINATION OF ESTIMATES OF POWER

When performing *n' independent trials for each sample, the results can be combined in order to obtain a power estimated with confidence interval reduced, which complies with the limits established in each monograph. There are several methods for combining repeated assays.*

Adopt simplifications, taking into account two aspects:

a) Correct estimates of the log of power (M') by power supposed (S_A before performing the combinations (M = M' + log S_A);

b) The estimates should be independent, i. e., obtained in separate tests.

8.8.1 WEIGHTED AVERAGE POWER AND LIMITS OF TRUST

Suppose that were analyzed results of n trials to provide n' values of M with confidence limits (in logarithms) associated with each value of M, obtained according to the equations 13 to 15 and 22 to 25. For each test, obtain the confidence interval logarithmic (L), by subtracting the lower limit of the upper. Calculate, also, a weighting (W) for each value of M from the equation 36, where t is the same value used in the calculation of the confidence interval:

$$W = \frac{4t^2}{L^2} \qquad (36)$$

For each trial, calculate the product WM and divide their sum by the sum of all the weights in order to obtain the logarithm of power weighted average (M), according to the equation 37:

$$\overline{M} = \sum_n WM / \sum_n W \qquad (37)$$

The Standard error of the mean power ($s\overline{M}$) is the square root of the reciprocal of the total weighting:

$$S\overline{M} = \sqrt{1/\sum_{n'} W} \quad (38)$$

Calculate the confidence limits approximate (a = 0.05), from the antilogarithm of the values obtained by the formula 39:

$$\overline{M} \pm ts_{\overline{M}} \quad (39)$$

You Get the value of t in Table 3, with degrees of freedom equal to the sum of the degrees of freedom of the variance of the error of individual tests.

This method approximate combination of satisfactory results when:

a) C is less than 1.1 for each of the n *' tests;*

b) The individual estimates of the power form a homogeneous set of agreement with the homogeneity test was performed by applying the statistic x2 This calculated raising himself to square the difference between each value of M in re1action to weighted average $\overline{M}$, multiplying this square by corresponding weighting (W) and summing the values for all the tests:

$$x^2_M = \sum_{n'} W (M - \overline{M}) \quad (40)$$

Se o valor de $\mathbf{c}^2_M$ calculated is less than the corresponding in Table 18 for (n' – 1) degrees of freedom, it is considered that there is no evidence to suspect the heterogeneity of powers. In this case, the average power and the calculated limits are correct.

If the value $\mathbf{c}^2_M$ formaiorque the Table 18, it is considered that the powers are heterogeneous, i. e., that the dispersion of the individual values of M is greater than expected, according to their respective confidence limits. In this case, do not apply the formulas 37 and 39, determine the origin of this heterogeneity and, if deemed appropriate, calculate M using semiponderacoes W':

$$W' = 1/(V + v) \quad (41)$$

Whereas

And v is the variance of the heterogeneity among trials and if it is calculated by the following equation:

$$V = 1/W = \frac{L^2}{4t^2} \quad (42)$$

When v varies in such a way that v calculated is negative number, you can calculate v approximate, omitting the term after the negative sign in equation 43.

$$V = \frac{\sum M^2 - (\sum M)^2 / n'}{n' - 1} - \frac{\sum V}{n'} \quad (43)$$

To calculate the average semiponderada (M), replace in equation 31 the values of W and ZW by the respective values of W' and ∑W':

$$\overline{M} = \sum_{n'} WM / \sum_{n'} W \quad (44)$$

You can consider this value of M near the center of a confidence interval of approximate size L'c, which is the square root of:

$$L_c'^2 = 4t^2 / \sum W \quad (45)$$

Whereas t, from Table 3, has degrees of freedom equal to the sum of degrees of freedom of the variance of the error of n ' individual tests.

In the special case *of the assay of heparin,* all the logarithms of power (M) has the same weighting and the confidence interval of logarithm of the estimation of power (M) is determined as follows:

Calculation of the variance of the error with n' – 1 degrees of freedom:

$$s^2 = \{\sum M^2 - (\sum M)^2/n'\}/n' - 1 \quad (46)$$

Determine the limit of confidence in plots (L)

$$L = \frac{2st}{\sqrt{n'}} \quad (47)$$

in which

$s = \sqrt{s^2}$, t (Table 3) with n – 1 degrees of freedom, n = number of individual estimates of power.

Calculate the confidence limits:

$$M_s = \overline{M} + 1/2L \quad (48)$$

$$M_i = \overline{M} - 1/2L \quad (49)$$

$$R_s = \text{antilog}M_s \quad (50)$$

$$R_i = \text{antilog}M_i \quad (51)$$

8.9 TABLES STATISTICS

Table 1 – Table G for outliers.

n	3	4	5	6	7						
G_1	0,976	0,846	0,729	0,644	0,586						
n	8	9	10	11	12	13					
G_2	0,780	0,725	0,678	0,638	0,605	0,578					
n	14	15	16	17	18	19	20	21	22	23	24
G_3	0,602	0,579	0,559	0,542	0,527	0,514	0,502	0,491	0,481	0,472	0,464

Table 2 – Test for groups containing outliers.

k	***Critical Value of R+ for interval of n observations each one, the significance level of a = 0.05***								
	2	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***
2	0,962	0,862	0,803	0,764	0,736	0,717	0,702	0,691	0,682
3	0,813	0,667	0,601	0,563	0,539	0,521	0,507	0,498	0,489
4	0,681	0,538	0,479	0,446	0,425	0,410	0,398	0,389	0,382
5	0,581	0,451	0,398	0,369	0,351	0,338	0,328	0,320	0,314
6	0,508	0,389	0,342	0,316	0,300	0,288	0,280	0,273	0,267
7	0,451	0,342	0,300	0,278	0,263	0,253	0,245	0,239	0,234
8	0,407	0,305	0,267	0,248	0,234	0,225	0,218	0,213	0,208
9	0,369	0,276	0,241	0,224	0,211	0,203	0,197	0,192	0,188
10	0,339	0,253	0,220	0,204	0,193	0,185	0,179	0,174	0,172

Table 3 – Student's t Distribution.

k	***Critical Value of (k+2) for interval of n observations each one, the significance level of a = 0.05***								
	2	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***
10	4,06	3,04	2,65	2,44	2,30	2,21	2,14	2,09	2,05
12	4,06	3,03	2,63	2,42	2,29	2,20	2,13	2,07	2,04
15	4,06	3,02	2,62	2,41	2,28	2,18	2,12	2,06	2,02
20	4,13	3,03	2,62	2,41	2,28	2,18	2,11	2,05	2,01
50	4,26	3,11	2,67	2,44	2,29	2,19	2,11	2,06	2,01

Table 3 – Student's t Distribution.t.

gl \ *α*	*0,9*	*0,8*	*0,7*	*0,6*	*0,5*	*0,4*	*0,3*	*0,2*	*0,1*	*,05*	*,02*	*,01*	*,001*
1	0,158	0,325	0,510	0,727	1,000	1,376	1,963	3,078	6,314	12,706	31,821	63,657	636,619
2	0,142	0,289	0,445	0,617	0,816	1,061	1,386	1,886	2,920	4,303	6,965	9,925	31,598
3	0,137	0,277	0,424	0,584	0,765	0,978	1,250	1,638	2,353	3,182	4,541	5,541	12,924
4	0,134	0,271	0,414	0,569	0,741	0,941	1,190	1,533	2,132	2,776	3,747	4,604	8,610
5	0,132	0,267	0,408	0,559	0,727	0,920	1,156	1,476	2,015	2,571	3,365	4,032	6,869
6	0,131	0,265	0,404	0,553	0,718	0,906	1,134	1,440	1,943	2,447	3,143	3,707	5,959
7	0,130	0,263	0,402	0,549	0,711	0,896	1,119	1,415	1,895	2,365	2,365	3,499	5,408
8	0,130	0,262	0,399	0,546	0,706	0,889	1,108	1,397	1,860	2,306	2,896	3,355	5,041
9	0,129	0,261	0,398	0,543	0,703	0,883	1,100	1,383	1,833	2,262	2,821	3,250	4,781
10	0,129	0,260	0,397	0,542	0,700	0,879	1,093	1,372	1,812	2,228	2,764	3,169	4,587
11	0,129	0,260	0,396	0,540	0,697	0,876	1,088	1,363	1,796	2,201	2,718	3,106	4,437
12	0,128	0,259	0,395	0,539	0,695	0,873	1,083	1,356	1,782	2,179	2,681	3,055	4,318
13	0,128	0,259	0,394	0,538	0,694	0,870	1,079	1,350	1,771	2,160	2,650	3,012	4,221
14	0,128	0,258	0,393	0,537	0,692	0,868	1,076	1,345	1,761	2,145	2,624	2,977	4,140
15	0,128	0,258	0,393	0,536	0,691	0,866	1,074	1,341	1,753	2,131	2,602	2,947	4,073
16	0,128	0,258	0,392	0,535	0,690	0,865	1,071	1,337	1,746	2,120	2,583	2,921	4,015
17	0,128	0,257	0,392	0,534	0,689	0,863	1,069	1,333	1,740	2,110	2,567	2,898	3,965
18	0,127	0,257	0,392	0,534	0,688	0,862	1,067	1,330	1,734	2,101	2,552	2,878	3,922
19	0,127	0,257	0,391	0,533	0,688	0,861	1,066	1,328	1,729	2,093	2,539	2,861	3,883
20	0,127	0,257	0,391	0,533	0,687	0,860	1,064	1,325	1,725	2,086	2,528	2,845	3,850
21	0,127	0,257	0,391	0,532	0,686	0,859	1,063	1,323	1,721	2,080	2,518	2,831	3,819
22	0,127	0,256	0,390	0,532	0,686	0,858	1,061	1,321	1,717	2,074	2,508	2,819	3,792
23	0,127	0,256	0,390	0,532	0,685	0,858	1,060	1,319	1,714	2,069	2,500	2,807	3,767
24	0,127	0,256	0,390	0,531	0,685	0,857	1,059	1,318	1,711	2,064	2,492	2,797	3,745
25	0,127	0,256	0,390	0,531	0,684	0,856	1,058	1,316	1,708	2,060	2,485	2,787	3,726
26	0,127	0,256	0,390	0,531	0,684	0,856	1,058	1,315	1,706	2,056	2,479	2,779	3,707
27	0,127	0,256	0,389	0,531	0,684	0,855	1,057	1,314	1,703	2,052	2,473	2,771	2,690
28	0,127	0,256	0,389	0,530	0,683	0,855	1,056	1,311	1,699	2,045	2,462	2,756	3,674
29	0,127	0,256	0,389	0,530	0,683	0,854	1,055	1,310	1,697	2,042	2,457	2,750	3,659
30	0,127	0,255	0,389	0,530	0,683	0,854	1,055	1,310	1,697	2,042	2,457	2,750	3,646
40	0,126	0,254	0,388	0,529	0,681	0,851	1,050	1,303	1,684	2,021	2,423	2,704	3,551
60	0,126	0,254	0,387	0,527	0,679	0,848	1,046	1,296	1,671	2,000	2,390	2,660	3,460
120	0,126	0,253	0,386	0,526	0,677	0,845	1,041	1,289	1,658	1,980	2,358	2,617	3,373
∞	0,126	0,256	0,385	0,524	0,674	0,842	1,036	1,282	1,645	1,960	2,326	2,576	3,291

gl = degrees of freedom; the = level of significance

Table 4 – Distribution F of Fisher for =0.05.

gl_1 Denominator	gl_2 Numerator									
	1	*2*	*3*	*4*	*5*	*6*	*8*	*12*	*24*	∞
1	161,40	199,50	215,70	224,60	230,20	234,00	238,90	243,90	249,00	254,30
2	18,51	19,00	19,16	19,25	19,30	19,33	19,37	19,41	19,45	19,50
3	10,13	9,55	9,28	9,12	9,01	8,94	8,84	8,74	8,64	8,53
4	7,71	6,94	6,59	6,39	6,26	6,16	6,04	5,91	5,77	5,63
5	6,61	5,79	5,41	5,19	5,05	4,95	4,82	4,68	4,53	4,36
6	5,99	5,14	4,76	4,53	4,39	4,28	4,15	4,00	3,84	3,67
7	5,59	4,74	4,35	4,12	3,97	3,87	3,73	3,57	3,41	3,23
8	5,32	4,46	4,07	3,84	3,69	3,58	3,44	3,28	3,12	2,93
9	5,12	4,26	3,86	3,63	3,48	3,37	3,23	3,07	2,90	2,71
10	4,96	4,10	3,71	3,48	3,33	3,22	3,07	2,91	2,74	2,54
11	4,84	3,98	3,59	3,36	3,20	3,09	2,95	2,79	2,61	2,40
12	4,75	3,88	3,49	3,26	3,11	3,00	2,85	2,69	2,50	2,30
13	4,67	3,80	3,41	3,18	3,02	2,92	2,77	2,60	2,42	2,21
14	4,60	3,74	3,34	3,11	2,96	2,85	2,70	2,53	2,35	2,13
15	4,54	3,68	3,29	3,06	2,90	2,79	2,64	2,48	2,29	2,07
16	4,49	3,63	3,24	3,01	2,85	2,74	2,59	2,42	2,24	2,01
17	4,45	3,59	3,20	2,96	2,81	2,70	2,55	2,38	2,19	1,96
18	4,41	3,55	3,16	2,93	2,77	2,66	2,51	2,34	2,15	1,92
19	4,38	3,52	3,13	2,90	2,74	2,63	2,48	2,31	2,11	1,88
20	4,35	3,49	3,10	2,87	2,71	2,60	2,45	2,28	2,08	1,84
21	4,32	3,47	3,07	2,84	2,68	2,57	2,42	2,25	2,05	1,81
22	4,30	3,44	3,05	2,82	2,66	2,55	2,40	2,23	2,03	1,78
23	4,28	3,42	3,03	2,80	2,64	2,53	2,38	2,20	2,00	1,76
24	4,26	3,40	3,01	2,78	2,62	2,51	2,36	2,18	1,98	1,73
25	4,24	3,38	2,99	2,76	2,60	2,49	2,34	2,16	1,96	1,71
26	4,22	3,37	2,98	2,74	2,59	2,47	2,32	2,15	1,95	1,69
27	4,21	3,35	2,96	2,73	2,57	2,46	2,30	2,13	1,93	1,67
28	4,20	3,34	2,95	2,71	2,56	2,44	2,29	2,12	1,91	1,65
29	4,18	3,33	2,93	2,70	2,54	2,43	2,28	2,10	1,90	1,64
30	4,17	3,32	2,92	2,69	2,53	2,42	2,27	2,09	1,89	1,62
40	4,08	3,23	2,84	2,61	2,45	2,34	2,18	2,00	1,79	1,51
60	4,00	3,15	2,76	2,52	2,37	2,25	2,10	1,92	1,70	1,39
120	3,92	3,07	2,68	2,45	2,29	2,17	2,02	1,83	1,61	1,25
∞	3,84	2,99	2,60	2,37	2,21	2,10	1,94	1,75	1,52	1,00

Table 5 – Distribution F of Fisher for = 0.01..

gl_2 Denominator	gl_1 Numerator									
	1	*2*	*3*	*4*	*5*	*6*	*8*	*12*	*24*	∞
1	4052	4999	5403	5625	5764	5859	5982	6106	6234	6366
2	98,50	99,00	99,17	99,25	99,30	99,33	99,37	99,42	99,46	99,50
3	34,12	30,82	29,46	28,71	28,24	27,91	27,49	27,05	26,60	26,12
4	21,20	18,00	16,69	15,98	15,52	15,21	14,80	14,37	13,93	13,46
5	16,26	13,27	12,06	11,39	10,97	10,67	10,29	9,89	9,47	9,02
6	13,74	10,92	9,78	9,15	8,75	8,47	8,10	7,72	7,31	6,88
7	12,25	9,55	8,45	7,85	7,46	7,19	6,84	6,47	6,07	5,65
8	11,26	8,65	7,59	7,01	6,63	6,37	6,03	5,67	5,28	4,86
9	10,56	8,02	6,99	6,42	6,06	5,80	5,47	5,11	4,73	4,31
10	10,04	7,56	6,55	5,99	5,64	5,39	5,06	4,71	4,33	3,91
11	9,65	7,20	6,22	5,67	5,32	5,07	4,74	4,40	4,02	3,60
12	9,33	6,93	5,95	5,41	5,06	4,82	4,50	4,16	3,78	3,36
13	9,07	6,70	5,74	5,20	4,86	4,62	4,30	3,96	3,59	3,16
14	8,86	6,51	5,56	5,03	4,69	4,46	4,14	3,80	3,43	3,00
15	8,68	6,36	5,42	4,89	4,56	4,32	4,00	3,67	3,29	2,87
16	8,53	6,23	5,29	4,77	4,44	4,20	3,89	3,55	3,18	2,75
17	8,40	6,11	5,18	4,67	4,34	4,10	3,79	3,45	3,08	2,65
18	8,28	6,01	5,09	4,58	4,25	4,01	3,71	3,37	3,00	2,58
19	8,18	5,93	5,01	4,50	4,17	3,94	3,63	3,30	2,92	2,49
20	8,10	5,85	4,94	4,43	4,10	3,87	3,56	3,23	2,86	2,42
21	8,02	5,78	4,87	4,37	4,04	3,81	3,51	3,17	2,80	2,36
22	7,94	5,72	4,82	4,31	3,99	3,76	3,45	3,12	2,75	2,31
23	7,88	5,66	4,76	4,26	3,94	3,71	3,41	3,07	2,70	2,26
24	7,82	5,61	4,72	4,22	3,90	3,67	3,36	3,03	2,66	2,21
25	7,77	5,57	4,68	4,18	3,86	3,63	3,32	2,99	2,62	2,17
26	7,72	5,53	4,64	4,14	3,82	3,59	3,29	2,96	2,58	2,13
27	7,68	5,49	4,60	4,11	3,78	3,56	3,26	2,93	2,56	2,10
28	7,64	5,45	4,57	4,07	3,75	3,53	3,23	2,90	2,52	2,06
29	7,60	5,42	4,54	4,04	3,73	3,50	3,20	2,87	2,49	2,03
30	7,56	5,39	4,51	4,02	3,70	3,47	3,17	2,84	2,47	2,01
40	7,31	5,18	4,31	3,83	3,51	3,29	2,99	2,66	2,29	1,80
60	7,08	4,98	4,13	3,65	3,34	3,12	2,82	2,50	2,12	1,60
120	6,85	4,79	3,95	3,48	3,17	2,96	2,66	2,34	1,95	1,38
∞	6,64	4,60	3,78	3,32	3,02	2,80	2,51	2,18	1,79	1,00

Table 6 – Order of doses in crossover trials..

Group	*Double Cruciate*		*Triple Crossover*	
	Phase I	*Phase II*	*Phase I*	*Phase II*
1	ρ_1	a_2	ρ_1	a_3
2	ρ_2	a_1	ρ_2	a_2
3	a_1	ρ_2	ρ_3	a_1
4	a_2	ρ_1	a_1	ρ_3
5	-	-	a_2	ρ_2
6	-	-	a_3	ρ_1

Table 7 - Example of order of doses in Latin square..

a_2	ρ_1	a_1	ρ_2
ρ_2	a_1	ρ_1	a_2
a_1	a_2	ρ_2	ρ_1
ρ_1	ρ_2	a_2	a_1

Table 8 - Formulas for tests with two doses of each preparation

	Default (P)	*1º sample (A)*	*Sample h -1 (Z)*
Lower Dose (sum of responses)	P_1	A_1	Z_1
Higher Dose (sum of responses)	P_2	A_2	Z_2
By preparation (sum of responses)	$P_1 + P_2 = P$	$A_1 + A_2 = A$	$Z_1 + Z_2 = Z$
Without Contrast linear	$P_2 – P_1 = L_P$	$A_2 – A_1 = L_A$	$Z_2 – Z_1 = L_Z$

Table 9 - Formulas for test with three doses of each preparation..

	Default (P)	*1º sample (A)*	*Sample h -1 (Z)*
Lower Dose (sum of responses)	P_1	A_1	Z_1
average Dose (sum of responses)	P_2	A_2	Z_2
higher Dose (sum of responses)	P_3	A_3	Z_3
By preparation (sum of responses)	$P_1 + P_2 + P_3 = P$	$A_1 + A_2 + A_3 = A$	$Z_1 + Z_2 + Z_3 = Z$
Quadratic contrast linear	$P_3 – P_1 = L_P$	$A_3 – A_1 = L_A$	$Z_3 – Z_1 = L_Z$
	$P_1 – 2P_2 + P_3 = Q_P$	$A_1 – 2A_2 + A_3 = Q_A$	$Z_1 – 2Z_2 + Z_3 = Q_Z$

Table 10 – validity Tests (analysis of variance).

Source of variation	*Degrees of freedom (gl)*	*Sum of squares reduced*	
		Testing of two doses	*Test of three doses*
Preparations	*h – 1*	$\frac{P^2 + A^2 + ... + Z^2}{2n} - K$	$\frac{P^2 + A^2 + ... + Z^2}{3n} - K$
Regression	*1*	$\frac{(L_P + L_A + ... + L_Z)^2}{2nh} - E$	$\frac{(L_P + L_A + ... + L_Z)^2}{2nh} - E$
Parallelism	*h – 1*	$\frac{(L^2_P + L^2_A + ... + L^2_Z)}{2n} - E$	$\frac{(L^2_P + L^2_A + ... + L^2_Z)}{2n} - E$
Quadratic	*1*	-	$\frac{(Q_P + Q_A + ... Q_Z)^2}{6nh} = Q$
Difference of Quadratics	*h – 1*	-	$\frac{(Q^2_P + Q^2_A + ... + Q^2_Z)}{6n} - Q$

Table 11 – Estimation of residual error.

Source of Variation	*Degrees of Freedom (gl)*	*Sum of squares reduced*		
		A Randomized to chance	*Randomized Blocks*	*Latin Square*
Treatments	*k - 1*	$\frac{P_1^2 + P_2^2 + ... + Z^2_d}{n} - K$	$\frac{P_1^2 + P_2^2 + ... + Z^2_d}{n} - K$	$\frac{P_1^2 + P_2^2 + ... + Z^2_d}{n} - K$
Blocks (queues)	*n - 1*	--	$\frac{F_1^2 + F_2^2 + ... + F_N^2}{k} - K$	$\frac{F_1^2 + F_2^2 + ... + F_N^2}{k} - K$
Blocks (columns)	*n - 1*	--	--	$\frac{C_1^2 + C_2^2 + ... + C_N^2}{k} - K$
Residual Error	Por diferença	*	*	*
TOTAL	*N - 1*	$\sum y^2 - K$	$\sum y^2 - K$	$\sum y^2 - K$

Obtained for all by subtracting from the sum of squares total reduced, all the other sums of squares reduced calculated for the corresponding design.

Table 12 – Table of t' for comparison between two-tailed (h-1) samples and a Standard for a confidence coefficient set of p =0.95.

gl_2	gl_1 = (h -1) = number of samples (excluding standard)								
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
5	2,57	3,03	3,29	3,48	3,62	3,73	3,82	3,90	3,97
6	2,45	2,86	3,10	3,26	3,39	3,49	3,57	3,64	3,71
7	2,36	2,75	2,97	3,12	3,24	3,33	3,41	3,47	3,53
8	2,31	2,67	2,88	3,02	3,13	3,22	3,29	3,35	3,41
9	2,26	2,61	2,81	2,95	3,05	3,14	3,20	3,26	3,32
10	2,23	2,57	2,76	2,89	2,99	3,07	3,14	3,19	3,24
11	2,20	2,53	2,72	2,84	2,94	3,02	3,08	3,14	3,19
12	2,18	2,50	2,68	2,81	2,90	2,98	3,04	3,09	3,14
13	2,16	2,48	2,65	2,78	2,87	2,94	3,00	3,06	3,10
14	2,14	2,46	2,63	2,75	2,84	2,91	2,97	3,02	3,07
15	2,13	2,44	2,61	2,73	2,82	2,89	2,95	3,00	3,04
16	2,12	2,42	2,59	2,71	2,80	2,87	2,92	2,97	3,02
17	2,11	2,41	2,58	2,69	2,78	2,85	2,90	2,95	3,00
18	2,10	2,40	2,56	2,68	2,76	2,83	2,89	2,94	2,98
19	2,09	2,39	2,55	2,66	2,75	2,81	2,87	2,92	2,96
20	2,09	2,38	2,54	2,65	2,73	2,80	2,86	2,90	2,95
24	2,06	2,35	2,51	2,61	2,70	2,76	2,81	2,86	2,90
30	2,04	2,32	2,47	2,58	2,66	2,72	2,77	2,82	2,86
40	2,02	2,29	2,44	2,54	2,62	2,68	2,73	2,77	2,81
60	2,00	2,27	2,41	2,51	2,58	2,64	2,69	2,73	2,77
120	1,98	2,24	2,38	2,47	2,55	2,60	2,65	2,69	2,73
∞	1,96	2,21	2,35	2,44	2,51	2,57	2,61	2,65	2,69

Table 13 – Totals and you employ in testing with a randomized double cruciate

	Default (P)	*Sample (A)*	*Total*
PHASE I			
Lower Dose (sum of responses)	P_{1I}	A_{1I}	-
Higher Dose (sum of responses)	P_{2I}	A_2	-
Total	P_I	A_I	$P_I + A_I = F_I$
PHASE II			
Lower Dose (sum of responses)	P_{1II}	A_{1II}	-
Higher Dose (sum of responses)	P_{2II}	A_{2II}	-
Total	P_{II}	A_{II}	$P_{II} + A_{II} = F_{II}$
For preparation (sum of responses)	P	A	$\sum y$
Linear Contrast			
PHASE I	$P_{2I} - P_{1I} = L_{PI}$	$A_{2I} - A_{1I} = L_{AI}$	$L_{PI} + L_{AI} = L_I$
PHASE II	$P_{2II} - P_{1II} = L_{PII}$	$A_{2II} - A_{1II} = L_{AII}$	$L_{PII} + L_{AII} = L_{II}$
TOTAL	$P_2 + P_1 = L_P$	$A_2 + A_1 = L_A$	$L_P + L_A = \sum L$

Table 14 – validity Tests in test double crossover.

Source of variation	*Degrees of freedom (gl)*	*Sum of squares reduced*
Parallelism	1	$\frac{L^2_P + L^2_A}{2n} - E$
Phases X Preparations	1	$\frac{P_I^2 + P_{II}^2 + A_I^2 + A_{II}^2}{n} - K$ (Fases – Preparações)
Regression Phases X	1	$\frac{L^2_I + L^2_{II}}{2n} - E$
Error I	Difference	Blocks - Parallelism - (Phases X Preparations) - (Phases X Regression)
Blocks (animals)	bl – 1	$\frac{B_1^2 + B_2^2 + ... + B_{2n}^2}{2} - K$
Preparations	1	$\frac{P^2 + A^2}{2n} - K$
Regression	1	$\frac{(L_P + L_A)^2}{N} - E$
Phases	1	$\frac{F^2_I + F^2_{II}}{2n} - K$
Phases X Parallelism	1	$\frac{L_{PI}^2 + L_{PII}^2 + L_{AI}^2 + L_{AII}^2}{n} - E$ Parallelism - (Phases X Regression)
Error II	Difference	Total - Blocks - Preparations - Regression - Stages - (Phases X Parallelism)
TOTAL	N - 1	$\sum y^2 - K$

$K = (\sum y)^2/N$

N = total number of responses

N = total number of replicas per dose included two phases

Bl = number of blocks (animals)

B = the sum of the two responses for each block (animal)

8

Table 15 – Constant used in the formula for the confidence limits.

Dose of each preparation (d)	*Number of samples tested (h – 1)*	*c'*
2	1	1
	2	3/2
	3	2
	4	5/2
	5	3
3	1	8/3
	2	4
	3	16/3
	4	20/3
	5	8

Table 16 – Probit corresponding to percentage.

%	*0*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
0	-	2,67	2,95	3,12	3,25	3,36	3,45	3,52	3,59	3,66
10	3,72	3,77	3,82	3,87	3,92	3,96	4,01	4,05	4,08	4,12
20	4,16	4,19	4,23	4,26	4,29	4,33	4,36	4,39	4,42	4,45
30	4,48	4,50	4,53	4,56	4,59	4,61	4,64	4,67	4,69	4,72
40	4,75	4,77	4,80	4,82	4,85	4,87	4,90	4,92	4,95	4,97
50	5,00	5,03	5,05	5,08	5,10	5,13	5,15	5,18	5,20	5,23
60	5,25	5,28	5,31	5,33	5,36	5,39	5,41	5,44	5,47	5,50
70	5,52	5,55	5,58	5,61	5,64	5,67	5,71	5,74	5,77	5,81
80	5,84	5,88	5,92	5,95	5,99	6,04	6,08	6,13	6,18	6,23
90	6,28	6,34	6,41	6,48	6,55	6,64	6,75	6,88	7,05	7,33
%	***0,0***	***0,1***	***0,2***	***0,3***	***0,4***	***0,5***	***0,6***	***0,7***	***0,8***	***0,9***
97	6,88	6,90	6,91	6,93	6,94	6,96	6,98	7,00	7,01	7,03
98	7,05	7,07	7,10	7,12	7,14	7,17	7,20	7,23	7,26	7,29
99	7,33	7,37	7,41	7,46	7,51	7,58	7,65	7,75	7,88	8,09

Table 17 – weighting Coefficients for probits (w)..

Probit	*0,0*	*0,1*	*0,2*	*0,3*	*0,4*	*0,5*	*0,6*	*0,7*	*0,8*	*0,9*
1	0,001	0,001	0,001	0,002	0,002	0,003	0,005	0,006	0,008	0,011
2	0,015	0,019	0,025	0,031	0,040	0,050	0,062	0,076	0,092	0,110
3	0,131	0,154	0,180	0,208	0,238	0,269	0,302	0,336	0,370	0,405
4	0,439	0,471	0,503	0,532	0,558	0,581	0,601	0,616	0,627	0,634
5	0,637	0,634	0,627	0,616	0,601	0,581	0,558	0,532	0,503	0,471
6	0,439	0,405	0,370	0,336	0,302	0,269	0,238	0,208	0,180	0,154
7	0,131	0,110	0,092	0,076	0,062	0,050	0,040	0,031	0,025	0,019
8	0,015	0,011	0,008	0,006	0,005	0,003	0,002	0,002	0,001	0,001

Table 18 -Values of x2 (a = 0.05).

gl	χ^2	*gl*	χ^2
1	3,84	9	16,92
2	5,99	10	18,31
3	7,81	11	19,67
4	9,49	12	21,03
5	11,07	13	22,36
6	12,59	14	23,69
7	14,07	15	25,00
8	15,51	20	31,41
		25	37,65

Table 19 – Matrix of responses in the test of antibiotics by design 5 x 1, after correction.

	Padrão					*Amostra*		
	P_1	**P_2**	**P_3**	**P_4**	**P_5**	**A**	**B**	**C**
Respostas	y_{11}	y_{21}	y_{31}	y_{41}	y_{51}	A_{31}	B_{31}	...
	y_{12}	y_{22}	y_{32}	y_{42}	y_{52}	A_{32}	B_{32}	...
	y_{13}	y_{23}	y_{33}	y_{43}	y_{53}	A_{33}	B_{33}	...
	y_{14}	y_{24}	y_{34}	y_{44}	y_{54}	A_{34}	B_{34}	...
	y_{15}	y_{25}	y_{35}	y_{45}	y_{55}	A_{35}	B_{35}	...
	y_{16}	y_{26}	y_{36}	y_{46}	y_{56}	A_{36}	B_{36}	...
	y_{17}	y_{27}	y_{37}	y_{47}	y_{57}	A_{37}	B_{37}	...
	y_{18}	y_{28}	y_{38}	y_{48}	y_{58}	A_{38}	B_{38}	...
	y_{19}	y_{29}	y_{39}	y_{49}	y_{59}	A_{39}	B_{39}	...
Total	$y_{1\cdot}$	$y_{2\cdot}$	$y_{3\cdot}$	$y_{4\cdot}$	$y_{5\cdot}$	$A_{3\cdot}$	$B_{3\cdot}$	

Ynz: n is the concentration (P1 to P5) and z is the response (1 to 9)

Anz: n is the concentration median (P3) and z is the response (1 to 9)

Table 20 – Table of Analysis of variance for the simple linear regression model - a randomized 5 x 1

$$y = b_0 + b_1 x$$

$$b_1 = \frac{\sum (x-\bar{x})(y-\bar{y})}{\sum (x-\bar{x})^2} \qquad b_0 = \bar{y} - b_1 \bar{x} \qquad r = \frac{\sum (x-\bar{x})(y-\bar{y})}{(N-1)\sqrt{\frac{(x-\bar{x})^2}{N-1}}\sqrt{\frac{(y-\bar{y})^2}{N-1}}}$$

Source of variation	***gl***	***Sum of squares***	***Mean Square***	***F computer***
Regressão	1	$SQ_{reg} = b_1\Sigma xy + b_0\Sigma y - (\Sigma y)^2/N$	$QM_{reg} = SQ_{reg}$	QM_{reg}/QM_{res}
Erro residual	N - 2	$SQ_{res} = \Sigma y^2 - b_1\Sigma xy - b_0\Sigma y$	$QM_{res} = \frac{SQ_{res}}{N} - 2$	---
Desvio linear	3	$SQ_{desv} = SS_{res} - SQ_{ep}$	$QM_{desv.} = \frac{SQ_{desv.}}{3}$	QM_{desv}/QM_{ep}
Erro puro (ep)	N - 5	$SQ_{ep} = \Sigma y^2 - (\Sigma yi)^2/k'$	$QM_{ep} = \frac{SQ_{ep}}{N} - 5$	---
Total	N - 1	$SQ_{reg} + SQ_{res}$	---	

$(\Sigma yi)^2 = (y_{11} + y_{12} + y_{13} + \ldots + y_{19})^2 + \ldots + (y_{51} + y_{52} + y_{53} + \ldots + y_{59})^2$

$\sqrt{\frac{(x-\bar{x})^2}{N-1}}$ = Sxx = standard deviation of the variable (x)

$\sqrt{\frac{(y-\bar{y})^2}{N-1}}$ = yy = standard deviation of the response variable. (Y)

8.10 EXAMPLES OF CALCULATING STATISTICAL TESTS APPLIED IN BIOLOGICAL

8.10.1EXAMPLE OF DIRECT TEST

Example 1: direct test with a sample.

Test of digital by method of cardiac arrest in guinea pig

The standard solution was used at a concentration of 0.0658 IU/mL. An equivalent dilution of sample was prepared from the power supposed to SA = 1.3 IU/100 mg.

The guinea pigs were perfused randomly with standard solution or sample, registering the volume precisely necessary to produce cardiac arrest in each animal.

The responses are given in Table 19.

Calculation of the estimate of power and confidence limits
Of equation 1:

M'= 1.3974 – 1.4089 = – 0.0115

Equation 6:

M = – 0.0115 + 1.3 = 0.1024 log

Of equation 5:

R = *antilog* 0.1024 = 1.2660

equation 3:

$$s^2_x = \frac{1}{22}\left[\left(1{,}4404^2 + \ldots + 1{,}3304^2 - \frac{19{,}5641^2}{14}\right) + \left(1{,}5317^2 + \ldots + 1{,}4072^2 - \frac{14{,}0890^2}{10}\right)\right]$$

$$= 1/22\ [27{,}3829 - 27{,}3396 + 19{,}8879 - 19{,}8500]$$

$$= 0{,}003691$$

Equation 2:

$s^2_M = 0{,}003691\ (1/14 + 1/10) = 0{,}000632$
$s_M = \sqrt{0{,}00632} = 0{,}0251$

Para α = 0,05 com 22 gl, t = 2,07 (**Table 3**)

Da equação 7:

$$\frac{R_s}{R_i} = anti\log[0{,}1024 \pm (2{,}07 \times 0{,}0251)]$$

$$R_s = anti\log[0{,}1024 + (2{,}07 \times 0{,}0251)] = 1{,}43$$

$$R_i = anti\log[0{,}1024 - (2{,}07 \times 0{,}0251)] = 1{,}12$$

The average estimate of the power of digital sample is 1.27 IU/100 mg. The confidence limits (p = 0.05) for the real power are 1.12 IU/100 mg and 1.43 IU/l00 mg.

Table 21 – Example 1: effective Doses to produce cardiac arrest

	Standard P		***Sample A***	
	Lethal Dose mL/kg	***Log lethal dose*** x_p	***Lethal Dose mL/kg***	***Log lethal dose*** x_A
	27,57	1,4404	34,02	1,5317
	25,97	1,4145	21,90	1,3404
	27,74	1,4431	28,33	1,4523
	30,94	1,4905	24,87	1,3957
	28,31	1,4519	27,56	1,4403
	27,29	1,4360	24,73	1,3932
	22,13	1,3450	21,67	1,3359
	23,63	1,3735	21,30	1,3284
	21,39	1,3302	29,10	1,4639
	22,13	1,3450	25,54	1,4072
	20,97	1,3216		
	29,23	1,4658		
	23,78	1,3762		
	21,40	1,3304		
Σx		19,5641		14,0890
$\bar{x}$		1,3974		1,4089
Σx^2		27,3829		19,8879
s^2		0,003331		0,004211
N		14		10

8.10.2 EXAMPLES OF QUANTITATIVE INDIRECT TESTS

Example 2: test with three doses, a completely randomized design.

Test of human chorionic gonadotropin by method of increase in weight of seminal vesicles

The doses used in the pattern were: *pl* = 1.0 IU/mL, p2 = 2.0 IU/mL and p3 = 4.0 IU/mL. Equivalent Doses of sample were prepared from power supposed SA = 3000 IU/mg.

The rats were injected subcutaneously with 0.20 mL of solution corresponding, for three consecutive days, a total of 0.6 mL/mouse.

The weights of the seminal vesicles are found in Table 22.

Table 22 – Example 2: weights of seminal vesicles (mg).

p_1	p_2	p_3	a_1	a_2	a_3
8,5	12,5	14,8	10,5	16,8	16,7
10,4	13,1	14,1	10,5	14,3	16,9
11,4	8,3	14,9	9,1	14,9	18,8
11,6	13,1	13,8	9,9	12,3	16,7
10,2	9,0	14,6	10,5	15,4	12,7
9,1	14,4	15,2	8,4	14,9	16,2
9,5	11,7	12,3	10,1	12,8	17,3
7,7	11,72*	15,5	10,1	10,0	12,8

* Lost Value replaced by mean of treatment.

Table 23 – Example 2: total and contrasts.

	Standard P	*Sample*
Lower Dose	$P_1 = 78,40$	$A_1 = 79,10$
Average Dose	$P_2 = 93,82$	$A_2 = 111,10$
Higher Dose	$P_3 = 115,20$	$A_3 = 128,10$
Preparation	$P = 287,42$	$A = 318,60$
Linear Contrast	$L_P = 36,80$	$L_A = 49,00$
Quadratic Contrast	$Q_p = 5,96$	$Q_A = 15,60$

Results obtained with the formulae in Table 9.

Table 24 – Example 2: analysis of variance.

Source of variation	*gl*	*Sum of squares*	*Square Middle*	*F*	*The*
Preparations	1	20,26	20,26		
Regression	1	230,05	230,05	79,05	<0,01
Parallelism	1	4,65	4,65	1,60	>0,05
Quadratic	1	0,97	0,97	0,33	>0,05
Difference of squares	1	4,84	4,84	1,66	>0,05
Treatments	5	260,77	52,15		
Error	41*	119,18	$s^2 = 2,91$		
TOTAL	47	379,95			

* Removed a degree of freedom has been replaced a lost value.

The sums of squares were obtained employing the formulas from Tables 9, 10 and 11..

N = 48

n = 8

K = $(\sum y)^2/N$ = 7 651,25

$\sum y^2$ = 8 031,21

$$\text{Preparations} = \frac{308^2 + 324^2}{8} - 24964{,}0 = 16{,}0$$

$$\text{Regression} = \frac{(24+24)^2}{2 \times 4 \times 2} = 144{,}0 = E$$

$$\text{Paralelism} = \frac{24^2 + 24^2}{2 \times 4} = 144{,}0 = 0$$

$$\text{Treatment} = \frac{142^2 + 166^2 + 150^2 + 174^2}{4} - 24964 = 160{,}0$$

$$\text{Rows} = \frac{156^2 + 150^2 + 161^2 + 165^2}{4} - 24964 = 31{,}5$$

$$\text{Columns} = \frac{160^2 + 156^2 + 161^2 + 155^2}{4} - 24964 = 6{,}5$$

Total = 8 7 031.21 – 651.25 = 379.95

Error = 379.95 – 260.77 = 119.18

Test Validity

Test complies with the conditions of validity:

a) Significant regression, F calculated 79.05 is greater than < critical value from Table 5 for = 0.0l, *g11 = 1 and g12 = 41;*

b) No significant deviation from parallelism, F computer< 1.60 is smaller than the critical value from Table 4 for = 0.05 *g11 = 1 and gl2 = 41e*

c) Deviation from linearity non significant, F = 0.33 and 1.66

Calculation of the estimate of power and confidence limits
Using formulae 10 to 15.

I = log 2,0 = 0,3010

T = 2.02 with 41 *gl of Table 3*

$$b = \frac{36{,}8 + 49{,}0}{2 \times 0{,}301 \times 8 \times 2} = 8{,}9$$

$$y_A = \frac{318{,}60}{24} = 13{,}2$$

$$\bar{y}_P = \frac{287{,}42}{24} = 11{,}97$$

$$M' = \frac{13{,}27 - 11{,}97}{8{,}90} = 0{,}1460$$

S_A = 3 000 log S_A = 3,4771

M = 0,1460 + 3,4771 = 3,6231

R = *anti* log 3,6231 = 4 198,56 UI/mg

$$C = \frac{230{,}05}{230{,}05 - 2{,}91(2{,}02)^2} = 1{,}05$$

C' = 8/3, da **Tabela 15**

$$\frac{M'_s}{M'_i} = 1{,}05 \times 1{,}146 \pm \sqrt{(1{,}05 - 1)\,[1{,}05(0{,}146)^2 + 8/3(0{,}3010)^2]}$$

M'_s = 0,2679

M'_i = 0,0381

Logarithm of the confidence limits of power

M_s = 0,2679 + 3,4771 = 3,7449

M_i = 0,0381 + 3,4771 = 3,5151

Confidence Limits of power

Rs = anti log 3.7445 = 5552,64 IU/mg = anti log 3.5151 = 3274,16 IU/mg

Example 3: test with three doses, the randomized complete block design.

Test of antibiotic using Petri plates

The doses used were: the default
P1 = 0.25 IU/mL, p2 = 0.50 IU/mL and p3 = 1.00 IU/mL.
Equivalent Doses of sample were prepared based on power supposed SA = 1 650 IU/mg.
The diameters of the inhibition halos are found in Table 25.

Table 25 – Example 3: diameter of inhibition halos.

Plates (Blocks)	*Standard P*			*Sample A*			*Total Block*
	p_1	p_2	p_3	a_1	a_2	a_3	
1	17,0	20,4	24,0	17,4	20,7	24,4	123,9
2	14,9	19,7	22,7	14,9	19,3	22,2	113,7
3	15,0	18,6	22,0	15,0	18,0	22,3	110,9
4	14,6	18,3	22,4	14,8	19,0	22,2	111,3
5	14,7	18,0	22,3	14,4	17,8	22,6	109,8
6	14,4	19,1	23,3	14,5	19,3	23,0	113,6
7	14,9	19,0	22,5	15,0	19,4	22,4	113,2

Table 26 – Example 3: total and contrasts.

	Padrão P	***Amostra A***
Lower Dose	$P_1 = 105,5$	$A_1 = 106,0$
Average Dose	$P_2 = 133,1$	$A_2 = 133,5$
Higher Dose	$P_3 = 159,2$	$A_3 = 159,1$
Preparation	P = 397,8	A = 398,6
Linear Contrast	$L_P = 53,7$	$L_A = 53,1$
Quadratic Contrast	$Q_P = -1,5$	$Q_A = -1,9$

Results obtained with Table 9 formula

Table 27 – Example 3: variance analysis

Source of variation	***gl***	***Sum of squares***		*Square Mean*	***A***
Preparations	1	0,0150	0,0150	0,09	>0,05
Regression	1	407,3657	407,3657	2396	<0,01
Parallelism	1	0,0129	0,0129	0,080	>0,05
Quadratic	1	0,1376	0,1376	0,81	>0,05
Difference of squares	1	0,0019	0,0019	0,01	>0,05
Treatments	5	407,53	3020,25		
Plates	6	22,18	3,70	21,8	< 0,01
Error	30	4,99	$s^2 = 0,17$		

The sums of squares were obtained employing the formulas from Tables 9.10 and 11.

N = 42

n = 7

K = $(\sum y)^2$ / N = 15101,26

$\sum y^2$ = 15 535,96

Preparations = $\frac{397,8^2+398,6^2}{21} - 15101,2610 = 0,0$

Regression = $\frac{(53,7 + 53,1)^2}{28} = 407,3657 = E$

Parallelism = $\frac{53,7^2 + 53,1^2}{14} - 407,3657 = 0,0129$

Quadratic = $\frac{[-1,5 + (-1,9)]^2}{84} = 0,1376 = Q$

Difference in quadratics = $\frac{-1,5^2 + (-1,9)^2}{42} - 0,1376 = 0,0019$

Treatments = $\frac{105,5^2 + 133,1^2) + \ldots + 159,1^2}{7} - 15101,261 = 407,53$

Blocks (Plates) = $\frac{123,9^2 + 113,7^2) + \ldots + 113,2^2}{7} - 15101,261 = 22,18$

Total = 15 535,96 – 15 101,261 = 434,7

Error = 434,7 – 22,18 – 407,53 = 4,99

Test Validity

Test complies with the conditions of validity:

a) Significant regression, F calculated 2390 is greater than theCritical value from Table 5 for α = 0,01, $gl_1 = 1$ e $gl_2 = 30$;

b) No significant deviation from parallelism, F calculated0.08 Is smaller than the critical value from Table 4 for α= 0,05, $gl_1 = 1$ and $gl_2 = 30$, and

c) No significant deviation from linearity, F calculated =0.81 And 0.01.

Calculation of the estimate of power and confidence limits

Use formulae 10 to 15.

I =1 Log00-log 0.50 = 0.301 t =

t = 2,04 con 30 gl de la Table 3.

b = $\frac{53,7 + 53,1}{28 \text{ x } 0,301} = 12,67$

$\bar{y}_A = \frac{398,6}{21} = 13,98$

$\bar{y}_P = \frac{397,8}{21} = 18,94$

$M' = \frac{18,92 - 18,94}{12,672} = 0,00315$

$S_A = 1650$ UI/mg

$M = M' + \log 1650 = 0,003157 + 3,217480 = 3,2206$

R = *anti* log 3,2206 = 1662

$C = 407,3657/[407,3657 - 0,17\ (2,04)^2] = 1,0017$

c' = 8/3, da **Table 15**

$M'_s = 0,0235$

M'i = -0,0171

Logarithm of the confidence limits of power

$M_s = 0,0235 + 3,2175 = 3,2410$

$M_i = -0,0\ 171 + 3,2175 = 3,2004$

Confidence Limits of power

$R_s =$ *anti* log 3,2410 = 1742 UI/mg

$R_i =$ *anti* log 3,2004 = 1586 UI/mg

Example 4: test with two doses, Latin square design.

Test of oxytocin – method of contraction of the isolated mice uterus

The administered doses of standard were: *pl* = 0.2 mL and p2 = 0.25 mL of a solution containing 0.02 IU/mL.

Equivalent Doses of the sample has been prepared on the basis of presumed power 10 IU/mL diluted 1:500.

Table 28 – Example 4: order of addition of the doses.

Queues	***Columns***			
	1	***2***	***3***	***4***
1	p_1	p_2	a_1	a_2
2	p_2	p_1	a_2	a_1
3	a_1	a_2	p_1	p_2
4	a_2	a_1	p_2	p_1

Table 29 – Example 4: records of contractions in mm.

Queues	***Columns***				***Total Filas***
	1	***2***	***3***	***4***	
1	38	43	35	40	$F_1 = 156$
2	38	30	44	38	$F_2 = 150$
3	39	45	37	40	$F_3 = 161$
4	45	38	45	37	$F_4 = 165$
Total Column	$C_1 = 160$	$C_2 = 156$	$C_3 = 161$	$C_4 = 155$	
Total doses	$P_1 = 142$	$P_2 = 166$	$A_1 = 150$	$A_2 = 174$	

Table 30 – Example 4: total and contrasts.

	Default	***Sample***
Lower Dose	$P_1 = 142$	$A_1 = 150$
Higher Dose	$P_2 = 166$	$A_2 = 174$
Preparation	P = 308	A = 324
Linear Contrast	$L_P = 24$	$L_A = 24$

Table 31 – Example 4: analysis of variance

Source of variation	*Gl*	*Sum of squares*	*Mean Square*	***F***	***A***
Preparapcao	1	16,0	16,0	1,65	> 0,05
Regression	1	144,0	144,0	14,89	< 0,01
Parallelism	1	0,0	0,0	0,00	> 0,05
Treatment	3	160,0			
Queues	3	31,5	10,5	1,08	> 0,05
Columns	3	6,5	2,2	0,23	> 0 05
Error	6	58,0	$s^2 = 9,67$		
Total	15	256,0			

The sums of squares were obtained employing the formulae of Tables 8.10 and 11.

N =16

n =4

$K = (\sum y)^2/N = 632^2/16 = 24\ 964$

$$\text{Preparations} = \frac{308^2+324^2}{8} - 24964{,}0 = 16{,}0$$

$$\text{Regression} = \frac{(24 + 24)^2}{2 \text{ x4 x } 2} = 144{,}0 = E$$

$$\text{Parallelism} = \frac{24^2 + 24^2}{2 \text{ x } 4} - 144{,}0 = 0$$

$$\text{Treatment} = \frac{142^2 + 166^2 + 150^2 + 174^2}{4} = 24964 = 160{,}0$$

$$\text{Rows} = \frac{156^2 + 150^2 + 161^2 + 165^2}{4} = 24964 = 31{,}5$$

$$\text{Columns} = \frac{160^2 + 156^2 + 161^2 + 155^2}{4} = 24964 = 6{,}5$$

Total= 25220 - 24964 = 256,0

Erro= 256,0 - 160,0 - 31,5 - *6,5* = 58,0

The analysis showed no significant differences (p > 0.05) between rows and between columns.

Test Validity

The Test complies with the conditions of validity:

a) Significant regression, F calculated 14.9 is greater than the critical value from Table 5 for a = 0.01, *gl1 = 1 and g12 = 6;*

b) No significant deviation from parallelism, F calculated 0.0. It is less than the critical value from Table 4 for a = 0.05, *g11 = 1 and gl2 = 6.* [1]

Calculation of the estimate of power and confidence limits
Using formulae 10 to 15

I = log 0,25 - log 0,20 = 0,0969

t = 2,45 com 6 *gl* of the table 3

$$b = \frac{24 + 24}{0{,}0969 \text{ x } 4 \text{ x } 2} = 61{,}9$$

$$\bar{y}_A = \frac{324}{8} = 40{,}5$$

$$\bar{y}_P = \frac{308}{8} = 38{,}5$$

$$M' = \frac{40{,}5 - 38{,}5}{61{,}91} = 0{,}0323$$

$S_A = 10$ $\log S_A = 1$

$M = 0{,}0323 + 1 = 1{,}1323$

R = *anti* log 1,0323 = 10,8 UI/mL = Estimated potency

$$C = \frac{144{,}0}{144{,}0 - 9{,}67 \text{ x } 2{,}45^2} = 1{,}67$$

c' = 1, of the **Table 15**

$$\begin{matrix} M'_s \\ M'_i \end{matrix} = 1{,}67 \text{ x } 0{,}0323 \pm \sqrt{(1{,}67 - 1{,}0)\,[1{,}67(0{,}0323)^2 + 1(0{,}09691)^2]}$$

$M'_s = 0{,}1402$

$M'_i = -0{,}0324$

Logarithm of the confidence limits of power

$M_s = 0{,}1402 + 1 = 1{,}1402$

$M_i = 0{,}0324 + 1 = 0{,}9676$

Confidence Limits of power

Rs = *anti* **log 1,1402 = 13,81 UI/mL**
R1 = *anti* **log 0,9676 = 9,28 UI/mL**

Example 5: test double crossover.
Assay of insulin in mice

The doses used were the standard p1 = 60 mIU/mL and p2 = 120 mIU/mL. Were prepared equivalent doses of sample, a1 = 60 mIU/mI and a2 = 120 mIU/mL from the power supposed S_A = 27.4 IU/mL.

The mice were injected with 0.1 mL of the solution corresponding to each 10 g of average weight, according to Table 6.

The responses are given in Table 30.

Table 32 – Example 5: concentration of blood glucose (mg/100 mL), 40 minutes after the injection.

Group 1			*Group 2*			*Group 3*			*Group 4*		
p_1	a_2	*total*	p_2	a_1	*total*	a_1	p_2	*total*	a_2	p_1	*Total*
37,1	16,6	53,7	32,4	32,4	80,8	36,8	17,0	53,8	30,9	52,1	83,0
35,2	40,1	75,3	35,2	35,2	103,0	53,2	24,9	78,1	27,8	59,4	87,2
43,1	33,9	77,0	35,3	35,3	108,4	71,2	58,2	129,4	35,4	39,1	74,5
41,3	16,2	57,5	32,9	32,9	78,1	37,1	24,8	61,9	49,8	79,0	128,8
54,2	33,2	87,4	31,9	31,9	65,0	45,9	22,7	68,6	28,2	37,3	65,5
41,4	13,1	54,4	51,2	51,2	113,6	82,2	42,7	124,9	49,9	51,1	101,0
48,6	32,7	81,3	38,2	38,2	114,4	64,8	33,9	98,7	28,3	59,5	87,8
57,8	50,4	108,2	39,7	39,7	89,8	49,1	37,6	86,7	39,6	55,8	95,4
71,1	47,3	118,4	37,0	37,0	110,8	44,1	10,4	54,5	32,2	40,6	72,8
60,8	26,1	86,9	38,9	38,9	103,5	64,7	34,7	99,4	55,1	68,2	123,3
78,2	50,9	129,1	42,6	42,6	97,2	88,0	61,6	149,6	40,6	61,4	102,0
76,1	54,4	130,5	30,4	30,4	80,0	90,1	60,3	150,4	43,5	52,8	96,3

Table 33 – Example 5: total and contrasts.

	Standard P	*Sample*	*Total*
PHASE I			
Lower Dose	$P_{1I} = 644,9$	$A_{1I} = 727,2$	
Higher Dose	$P_{2I} = 445,7$	$A_{2I} = 461,3$	
TOTAL	$P_I = 1090,6$	$A_I = 1188,5$	$F_I = 2279,1$
PHASE II			
Lower Dose	$P_{1II} = 656,3$	$A_{1II} = 704,9$	
Higher Dose	$P_{2II} = 428,8$	$A_{2II} = 414,9$	
TOTAL	$P_{II} = 1085,1$	$A_{II} = 1119,8$	$F_{II} = 2204,9$
Preparation contrast Linear	$P = 2175,7$	$A = 2308,3$	$\sum y = 4484,0$
PHASE I	$L_{PI} = -199,2$	$L_{AI} = -256,9$	$L_I = -465,1$
PHASE II	$L_{PII} = -227,5$	$L_{AII} = -290,0$	$L_{II} = -517,5$
TOTAL	$L_p = -426,7$	$L_A = -555,9$	$\sum L = -982,6$

Results obtained with the formulas of Table 13

Table 34 – Example 5: analysis of variance.

Source of variation	*gl*	*Sum of squares*	*Mean Square*	*F*	*A*
Parallelism	1	173,88	173,88	0,53	> 0,05
Phases x Preparations	1	41,61	41,61	0,13	> 0,05
Phases x regressions	1	28,60	28,60	0,09	> 0,05
Error I	44	14 545,64	330,58		
Blocks	47	14 789,73	314,67		
Preparations	1	183,15	183,15	3,01	> 0,05
Regression	1	10 057,32	10 057,32	165,52	< 0,01
Phases	1	57,35	57,35	0,94	> 0,05
Phases x Parallelism	1	0,19	0,19	0,00	> 0,05
Error II	44	2 673,39	60,76		
TOTAL	95	27 761,13			

The sums of squares were obtained employing the formulae of Tables 13 and 14.

N = 96

n = 24

b1 = 48

K = $(\sum y)^2/N$ = 4 484,0^2/96 = 209 440,17

$\sum y^2$ = 237 201,30

Total = 237 201,30 – 209 440,17 = 27 761

Blocks = $\frac{448459,8}{2}$ – 209440,17 = 14789,73

Preparation = $\frac{2175,7^2+2308,3^2}{48}$ – 209440,17 = 183,15

Phase = $\frac{2279,1^2+2204,9^2}{48}$ – 209440,17 = 57,35

Regression = $\frac{[(-426,7) + (-555,9)]^2}{96}$ = 10057,32 = *E*

Paralleism = $\frac{(-426,7)^2 + (-555,9)^2}{48}$ = 10057,32 = 17

Phase x Regression = $\frac{(-465,1)^2 + (-517,5)^2}{48}$ = 10057,32 = 28

Phase x parallelism =

$\frac{(-199,2)^2 + (-227,5)^2 + (-265,9)^2 + (-290,0)^2}{24}$ = 10057,32 = 28

Phase x preparations =

$\frac{(-1090,6)^2 + (-1085,1)^2 + (1188,5)^2 + (1119,8)^2}{24}$ = 10057,32 = 28

Error I = 14 789,73 – 173,88 – 41,61 – 28,60 = 14 545,64

Error II = 27 761,13 – 14 789,73 – 183,15 – 10 057,32 – 53,35 – 0,19 = 2 673,39

Test Validity

Test meets the conditions of validity:

a) Significant regression, F calculated 165.52 is greater than the critical value from Table 5, for a = 0.01, gll = 1 and *gl2 = 44;*

b) Parallelism is not significant, F calculated 0.53 is smaller than the critical value from Table 4, for a = 0.05, g11 = 1 and gl2 = 44; and

c) None of the three interactions was significant – the three F values calculated: 0.13.0.09 and 0.00 were smaller than the critical value from Table 4 for a = 0.05, *g11 = 1 and gl2 = 44.*

Calculation of the estimate of power and confidence limits
Using formulae 10 to 15.

I= log 120 – log 60 = 2,0792 – l,77820,301

t = 2,01 con 44 *gl* of the**Table 3**

b = $\frac{(-426,7) + (-555,9)}{24 \text{ x } 2 \text{ x } 0,301}$ = 68,01

$\bar{y}_A = \frac{2175,7}{2 \text{ x } 24} = 48,09$

$\bar{y}_P = \frac{2175,7}{2 \text{ x } 24} = 45,33$

$M' = \frac{48,09 - 45,33}{-68,01} = 0,0406$

S_A = 27,4 log S_A = 1,4377

M = - 0,0406 + 1,4377 = 1,3791

Estimated potency: R = *anti*log 1,3971 = 24,95 UI/mL

C = $\frac{10057,32}{[10057,32 - 60,76(2,01)^2]}$ = 1,025

c' = 1 of the **Table 15**

$\frac{M'_s}{M'_i} = 1,025(-0,0406) \pm \sqrt{(1,025 - 1)\,[1,025(-0,0406)^2+1(0,301)^2]}$

M'_s = 0,0064
M'_i = - 0,0064

Confidence Limits of power

R_s = anti *log* 1,4441 = 27,80 UI/mL
R_i = anti *log* 1,3481 = 22,29 UI/mL

Example 6: moving average

Assay of heparin by method of inhibition of coagulation of sheep plasma citratado

The doses used in the pattern, in mL, were: = p1 = 0.78; p2 = 0.76; p3 = 0.74; p4 = 0.72; p5 = 0.70 and p6 = 0.68. Equivalent Doses (a) of the sample were prepared from power supposed SA = 140.6 IU/mg.

The test was developed as described in the method of evaluation of heparin in this volume.

Three trials were performed. For example the calculation of *M, only if will develop the test No* 1.

The degree of coagulation are found in Table 35.

Table 35 – Example 6: degrees of coagulation = y.

Tube	*Standard P*		*Sample*	
	P (mL)	*Y*	*The (mL)*	*Y*
1	0.78	0.00	0.78	0.00
2	0.76	0.00	0.76	0.25
3	0.74	0.50	0.74	0.75
4	0.72	0.75	0.72	1.00
5	0.70	1.00	0.70	1.00
6	0.69	1.00	0.68	1.00

Calculation of the estimate of power and confidence limits
Use formulas 27.28 and 40 to 45.

x_{iP} = 0,8691

y_{iP} = 0,8572

$x_{(i+1)}$ = 0,8691 $y_{(i+1)}$ = 0,750

$$x_P = 0,8691 + (0,4171 - 0,5)\ \frac{0,8575 - 0,8691}{0,417 - 0,750} = 0,8661$$

$x_{iA} = 0,8807$ $y_{iA} = 0,333$

$$x_A = 0,8807 + (0,333 - 0,5)\ \frac{8691 - 0,8807}{0,333 - 0,667} = 0,8749$$

$x_{(i+1)A} = 0,8691$ $y_{(i+1)A} = 0,667$

$S_A = 140,6$ UI/mg

$M_1 = 0,8661 - 0,8749 + \log 140,6 = 2,1392$

Assuming that other two tests carried out on the same sample provided the estimates:

$M_2 = 2,1995$ y $M_3 = 2,1805$, calcular $\overline{M}$

$\overline{M} = (2,1392 + 2,1995 + 2,1805)/3 = 2,1731$

R = anti log $\overline{M}$ = 149,0 UI/mg

= (2,1392+2,1995+2,1805)/3 = 2,1731 = antilog
M = 149,0 UI/mg

Calculate the variance of error:

$s^2 = \{14,1686 - 42,4999/3\}/2$

$s^2 = 0,001$

$s = \sqrt{0,001} = 0,0316$

n' = 3

t = 4,3 **(Table 3** *gl* =2)

Calculate the confidence interval:

$$L = \frac{2x0,0316x4,3}{1,7321} = 0,1569$$

L/2 = 0,0784

$M_s = 2,1731 + 0,0784 = 2,2515$

$M_i = 2,1731 - 0,0784 = 2,0947$

$R_s = 178,4$

$R_i = 124,4$

Table 36 -Example 6: medium 511.

Tube	*Padrão* **P**			*Amostra* **A**		
	Log dose (mL × 10) x_P	***Medium log dose*** x_{iP}	***Medium grade coagulation*** y_{iP}	***log dose (mL × 10)*** x_A	***Medium log dose*** x_{iA}	***Medium grade coagulation*** y_{iA}
1	0,8921	-	-	0,8921	-	-
2	0,8808	0,8807	0,167	0,8808	0,8807	0,333
3	0,8692	0,8491	0,417	0,8692	0,8691	0,667
4	0,8572	0,8572	0,750	0,8573	0,8572	0,917
5	0,8450	0,8450	0,917	0,8451	0,8450	1,000
6	-	-	-	0,8325	-	-

Example 7: microbiological test with 5 doses of standard and a dose of sample (5 x 1)

Test of antibiotic using Petri plates – microbiological Assay of benzathine Benzylpenicillin powder for injectable.

The doses used were: the default

0.15 IU/mL; 0.30 IU/mL; 0.60 IU/mL; 1.20 IU/mL; 2.40 IU/mL

Equivalent Doses of the sample were prepared based on power supposed

SA of 600,000 IU/vial

Table 37 – Example 7: readings of inhibition halos.

	P_1	P_3	P_2	P_3	P_4	P_3	P_5	P_3
Diameter of Inhibition halos	13,87	19,88	16,24	19,54	23,47	19,21	27,41	19,32
	12,95	20,60	16,35	19,85	23,04	18,97	27,62	19,61
	13,08	20,43	16,88	19,86	23,19	19,39	26,67	19,72
	12,86	19,85	15,34	18,49	23,04	19,68	27,50	19,65
	13,24	20,07	15,98	19,06	22,65	19,14	27,41	19,27
	13,08	20,06	15,50	19,20	23,01	19,65	26,53	20,04
	12,88	19,75	16,26	19,96	23,99	19,81	27,30	19,25
	13,39	20,30	16,70	19,70	23,85	19,72	27,49	19,53
	13,31	20,30	16,70	19,95	23,82	19,55	27,27	19,90
Media	13,184	20,138	16,217	19,512	23,340	19,458	27,244	19,588

Average of P_3 (36 scans): 19,674

Table 38 – Example 7: readings of inhibition halos after correction.

	P_1	P_2	P_3	P_4	P_5
	x_1 = - 0,82391	x_2 = - 0,52288	x_3 = - 0,22185	x_4 = 0,079181	x_5 = 0,380211
Diameter of Inhibition halos	13,406	16,402	19,674	23,686	27,496
	12,486	16,512	19,674	23,256	27,706
	12,616	17,042	19,674	23,406	26,756
	12,396	15,502	19,674	23,256	27,586
	12,776	16,142	19,674	22,866	27,496
	12,616	15,662	19,674	23,226	26,616
	12,416	16,422	19,674	24,206	27,386
	12,926	16,862	19,674	24,066	27,576
	12,846	16,862	19,674	24,036	27,356
$(\Sigma yi)^2$	13106,59	21729,12	31352,37	44945,7	60503,21

X = concentration of the antibiotic in logarithm

Totais

N = 45

Σ = -9,983205

Σ = 896,936

Σ^2 = 19.076,73

$\Sigma(\Sigma^2)/9$ = 19.070,78

Σ = -100,374

$\sum(-\bar{x})(-\bar{y})$ = 98,61069

$\sum(-\bar{x})^2$ = 8,155715 $\quad\quad$ $\sum(-\bar{y})^2$ = 1.199,081

$\sqrt{\frac{\sum(x-\bar{x})^2}{N-1}}$ = 0,4305 $\quad\quad$ $\sqrt{\frac{\sum(y-\bar{y})^2}{N-1}}$

$_0$ = 22,61426 $\quad\quad$ $_1$ = 12,09086

Table 39 – Example 7: analysis of variance.

b1 = 12,09 b0 = 22,61 r = 0,997				
Source of variation	*Gl*	*Sum of squares*	*Mean Square*	*F calc.*
Regression	1	1,192.3	1192,3	5691,5
Residual Error	43	6.80	0.2	---
Deviation from linearity	3	0.85	0.3	3
Pure Error	40	5.95	0.1	---
Total	44	1,199.1	---	

Table 40 – Example 7: reading of the samples.

A_1	P_3	A_2	P_3	A_3	P_3
19.66	19.78	19.57	18.73	18.69	18.57
19.49	19.45	18.91	19.12	19.04	18.89
19.94	19.50	19.02	19.70	19.28	19.12
19.38	19.68	19.41	19.55	19.38	19.14
19.88	19.90	19.32	19.38	19.22	19.40
19.88	19.91	19.55	19.74	19.22	19.10
19.74	19.45	19.41	19.33	20.03	19.45
19.15	19.04	19.47	19.68	19.33	19.45
19.45	19.32	19.48	19.46	19.45	19.45
$\sum y_i^2 = 31.176,96$	$\sum y_i^2 = 30.986,56$	$\sum y_i^2 = 30.324,74$	$\sum y_i^2 = 30.516,6$	$\sum y_i^2 = 30.150,85$	$\sum y_i^2 = 29.780,40$

Table 41 – Example 7: differences in responses paired or X = (yA – yP) / b (formula 16).

X_1	X_2	X_3	X_4
- 0,0099256	0,0694789	0,0099256	- 0,0198511
0,0033085	- 0,0173697	0,0124069	- 0,0256410
0,0363937	- 0,0562448	0,0132341	0,0330852
- 0,0248139	- 0,0115798	0,0198511	- 0,0281224
- 0,0016543	- 0,0049628	- 0,0148883	0,0066170
- 0,0024814	- 0,0157155	0,0099256	0,0016543
0,0239868	0,0066170	0,0479735	- 0,0281224
0,0090984	- 0,0173697	- 0,0099256	0,0645161
0,0107527	0,0016543	0,0000000	- 0,0264682
Tx = 0,044665	Tx = – 0,04549	Tx = 0,088503	Tx = – 0,02233
$Tx^2/9 = 0,000222$	$Tx^2/9 = 0,00023$	$Tx^2/9 = 0,00087$	$Tx^2/9 = 0,0000554$

$\Sigma X^2 = 0,024058 \quad \Sigma (Tx^2/9) = 0,001377$

$t = 2,042 \qquad k' = 9 \quad f = 9 \quad n = 32$

$$S_M^2 = \frac{0,024058 - 0,001377}{32} = 0,00071 \text{ (fórmula 19)}$$

$s = 0,02662$

½ $L = 0,01812$ (formula 20)

Table 42 – Example 7: logarithm of the power ratio and confidence limits for the samples TO^ A2, A3 and A4.

	A_1		A_2		A_3		A_4	
Logarithm of the power ratio (log)	0,004963		- 0,00505		0,009833		- 0,00248	
M'_{AS} e M'_{AI}	0,02308	- 0,01316	0,01307	0,01564	0,01564	0,02795	0,01564	- 0,02060

Calculation of the estimate of power and limits of confianc to sample A1:

Using the formula 17 and 20 to 25

$M'(A_1) = \Sigma X_1/9 = 0,004963$

$M = M' + \log 600.000 = 5,78311$

$R = anti\log 5,78311 = 606.895,97$ UI/frasco

$M'_{as}(A_1) + ½ L = 0,004963 + 0,01812 = 0,02308$

$M'_{Ai}(A_1) - ½ L = 0,004963 - 0,01812 = -0,01316$

The Logarithm of the confidence limits of power

$M_{As}(A_1) = 0,02308 + \log 600.000 = 5,80123$

$M_{Ai}(A_1) = -0,01316 + \log 600.000 = 5,76499$

Confidence Limits of power

$R_s = anti\log 5,80123 = 632.746,9$ UI/frasco

$R_i = anti\log 5,76499 = 582.091,5$ UI/frasco

Table 43 – Example 7: coefficient of Variation (formula 18).

	A1	A2	A3	A4
Standard Deviation (s)	0,269	0,232	0,356	0,229
Media	19,62	19,35	19,24	19,26
Coefficient of variation (CV)	1,37	1,20	1,85	1,19

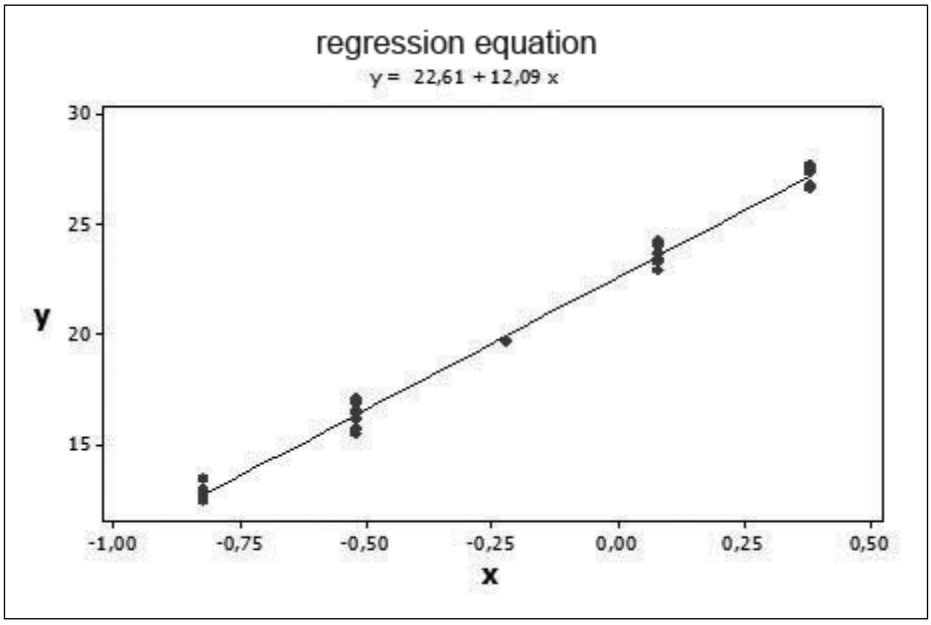

Figure 1 – Example 7: Graph the regression curve

8.10.3 EXAMPLE OF INDIRECT TEST "ALL OR NOTHING"

Example 8: Test of two doses, dichotomous probit method simplified

Assay of insulin by method of convulsion in mice

The doses used in the pattern were pt = 18 mIU/ mice and *p2 = 30 mIU/mouse.*

Equivalent Doses of sample (a; = 18 mIU/mice and *a2 = 30 mIU/mice) were prepared based on power supposed SA* = 40 IU/mL.

The mice were injected subcutaneously with 0.25 mL/ mouse of their solution. Previously were divided at random into four groups, which received, respectively,

Table 44 – Example 8: Responses (% of mice into convulsions).

	Standard P		*Sample*	
	PI	*P2*	*a1*	*a2*
Number of mice injected (n)	30	28	28	24
Number of mice in convulsion	9	17	11	18
Percentage of responses (%)	30.0	60.7	39.3	75.0

$$K = \frac{(P + A)^2}{k} = \frac{20{,}15^2}{4} = 101{,}5056$$

$$\text{Preparaciones} = \frac{\quad}{2} - 101{,}5056 = 0{,}1056$$

$$\text{Regresión} = \frac{(0{,}79 + 0{,}94)^2}{} = 0{,}7482 = E$$

$$\text{Paralelismo} = \frac{0{,}79^2 + 0{,}94^2}{2} = 0{,}748 = 0{,}0056$$

Test Validity

The test meets the conditions of validity:

Significant regression, F calculated 12.15 is greater than The Critical value from **Table** 5**,** for p = 0.01, gl_1 *= 1 and* gl_2 =Infinity; and

No significant deviation from parallelism, F calculated 09 Is less than the critical value from **Table 4,** for p = 0.05; gl_1 *= 1 and* gl_2 *= infinity.*

Table 45 – Example 8: Processing in probit, totals, and contrasts.

	Standard P		***Semple A***	
	p_1	p_2	a_1	a_2
Probito (**Table 16**)	$P_1 = 4{,}48$	$P_2 = 5{,}27$	$A_1 = 4{,}73$	$A_2 = 5{,}67$
Weighting w **(Table 17**)	0,576	0,619	0,540	0,540
Preparation	P = 9,75		A = 10,4	∑y = 20,15
Contrast linear	$L_p = 0{,}79$		$L_A = 0{,}94$	∑L = 1,73

Results obtained with the formulas of Table 8.

Table 46 - Example 8: Analysis of variance

Source of variation	***gl***	***Sum of squares***	***Mean Square***	***F***	***p***
Preparation	1	0,1056	0,1056	1,71	> 0,05
Regression	1	0,7482	0,7482	12,15	< 0,01
Parallelism	1	0,0056	0,0056	0,09	> 0,05
Erro	∞		$s^2 = 0{,}0616$		

The sums of squares were obtained employing the formulas of Table 10, taking n = 1..

Calculation of the estimate of power and confidence limits

Using formulae 10 to 15.

I= log 30 – log 18 = 1,4771 – l,2553= 0,2219

t = 1,96 con *gl=infinito y p=0,05* de (la **Tabla 3)**

$$b = \frac{0,79 + 0,94}{2(0,2219)}$$

$$\bar{y}_P = \frac{\quad}{2} = 4,87$$

$$\bar{y}_A = \frac{10,4}{2}$$

$$M' = \frac{5,20 - 4,87}{3,9318} = 0,0839$$

$S_A = 40,0$

$\log S_A = 1,6021$

$M = 0,0839 + 1,6021 = 1,6860$

R_A = antilog 1,6860 = 48,53 UI/ml= Estimated potency:

$$C = \frac{0,7482}{[0,7482 - 0,0616(1,96)^2]} = 1,4625$$

c' = 1 (of the Table **15**)

$$\begin{matrix} M'_s \\ M'_i \end{matrix} = 1,4625 \times 0,0839 \pm \sqrt{0,4625[1,425(0,0839)^2+(0,2219)^2]}$$

$$\begin{matrix} M'_s \\ M'_i \end{matrix} = 0,1227 \pm 0,1658$$

M'_s=0,2885

M'_i = 0,0431

The Logarithm of the confidence limits of power

$M_s = 0,2885 + 1,6021 = 1,8906$

$M_i = 0,0431 + 1,6021 = 1,5590$

Confidence Limits of power

R_s = 77,73 UI/mL

R_i = 36,22 UI/ mL

Using the complete method of probit analysis, obtained- if an estimate of power of 48.48 with limits of 35.9 and 75.92 IU/mL.

8.10.4 EXAMPLE OF COMBINATION OF ESTIMATES OF POWER

Example 9: Combination of estimates of power

Combination of tests of corticotropin by method of depletion of ascorbic acid in rats supra-renal hipofisectomizadas

Three independent trials of the same sample were performed according to the procedure described in Combination of Estimates of Power (8.8). The results of the tests are shown in Table 47.

Table 47 – Example 9: Data for combination of powers.

	Test 1	*Test 2*	*Test 3*
M	**1.24797**	**1.25164**	**1.42193**
L	**0.29097**	**0.90082**	**0.11555**
T2	**4.1209**	**4.1209**	**4.2025**
Gl	**34**	**33**	**27**

Calculating the weighted average power

$$\overline{M} = \frac{\sum MW}{\sum W} = \frac{2058,6174}{1474,0148} = 13966$$

R = *anti* log 1,3966 = 24,9

Test of homogeneity of log of estimates of power.

$x^2_M = \sum W(M - \overline{M})^2 = 5,5$

x^2_M = 2p = 0,05 (**Table 18**) = 5,9

As χ^2 calculated is less than the critical value, you do not have Elements to suspect heterogeneity.

Calculation of the confidence limits

$$S_{\overline{M}} = \sqrt{1/\sum W} = \sqrt{1/1474,0198 = 0,0260}$$

$$\begin{matrix} M'_s \\ M'_i \end{matrix} = \overline{M} \pm 1,98 \times 0,0260$$

$M_s = 1,4226$

$M_i = 1,3700$

$R_s = 26,5$

$R_i = 23,5$

9 RADIOPHARMACEUTICALS

GLOSSARY

Specific Activity (or radioactivity specifies):

Radioactivity radionuclide related to earth of the unitary element or compound. It is commonly referred to the activity of 1 g of substance specified in the monograph:

$$S = \frac{N \times 0{,}693}{W \text{ ou } M \times T_{1/2}} \text{desintegrações/s/g}$$

Whereas:

S = specific radioactivity;
N = number of Avogadro;
W = atomic weight;
M = molecular weight.

Not radioactive Components for marking: preparation or set of reagents that must be reconstituted or combined with a radionuclide for the synthesis of the radiopharmaceutical end, before the administration to the patient. May come in the form of lyophilized reagents or other substances and are more commonly known as "kits" for marking.

Radioactive Concentration: the concentration of the solution is the radioactivity radioactivity radionuclide contained in unit volume and generally referred to as activity per 1 mL. As occurs with all specifications involving radionuclides, it is necessary to state the date and, in the case of radionuclides with short half-life, the time in which the radioactivity concentration was determined.

Waterway: stable isotope of radionuclide in question, added to the preparation radioactive chemical form identical to that in which the radionuclide is present.

Radiative decay: the nuclei of radioactive elements – radionuclides – suffer loss of particles and/or energy according to their own characteristics. These characteristics include the speed of decay and the type of issue. The particulate emission of cores determines modification of its number of earth. When the particles emitted is the bearer of positive or negative charge the nucleus undergoes change of atomic number and, consequently, the number of electrons in the atom eletrosfera that corresponds to it, determining changes in chemical properties of the atom. The radioactivity decays in reason exponential, that is characteristic for each radionuclide. The activity at any time can be calculated by exressao

$$A = A_0 e^{-\lambda t}$$

Whereas:

A = activity at time t;
A_0 = initial activity;
λ = Constant of decay – also called for constant of disintegration or constant transformation,
And., the fraction of radioactive atoms that undergo transformations in unit of time, provided that this is a short time in comparison with the half-life physics;
t = time elapsed;
e = base of logarithms natural and base.

Disintegration: transformation in which the nucleus emits one or more particles.

Generator: system that incorporates a radionuclide father who, by decay, produces a radionuclide son that can be removed by elution or by some other method to be used as an integral part of a radiopharmaceutical.

Isotopes: nuclides of a same chemical element whose nuclei have the same atomic number and atomic mass different.

Starting Material: all constituents that are used in the preparation of radiopharmaceuticals.

Biological Half-life: time required for a body remove, by eliminating biological, half the quantity of a substance administered.

Effective Half-life: Time NECESSARY For a Radionuclide in an organism decrease their activity by half as a combined result of the elimination of biological and radioactive decay. The effective half-life is important for the calculation of the optimal dose of radiopharmaceutical to be administered and in monitoring the amount of exposure to radiation.

Can be calculated from the formula: in which:

$$T_{1/2e} = \frac{T_{1/2p} \text{ x } T_{1/2b}}{T_{1/2p} + T_{1/2b}}$$

$T_{1/2e}$ = time of effective half-life of the radionuclide.
$T_{1/2p}$ = time of physical half-life of the radionuclide.
$T_{1/2b}$ = time of biological half-life of the radionuclide.

Half-life physics: time required for half of a population of atoms of a radionuclide can deteriorate to another nuclear form. The half-life is related to the decay constant (λ) by the equation:

$$T_{1/2} = \frac{0{,}693}{\lambda}$$

Neutrino: speck of difficult detection, with negligible mass, neutral, but endowed with energy, issued at the same time the emission of beta particles. The sum of the energies

of the particles beta and neutrino corresponds to value quantified for each process of disintegration beta.

Nuclides: species of atom characterized by the constitution of its nucleus, in particular by its number of protons and neutrons, and also by their state of nuclear energy.

Precursors or raw material for synthesis: generally, these precursors are not produced on a large scale. Some precursors are synthesized by the laboratory for the production of radiopharmaceuticals, others are provided by laboratories specialized producers. Tests for identity, for chemical purity and testing must be carried out by means of validated procedures. When lots of precursors are supported using the certificates of analysis, appropriate evidence should be established to demonstrate the reliability of the analysis of the supplier and at least an identity test should be performed. It is recommended to test materials precursors prior to its use in the routine production of radiopharmaceutical agent, to ensure that under production conditions specified, the precursor enables the preparation of radiopharmaceutical in quantity and quality specified.

Radionuclide Purity: it is the ratio, expressed as a percentage, of the radioactivity of the radionuclide in relation to the total radioactivity of the radiopharmaceutical. The impurities radionuclidicas relevant are listed, with their limits, individual monographs.

Radioactivity Specifies: the radioactivity of a radionuclide per unit mass of element or chemical product of interest.

Total Radioactivity: the radioactivity of the nuclide per unit mass of element or chemical product of interest.

Radioisotopes: radioactive isotopes or radionuclides. Isotopes are unstable which suffer from radioactive decay and transmute into new element. Are atoms that have disintegrated by emission of corpuscular radiation (particles) or electromagnetic. All radioisotope is characterized by its long half-life (T1/2), expressed in units of time (seconds, minutes, hours, days and years) and by the nature and energy of the radiation. The energy can be expressed in eletronvolts (eV), kilo-eletronvolts (keV) or mega-eletronvolts (Sem).

Chemical Purity: it can be understood as the ratio expressed as a percentage of the mass of the molecule of the compound of interest in its chemical status indicated, in relation to the total weight of the preparation. The chemical impurities that are relevant are listed with their limits in individual monographs.

Radiochemical Purity: it can be understood as the ratio expressed in percentage of radioactivity of the radionuclide of interest in its chemical status indicated, in relation to the total radioactivity of radiopharmaceutical preparation. The impurities radioquimicas relevant are listed, with their limits, individual monographs.

INTRODUCTION

Radiopharmaceuticals are pharmaceutical preparations with diagnostic or therapeutic purpose which, when ready for use, contains one or more radionuclides.

The radiopharmaceuticals include, also, the non-radioactive components for marking and the radionuclides, including components extracted for radionuclide generators.

The production of radiopharmaceuticals should meet the requirements of Good Manufacturing Practices (GMP) for Radiopharmaceuticals, in addition to meet the specifications for pharmacopoeial. The radiotracers has its production, supply, storage, use and dump regulated by national legislation.

The radiopharmaceutical agent contains the radionuclide in one of the following forms:

- As an element atomic or molecular;
- As an ion;
- Included or connected to the organic molecules, by process of chelation or by covalent binding.

The ways of obtaining radionuclides, used in the production of radiopharmaceuticals are:

- Bombardment of neutrons in nuclear reactors;
- Bombardment with charged particles in particle accelerators;
- Nuclear fission of heavy nuclides after bombardment with neutrons exist or with particles;
- Systems radionuclide generators that involve physical or chemical separation of a radionuclide son, shorter half-life than the parent radionuclide.

STORAGE

The radiopharmaceuticals must be kept in sealed containers and in place sufficiently protected to prevent irradiation of personnel per primary or secondary emissions, in accordance with national and international regulations on handling of radioactive substances.

STABILITY

The preparations of radiopharmaceuticals tend to be less stable than their corresponding inactive, occurring its decomposition by radiolysis and, therefore, should be used in the short term. The effects of primary radiation include the disintegration of radioactive atom and the decomposition of molecules when the energy fraction of particles emitted or gamma ray is absorbed by these molecules.

The stability of radiopharmaceuticals depends on many factors, including the energy and the nature of the radiation, the specific activity and the time of storage. The effects of primary radiation may induce side effects

Due to the formation of excited species, that can degrade other molecules, for example, the solvents or preservatives.

Also, it should be considered susceptibility to oxidation and reduction of small quantity of chemical species present. The initial exclusion of all traces of agents of oxidation and reduction is not always sufficient because such agents may form-if continuously by effects of radiation. During storage, containers and solutions can darken due to the radioactivity emitted. This fact does not necessarily indicate the deterioration of preparation.

Wood Preservatives

Injectable radiopharmaceutical Preparations are usually packaged in multidose containers. The preservatives antimicrobials can be decomposed by the influence of radiation and this restricts its use for some injectable radiopharmaceuticals. Therefore, the requirement that injectable preparations containing an antimicrobial preservative suitable, in adequate concentration, does not necessarily apply, the radiopharmaceutical preparations.

The radiopharmaceutical preparations injectables with period of useful life greater than one day, and that does not contain an antimicrobial preservative should be supplied in single-dose vials. If, however, the preparation is supplied in a container multidose, must be used within 24 hours after the withdrawal of the first dose, aseptically.

The injectable radiopharmaceutical preparations for which the period of useful life is greater than one day and containing antimicrobial preservative can be supplied in multidose containers. After the withdrawal of the first dose, so aseptic, the container must be stored at a temperature in the range of 2 ºC to 8ºC and the content used within 7 days.

DILUTION

If necessary make dilution it is preferable to use vehicles of the same composition as those present in the preparation. In the case of injectable radiopharmaceuticals must be used solutions and materials sterile, free of particles and traces of organic matter.

The quantity of radioactive material present in the preparation is often too small to be measured by chemical or physical methods available.

Whereas the formula in which:

$$S_{max} = \frac{1{,}16 \times 10^{20}\ \text{Bq g}^{-1}}{W \times T_{1/2}}$$

S = Maximum specific activity, Max

W = atomic weight,

$T_{1/2 =}$ half-life time in hours.

It appears that, for example, for solution of sodium pertechnetate (99mTc) with the radioactive concentration of 37 MBq (1 mCi) per mL, the concentration of sodium pertechnetate may be as low as 3 x 10-10 g mL-1.

The behavior of masses so small in very dilute solutions may require the addition of inert carrier to limit to adsorption to the surface of the container as well as facilitate the chemical reactions for the preparation of radiopharmaceuticals.

BIOLOGICAL CONTROL

Sterility

Injectable Radiopharmaceuticals must be prepared in accordance with the GMP requirements so as to ensure sterility, given the criteria of the *sterility Test* (5.5.3.2.1). Because of radioactive characteristics of preparations, it is not practicable to delay release of some pharmaceutical products radioactive per account of sterility test. In such cases, the results of sterility tests only provide retrospective evidence confirmatory for sterility assurance, therefore, depends on the initial methods established in manufacturing and in the procedures of validation/ certification. In the case of radiopharmaceuticals prepared in small batches and for which the execution of the sterility test shows high degree of radiological risk, the amount of sample required in sterility test should be considered. If the radiopharmaceutical preparation is sterilized by filtration or processed aseptically, process validation is required.

Bacterial Endotoxins

When specified, a monograph individually for a radiopharmaceutical preparation requires compliance with the test for bacterial endotoxins, described in Biological Methods – *Bacterial Endotoxins* (5.5.2.2). In performing the test must be taken the necessary precautions to limit the irradiation of staff that performs the test.

The limit of bacterial endotoxin is indicated in the monographs of radiopharmaceuticals. The validation of the test is necessary to exclude any interference due to the nature of the radiopharmaceutical. Level of radioactivity must be standardized as some types of radiation and radionuclides, especially high levels of activity, may interfere with the test. The pH of some radiopharmaceutical preparations should be adjusted to pH 6.5 – 7.5 to promote optimal outcomes.

When the nature of the preparation radiopharmaceutical chemistry result in an interference by inhibition or potentiation and is not possible to eliminate the factor interfering, in accordance with the test for bacterial endotoxins should be specified. In some cases it is difficult to conclude the test before the release of the batch for use, when the half-life of the radionuclide in the preparation is short. The test then if constitutes a quality control of production.

PERIOD OF VALIDITY

Date limit specified by the manufacturer for the use of a radiopharmaceutical, before and after reconstitution and/or radioactive labeling of the product, taking into account degradation products chemical, radiochemical and radionuclidicos, being kept the conditions of storage and transport established.

RADIOACTIVITY

Property that certain nuclides must emit radiation by spontaneous transformations of their cores. Generally the term "radioactivity" is used to describe the phenomenon of radioactive decay and to express the physical quantity (activity) of this phenomenon. The activity of a preparation is the number of nuclear transformations per unit of time that occur in preparation. These transformations may involve the emission of charged particles, electron capture or isomeric transition. The charged particles emitted by the nucleus may be alpha particles (helium nuclei, earth number 4) or beta particles (electrons of negative charge or positive, respectively -1P – negatron or +1P – positron). The emission of beta particles is accompanied by the emission of neutrino.

The emission of charged particles can be accompanied by gamma rays, which, also, are issued in isomeric transition. This emission of gamma rays can be partially replaced by ejection of electrons, known as internal conversion electrons. This phenomenon, as well as the process of capturing electrons, cause secondary emission of X-rays, due to the reorganisation of electrons in an atom. This issue secondary cause, also, the ejection of electrons of low energy electrons known as Auger. X-Rays, possibly accompanied by gamma rays, are issued in case of capture of electrons. +1P Particles are annihilated in contact with another electron (-1e) present in the matter, being this process accompanied by the emission of two photons range, each one with energy of 511 keV, generally issued at 180º one another and that is called radiation from annihilation.

The poderpenetrante of each radiation varies considerably according to their nature and energy. Alpha Particles are completely absorbed by thicknesses of solids or liquids that vary from a few tens of micrometers; beta particles are absorbed completely in the thickness of a few millimeters to several centimeters. Gamma Rays are not completely absorbed, but only mitigated, and a ten-fold reduction may require, for example, some centimeters of lead. The more dense is the absorbent, lesser is the range of alpha and beta particles and the greater the attenuation of gamma rays.

Measurement of radioactivity

The absolute measurement of radioactivity in a sample can be performed if the schema of the nuclide decay is known, but in practice many corrections are required to obtain accurate results. For this reason it is common perform measurements using a standard primary source.

Primary Standards may not exist for radionuclides with short half-life, such as for example, positron emitters. The measurement instruments are calibrated using appropriate standards for particle emitting radionuclides.

The counter Incorporating Geiger-Mueller Tubes can be used to measure beta emitters and beta-gamma. Scintillation Counters, semiconductors or ionization chambers can be used to measure gamma rays. Low energy beta-Emitters need liquid scintillation counter.

In this case, the sample is dissolved in the solution of one or more (usually two) organic substances (scintillators fluorescent primary and secondary), which convert part of energy of disintegration in light photons, which are detected and converted into electrical impulses in photomultiplier. When using the liquid scintillation counter, comparative measures must be corrected due to the effects of interference of light. Direct Measurements should be made under conditions that ensure that the geometric conditions are constant (identical volumes of containers and solutions).

Whatever the equipment used is essential that if work in geometric conditions extremely well defined, so that the radioactive source is always in the same position and, consequently, its distance from the measuring device is constant and remains the same, while the sample is replaced by default.

All the measures of radioactivity must be corrected by subtracting the activity of the background radiation, due to the radioactivity of the means and the spurious signals generated on the machine itself. In certain equipment, in which the count is done at high levels of activity, the correction may be necessary by reason of losses by coincidence, due to the time resolution of the detector and associated electronic equipment.

For accounting system with fixed dead time (t), after each count the correction is given by the equation:

$$N = \frac{N_0}{1 - N_{0\tau}}$$

N = rate of actual count per second;

N_0 = count rate measured per second;

τ = time dead in seconds.

In certain equipment, the correction is done automatically. Corrections of loss by coincidence must be made before the corrections for background radiation.

In determinations of radioactivity there are variations statistics because they are related to the probability of nuclear decay. A sufficient number of counts should be done to compensate for variations in the number of disintegration per unit of time. At least 1.000 counts are necessary to obtain standard deviation of no more than 1 %.

The activity decays in reason exponential, that is characteristic of each radionuclide. His determination it is only true in reference specified. The activity at other times can be calculated from the equation exponential decay or by table or, even, can be obtained graphically the curve established for each radionuclide. All determinations of activity must be accompanied by a statement of the date and, if necessary, the time at which the measurements were made. The measurement of the sample activity in solution is calculated in relation to its original volume and expressed per unit volume – concentration radioactive.

The activity decays in reason exponential, that is characteristic of each radionuclide. His determination

Units of Radioactivity

In the International System (SI) the radioactivity is expressed in becquerels (Bq) which means a transformation per second. The historical unity of activity is the curie (Ci) which is equivalent to 3.7 x 1010 Bq.

The conversion factors between becquerel and curie and its submultiples are indicated in Table 1.

Table 1 – Units of radioactivity used in radiopharmacy and conversions between SI units and historical units.

Number of atoms processed per second	*SI Unit: becquerel (Bq)*	*Historical Unity: curie (Ci)*
1	1 Bq	27 Picocurie (pCi)
1000	1 Kilobecquerel (KBq)	27 Nanocurie (nCi)
1 x10^6	1 Megabecquerel (MBq)	27 microcurie (|Ci)
1 x10^9	1 Gigabecquerel (GBq)	27 Millicurie (mCi)
37	37 Bq	1 (nCi)
37.000	37 KBq	1 (μCi)
3,7 x 10^7	37 MBq	1 (mCi)
3,7 x 10^{10}	37 GBq	1 Ci

Identification of radionuclides

The radionuclide is, generally, identified by half- physical life or by nature and energy of the radiation or radiation, or both.

As Far As the half-life time the half-life is measured with the aid of detection apparatus such as ionization chamber, Geiger counter- Muller, scintillation counter or semiconductor detector. The amount of radioactivity, considered the experimental conditions, must be sufficiently high to allow detection during several half-lives presumed, but not too high, to avoid the phenomenon of loss by coincidence due, for example, the dead time of the equipment.

The radioactive source is prepared so as to avoid losses during its handling. Liquid Samples must be contained in sealed flasks or tubes. Solid Products should be protected by cover of adhesive sheet of cellulose acetate, or other material whose mass per unit area is contemptible to avoid attenuation of significant amount of radiation in a study. The same source is so far as geometric conditions identical and at intervals that correspond usually half the half-life and by time corresponding to approximately 3 half-lives. The correct operation of the equipment is checked by means of the use of a permanent source and the variations of the count are corrected, if necessary, as described in Measurement of radioactivity.

Moth-if a curve throwing them if the time in the abscissa axis and plotting the logarithm of the number of counts per unit time, or the electric current, depending on the type of equipment used. The half-life calculated from this curve should meet the specification described in the monograph.

Determination of the nature and the radiation energy

The nature and the energy of the emitted radiation can be determined by various procedures that include the elaboration of curve of attenuation and the use of spectrophotometry. The curve of attenuation is generally used for the determination of the energy of the beta radiation and the spectrometry is used primarily for determining the energy of gamma radiation.

The curve of attenuation is designed for pure beta emitters or for beta emitters-range when there is no availability of gamma ray spectrometer. This method of determining maximum energy of beta radiation provides only approximate values.

The source, mounted conveniently to provide geometrical conditions constant, is placed in front of the thin window counter INCORPORATING GEIGER-MUELLER TUBES and protected as described in Measurement of the half-life time. The count of the source, then, is measured. Between the source and the counter are placed at least six absorbers aluminum, growing mass per unit area, until the count rate is not affected by the addition of additional absorbers. The absorbers are inserted so that the geometric conditions are kept constant.

It Builds a curve by placing in abscissa coordinate the mass per unit area of the absorber expressed in mg cm-2 and in sorted, the logarithm of the number of counts per unit time for each one of the absorbers used. Identical Curve is drawn up using the default. The attenuation coefficient of mass is calculated in relation to the median, practically straight, curves.

The attenuation coefficient of the mass, expressed in cm^2 mg^{-1}, depends on the energy of the emission beta and the physical and chemical properties of the absorber. This allows for the identification of emission and the beta coefficient is calculated, from the curves constructed as described previously, by the expression:

$$\mu m = \frac{2{,}303}{m_2 - m_1} (\log A_1 - \log A_2)$$

Whereas:

M_1 = mass per unit area of the absorber, lighter; m2 = mass per unit area of the absorber, heavier (measure m1 and m2 within the rectilinear portion of the curve).

A_1 = count rate for mass per unit area of mL;

A_2 = count rate for mass per unit area of m2.

The attenuation coefficient thus calculated shall not differ by more than 10% of the coefficient obtained under identical conditions with the pattern of the same radionuclide.

The gamma spectrometry is used to identify radionuclides by energy and X-ray intensity or range. It is based on the property that certain substances (scintillators) must emit light when they interact with electromagnetic radiation. The number of photons produced is proportional to the energy absorbed by the scintillator. The light is transformed into electrical impulses of amplitude approximately proportional to the energy dissipated by photons range.

With the analysis of impulses per output percentage is obtained, with the aid of the analyzer of pulses, the energy spectrum of the source. In the spectra of scintillation gamma rays there are one or more characteristic peaks corresponding to the energies of gamma radiation at source. These spikes are accompanied by other, more or less wide, due to the secondary effects of radiation in scintillator or the material around it. The shape of the spectrum varies according to the equipment used, making it necessary calibrate it with the aid of standard radionuclide in question.

The spectrum of gamma rays of the radionuclide that emits is own him, being characterized by the number of gamma rays of energy individualized produced by transformation. This property can be used to identify which radionuclides are present in the source and the quantities of each one of them. Allows, also, assess the degree of impurities present, by detection of peaks strangers those expected.

The preferred detector for gamma-ray spectrometry is a semiconductor detector of germanium activated with lithium. The scintillation detectors of sodium iodide activated with thallium, although presenting lower resolution, also, can be used. The output of each of these detectors occurs in the form of electrical pulses, the amplitude of which is proportional to the energy of gamma rays detected. After amplification, these pulses are analyzed in multichannel analyzer, which provides the energy spectrum range of the source. The relationship between energy range and the channel number can be easily established using sources of gamma rays of energy known. The detection system should be calibrated, since the efficiency of the detector is a function of the energy of gamma radiation, the shape of the source and the distance from the source to the detector. The efficiency of detection can be measured using a calibrated source of radionuclide in question or, to work more generic, can be constructed a curve of efficiency versus energy range from a series of calibrated sources of various radionuclides.

The use of low-resolution detector may bring some difficulty in identifying the impurities, because peaks in spectrum can not be resolved well. In this case, it is recommended to determine the half-life by repeated measures of the sample.

If, in a source, the impurity radioactive half-life different is present, it is easily detectable by the identification of characteristic peaks, whose amplitudes decrease in rates different from those of the radionuclide expected. The determination of the half-life of interfering peaks by repeated measurements of the sample will help in the identification of the impurity. It is possible to establish the rate of decay of radioactivity using gamma spectrometry provided that the peaks decrease in amplitude as a function of half-life.

Information about the physical characteristics of radionuclides of importance in the production of radiopharmaceuticals are provided in Table 2.

PURITY RADIONUCLÍDICA

To establish the purity of radionuclide preparation, the radioactivity and the identity of each radionuclide present must be known. The method most commonly used to examine the radionuclide purity is the gamma spectrometry. It is not a method fully precise because the impurities alpha and beta-broadcasters are generally not detectable and, when employees are detectors of sodium iodide, the peaks due to impurities are often masked by the radionuclide spectrum main.

In the monograph are laid down the general requirements for the radionuclide purity (for example, the spectrum of gamma rays should not differ significantly from that of the standard font) and can establish limits for impurities radionuclidicas specific (for example, molybdenum-99 in technetium-99m). These requirements are necessary although they alone are not sufficient to ensure that the purity of radionuclide preparation is suitable for human use. The manufacturer must analyze their products, especially the preparations of radionuclides with short half- life, regarding the presence of impurities of long half-life, after appropriate periods of decay. In This way, information can be obtained about the appropriateness of manufacturing processes and control procedures.

Due to the differences in half-lives of different radionuclides present in pharmaceutical preparations, the radionuclide purity changes with time. The specification of radionuclide purity must be guaranteed throughout the period of validity. Sometimes it is difficult to perform this test before release for use of a batch produced, when the half-life of the radionuclide in the preparation is short. The test is whether, in this case, a quality control of production.

PURITY RADIOQUÍMICA

The determination of radiochemical purity requires the separation of different chemical substances containing the radionuclide and the estimate of the percentage of radioactivity associated with the chemical substance declared.

In the determination of radiochemical purity can be used analytical methods of separation, such as chromatographic methods (paper chromatography, thin-layer, molecular exclusion chromatography, gas chromatography or liquid of high efficiency), electrophoresis and extraction by solvents.

In chromatography, the volume of sample to be used depends on the technique used. It is preferable not to dilute the preparation in analysis, but it is important to use quantity of radioactivity such that loss of count by coincidence will not occur during the measurement of radioactivity.

Whereas the masses very small from radioactive material applied to the chromatograms, the use of carriers is sometimes necessary and they can be added when the monograph so prescribe.

After the development of chromatography in paper or thin layer, the support is dry and the positions of radioactive areas are detected or by autorradiografia or by measurement of radioactivity along the chromatogram, with aid of counters properly collimated, or by cutting the tape and count of each portion.

The positions of the spots or areas allow chemical identification by comparison with solutions of the same chemicals (not radioactive), visualized by reaction of color or examination under ultraviolet light. The display by the reaction of the sample color direct radioactivity is not always possible or desirable, since the revelation may cause diffusion of radioactive substance in addition to the spots or areas identified.

Measurements of radioactivity may be made by integration, using automatic equipment or digital counter. The proportions of areas below the peaks provide the relations of radioactive concentrations of chemical substances. When the tapes are cut into portions, the reasons of quantities of radioactivity measurements provide the proportions of radioactive concentrations of chemical species.

As the radiochemical purity may change with time, mainly because of the decomposition by radiation, the test result should indicate that the product presents values specified during the whole period of validity of the radiopharmaceutical.

SPECIFIC ACTIVITY

The specific activity is calculated by dividing the concentration (radioactive radioactivity per unit of volume) with the concentration of the chemical substance in analysis, after checking that the radioactivity is due only to the radionuclide radionuclide (purity) and the chemical species (radiochemical purity) in question.

The specific activity changes with time, and should be expressed having as reference the date and, if necessary, the time. The specification must be guaranteed during the entire period of validity of the radiopharmaceutical.

Table 2 – Information about the physical characteristics of radionuclides of importance in the production of radiopharmaceuticals.

Radionuclide	*Half-life Physics*	*Type of decay*	*Energy (MeV)*	*Probability of transition (%)*	*Photon Energy (MeV)*	*Photons Emitted (%)*	*Transitions Internally Converted (%)*
Caesium- 137	30,1 a	β^+	0,512	94,6	Via 2,6 min ^{137m}Ba	85,1	9,5
			1,174	5,4	0,662	8 (Ba K X-Ray)	
					0,032-0,038		
Carbon-11	1223,1s	β^+	0,960	99,76	0,511	From annihilation	
Carbon-14	5730 a	β^-	0,158	100	-	-	
Cromio-51	27,7d	c.e.		100	0,320	9,83	
					0,005-0,006	~22 (V K Ray X)	
Cobalt-57	270d	c.e.		100	0,114	9,4	
					0,122	85,2	
					0,136	11,1	
					0,570	0,02	
					0,692	0,16	
					other	Low intensity	
					0,006-0,007	~55% (Fe K X-Ray)	
Cobalt-58	70,8d	β^+	0,475	15,0	0,511	β^+	
		c.e.		85	0,811	99,4	
					0,864	0,7	
					1,675	0,5	
					0,006-0,007	~26 (Fe K X-Ray)	
Cobalt-60	5,27a	β^-	0,318	99,9	1,173	99,86	0,02
			1491	0,1	1,333	99,98	0,01
					other	<0,01	
Disprosio-165	2,32h	β^+, γ	0,205	0,1	0,046	2,5	
			0,290	1,6	0,047	4,6	
			1,190	14,6	0,053	1,8	
			1,285	83,4	0,094	3,5	
					0,279	0,5	
					0,361	0,8	
					0545	0,16	
Erbio-169	9,4d	β^+, γ	0,341	45	0,008	0,15	
			0,350	55			
Fluorine-18	111min	β^+, K	0,649	97	0,511	From annihilation	

Table 2 (continued)

Radionuclide	*Half-life Physics*	*Type of decay*	*Energy (MeV)*	*Probability of transition (%)*	*Photon Energy (MeV)*	*Photons Emitted (%)*	*Transitions Internally Converted (%)*
Galio-67	78,3h	c.e.		100	0,091	3,6	0,3
					0,185	23,5	0,4
					0,209	2,6	0,02
					0,300	16,7	0,06
					0,394	4,4	0,01
					0,494	0,1	
					0,704	0,02	
					0,795	0,06	
					0,888	0,17	
					0,008-0,010	43 (Zn X-Ray)	
					Track 9,2 μs ^{67m}Zn		
					0,093	37,6	32,4
					0,008-0,010	13 (Zn X-Ray)	
Holmium-166	27,3h	β^-, γ	0,191	0,2	0,007	7,6	
			0,394	1	0,048	2,8	
			1,773	48	0,049	5,0	
			1854	51	0,055	2,0	
					0,080	6,2	
					1,379	0,9	
					1,581	0,18	
						0,12	
					1,662		
Indium-111	2,81 d	c.e.		100	0,172	89,6	10,4
					0,247	94	6
Indium-113 m	99,5 min	t.i.		100	0,392	64,9	35,1
					0,024-0,028	24 (In K X-Ray)	
Iodine-123	13,2 h	c.e.		100	0,159	83,0	16,3
					0,347	0,10	
					0,440	0,35	
					0,506	0,26	
					0,529	1,05	
					0,539	0,27	
					0,027-0,032	~86 (Te K X-Ray)	

Table 2 (continued)

Radionuclide	*Half-life Physics*	*Type of decay*	*Energy (MeV)*	*Probability of transition (%)*	*Photon Energy (MeV)*	*Photons Emitted (%)*	*Transitions Internally Converted (%)*
Iodine-124	4,1d	β^-, γ, K	1,53		0,511 and	From annihilation	
			2,13		0,605	66	
					0,644	12	
					0,730	14	
					1,320	1,0	
					1,510	4,2	
					1,695	14	
					2,09	2,0	
					2,26	1,5	
Iodine-125	60,0d	c.e.		100	0,035	7	93
					0,027-0,032	138 (Te K X-Ray)	
Iodine-131	8,06d	β^-	0,247	1,8	0,080	2,4	3,8
			0,304	0,6	0,284	5,9	0,3
			0,334	7,2	0,364	81,9	1,7
			0,606	89,7	0,637	7,2	
			0,806	0,7	0,723	1,8	
				1.3% OF ^{131}I decays Track 12.^{131}mXe			
(Xenon-131m)		t.i.		100 (Percent relative to Disintegration of ^{131m}Xe)	0,164	2	98
Iron-59	44,6d	β^-	0,084	0,1	0,143	0,8	
			0,132	1,1	0,192	2,8	
			0,274	45,8	0,335	0,3	
			0,467	52,7	0,383	0,02	
			1,566	0,3	1,099	55,8	
					1,292	43,8	
					1,482	0,06	
Kriptonio-81m	13s	γ	-	-	0,193	82	
Lutetium Isotope-177	6,71d	β^-, γ	0,175	12,3	0,071	0,16	
			0,384	9,0	0,113	6,3	
			0,497	78,7	0,208	11,0	
					0,250	0,2	
					0,321	0,2	

Table 2 (continued)

Radionuclide	*Half-life Physics*	*Type of decay*	*Energy (MeV)*	*Probability of transition (%)*	*Photon Energy (MeV)*	*Photons Emitted (%)*	*Transitions Internally Converted (%)*
Molybdenum-99	66,2h	β^-	0,454	18,3	0,041	1,2	4,8
			0,866	1,4	0,141	5,4	0,7
			1,232	80	0,181	6,6	1,0
			other	0,3	0,366	1,4	
					0,412	0,02	
					0,529	0,05	
					0,621	0,02	
					0,740	13,6	
					0,778	4,7	
					0,823	0,13	
					0,961	0,1	
					Track 6.02.^{99m}Tc		
					In equilibrium	~0	93,9
					0,002	83,9	10
					0,141	0,03	0,8
					0,143		
Nitrogen-13	At l0 min working	β^+	1,19		0,511	From annihilation	
Oxygen-15	2,04s	β^+	1,723		0,511	From annihilation	
Phosphorus-32	14,3d	β^+	1,709	100			
Rhenium-186	88,9h	β^-, γ, K	0,939	22	0,123	0,7	
			1,076	71	0,137	9,5	
					0,631	1,9	
					0,768	0,8	
Rhenium-188	18h	β^-, γ	1,964	25,3	0,155	14,9	
			2,119	71,4	0,477	1,0	
					0,633	1,2	
					0,635	0,14	
					0,673	0,11	
					0,829	0,41	
					0,931	0,56	
					1,13	0,7	
					1,306	0,01	

Table 2 (continued)

Radionuclide	*Half-life Physics*	*Type of decay*	*Energy (MeV)*	*Probability of transition (%)*	*Photon Energy (MeV)*	*Photons Emitted (%)*	*Transitions Internally Converted (%)*
Rubidium-81	4,7h	β^+, γ, K	0,33	0,253			
			0,58	0,450			
		Daughter ^{81m}Kr	1,05	1,10			
Samarium-153	47h	β^-, γ	0,26	0,1	0,0058	11,8	
			0,632	34,1	0,0409	17,2	
			0,702	44,1	0,0415	31,2	
			0,81	21	0,0470	12,2	
					0,0696	5,1	
					0,103	28,3	
					0,422	0,2	
Selenium-75	118,5d	c.e.		100	0,066	1,1	
					0,097	2,9	
					0,121	15,7	
					0,136	54	
					0,199	1,5	
					0,265	56,9	
					0,280	18,5	
					0,401	11,7	
					other	<0,05 cada	
					0,010-0,012	~50 (as K X-Ray)	
					Track 16,4 ms ^{75m}As		
					0,024	0,03	5,5
					0,280	5,4	
					0,304	1,2	0,1
					0,010-0,012	~2,6 (as K X-Ray)	
Stroncio-89	50,5d	β^-, (γ)	0,582	0,01	0,909	0,01	
			1,491	99,98			
Technetium-99m	6,02h	t.i.		100	0,002	~0	99,1
					0,141	88,5	10,6
					0,143	0,03	0,87
		Daughter ^{99}Tc					

Table 2 (continued)

Radionuclide	*Half-life Physics*	*Type of decay*	*Energy (MeV)*	*Probability of transition (%)*	*Photon Energy (MeV)*	*Photons Emitted (%)*	*Transitions Internally Converted (%)*
Thallium-201	73,5h	c.e.		100	0,031	0,29	10,1
					0,032	0,25	9,6
					0,135	2,9	8,9
					0,166	0,13	0,2
					0,167	8,81	16
Aluminum-113	115d	c.e.		100	0,255	21	0,1
					0,024-0,028	73 (In K X-Ray)	
		Daughter ^{131m}In					
Tritium (^{3}H)	12,35a	β^-	0,0186	100			
Tungsten-188	69,5d	β^-,	0,3	0,227			
				0,291			
		Daughters $^{188m+188}$Re					
Xenon-131m	11,9d	t.i.		100	0,164	2	98
					0,029-0,035	~52 (Xe K X-Ray)	
Xenon-133	5,25d	β^-	0,266	0,9	0,080	0,4	0,5
			0,346	99,1	0,081	36,6	63,3
					0,160	0,05	
					0,030-0,036	~46 (Cs K X-Ray)	
Xenon-133m	2,26d	t.i.		100	0,233	8	92
					0,029-0,035	~59 (Xe K X-Ray)	
		Daughter ^{133}Xe					
Yterbio-169	32,0d	c.e.		100	0,021	0,21	12,3
					0,063	45,16	50,4
					0,094	0,78	12,3
					0,110	3,82	56,2
					0,117	0,04	
					0,118	1,90	3,2
					0,131	11,42	13,5
					0,177	17,31	17,7
					0,198	26,16	25,7
					0,240	0,12	
					0,261	1,74	
					0,308	11,04	0,7
Ytrio-90	64,5h	β^-	2,281	100	-	-	

10 PHARMACEUTICAL EQUIVALENCE AND BIOEQUIVALÊNCIA MEDICINES

INTRODUCTION

In this chapter are discussed scientific and technical aspects related to tests of Pharmaceutical Equivalence, Dissolution, Bioavailability and Bioequivalence applicable to medicinal products, with emphasis on the solid pharmaceutical forms of immediate release (FFSLI) for oral use and suspensions, in the context of the interchangeability between medicines. Biological Medicinal Products (vaccines, sera, blood derivatives, etc), biotechnology, radiopharmaceuticals and phytotherapies require other considerations and, therefore, not be addressed.

The acts of registration and post-registration of medicinal products are the responsibility of the National Health Surveillance Agency (Anvisa). The scientific and technical aspects presented in this chapter are in line with the criteria adopted internationally and with the technical regulations in force in Brazil on topics related.

The generic medicines were implanted in Brazil in 1999, while in 2003 he published-if technical regulations specifies for the record and for the adequacy of the record of similar drugs that were already sold in the country. The similar drugs that were on the market had been registered according to standards that allowed its health record by means of the concept of similarity to a drug previously registered, without the need for the submission, on the occasion of the record, the results of tests in vitro or in vivo related to proof of effectiveness and safety. The new regulations for similar drugs published in 2003 are intended to establishment of isonomy of criteria for registration and renewal of registration of medicines not innovative (generic and similar), having as a basis the precepts of guarantee of quality, efficacy and safety.

The Pharmaceutical Equivalence and Bioequivalence criteria are applicable to generic medicines and similar. For the record of a generic drug, the pharmaceutical industry must request the National Health Surveillance Agency (Anvisa) the indication of the reference medicinal product for the attainment of necessary tests to the formulation development, of the pharmaceutical form and the manufacturing process, establishing the conditions for the stability tests and the specifications of the medicinal product in order to prove its Pharmaceutical Equivalence (in vitro) and Bioequivalence (in vivo) with the reference medicinal product, an indispensable condition for the attestation of Therapeutic Equivalence between the candidate and the generic reference drug (usually the drug innovative whose bioavailability is known and the clinical efficacy and safety have been proven by occasion of health record).

The Therapeutic Equivalence between the generic and the reference medicinal product enables the interchangeability between them, which, also, is known as generic substitution in the dispensing of medication by the pharmacist. When two drugs are considered Equivalent Therapeutic it is assumed that both will submit the same efficacy and safety to be administered to the body, as well as the same potential to cause adverse effects.

If a pharmaceutical industry wishing to register a new similar medicinal product, must check the technical regulations in force. For similar drugs already marketed, the technical regulations pertinent presents a timeline of adequacy based on the criterion of health risk, according to which up to the year 2014 all similar drugs from the market will have if appropriate the same criteria required for the registration of generic medicines.

PHARMACEUTICAL EQUIVALENCE

The Pharmaceutical Equivalence corresponds to prove that two medications are equivalent with respect to the results of in vitro tests. By definition, Pharmaceutical Equivalents are medicines that contain the same drug, that is, even salt or ester of the same molecule therapeutically active, same pharmaceutical form and route of administration and are identical in relation to power or concentration. Must be formulated to comply with the same specifications updated Brazilian Pharmacopoeia and, in the absence of these, with the other codes authorized by current legislation, or even with other applicable standards of quality; related to identity; dosage; purity; power; uniformity of content; time of disintegration and dissolution rate, when it is the case. However, they may differ in characteristics such as shape, release mechanisms, packaging, excipients, term of validity and, within certain limits, labelling.

The studies of Pharmaceutical Equivalence are intended to assess the quality of medicines by means of comparative analysis between the drug test and the reference medicinal product and must necessarily be carried out by laboratories authorized by Anvisa. In addition, the studies should be carried out on samples within the term of validity, using chemical reference substances of the Brazilian Pharmacopoeia, inventoried by means of Resolution of Directors of Anvisa or originating from other pharmacopoeias. In the absence of these substances, it is recognized that the use of chemical substances to work with identity, content and profile of impurities duly determined.

The analytical methods used for the assessment of the quality of medicinal products have considerable

importance in the study of Pharmaceutical Equivalence. Should be used, preferably, the analytical methods described in the monograph of individual medicinal product present in Brazilian Pharmacopoeia, being that, in the absence of this, it allows the use of methods included in other pharmacopoeias authorised by existing legislation. When there are no monographs for the product in official pharmacopoeia, the study should be performed using validated analytical methods, complementing it with the tests described in general methods of Brazilian Pharmacopoeia. In the event that the analytical method is provided by the manufacturer of the drug, the parameters of precision, accuracy and linearity should be determined by laboratory authorized by Anvisa where the study is being performed.

The Pharmaceutical Equivalence tests must be carried out simultaneously in the generic medicinal product candidate, or similar medicinal product, and on the reference medicinal product, and are based on a comparison of the results obtained with both.

It is important to emphasize that the medicinal product under test should not be developed and formulated to be greater than the reference drug, but rather to present the same characteristics related to the release of the drug and the quality already established for the reference medicinal product. The demonstration of Pharmaceutical Equivalence between the two drugs is an indication that the candidate generic or similar, you can submit the same efficacy and safety of the reference medicinal product.

ABSOLUTE BIOAVAILABILITY, BIOAVAILABILITY, RELATIVE BIOAVAILABILITY AND BIOEQUIVALÊNCIA

The Bioavailability (BD) is defined as the speed and extent of absorption of a drug, from a pharmaceutical form that becomes available, to exert the desired pharmacological effect. Depending on the purpose and the design employed in the study determines how-to absolute Bioavailability (BDA) of a drug or the Relative Bioavailability (BDR) between medicines.

The BDA applies to innovative medicines that are developed as pharmaceutical forms for administration by tracks extravascular. In general, corresponds to a crossover clinical trial, conducted in healthy volunteers, consisting of two periods separated by an interval of time called *washout.* In the first period, the volunteers are randomly distributed into two groups (A and B). The volunteers from the group TO receive the drug in test per track extravascular, while the volunteers group B administers, if possible, the same dose of medication intravenously. The biological fluid collections are carried out in accordance with the procedures established in advance and, after the interval of *washout,* it starts the second period by repeating the procedures with the same volunteers, reversing the groups. The concentrations of the drug in the samples are quantified using bioanalytical method validated, which allows the construction of curves of concentration *versus* time to perform the calculations of pharmacokinetic parameters concerning the bioavailability.

When given to a pharmaceutical form administered by the oral route, for example, the PDA corresponds to systemic fraction calculated in relation to the dose administered intravenously, whose bioavailability is, by definition, equal to 100 %. If it is possible to administer the same dose of medication by oral routes and intravascular and BDA calculated is equal to 80 %, this means that the use of the dose by oral route is not complete, and there is loss of 20% which may be related to the characteristics of the drug elimination (pre- systemic) or formulation.

The calculation of bioavailability is carried out using the following pharmacokinetic parameters: a) area under the curve for concentrations of the drug in liquid biological *versus* time (ASC_{0-t}), which expresses the amount of drug absorbed, i. e. the extent of absorption; (b) maximum concentration achieved after administration of the dose (C_{max}), which is related to the speed of the process of absorption and occurs at time called T max (Figure1).

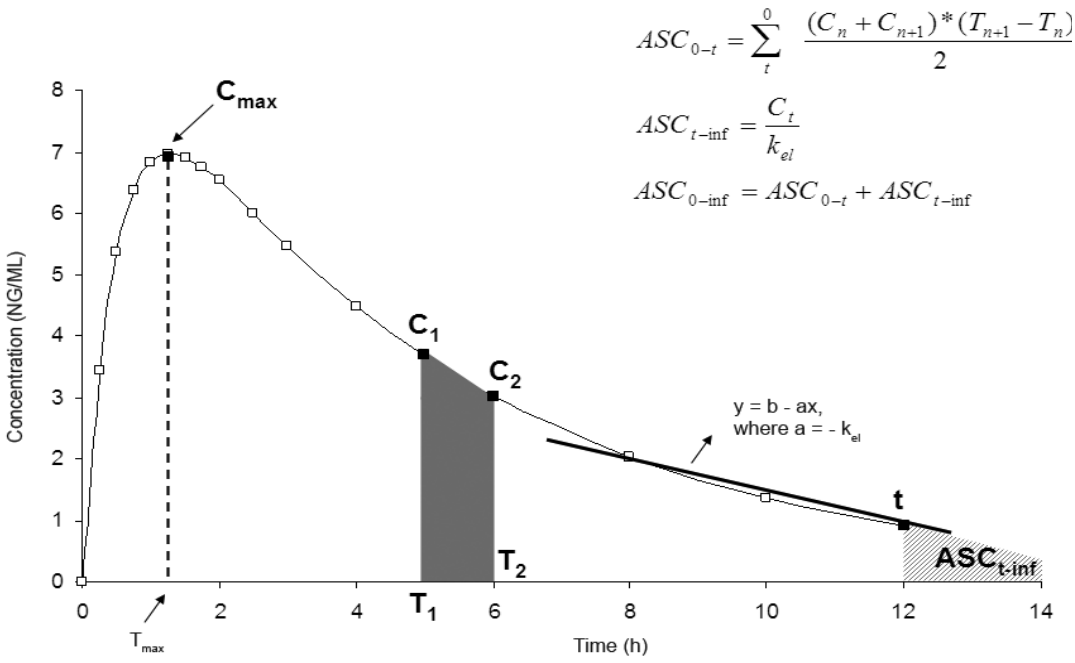

Figure 1 – Representation of the curve of plasma concentrations of the drug as a function of time after administration of a dose of medication via extravascular.

In the case of a generic medicinal product or a similar medicinal product in adequacy, the development of the formulation must be directed to obtaining an Equivalent Pharmacist who, *in vivo, does not present* significant differences in relation to the bioavailability of the reference medicinal product, which is evaluated using- if a study of BDR properly planned and an acceptance criterion applies.

The Bioequivalence (BE) corresponds to a particular case of BDR and involves acceptance criterion and statistical analysis that enable complete on the comparison of bioavailability between two medicines with risk previously established. Two medications are considered bioequivalent and, therefore, interchangeable, when the Confidence Intervals (CI) of 90% calculated for the reasons of geometric averages $ASC_{0\text{-}t\,(T)}$ / $ASC_{0\text{-}t}$ (R) and C (T) / C (R) are among; 80 and 125 %, considering T as the drug in test and R as the reference drug, criterion adopted internationally for the acceptance of bioequivalence.

FACTORS RELATED TO BIOAVAILABILITY AND THE DISSOLUTION OF MEDICINES

In a general way, the main factors that can change the bioavailability of drugs are related to the individual (age, sex, body weight, pathophysiological factors associated) and the characteristics of the drug (drug, formulation and manufacturing process). In the case of factors related to the individual, their influence should be minimized to the maximum, which occurs when the planning of the test of bioavailability is well executed, by means of inclusion and exclusion criteria as well defined, the selection of a group of volunteers representative in relation to the population for the study and the use of an experimental design appropriate.

Among the factors related to the medicinal product are cited: chemical nature of the drug; solubility; particle size; polymorphism; type and quantity of excipients; mixing time and drying; technique of granulation and compression; instability of the drug. In this respect, it is considered essential to the carrying out of studies of pre-formulation and increase in scale for obtaining a stable formulation, to be administered by means of a pharmaceutical form and a track suitable for therapeutic objective. Thus, the professional involved in the development farmacotécnico should widely known the physico-chemical characteristics, pharmacokinetic and pharmacodynamic properties of the drug, selecting, also, the adjuvants farmacotecnicos (excipients) most suitable, besides the best unitary operation involved in manufacturing.

Among the pharmaceutical forms most commonly used in therapy, the solid forms of oral use are those that may cause potential problems of bioavailability due to the characteristics of the drug, formulation, the processes employed in manufacturing and the route of administration. In these cases, after the administration, the process of dissolution of the drug is fundamental for who he is in solution and can be absorbed, and may be a limiting factor for absorption. However, the suspension of use oral or intramuscular, also, can cause problems, since the process of dissolution of the drug, also occurs and suffers the influence of the factors mentioned above.

DISSOLUTION PROFILE

The dissolution profile can be defined as an in vitro assay that allows the construction of the curve of percentage of drug dissolved in function of time, employing-if, generally, the conditions laid down in dissolution test described in the monograph of the medicinal product entered in Brazilian Pharmacopoeia or, in his absence, in other compendia authorized by current legislation.

For completion of the dissolution profile, in the absence of method of dissolution farmacopeico, the company registrant must develop appropriate analytical method to evaluated product in accordance with the parameters described in existing legislation.

The evaluation of the dissolution profile is applicable in cases of development of formulations, quality control batch-to-batch, exemption from the study of bioequivalence for lower doses (when the study of bioequivalence is carried out with the highest dosage and the profiles of dissolution for the measurements considered are similar to the profile of the biolote, formulations of these dosages are proportional and pharmacokinetics is linear within the range between the highest and the lowest dosage) and changes post-registration.

In the case of medicines that will be submitted to the study of bioequivalence, the assessment of comparative dissolution profile in relation to the reference drug is indispensable for the understanding of the behavior of the formulations. When the profiles of dissolution are similar, according to the applicable criteria, there is an indication that the drug test might be is bioequivalent to the reference drug. However, the method of dissolution should be discriminative, allowing to detect significant changes in formulations and in manufacturing processes.

11 WATER FOR PHARMACEUTICAL USE

INTRODUCTION

In this chapter are considered as water for pharmaceutical use the various types of water employed in the synthesis of drugs; in the formulation and production of medicines; in testing laboratories; diagnostics and other applications, related to the area of health, including as the main component in the cleaning of utensils, equipment and systems.

The chemical structure of water is peculiar, with a dipole moment and great ease in forming hydrogen bonds. These properties make the water is an excellent way to solubilize, absorb, adsorb or suspend several compounds, including to wash contaminants and undesirable substances, which will change the purity and efficacy of a pharmaceutical product.

In the face of their characteristics, the processes of purification; storage and distribution should ensure that the specifications for pharmacopoeial are met, maintained and controlled appropriately.

The requirements for water quality will depend on its purpose and employment, and the choice of the purification system is intended to meet purity established. The user is responsible for the selection of the type of water appropriate to their goals, as well as by controls and checks necessary, at intervals that ensure the maintenance of the desired quality. He must ensure that the system provides adequate performance and ability to provide water to the level of quality established, to meet specified parameters corresponding monographs.

In this chapter is not just the theme and there is not the purpose of replacing the legislation, guides or official monographs already existing on water for pharmaceutical purposes. It has as purpose present subsidies that enable users to a better understanding of basic points concerning the quality of water at the time of obtaining and during distribution and use.

The control of contamination of water is crucial, since the water has great ability to aggregate various compounds and, also, to defile herself again after purification. The water contaminants are represented by two large groups: chemical and microbiological testing.

Chemical Contaminants

The organic and inorganic contaminants have different origins: the power supply; the extraction of materials with which it comes in contact; the absorption of gases in the atmosphere; of polluting waste, or waste products used in cleaning and sanitizing of equipment, among many others. Included here are the bacterial endotoxin, resulting from micro-aquatic organisms Gram-negative, critical contaminants that must be properly removed.

These contaminants can be evaluated, mainly, by the tests of total organic carbon (TOC – 2.5.30) and conductivity (2.5.24). The conductivity, measured in microsiemens/cm, it is recommended to evaluate water with large quantity of ions and its reciprocal, the resistivity, in megohm. cm, is measured when there is a low concentration of dissolved ions.

The majority of organic compounds can be removed by reverse osmosis, however, those with low molecular weight demand of additional techniques, such as the ion exchange resin, activated charcoal or oxidation by ultraviolet or ozone, to be removed.

The limits for the parameters of organic and inorganic chemical contaminants are intended to protect the health and avoid that chemical compounds critics may interfere in the phase of pre-treatment of water systems, whereas, subsequently, can be difficult to remove.

Microbiological Contaminants

Are represented mainly by bacteria and present a great challenge to the quality of the water. They originate from the own microbiota of the source of water and, also, of some equipment for purification. Can arise, also, due to the procedures for cleaning and sanitizing inadequate, that lead to the formation of biofilms and, as a consequence, they install a continuous cycle of growth from organic compounds that, in the last analysis, are the own nutrients for the micro

The bacteria can affect the quality of water by disabling reagents or change substrates by enzymatic action, increasing the content in TOC, change the base line (background noise) in spectral analyzes and produce pyrogens and endotoxins.

The bacterial count is reported in colony forming units per milliliter (CFU/mL) and, in general, increases with the time of storage of water. The contaminants are more frequent nonfermenting gram-negative, mainly of the genera Alcaligenes, Pseudomonas, Escherichia, Flavobacterium, Klebsiella, Enterobacter, Aeromonas and Acinectobacter.

The microbiological standard is specified, in parallel to the chemical contaminants, and consists in the absence of total and thermotolerant coliforms (pathogenic micro-organisms of fecal origin), in addition to enterovirus, Cysts and oocysts of protozoa, such as Giardia sp and Cryptosporidium sp in a sample of 100 mL.

To meet these limits, the treatment stations use disinfection processes with chemical substances containing chlorine

or other oxidants, employed for decades, and considered relatively safe for humans. However, these oxidants can react with the organic material of natural origin and generate secondary products of disinfection, such as trihalomethanes, chloramines or still leave residues of own disinfectants. These undesirable products require special attention, on the part of legislators and users.

The chloramines, in particular, can damage irreversibly a equipment dechlorification process is concluded an integral part of a purification system, in addition to presenting risk of formation and release of ammonia.

In addition to these two fundamental groups of contaminants, there are particulate matter, composed of silica, residues of piping or colloids and that, in addition to being a risk to the quality of the purified water, can cause clogs and seriously impair the purification process, by reducing its performance, or even cause irreversible damage to the equipment. Can be detected by filtration, combined with gravimetry or microscopy. But in general it is not necessary to identify the type of particles, just remove it.

In this chapter are addressed some considerations about the main purification systems normally used in the production of water for pharmaceutical use; its main applications; monitoring and maintenance. Covers, also, the parameters of purity for those types of water that are not covered by existing legislation.

TYPES OF WATER

Basically, there are three types of water for pharmaceutical use: the purified water (AP); the water for injectables (API) and the water ultrapurificada (AUP), whose monographs are in this Pharmacopoeia. Official compendia of other countries or international shall specify, in addition to these, other types of water, such as: wrapped in sterile flasks, or bacteriostatic, for irrigation or inhalation. However, all possess characteristics of purity similar to fundamental types already mentioned.

In addition it is important to comment, also, on the drinking water and water reagent, which are widely used and have direct application in pharmaceutical plants, mainly in general procedures for cleaning. Thus, they are considered the five types of water below, in relation to their main characteristics and suggestions for implementation. The specific monographs, when available, detail the parameters of purity for each type.

Drinking Water

As a guideline, the fundamental starting point for any process of purification of water for pharmaceutical purposes is the drinking water. This is achieved by treating the water withdrawal of watersheds, by means of appropriate procedures to meet the specifications of Brazilian legislation relating to physical parameters, chemical, microbiological and radioactive, for a given pattern of potability and, therefore, has no monograph specifies in this compendium.

The drinking water is employed, normally, in the initial stages of cleaning procedures and as a source of obtaining water from the highest degree of purity. Can be used, also, in thermal conditioning of some gadgets and ingredients in the synthesis of intermediaries.

The strict control and maintenance of conformity of parameters of drinking water are fundamental, critical and responsibility of the user of the system of purification that will be supplied. The control must be periodically to ensure that the system of purification used is appropriate for the conditions of the power supply and that there was no change in the quality of water supplied. However, the majority of applications requires additional treatment of drinking water, whether by deionizacao, distillation, ion exchange, reverse osmosis, isolated or hookups, or another appropriate process to produce purified water, free from interference from contaminants that can affect the quality of the medicinal products produced. Another variant of the drinking water is the water reagent, described below, with informative nature, because it does not have specific monograph.

Reagent Water

It is produced by one or more processes, such as simple distillation, filtration, deionizacao descloracao or another, appropriate to the specific characteristics of its use. Usually the water reagent is employed in cleaning materials and some equipment and in the final stage of the synthesis of active ingredients and excipients. Also, has an application for the supply of equipment, autoclaves, bath- mary and in histology. Measures must be taken to avoid microbial proliferation in points of movement, distribution and storage. The main parameters that characterize the water reagent are: conductivity of 1.0 to 5.0 |the/cm (resistivity > 0.2 MW-cm) and total organic carbon (TOC) < 0.20 mg/L.

Purified Water (AP)

The purified water is produced from the drinking water or water reagent and must meet the specifications set forth in the monograph. Does not contain any other substance added. It is obtained by a combination of purification systems, in a logical sequence, such as multiple distillation; ion exchange; reverse osmosis; eletrodeionizacao; ultra filtration, or another process able to meet, with the desired efficiency, the limits specified for the various contaminants.

It is used as an excipient in the production of pharmaceutical forms not parenteral and in formulations magistral, provided that there is no recommendation of greater purity in its use or which does not need to be apirogenica. Also, can be used in washing of material, preparation of solutions reagents, culture media, buffers, various dilutions, microbiology in general, clinical analysis, techniques by Elisa or radioimmunoassay, various applications in most

laboratories, mainly in qualitative or quantitative analyzes less demanding (determinations in percentage).

It is used in tests and determinations that indicate the employment of water, unless it is otherwise specified in the level of purity required, such as for example, some analytical methods and instrumental analyzes that require water apirogenica or chemical purity higher. Can be used in liquid chromatography, high-efficiency when confirmed that your employment does not affect the accuracy or precision of the results.

Depending on the application, can be sterile, without necessarily reaches the limit of bacterial endotoxins established for Water for *Injectables*.

Need monitoring of counting the total viable aerobic organisms, in production and storage, since that has no growth inhibitor added. Minimally, it is characterized by conductivity of 0.1 to 1.3 |the/cm to 25.0 ºC (resistivity > 1.0 MW-cm) and TOC < 0.50 mg/L, endotoxins < 0.25 IU of endotoxin/mL and total count of bacteria < 100 CFU/mL, unless specified differently. The whole system of obtaining; storage and distribution should be properly validated and monitored regarding the parameters of conductivity and microbial count.

Although it is a microbial count specified maximum of 100 CFU/mL in the monograph, each installation or installation productive should establish your limit of alert or action, if the specific characteristics of use are more restrictive, and define appropriate thresholds.

Water ultrapurificada (PU)

The water ultrapurificada has low ionic concentration, low microbial load and low level of TOC. This modality of water is required in more demanding applications, mainly in testing laboratories, for dilution of reference substances, in quality control and final cleaning of equipment and utensils used in processes that come into direct contact with the sample that requires water with this level of purity. It is ideal for methods of analysis which require minimal interference and maximum precision and accuracy. The use of water ultrapurificada in quantitative analyzes of low levels of analyte is essential for obtaining accurate analytical results. Other examples of application of water ultrapurificada are: analyzes of residues, among them the traces of mineral elements, endotoxins, preparations of calibrators, controls, reference chemical, atomic absorption spectrometry in general, ICP/IOS, ICP/MS, mass spectrometry, enzymatic procedures, gas chromatography, liquid chromatography of high efficiency (determination of residues in ppm or ppb), methods in molecular biology and cell culture etc. must be used at the time in which it is produced, or the same day of collection.

The laboratory must use the same type of water required for the final reading of the analysis in the preparation of the samples, in obtaining the standard curve, controls, preparation of solutions, whites, final flushing of the material and in the entire glassmaker that

Will be in direct contact with the sample, where it is appropriate.

The water ultrapurificada is characterised by conductivity of 0.055 to 0.1 to |the/cm to 25.0 ºC ± 0.5 ºC (resistivity > 18.0 MW-cm), TOC < 0.05 mg/L (some cases < 0.03 mg/L), endotoxin < 0.03 IU of endotoxin/mL and total count of bacteria < 1 CFU/100 mL.

Water for Injectables (API)

Water for Injectable is used as an excipient in the preparation of pharmaceutical products parenteral of small and large volume, in the manufacture of active principles of parenteral use, sterile products, other products that require the control of endotoxin and are not subjected to the subsequent step of removal, as well as in cleaning and preparation of processes, equipment and components that come into contact with the parenteral forms in the production of drugs. This modality includes, also, the sterile water for injection, used in parenteral administration and sterile water for injection, which is packaged in airtight flask and sterilized by heat treatment.

The process of purification of first choice is the distillation, in equipment whose internal walls are manufactured in appropriate metal, such as stainless steel AISI 316L, neutral glass or quartz Alternatively, the API, also, can be obtained by a process equivalent to or higher than the distillation for removal of chemical contaminants and micro-organisms, provided that it is validated and monitored regarding the parameters established. The water supply should be, at the very least, drinking and, in general, it will need to be pre-treated to feed the equipment. The process is thus specified by reason of robustness that such equipment present in terms of operation and performance.

The system of production, distribution and storage of the water must be validated and appropriate, so as to prevent the microbial contamination and the formation of bacterial endotoxins. Must meet the requirements laid down in specific monograph. The control will be more rigorous when the application is for injectables, which does not allow the occurrence of microbial contamination, nor of endotoxins. The addition of one or more antimicrobial agents to sterile purified water causes the water bacteriostatic sterile, which is used as a diluent for some parenteral preparations, packed in individual doses.

Another variety of water is the water of hemodialysis, which is treated to obtain the maximum reduction of chemical and microbiological contaminants. Has regulation itself, with quality specifications and frequency specific to the control, and is not covered in this pharmacopoeia.

The water for injectables should meet the physical chemical tests recommended for purified water, in addition to the tests of total count of bacteria < 10 CFU/ 100 mL, sterility, particulate and bacterial endotoxins, whose maximum value is 0.25 IU of endotoxin/mL.

Table 1 – Types of water for pharmaceutical use and quality parameters.

Type of Water	*Characteristics*	*Critical Parameters suggested*	*Application Examples*
Drinking Water	Obtained from springs or the network of public distribution.	Has specific legislation.	General Cleaning and power supply systems for treatment.
Reagent Water	Drinking Water treated by deionizacao or another process. Has low requirement of purity.	Conductivity from 1 to 5.0 |. s/cm to 25.0 ºC ±0.5 ºC (resistivity > 0.2 MD-cm) TOC < 0.20 mg/L	Washing of material, supply of equipment, autoclaves, water-bath, histology, miscellaneous uses.
Purified Water	Varying Levels of organic contamination and bacterial. Requires care to avoid chemical and microbiological contamination. Can be obtained by reverse osmosis or by a combination of techniques of purification from the drinking water or reagent.	Conductivity of 0.1 to 1.3 |j. S /cm to 25.0 ºC ±0.5ºC (resistivity > 1.0 MQ-cm); TOC < 0.50 mg/L; Total Count of bacteria <100 CFU/mL Absence of Pseudomonas and other pathogens.	Production of medicines and cosmetics in general, pharmacies, washing of material, preparation of solutions reagents, culture media, buffers, dilutions, microbiology in general, clinical analysis, techniques by Elisa, radioimmunoassay, various applications in most laboratories, mainly in qualitative or quantitative analyzes less demanding (in %). In HPLC (in %).
Water for injectables	Purified Water treated by distillation or similar process.	Meets the chemical requirements of the purified water and requires control of endotoxin, particles and esterilida< 0.25 IU of endotoxin/mL; TOC < 0.50 mg/L.	As vehicle or solvent of injectables, manufacture of active principles of parenteral use, final washing of equipment, piping and containers used in parenteral preparations. Used as a diluent for parenteral preparations.
Water ultrapurificada	For analyzes that require minimal interference and maximum precision and accuracy. Low ionic concentration, low microbial load and low level of total organic carbon. Purified Water treated by complementary process.	Conductivity of 0.055 to 0.1 |j. S /cm to 25.0 ºC ±0.5 °C (Resistivity > 18.0 MQ-cm) TOC < 0.05 mg/L (some cases < 0.003 mg/L) total Count of mesophiles < 1 CFU/ 100mL (if used for pharmaceutical purposes).	Dosage of mineral or organic waste, endotoxins, preparations of calibrators, controls, SQR, atomic absorption spectrometry, ICP/IOS, ICP/MS, mass spectrometry, enzymatic procedures, gas chromatography, HPLC (ppm or ppb), molecular biology and cell culture etc. Eventualmente in pharmaceutical preparations that require water of high purity

TOC = total organic Carbon;

JFC/100 mL = colony forming Units; microbiological population viable

Some parameters of quality and suggestions of applications are recorded in Table 1, for each type of water for pharmaceutical use

SYSTEMS OF PURIFICAÇAO WATER – PURIFICATION TECHNOLOGIES

The design, installation and operation of systems for the production of purified water, water for injectable and water ultrapurificada have components, controls, and similar procedures. The difference lies in the presence of parameter bacterial endotoxin in water for injectable and in their methods of preparation, specifically in the last stage. These similarities of quality parameters make it possible to establish a common basis for the design of systems for the obtaining of AP, API or AUP, being the critical point differential, the degree of control over the system and the final stages of purification required to remove bacteria, bacterial endotoxin and reduce the conductivity.

The processes for obtaining employ sequential unitary operation – the stages of purification – which are focused on the removal of certain contaminants and protection of subsequent stages of purification. It is noted that the final unitary operation for obtaining water for injectables is limited to distillation or other equivalent process or higher, in the removal of chemical contaminants, as well as micro-organisms and their components. The technology of distillation is consecrated by his long history of reliability and can be validated for production of water for injectables. However, other technology or combination of technologies can also be effective and validated for this purpose. The ultrafiltration placed in a sequence after other technologies for purification of chemical contaminants can be suitable for the production of water for injectables if demonstrate the same efficiency and reliability of distillation, validation. Nowadays, with the availability of new materials for technologies such as reverse osmosis and ultrafiltration, which allows you to operate and sanitize at higher temperature, for microbial reduction, there are new and promising applications validated to produce water for injectables.

The project to install a water purification system must take into account the quality of the water supply and water to desired end, the flow required, the distance between the production system and the points of use, the layout *(layout)* of the piping and connections, the material employed, consisting of technical assistance and maintenance and the appropriate instruments for monitoring.

The technologies of purification described here are intended to remove contaminants in various stages of the sequence of purification. The choice and the order in which they are applied will depend mainly on the quality of drinking water entry, who will determine which stages will be needed effectively. The main technologies are presented below in a sequential order logic, but not all are necessarily compulsory and are used as the quality of the input water and the type of water that is searching for.

Pre-filtration

Also, known as filtration of depth filtration or initial, is intended to remove contaminants in particulate size range between 5 and 10 ^m, essentially to protect subsequent technologies, using sand filters or combination of filters.

Adsorption by activated charcoal

This technology employs the adsorption capacity of charcoal activated on contact with organic compounds or contaminants, such as chloramines. In addition, remove oxidizing agents by chemical reduction, in particular the free chlorine, which affects other technologies based on membrane, such as reverse osmosis or ultrafiltration.

The removal of agents sanitizers delivers the bacterial growth and the formation of biofilm, which implies the need of sanitization own activated charcoal, with direct steam or hot water, for example, and the control of particulate and microbial count of their effluent.

Treatment with chemical additives

The use of chemical additives refers to those who are intended to adjust the pH or to remove carbonates and ammonia, for the protection of other technologies, among them the reverse osmosis.

As chemical additives may be employed: ozone, commonly used in the control of micro-organisms and the metabisulphite, applied as a reducing agent for free chlorine, in replacement of the charcoal activated.

The chemical additives are, necessarily, removed at some later stage of purification and can not leave residue in water end.

Treatment with abrandadores

In cases in which the feed water is “hard”, it becomes necessary to use the abrandadores. This technology employs regenerating resins of ionic exchange, that capture the ions of calcium and magnesium, and release sodium ions in the water. The slowdown is used in the protection of sensitive technologies the incrustation, such as reverse osmosis.

Here, too, there is a concern with the formation of biofilm and it is necessary to control the microbial count, with frequent regeneration, recirculation or other ways of reducing microbial count.

Deionizacao and eletrodeionizacao continues

The deionizacao and eletrodeionizacao continues are effective technologies for the removal of dissolved inorganic salts. The systems of deionizacao, also known as conventional deionizacao, produce purified water for routine use, by means of ion exchange resins for specific cations or anions. Are organic polymers, usually sulpho groups, in the form of small particles. The cationic resins

capture ions releasing the ion H+ in water and the anionic release OH-. Are a. c. with acids and bases, respectively. This process alone does not produce water of high purity, by there is leakage of small fragments of the resin, ease of microbial growth and by be low removal of organic.

The systems of eletrodeionizacao continues combine cationic and anionic resins with membranes semi-permeable for a long time and the application of an electric field, thereby promoting the removal of ions in a continuous manner, that is, without the need to stop for regeneration.

In both cases it is necessary to have a control over the generation of particles arising from successive regenerations, as well as micro-organisms. This can be accomplished by controlling the regenerations, in the case of deionizacao, using recirculation of water and applying-if UV radiation for the control of micro-organisms in the output, whose effectiveness needs to be proven.

Reverse Osmosis

The reverse osmosis is a purification technology based on semi permeable membranes and with special properties of removal of ions; micro-organisms and bacterial endotoxins. Removes 90 to 99% of most contaminants. However, various factors, such as pH; differential pressure across the membrane; temperature; type of polymer membrane and the actual construction of the cartridges for reverse osmosis may significantly affect this separation.

The membranes for reverse osmosis equipment must be properly controlled as the formation of encrustations from salts of calcium, magnesium and other, and biofilm, source criticism of microbial contamination and endotoxins. For this reason it is essential that we provide a system of pre-treatment before the reverse osmosis, which remove particles and oxidizing agents, and, in parallel, must make it, periodically, the sanitization of the system. This practice helps to increase the life of the membranes and reduces the frequency of its regeneration.

There are, also, the systems for reverse osmosis of double step, in which the purified water by first stage feeds the second stage, enhancing and complementing the purification.

Ultrafiltration

Ultrafiltration Systems are often used in water systems for pharmaceutical use, for removal of endotoxins. The ultrafiltration is carried out by using a special membrane with the property to retain molecules as its molecular weight and estereoquimica. It is called Cutting Nominal Molecular Weight *cut off* the range used for the separation of particles, characterized by the size of the molecular weight. In the removal of endotoxins are used filters in the range of 1.000 Da, which retains molecules with molecular weight, greater than or equal to 1.000 Da.

This technology can be used in a final step or middle of the purification system, provided that validated, and, in the same way that the reverse osmosis, requires a pre-treatment, an appropriate control of operating conditions and appropriate procedures for cleaning and sanitizing, to maintain the quality of water as established.

Filtration with electrostatic charge

This type of filtration employs positive charges on the surface of the membranes and is intended to reduce the levels of endotoxWhereas have electrical nature. Exhibit a marginal capacity for removal of micro-organisms, but its greater efficiency is due to the removal of endotoxins. It Presents an important limitation: when the loads are fully inhibited, by saturation by the capture of endotoxins, removing it paralyzes. For this reason, filters with electrostatic charge are extremely difficult to validate, given this unpredictability, regarding the time at which effectively not more retains these contaminants.

Microfiltration – retention of micro-organisms

This technology uses microporous membrane, with a specification of pore size 0.2, or 0.22 ^m. Must be validated as the retention, by means of a bacteriological test, which determines the value of the logarithmic reduction of micro-organisms in the membranes. The model used currently employs a suspension of *Brevundimonas diminuta* to 107 CFU /cm2 of filter area, and tests the sterility of the filtrate. Even that the membrane is specified as 0.2 or 0.22 ^m pore size, not necessarily will be sterilizing agent, if you do not produce a sterile filtered through this test, i. e., a value of logarithmic reduction equal to 7. If the logarithmic reduction obtained is not of the order of seven, the membrane can be used to reduce the microbial flora, but not serves to sterilize.

The microfiltration is applied, also, in filtration of gases, or ventilation of storage tanks, to avoid contamination of water contained therein. In these cases, we use hydrophobic membranes, for which the filter operate without accumulation of condensed water from the moisture in the air itself.

Ultraviolet Radiation (UV)

The UV radiation is used in water purification systems at two wavelengths: 185 nm + 254 nm, that promote two effects:

- 185 NM + 254 nm – Oxidation of organic compounds and consequent reduction of its concentration, to meet the limits of AP, AUP and API;
- 254 NM – germicide Action in various points of the sequence of purification, where it is necessary to reduce the microbial count.

For the oxidation of the organic water must be in the final stage of purification, and such removal will be more effective as lower the load of contaminants. Another limitation is the presence of particles, which may be

deposited in surface of the bulb, decreasing the intensity of radiation, and impair the efficiency of the method. You should also consider the depth / thickness of the bed of water, the flow of water in place of radiation and the power and time of use of the radiation source.

Distillation

In industrial installations can be distillers simple, multiple effects and compression of steam, which are used, in general, for production systems of large volumes. The feed water for such equipment requires controls different from those used in reverse osmosis. In this case, the concentration of silicates is critical, as in any system of steam generation. Another important aspect is the possibility of transport of volatile compounds in condensate. This is especially important with regard to organic impurities, such as trihalomethanes and gases dissolved in water, such as carbon dioxide and ammonia. Thus, the control of drinking water input, as mentioned on the feed water for purification systems, is fundamental.

DISTRIBUTION, SANITIZAÇÃO, STORAGE AND VALIDATION

Distribution

The design of the distribution system must take into account the constant recirculation of the purified water and maintaining the temperature of the water contained in the tank. If necessary, should count with a heat exchanger to provide colder water to points of use.

Piping, valves, instruments and other devices must have construction and sanitary finish, in order not to contribute to that occurring microbial contamination and be sanitized.

Not filters should be used for microbiological retention in output, or the return of distribution systems, because they are repositories of micro-organisms retained and, therefore, a critical source for the formation of endotoxins.

The points of use should be designed so as to avoid dead volumes and allow the water recirculate fully in them when they are closed.

Sanitization

There are Various methods of sanitization of production systems, storage or distribution

The material of construction of the system must be resistant to agents employed and the temperature used in the process is critical. It is common to use temperatures of 80 ºC or 65ºC, with continuous circulation of water. However, to prevent the formation of biofilms is usually employed a combination of heat and chemical agents in sanitization. The sanitization procedure must be properly validated.

As chemical agents, are generally used as the oxidant, halogenated compounds, hydrogen peroxide, ozone or a combination of these. The frequency of the sanitization is determined by the history of the results of the monitoring and the curves of tendency, so that the system can operate without exceeding the limit of alert.

Storage

The conditions of storage shall be appropriate to the quality of the water. The water ultrapurificada should not be stored for a period exceeding 24 hours.

The fundamental guideline for the storage of purified water, water ultrapurificada, or water for injectables is taking into account that the greater the degree of purification of water, the more quickly it tends to recontaminar.

Thus, the water must be kept in constant recirculation, by means of its distribution system, where applicable. The first portions of water produced by a system of purification that has been inactive for more than four hours should be despised, in proportion to the dead volume of the container. These variables must be validated for the specific conditions of each system, as well as, established the parameters to be evaluated in the validation.

The reservoir used for its maintenance must be appropriate to the intended use, composed of inert material, clean and does not serve as a source of contamination to the content. The material of construction must submit appropriate characteristics and roughness to hinder the adhesion of waste, the formation of biofilm and corrosion by agents sanitizers. The 316L stainless steel eletropolido, with surface roughness less than 0.5 microRA, is the most frequent choice to meet these requirements. The reservoir must be protected from sources of heat and light unfit and geometry must allow its total depletion by fund, without dead volumes.

Appropriate Procedures must be adopted to avoid contamination by particulate matter, organic or micro-organisms. Must have a filter of “breath” / ventilation to avoid contamination of the tank volume by air intake / moisture, contaminated and avoid a recontamination by this track.

In particular, but not exclusively, water reservoirs for injectables should be encamisados, to keep the water circulating in higher temperature to 80º C, which significantly restricts the bacterial growth.

Validation

The fundamental purpose of validation is to ensure the reliability of a water purification system, involving their obtaining, storage, distribution and quality at the point of use.

The validation includes the qualification of project (QP); installation (QI); the operation (QO) and performance (QD).

The validation plan for a water system involves the following steps:

a) Knowing the standard of quality of the power supply;

b) Establish the standard of quality of the purified water;

c) Define the technologies for purification and their sequence, from the quality of the input water;

d) Select the materials of construction of the systems of production, storage, distribution and monitoring of points of use;

e) Develop protocols for qualification of design, installation, operation and performance;

f) Establish the critical parameters, levels of alert and action and the periodicity of sanitization and monitoring;

g) Establish a plan for maintaining the validation, which include mechanisms for the control of changes in water systems and provide subsidies for a preventive maintenance program.

The qualification protocols must be previously approved prior to their implementation.

MONITORING OF WATER QUALITY

The process employed in the production of water for pharmaceutical use must be validated, and systematically, the parameters set out in the legislation and in the monographs specific to each type of water should be checked.

The monitoring of water quality must cover all the critical points and representative of the system, in accordance with the established planning, so consistent and continuous.

Thus, procedures should be established and operational sanitization, a program of comprehensive monitoring, with preventive maintenance and a system of control of changes, that determine carefully if the system will need to be revalidated after any modification.

The seasonal issues that can affect the quality of the water from the source of supply must be considered in drafting the plan. The frequency of collection of samples is defined in validation of the system, as well as the tests necessary to ensure the maintenance of water quality required. Any changes to the original plan should be re evaluated.

The equipment and apparatus used in the verifications shall be capable of providing the reading in range required for purity established. The equipment used must be properly calibrated.

The checks performed should be recorded in the proper form, which is included, at least, the parameter (s) measured (s), the date of the measurement, the value obtained, the range of acceptance and the responsible for reading. The staff who performs this task must know the sampling plan and the methods used, as well as the limits of alert and action established. If the user outsource this control, must ensure that the contractor complies with the requirements and procedures set forth.

The data obtained are compared with the typical specifications and limits of alert and action. These are set by the user, who knows both the history of the system of purification and distribution, such as the quality requirements for a given application, and based on validation.

In most applications, the monitoring of water for pharmaceutical use is based on microbiological control and parameters which ensure the maintenance of water quality desired. In general, it is not necessary to identify the micro-organisms present, but yes, proceed to the total count of bacteria, by means of suitable method to cover a wide range of organisms. Samples containing agents sanitizers must be neutralized before carrying out the analysis. The microbiological assays should be performed after a short interval of time of sample collection, or this should be cooled properly and for a certain time, to preserve the original features.

The physico-chemical monitoring accompanies, mainly, the conductivity and the total organic carbon, which can also be measured in line. These tests cover a wide range of inorganic contaminants. If the sample is not analyzed then the collection, must be preserved and stored under conditions that guarantee its integrity and conservation and by appropriate period. Depending on the required application critical parameters to be monitored may vary.

The user must define the limits of alert and action, so as to avoid the use of the product with quality specification below that required for a given application. The alert threshold indicates a deviation warning of quality and not necessarily requires a corrective measure. Can be established on the basis of a statistical analysis of historical trends, using two standard deviations, for example, or about 70% of the action limit, or 50% of the count of the number of viable units, whichever is less. The action limit indicates that the deviation of the quality parameters exceeded the tolerable and requires interruption of activity for the correction.

12 CHEMICAL SUBSTANCES REFERENCE

According to the WHO definition, reference standards of pharmacopoeial assays (PRef) are products of uniformity recognized, intended for use in trials where one or more of its properties will be (ao) compared (s) with a (s) of the substance under examination. Have a degree of purity suitable to the use to which they are intended.

The PRef is established and distributed by authorities for pharmacopoeial, whose value assigned to one or more of its properties is accepted without requiring comparison with another standard, intended for use in specific tests described for pharmacopoeial monographs. Include chemical reference substances, biological products, plant extracts and powders, radiopharmaceuticals, among others. The expression used is related more: Reference Chemical Farmacopeica. Is established and distributed by authorities for pharmacopoeial, and is widely recognized as having degree of purity appropriate, within a specific context and whose value, when used as analytical reference, is accepted without requiring comparison with other chemical substance.

CHEMICAL REFERENCE BRAZILIAN PHARMACOPOEIA (SQR-FB)

It is established and distributed by Direction of Brazilian Pharmacopoeia, following the principles of the WHO, and officialized by Anvisa, being its use mandatory in all national territory. In the absence of a SQR-FB is allowed the use of SQR established by other pharmacopoeias recognized, as current legislation.

CHEMICAL TO WORK

It is determined by comparison with a SQR Farmacopeica, by means of tests pharmacopoeial assays, or properly validated and registered by own lab that will use it. In this situation, should be kept records and performed analytical controls periodicals, employing a SQR Farmacopeica.

CHEMICAL CHARACTERIZED

SQR used in absence of a SQR Farmacopeica. This SQR must be characterized by means of appropriate tests and the values obtained should be properly documented.

GENERAL ASPECTS OF CHEMICAL REFERENCE SUBSTANCES BRAZILIAN PHARMACOPOEIA (SQR-FB)

The Chemical Reference Substances of the Brazilian Pharmacopoeia (SQR-FB) are patterns of reference pharmacopoeial assays, whose production is under the coordination of the Committee Thematic Technical Reference Material (CTT – MR) Brazilian Pharmacopoeia, in line with the guidelines of the Commission of the Brazilian Pharmacopoeia.

The SQR-FB are established and monitored in accordance with the principles of the WHO, with the collaboration of public and private laboratories, by means of interlaboratory studies that use an analytical protocol previously developed and validated, leading to a high-quality product, whose value assigned to one or more of its physical properties and/or chemical does not require comparison with another SQR.

Analytical Methods often use sophisticated equipment to facilitate the accuracy and speed of the procedure used, basing it on measures relating, which require reference standards for obtaining the results.

The SQR-FB are developed to assist in the execution of tests described in the monographs of FB. Its degree of purity can vary according to the test in which it was intended. The declared value is specific to the test described in FB.

The SQR-FB should be stored and handled properly in order to obtain reliable results when used. Bottles should be stored in original, closed and in conditions of temperature and humidity in accordance with the specifications listed on the label and/or certificate of analysis.

The quantities supplied in each bottle of SQR-FB are suitable for a number of analyzes, in order to avoid problems with the excessive exposure of the material. However, the quantities and their value are intended stimulate the direct use of SQR-FB, without the necessity of establishment of standards derived.

When indicated drying the material before use, this procedure will never performed in its original packaging, but by transferring part of the material to another container. After use, the dried material should not be returned to the original container, avoiding possible contamination.

The validity of a given batch must be accompanied by the user through the website of the Brazilian Pharmacopoeia in the internet, which will inform the current batch, the withdrawal of lots in use and the availability of new lots. There are, also, the information for the acquisition of reference standards of pharmacopoeial assays.

13 COLORING SUBSTANCES

Dye Substance is any compound organic or inorganic, natural or synthetic that, regardless of having or not pharmacological activity, is added the pharmaceutical forms with the single purpose of cora them or to change its original color.

The dyes used are of two types:

- Coloring matter;
- Pigments.

The basic difference between pigments and dyes is in particle size and solubility in the environment in which it is inserted. The pigments have, in general, larger particle size and are insoluble in water, while dyes are soluble molecules in water. You can assert that the dyes are employed in solutions and pigments in suspensions. In addition, the pigments have greater chemical and thermal stability that the dyes.

The solubility of the dye may be determined by the presence of certain chemical groups in the structure of the compound, which may cause the differentiations between pigments and dyes.

The dyes used are, in their majority, of synthetic origin and can, in general, be classified into one of seven chemical groups, described below:

- Group Indigoide;
- Group Xanthine;
- Group Lead:
- Group Nitrocellulose;
- Group Trifenilmetano;
- Group Quinolone;
- Group Anthraquinone;

The dyes can also be subdivided into azo dyes (those that contain groupings -N=N-) and non-azo (which belong to a wide variety of chemical classes). Most of The coloring matter of more frequent use is not of type azo, being erythrosine, the indigo carmine / and the yellow of quinoline the three most widely known.

The pigments, two types are used: iron oxide (black, red and yellow), and titanium dioxide, which is white and it is also used in the coating of tablets, to prevent photodegradation of components of the formulation sensitive to light, or even to obtain wrappings of opaque capsules.

The dyes can be classified, according to the

Food and Drug Administration (FDA) in:

- Coloring agents designated as FD&C can be used in food, drugs and cosmetics;
- Coloring agents designated as D&C are authorised for use in drugs and cosmetics;
- Dyes D&C for external use feature employment restricted to medicines and cosmetics applied externally;

Medicinal products intended for application by oral, rectal, vaginal or skin may be added coloring substances listed in relation to the following (Table 1) or a mixture of these substances in cases and in quantities compatible with the good manufacturing practices pharmaceutical. The coloring substances employed must meet the requirements outlined in their respective monographs.

Tabela 1 – Relação de corantes permitidos.

Color	*Substance*	*CAS*	*Synonym*	*Title in English*	*Description (IUPAC)*	*Color Inaex*	*Reference 21 CFR*	*Reference Union European*	*Uses, restrictions and requirements*
YELLOW	YELLOW TWILIGHT	*2783-94-0*	YELLOW 6 INS 110	FD&C YELLOW #6	DISODIUM (5E) -6-OXO-5- (4-SULFONATOPHENYL) HYDRAZINYLIDENE] NAPHTHALENE-2-PERFLUOROOCTANE SULFONATE	15985	741706	E110	Used in food, cosmetics and medicines in general. Not allowed to use in the area of the eyes, in surgical sutures and injectable pharmaceutical forms.
YELLOW	YELLOW TWILIGHT, ALUMINUM LACQUER	*15790-07-5*	6 YELLOW LACQUER ALUMINUM INS 110	FD&C YELLOW #6 ALUMINUM LAKE	ALUMINUM 6-OXIDE-5-(4-SULFONATOPHENYL) DIAZENYLNAPHTHALENE-2-PERFLUOROOCTANE SULFONATE	15985:1	741706	E110	Used in food, cosmetics and medicines in general. Not allowed to use in the area of the eyes, in surgical sutures and injectable pharmaceutical forms.
YELLOW	QUINOLINE YELLOW	*8004-92-0*	YELLOW 10	D&C YELLOW # 10; QUINOLINE YELLOW	2- (2-QUINOLYL) -l. 3-INDANDIONE DISULFONIC ACID DISODIUM SALT	47005	741710	E104	Used in cosmetics and medicines in general. Not allowed to use in the area of the eyes, in surgical sutures and injectable pharmaceutical forms.
YELLOW	YELLOW, QUINOLINE, ALUMINUM LACQUER	*68814-04-0*	YELLOW 10 ALUMINUM LACQUER	D&C YELLOW #10 ALUMINUM LAKE; QUINOLINE YELLOW ALUMINUM LAKE	ALUMINUM; 2- (2-QUINOLYL) – 1,3-INDANDIONE DISULFONIC ACID DISODIUM SALT	47005:1	741710	E104	Used in cosmetics and medicines in general. Not allowed to use in the area of the eyes, in surgical sutures and injectable pharmaceutical forms.
YELLOW	YELLOW OF QUINOLINE SOLUBLE	*92874-95-8*	YELLOW 11	D&C YELLOW# 11	2-QUINOLIN-2-YLINDENE-1,3-DIONE	4700	741711	N/C	Used in cosmetics and medicines for topical application. Not allowed to use in the area of the eyes, in surgical sutures and injectable pharmaceutical forms.
YELLOW	YELLOW FLUORESCEÍNA	*6417-85-2*	FLUORESCEÍNA; YELLOW 7	D&C YELLOW # 7; FLUORESCEINE	3 ‘,6 ‘ -DIHYDROXYSPIRO[2- BENZOFURAN-3,9 ‘-XANTHENE) -1 -ONE	45350:1	741707	N/C	Used in cosmetics and medicines for topical application. Not allowed to use in the area of the eyes, in surgical sutures and injectable pharmaceutical forms.

Table 1 – Relationship of permitted colors.

Color	*Substance*	*CAS*	*Synonym*	*Title in English*	*Description (IUPAC)*	*Color Inaex*	*Reference 21 CFR*	*Reference Union European*	*Uses, restrictions and requirements*
YELLOW	CURCUMIN	*458-37-7*	YELLOW CURCUMIN; CÚRCUMA	TURMERIC OLEORESIN	(1E,6E) -1.7-BIS (4-HYDROXY-3-METHOXYPHENYL) HEPTA-1.6-DIENE-3,5-DIONE	75300	73615	E100	Pemiitidas for all types of products, including foods
YELLOW	IRON OXIDE YELLOW	*51274-00-1*	IRON OXIDE YELLOW	YELLOWIRON OXIDE	IRON OXIDES OBTAINED BY SUMMARY, INCLUDING ITS HYDRATED FORMS OR COMBINATIONS OF MORE THAN ONE OF THESE OXIDES	77492	731200	E172	Used in medicines for oral administration (not exceeding daily dose of 5 mg of Fe) and topical use not permitted in the area of the eyes, in surgical sutures and injectable pharmaceutical forms.
YELLOW	RIBOFLAVIN	*83-88-5*	VITAMIN B2 LACTOFLAVINA	RIBOFLAVIN	7,8-DIMETHYL-10- (2S,3S,4R) - 2,3,4,5-TETRAHYDROXYPENTYL] BENZO (G) PTERIDINE-2,4-DIONE	N/C	73450	E101	Allowed for all types of products, including foods
YELLOW	TARTRAZINE	*1934-21-0*	YELLOW TARTRAZINE; YELLOW 5 INS 102	FD&C YELLOW #5	TRISODIUM (4E) -5-OXO-l- (4-SULFONATOPHENYL) -4- (4-SULFONATOPHENYL) HYDRAZINYLIDENE] PYRAZOLE -3-CARBOXYLATE	19140	741705	E102	Used in food, cosmetics and medicines for internal and external use. Not allowed in the area of the eyes, in surgical sutures and injectable pharmaceutical forms.
YELLOW	TARTRAZINE LACA OF ALUMINUM	*12225-21-7*	TARTRAZINE YELLOW LACQUER ALUMINUM; YELLOW 5 LACQUER ALUMINUM INS 103	FD&C YELLOW #5 ALUMINUM LAKE	ALUMINUM; 4- [3-CARBOXY-5-OXO-1- (4-POLYANILINE OPHEN YL) – 4H-PYRAZOL-4-YL) DIAZENYL] BENZENESULFONATE; 4- [3-CARBOXY-5-OXO-1- (4-SULFOPHEN YL) -4H-PYRAZOL-4-YL] DIAZENYL] BENZENESULFONATE	19140:1	741705	E102	Used in food, cosmetics and medicines for internal and external use. Not allowed in surgical sutures and injectable pharmaceutical forms.

Table 1 (continued)

Color	*Substance*	*CAS*	*Synonym*	*Title in English*	*Description (IUPAC)*	*Color Inaex*	*Reference 21 CFR*	*Reference Union European*	*Uses, restrictions and requirements*
BLUE	BLUE BRIGHT	*3844-45-9*	AZULN. 1 INS 133	FD&C BLUE #1	DISODIUM 2- [4- (ETHYL- (3-SULFONATOPHENYL) METHYL) AMINO) PHENYL) – (4- (ETHYL- (3-SULFONATOPHENYL) METHYL] AZANIUMYLIDENE] CYCLOHEXA- 2,5-DIEN- 1-YLIDENE] METHYL] BENZENESULFONATE	CI 42090	741101	E133	Used in medicinal products for internal and external use cosmetics and food. Not allowed in the area of the eyes, in surgical sutures and injectable pharmaceutical forms.
BLUE	BRIGHT BLUE, ALUMINUM LACQUER	*68921-42-6*	ALUMINUM LACQUER AZULN. 1INS 133	FD&C BLUE #1 ALUMINUM LAKE	3- [ETHYL- (4- [4- (ETHYL- (3- SULFOPHEN YL) METH YL] AMINO) PHENYL) – (2- POLYANILINE OPHEN YL) METHYLIDENE] CYCLOHEXA-2,5-DIEN-l- YLIDENE] AZANIUMYL] METHYL] BENZENESULFONATE	CI 42090:2	741101	E133	Used in medicinal products for internal and external use cosmetics and food. Not allowed in surgical sutures and injectable pharmaceutical forms.
BLUE	BLUE INDIGOTINA	*860-22-0*	AZULN. 2; INDIGOTINA; INDIGO CARMIN; INS 132	FD&C BLUE *#2*	DISODIUM (2E) -3-OXO-2- (3-OXO- 5-SULPHONATE-1H- INDOL- 2-YLIDENE) -1H- INDOLE-5- PERFLUOROOCTANE SULFONATE	73015	741102	E132	Used in food, cosmetics and medicines for oral administration
BLUE	BLUE OF INDIGOTINA, ALUMINUM LACQUER	*16521-38-3*	AZULN. 2 LACQUER ALUMINUM INS 132	FD&C BLUE *#2* ALUMINUM LAKE	ALUMINUM (2E) -3-OXO-2- (3-OXO-5-SULFO-1H-INDOLE- 2-YLIDENE) -1H-INDOLE- 5-SULFONIC ACID	73015	741102	E132	Used in food, cosmetics and medicines for oral administration
BLUE	PATENT BLUE, SALT PURIFIE	*3536-49-0*	PATENT BLUE V; ACID BLUE 3;	ACID BLUE 3	ETHANAMINIUM, N- (4- [4- (DIETHYLAMINO) PHENYL) (5-HYDROXY-2,4- DISULFOPHENYL) METHYLENE) – 2,5-C YCLOHEXADIEN-1- YLIDENE) -N-ETHYL-, INNER SALT, CALCIUM SALT (2:1)	42051	1356	E131	Allowed for all types of products.

Tabela 1 (continuação)

Color	*Substance*	*CAS*	*Synonym*	*Title in English*	*Description (IUPAC)*	*Color Inaex*	*Reference 21 CFR*	*Reference Union European*	*Uses, restrictions and requirements*
BLUE	PATENT BLUE, SODIUM SALT	*129-17-9*	ACID BLUE 1; FOOD BLUE 3; CARMINE BLUE; PATENT BLUE VS	ACID BLUE 1	ETHANAMINIUM, N- (4- [4- (DIETHYLAMINO) PHENYL] (2,4-DISULFOPHENYL) METHYLENE) -2,5- CYCLOHEXADIEN-1- YLIDENE) -N-ETHYL-, INNER SALT, SODIUM SALT (1:1)	42045	1355	El 31	Pemiitido for all types of products.
BLUE	CHLORIDE METILTIONÍNIO	*61-73-4*	METHYLENE BLUE	METHYLTHIONINIUM W EMULSIONS; BASIC BLUE 9	3,7-BIS (DIMETHYLAMINO) PHENOTHIAZIN-5- Rodamine B STAINING W EMULSIONS	52015 Dated			Used in medicinal products, including such as contrast.
WHITE	CALCIUM CARBONATE	*72608-12-9*	CALCIUM CARBONATE	CALCIUMCONCEN-TRATION CARONATE	CALCIUM CARBONATE	72220	731070	N/C	Used in medicines. Allowed for general use, not being allowed to use in the area of the eyes, in surgical sutures and injectable pharmaceutical forms.
WHITE	TITANIUM DIOXIDE	*13463-67-7*	TITANIUM DIOXIDE	TITANIUM DIOXIDE	DIOXOTITANIUM	77891	731575	E171	Used in medicines. Allowed for general use including the eye area, not being allowed to use in surgical sutures, injectable pharmaceutical forms.
ORANGE	ANNATTO	*8015-67-6*	ORANGE N. 4	ANNATTO	(2E,4E,6E,8E, 10E, 12E, 14E, 16Z, 18E) – 4,8,13,17-TETRAMETHYLI COSA-2,4,6,8,10,12,14,16,18-NONAENEDIOIC ACID	75120	731030	E160B	Used in medicinal products for external use (including eye area) and internal (excluding eye area). Not allowed to use in surgical sutures and injectable pharmaceutical forms.

Tabela 1 (continuação)

Color	*Substance*	*CAS*	*Synonym*	*Title in English*	*Description (IUPAC)*	*Color Inaex*	*Reference 21 CFR*	*Reference Union European*	*Uses, restrictions and requirements*
ORANGE	BETA CAROTENE	*9000-07-1*	ORANGE FOOD 5	BETACAROTENE	1,3,3-TRIMETH YL-2- (1E,3E,5E,7E,9E, 11AND,13AND,15E,17E) – 3,7,12,16-TETRAMETHYL-18- (2,6,6-TRIMETHYLCYCLOHEXEN-1-YL) OCTADECA-1,3,5,7,9,11,13,15,17-NONAENYL]CYCLOHEXENE	GRD 40,8 billion	731095	E160E	Used in medicinal products for external use (including eye area) and internal (excluding eye area). Not allowed to use in surgical sutures and injectable pharmaceutical forms.
ORANGE	BETA-APO-8 ‘CAROTENAL	*4172-46-7*	INS 160E	ALL-TRANS-BETA-APO-8’ -C AROTEN AL	(2E,4E,6E,8E,10E,12E,14E,16E) - 2,6,11,15-TETRAMETHYL-17- (2,6,6-TRIMETHYLC Y CLOHEXEN -1 – YL) HEPTADECA-2,4,6,8,10,12,14,16-OCTAENAL	40820	N/C	E160E	Used in medicinal products for external use (including eye area) and internal (excluding eye area). Not allowed to use in surgical sutures and injectable pharmaceutical forms.
ORANGE	ORANGE SOLAR	*633-96-5*	ORANGE PERSIA	D&C ORANGE # 5	SODIUM 4- (2E) -2- (2-OXONAPHTHALEN -1 – YLIDENE) HYDRAZINYL] BENZENESULFONATE	15510	741255	N/C	Used in cosmetics and medicines for external use (including eye area) not exceeding daily dose of the drug of 5mg. Not allowed to use in surgical sutures and injectable pharmaceutical forms.
MARRON	CARAMEL	*8028-89-5*	NATURAL MARRON 10	CARAMEL	IN			E150A	Used in topical medications and oral administration. Not allowed to use in the area of the eyes, in surgical sutures and injectable pharmaceutical forms.
BLACK	IRON OXIDE BLACK	*12227-89-3*	IRON OXIDE BLACK	BLACK IRON OXIDE	IRON OXIDES OBTAINED BY SUMMARY, INCLUDING ITS HYDRATED FORMS OR COMBINATIONS OF MORE THAN ONE OF THESE OXIDES	77499	731200	E172	Used in topical medications and oral administration (not exceeding daily dose of 5 mg of Fe). Not allowed to use in the area of the eyes, in surgical sutures and injectable pharmaceutical forms.

Tabela 1 (continuação)

Color	*Substance*	*CAS*	*Synonym*	*Title in English*	*Description (IUPAC)*	*Color Inaex*	*Reference 21 CFR*	*Reference Union European*	*Uses, restrictions and requirements*
GREEN	CHLOROPHYLL	*1406-65-1*	CHLOROPHYLL INS 1401	CHLOROPHYLL	MIXTURE OF CHLOROPHYLLS To EB. CHLOROPHYLL A: C55H72MGN405, ESTER FITÍLICO OF COMPLEX THERMOPHOSPHATE [(1,3,5,8-TETRAMETHYL-4-ETHYL-2-VINYL-9-OXO-10-METHOXYCARBONYL) FORBINIL]- 7-PROPIONATE. CHLOROPHYLL B: C55H70MGN406, ESTER FITÍLICO OF COMPLEX THERMOPHOSPHATE OF [(1.5,	75,810	N/C	AND 140 (1)	Used in medicines.
GREEN	CHLOROPHYLL	*48240-36-4*	INS 140II	CHLOROPH YLLIN S	MAGNESIUM; 3- (18- (DIOXIDOMETHYLIDENE) – 8-ETHENYL-l 3-ETHYL-3,7,12,17- TET RAMETHYL-20- (2-OXIDE- 2-OXOETHYL) -2,3-DIHYDROPORPHYRIN-23- ID-2- YLJPROPANOATE; HYDRON	75,810	N/C	AND 140 (11)	Used in medicines.
GREEN	GREEN BRIGHT	*4403-90-1*	ALIZARIN GREEN	D&C GREEN # 5	DISODIUM 5-METHYL-2- [4- (4-METHYL-2-SULFONATOANILINO) -9,10-DIOXO ANTHRACEN-1-YL) AMINO] BENZENESULFONATE	61570	741205	N/C	Used in cosmetics and medicines for external use (including eye area). Not allowed to use in surgical sutures and injectable pharmaceutical forms.
GREEN	SOLID GREEN	*2353-45-9*	GREEN FOOD 3	FD&C GREEN # 3	DISODIUM 2- [4- (ETHYL- (3-SULFON ATOPHENYL) METH YL) AMINO] PHENYL) – (4- (ETHYL- (3SULFONATOPHENYL) METHYL] AZANIUMYLIDENE] CYCLOHEXA-2,5-DIEN-1-YLIDENE) METHYL) -5-HYDROXYBENZENESULFONATE	42053	741203	N/C	Used in foods and medicines for external use (including eye area). Not allowed to use in surgical sutures and injectable pharmaceutical forms.

Tabela 1 (continuação)

Color	*Substance*	*CAS*	*Synonym*	*Title in English*	*Description (IUPAC)*	*Color Inaex*	*Reference 21 CFR*	*Reference Union European*	*Uses, restrictions and requirements*
GREEN	GREEN SOLUBLE	*128-80-3*	GREEN ANTHRAQUINONE	D&C GREEN # 6	1,4-BIS (4-METHYLANILINO) ANTHRACENE-9,lC) -DIONE	61565	741206	N/C	Used in cosmetics and medicines for external use (including eye area). Not allowed to use in surgical sutures and injectable pharmaceutical forms.
GREEN	GREEN SOLVENT	*6358-69-6*	GREEN PIRANINA	D&C GREEN # 8	TRISODIUM 8-HYDROXYPYRENE- 1,3,6-TRISULFONATE	59040	741208	N/C	Used in cosmetics and medicines for external use (including eye area). Not allowed to use in surgical sutures and injectable pharmaceutical forms. No more than 0.01% of the dose.
RED	AMARANTH	*915-67-3*	BORDEAU S INS 123	AMARANTH; D&C RED 2; ACID RED 27, TRISODIUM SALT	TRISODIUM (4Z) -3-OXO-4- (4-SULFONATONAPHTHALEN-1-YL) HYDRAZINYLIDENE] NAPHTHALENE-2,7-DISULFONATE	16185	N/C	E123	Allowed for all types of products.
RED	GRAIN AMARANTH, ALUMINUM LACQUER	*12227-62-2*	BORDEAU S ALUMINUM LACQUER	AMARANTH ALUMINUM LAKE; PIGMENT RED 193; ACID RED 27 ALUMINUM LAKE; FD AND C RED. 2 ALUMINUM LAKE	ALUMINUM; TRISODIUM (4Z) -3-OXO-4- (4- SULFONATONAPHTHALEN- 1-YL) HYDRAZINYLIDENE] NAPHTHALENE-2,7- DISULFONATE	16185:1	N/C	E123	Allowed for all types of products.
RED	AZORRUBINA	*3567-69-9*	CARMOISINA	CARMOISINE	DISODIUM (3Z) -4-OXO-3- (4-SULFON ATON APHTH ALEN-1-YL) HYDRAZINYLIDENE]NAP HTH ALENE-1 -SULFON UNTIL	14720	N/C	E122	Allowed for all types of products.
RED	AZORRUBINA, ALUMINUM LACQUER	*84041-67-8*	CARMOISINA ALUMINUM LACQUER	CARMOISINE ALUMINUM LAKE	DISSÓDICO ACID SALT OF 2- (4 ‘ -SULFO-1 ‘ -NAFTILAZO) – 1 -NAPHTHOL-4-SULFÔNICO	14720	N/C	E122	Allowed for all types of products.

Tabela 1 (continuação)

Color	*Substance*	*CAS*	*Synonym*	*Title in English*	*Description (IUPAC)*	*Color Inaex*	*Reference 21 CFR*	*Reference Union European*	*Uses, restrictions and requirements*
RED	ERYTHROSINE	*16423-68-0*	ERYTHROSINE; RED No. 3; ERYTHROSINE SODIUM (INN) INS 127	FD&C RED # 3; ERYTHROSINE	DISODIUM 2- (2,4,5,7-TETRAIODO-3-OXIDE-6-OXOXANTHEN-9-YL) BENZOATE	45430	741303	E127	Used in food, cosmetics and medicines for oral administration
RED	ERYTHROSINE, ALUMINUM LACQUER	*12227-78-0*	ERYTHROSINE ALUMINUM LACQUER; RED No 3 ALUMINUM LACQUER; ERYTHROSINE SODIUM (INN) ALUMINUM LACQUER INS 127	FD&C RED #3 ALUMINUM LAKE	DIALUMINUM; l',3 ',6 ',8 ' -TETRAIODO-3- OXOSPIRO[2-BENZOFURAN- 1,9 ' -XANTHENE] -2 'T-DIOLATE; l',3 \6 ',8 ' -TETRAIODO-3-OXOSPIRO[2-BENZOFURAN-1,9 '-XANTHENE) -2 ',7 ' -DIOLATE	45430	741303	E127	Used in food, cosmetics and medicines for oral administration
RED	IRON OXIDE RED	*1309-37-1*	IRON OXIDE RED	RED IRON OXIDE	IRON OXIDES OBTAINED BY SUMMARY, INCLUDING ITS HYDRATED FORMS OR COMBINATIONS OF MORE THAN ONE OF THESE OXIDES	77491	73.1200	E172	Used in medicines for oral administration (not exceeding daily dose of 5 mg of Fe) and topical use. Not allowed in the area of the eyes, in surgical sutures and injectable pharmaceutical forms.
RED	PIGMENT RED 63	*74336-37-1*	RED 34	D&C RED # 34	CALCIUMCONCENTRATION (4Z) -3-OXO-4- [(1 – SULFON ATON APHTH ALEN-2-YL) HYDRAZINYLIDENE]NAPH THALENE-2-CARBOXYLATE	15880	741334	N/C	Used in cosmetics and medicines for external use. Not allowed in the area of the eyes, in surgical sutures and injectable pharmaceutical forms.
RED	PONCEAU SX	*4548-53-2*	RED 4	FD&C RED #4	DISODIUM (3E) -3-(Z4-DIMETHYL-5-SULFONATOPHENYL) HYDRAZINYLIDENE] -4-OXONAPHTHALENE-1-PERFLUOROOCTANE SULFONATE	14700	741304	N/C	Used in food, cosmetics and medicines for external use. Not allowed in the area of the eyes, in surgical sutures and injectable pharmaceutical forms.

Tabela 1 (continuação)

Color	*Substance*	*CAS*	*Synonym*	*Title in English*	*Description (IUPAC)*	*Color Inaex*	*Reference 21 CFR*	*Reference Union European*	*Uses, restrictions and requirements*
RED	RED 27	*13473-26-2*	The RED PHLOXINE	D&C RED #27	DISODIUM 2 ‘,4 ‘,5 ‘,7 ‘ -TETRABROMO--4,5,6,7-TETRACHLOR 0-3 -0X0SPIR0 (2-BENZ0FURAN-1:9 ‘ -XANTHENE) -3 ‘,6 ‘ -DIOLATE	45410:2	741327	N/C	Used in cosmetics and medicines in general. Not allowed in the area of the eyes, in surgical sutures and pharmaceutical forms
RED	RED 30	*2379-74-0*	RED 30	D&C RED # 30; INDANTHREN BRILLIANT PINK R	(2Z) -6-CHLORO-4-2- (6-CHLORO-4.4-METH YL-3-OXO-1 – BENZOTHIOPHEN-2-YLIDENE) -4- METHYL-1-BENZOTHIOPHEN-3-ONE	73360	741330	N/C	Used in cosmetics and medicines in general. Not allowed in the area of the eyes, in surgical sutures and pharmaceutical forms
RED	RED 33	*3567-66-6*	RED ESPANICO	D&C RED #33	DISODIUM (3E) -5-AMINO-4-OXO-3- (PHENYLHYDRAZINYLIDENE) NAPHTHALENE-2,7-DISULFONATE	17200	741333	N/C	Used in cosmetics and medicines for oral administration and topic. Not allowed in the area of the eyes, in surgical sutures and injectable pharmaceutical forms. Do not exceed more than 0.75 mg of daily dose of dose when used in dental medicine.
RED	RED 40	*25956-17-6*	FD&C RED No. 40; RED ALURAAC INS 129	FD&C RED #40	DISODIUM (5E) -5- (2-METHOXY-5-METHYL-4-SULFONATOPHENYL) HYDRAZINYLIDENE]-6-OXONAPHTHALENE-2-PERFLUOROOCTANE SULFONATE	16035	741340	E129	Used in food, cosmetics and medicines in general. Not allowed in the area of the eyes, in surgical sutures and pharmaceutical forms
RED	RED 40, ALUMINUM LACQUER	*68583-95-9*	RED 40 LACA OF ALUMINUM; ALUMINUM LACQUER RED ALURAAC	FD&C RED #40 ALUMINUM LAKE	LACA OF ALUMINUM OR CALCIUM AND ALUMINUM, IN 7503 DISSÓDICO SALT OF 6-HYDROXY-5- (2-METHOXY-5-METHYL-4-SULFOPHENYL) AZO-2-NAFTALENOSSULFÔNICO	16035:1	741340	E129	Used in food, cosmetics and medicines in general. Not allowed in surgical sutures and pharmaceutical forms

Tabela 1 (continuação)

Color	*Substance*	*CAS*	*Synonym*	*Title in English*	*Description (IUPAC)*	*Color Inaex*	*Reference 21 CFR*	*Reference Union European*	*Uses, restrictions and requirements*
RED	RED 7, LACA PURIFIE	*5281-04-9*	7 RED LACQUER OF CALCIUM; RED LACQUER LITHIUM RUBIM PURIFIE	D&C RED #7 CALCI-UMCONCENTRATION LAKE; D&C RED # 7	CALCIUMCONCENTRATION (4Z) -4- (4-METHYL-SULFONATOPHENYL) HYDRAZINYLIDENE]-OXONAPHTHALENE-2-CARBOXYLATE	15850:1	741307	N/C	Used in cosmetics and medicines in general. The total of D&C Red no. 6 should not be more than 0.01% in 5mg/ daily dose of medication
RED	RED BEET, BETANIN	*7659-95-2*	BEETROOT RED; BETANIN INS 162	BEET POWDER BEETROOT RED	(2S) -4- (2- (2S) -2-C ARBOXY-6-HYDROXY-5- (2S,3XT,4S,5S,6R) -3,4,5-TRIHYDROXY-the-(HYDROXYMETHYL) OXAN-2-YL] OXY-2,3-DIHYDROINDOL-1-YL) ETHENYL) -2,3-D IHYDROPYRIDINE-2,6-DICARBOXYLICACID	N/C	N/C	E162	Used in foods
RED	COCHINEAL RED	*1390-65-4*	CARMIN COCHINEAL; CARMIN; NATURAL RED 4 INS 120	CARMINE	3,5,6,8-TETRAHYDROXY-METHYL-9,10-DIOXO-7- (3,4,5-TRIHYDROXY-the- (HYDROXYMETHYL) OXAN-2-YL) ANTHRACENE-CARBOXYLIC ACID)	75470	731100	E120	Allowed for all types of products.
RED	RED EOSIN	*62342-51-2*	RED 21	D&C RED # 21	2 ‘,4 ‘,5 ‘,7 ‘ -TETRABROMO--3 ‘,6 ‘ -DIHYDROXYSPIRO (2-BENZOFURAN-3,9 ‘-XANTHENE) -l-ONE	YEL-low:2	741321	N/C	Used in cosmetics and medicines in general. Not allowed in the area of the eyes, in surgical sutures and pharmaceutical forms
RED	RED EOSIN PURE	*95917-83-2*	RED 22	D&C RED # *22*	DISODIUM 2- (2,4,5,7-TETRABROMO--3-OXIDE-6-OXOXANTHEN-9-YL) BENZOATE	YEL- low	741322	N/C	Used in cosmetics and medicines in general. Not allowed in the area of the eyes, in surgical sutures and pharmaceutical forms

Tabela 1 (conclusão)

Color	*Substance*	*CAS*	*Synonym*	*Title in English*	*Description (IUPAC)*	*Color Inaex*	*Reference 21 CFR*	*Reference Union European*	*Uses, restrictions and requirements*
RED	RED PERMANENT	*70632-40-5*	RED 36	D&C RED # 36	(Lz) -l- (2-CHLORO-4-4-NITROPHENYL) HYDRAZINYLIDENE] NAPHTHALEN-2-ONE	12085	741336	N/C	Used in cosmetics and medicines for external use. Not allowed in the area of the eyes, in surgical sutures and injectable pharmaceutical forms. No more than 1.7 mg/daily dose of the drug prescribed for less than 1 year, or 1.0 mg/daily dose of drugs prescribed for more than 1 year.
RED	RED PONCEAU 4R	*2611-82-7*	PONCEAU 4R; RED 2 INS 124	PONCEAU 4R	TRISODIUM (8Z) -7-OXO-8- (4-SULFON ATON APHTH ALEN-1-YL) HYDRAZINYLIDENE]IN PHTHALENE-L3-DISULFONATE	16255	N/C	E124	Allowed for all types of products.
RED	RED PONCEAU 4R ALUMINUM LACQUER	*15876-47-8*	PONCEAU 4R LACA OF ALUMINUM; ALUMINUM LACQUER RED 2	PONCEAU 4R ALUMINUM LAKE	LACA OF ALUMINUM OR CALCIUM AND ALUMINUM, IN 7503 SAL TRISSÓDICO ACID 1 – (4 ‘ -SULFO-1 ‘ -NAFTILAZO) -2-NAPHTHOL-6.8-DISSULFÔNICO	16255	N/C	E124	Allowed for all types of products.
RED	RED Ruby	*5858-81-1*	RED 6	D&C RED # 6	DISODIUM (4E) -4- (4-METHYL-2-SULFONATOPHENYL) HYDRAZINYLIDENE] -3-OXONAPHTHALENE-2-CARBOXYLATE	15850	741306	N/C	Used in cosmetics and medicines in general. The total of D&C Red no. 7 should not be more than 0.01% in 5mg/ daily dose of medication
RED	RED SCARLET	*85-86-9*	RED 17	D&C RED # 17	(Lz) -l- (4-PHENYLDIAZENYLPHENYL) HYDRAZINYLIDENE] NAPHTHALEN-2-ONE	26100	741317	N/C	Used in medicinal products for external use. Not allowed in the area of the eyes, in surgical sutures and injectable pharmaceutical forms.
VIOLET	VIOLET ALIZARIN	*81-48-1*	VIOLET 2	D&C VIOLET *#2*	1 -HYDROXY-4-(4-METHYLANILINO) ANTHRACENE-9, ÍO-DIONE	60725	741602	N/C	Used in medicinal products for external use. Not allowed in the area of the eyes, in surgical sutures and injectable pharmaceutical forms.

14 REAGENTS

14.1 INDICATORS AND INDICATOR SOLUTIONS

Indicators are dyes used to indicate the end point of a volumetric analysis or to assess the pH of solutions not stained. The indicators most frequently used are listed in Table 1, in ascending order of the lower limit of its range transition of pH. Then, are described the indicators and indicator solutions (SI) used in the Pharmacopoeia.

Table 1 – Indicators of more frequent use.

Indicator	*Range of transition*	*Color Change*
Malachite Green	0.0 To 2.0	Yellow green
Cresol Red	0.2 To 1.8	Red to yellow
Metacresol Purple	0.5 To 2.5	Red to yellow
Tropeolina OO	1.0 To 2.8	Red to yellow
Thymol Blue	1.2 To 2.8	Red to yellow
Yellow naphthol	2.0 To 3.2	Colorless to yellow
Yellow trifluorated	2.8 To 4.6	Red to yellow
Bromophenol Blue	2.8 To 4.6	Yellow to blue-violet
Methyl Orange	2.9 To 4.0	Red to yellow
Methyl Red	3.0 To 4.4	Red to yellow
Red of Congo	3.0 To 5.0	Blue red
Bromocresol Green	3.6 To 5.2	Yellow to blue
Resazurin	5.0 To 7.0	The Pinkish violet
Litmus	5.0 To 8.0	Red to blue
Bromocresol Purple	5.2 To 6.8	Yellow to blue-violet
BROMOTHYMOL Blue	6.0 To 7.6	Yellow to blue
Phenol Red	6.8 To 8.4	Yellow red
Cresol Red	7.2 To 8.8	Yellow red
Metacresol Purple	7.5 To 9.2	Yellow violet
Thymol Blue	8.0 To 9.6	Yellow to blue
Phenolphthalein	8.3 To 10.0	Colorless to intense violet
Nile Blue The	9.0 To 13.0	Blue red
Thymolphthalein	9.3 To 10.5	Colorless to blue
Alizarin Yellow GG	10.0 To 12.0	Pale Yellow to brown
The Treopeolina	11.0 12.7	Yellow to orange
Yellow titan	12.0 To 13.0	Yellow red

Methyl Orange (CI 13025)

(CAS 547-58 -0)

Formula and molecular mass – $C_{14}H_{14}N_3NaO_3S$ – 327,34

Description – an orange-yellow crystalline Powder.

Solubility Slightly soluble in water and practically insoluble in ethanol 96% (v/v).

Methyl Orange SI

Preparation – Dissolve 0.1 g in 100 mL of ethanol 20% (v/v).

PH Range – 2.9 – 4.0

Color Change – Provides red coloring in the midst moderately acid and yellow coloration in the midst weakly acid and alkaline.

Sensitivity Test – A mixture of 0.1 mL of indicator solution with 100 mL of carbon dioxide free water color is yellow. Are required not more than 0.1 mL of 0.1 *M hydrochloric acid* to determine the color change to red.

Methyl Orange solution,

Preparation – Dissolve 20 mg of methyl orange and 0.1 g of bromocresol green in 1 mL of sodium hydroxide 0.2 *M* and complete with water until the volume of 100 mL.

PH Range – 3.0 – 4.0

Color Change – Provides coloring orange in moderately acidic solutions and green coloring-oliva in weakly acidic and alkaline solutions.

Xylenol Orange

(CAS 3618-43 -7)

Formula and molecular mass – $C_{31}H_{28}N_2Na_4O_{13}S$ – 760,59

Description – reddish brown crystalline Powder. *Solubility* Soluble in water and ethanol.

Xylenol Orange SI

Preparation – Dissolve 0.1 g in 100 mL of ethanol. *Color Change* – In acidic medium presents yellow color- pale. Reacting with certain metals (such as lead and zinc), form complex of intense red color. In the presence of excess edetate disodium acquires color yellow.

Alizarin

(CAS 130-22 -3)

Formula and molecular mass – $C_{14}H_7NaO_7S.H_2O$ – 360,26

Description – Po yellow-orange.

Solubility Easily soluble in water and in ethanol.

Alizarin SI

Preparation – **Dissolve 0.1 g in 100 mL of water.**

Alizarin Yellow GG (CI 14025)

CAS 584-42 -9)

Formula and molecular mass – **$C_{13}H_{8\ N3}not_5$ – 309.21**

Description – **yellow Powder.**

Solubility Slightly soluble in cold water and soluble in hot water.

Alizarin Yellow GG SI

Preparation – Dissolve 0.1 g in 100 mL of water.

PH Range – **1**0.0 – 12.0

Color Change – Provides coloration pale yellow in weakly alkaline solutions and brown coloring in strongly alkaline solutions.

Yellow trifluorated (CI 11020)

(CAS 60-11 -7)

Formula and molecular mass – $C_{14}H_{15}N_3$ – 225,29

Description – yellow crystals.

*Solubility***:** *Insoluble in water, soluble in ethanol, benzene, chloroform, diethyl ether and dilute mineral acids.*

Yellow trifluorated SI

Preparation – Dissolve 0.2 g in 100 mL of ethanol 90% (v/v).

PH Range – 2.8 – 4.6

Color Change – Provides red coloration in moderately acidic solutions and yellow coloration in weakly acidic and alkaline solutions.

Test of homogeneity – Prepare solution 0.01% (p/v) in methylene chloride and apply 0.01 mL of this solution into cromatoplaca silica-gel G. Using as eluent methylene chloride. The chromatogram should show a single stain.

Sensitivity Test – Prepare solution of 2 g of ammonium chloride in 25 mL of water free of carbon dioxide. This solution, added to 0.1 mL of yellow trifluorated ITSELF, must submit color yellow. The color changes to red by adding no more than 0.1 mL of 0.1 M hydrochloric acid

Yellow metanila (CI 13065)

(CAS 587-98 -4)

Formula and molecular mass – $C_{18}H_{14}N_3NaO_3S$ – 375,38

Description – brownish yellow Powder.

Solubility – Soluble in water and in ethanol.

Yellow metanila SI

Preparation – Dissolve 0.1 g in 100 mL of methanol.
Color Change – In titrations carried out in a non-aqueous medium changes the coloring of yellow (basic means) for indigo carmine (acidic).

Sensitivity Test – Dissolve 0.1 mL of yellow metanila SI in 50 mL of glacial acetic acid anhydrous. This solution must submit coloring red-pink. Add 0.05 mL of 0.1 M perchloric acid staining should change to violet.

Yellow naphthol (CI 10315)

(CAS 887-79 -6)

Formula and molecular mass – $C_{10}H_5N_2NaO_5$ – 256,16

Description – Powder or crystals yellow-orange. – Solubility Easily soluble in water and slightly soluble in ethanol 96% (v/v).

PH Range – 2.0 – 3.2

Color Change – Provides colorless solution in strongly acid medium and yellow coloration in solutions less acidic.

Yellow titan (CI 19540)

(CAS 1829-00 -1)

Formula and molecular mass – $C_{28}H_{19}N_5Na_2O_6S_4$ – 695,72

Description – Po brown-yellowish.

Solubility Easily soluble in water and in ethanol.

Yellow titan SI

Preparation – Dissolve 0.05 g in water and make up the volume to 100 mL.

PH Range – 12.0 – 13.0

Color Change – In acidic solutions and moderately alkaline provides yellow coloration. In strongly alkaline solutions presents red color.

Sensitivity Test – Prepare mixture of 10 mL of water, 0.2 mL of the standard solution of magnesium (10 ppm of Mg) and 10 mL of sodium hydroxide M. Add 0.1 mL of yellow titan ITSELF. Prepare evidence in white in a similar manner, but omitting the standard magnesium. Compare the two solutions: intense pink color develops in comparison to proof in white.

Yellow titan, paper

Preparation – Impregnate filter paper with common solution of yellow titan ITSELF. TO air Dry at ambient temperature.

Starch (soluble Starch)

(CAS 9005-84 -9)

Molecular Formula – $(C_6H_{10}O_5)_x$

Description – white or almost white Powder.

Starch SI

Specification – Solution of soluble starch 2% (w/v) in hot water. The solution can provide small opalescence.

Sensitivity Test – Mix 1 mL of the starch ITSELF, 20 mL of water, approximately 50 mg of potassium iodide and, finally, 0.05 mL of iodine 0.01 *M.* There is development of blue color.

Starch iodetado SI

Preparation – Impregnate filter paper with starch ITSELF, freshly-prepared, plus 0.5 g of potassium iodide.

Starch iodide-free SI

Preparation – Crush 1 g of soluble starch with 5 mL of water and add, with constant stirring, boiling water sufficient for 100 mL.

Stability – Prepare immediately before use. Starch iodetado, paper

Preparation – Impregnate filter paper with starch ITSELF, freshly-prepared, plus 0.5 g of potassium iodide.

Bromophenol Blue

(CAS 115-39 -9)

Formula and molecular mass – $C_{19}H_{10}Br_4O_5S$ – 670,02

Description – Po yellow-orange of course.

Solubility – Very slightly soluble in water, slightly soluble in ethanol and readily soluble in solutions of alkali hydroxides.

Bromophenol Blue SI

Preparation – Dissolve, warming softly, 0.2 g of bromophenol blue in 3 mL of 0.1 M sodium hydroxide and 10 mL of 96% ethanol (v/v). Let cool and make up the volume to 100 mL with 96% ethanol (v/v).

PH Range – 2.8 – 4.6

Color Change – Provides color yellow in moderately acidic solutions and color blue-violet in weakly acidic and alkaline solutions.

BROMOTHYMOL Blue

(CAS 76-59 -5)

Formula and molecular mass – $C_{27}H_{28}Br_2O_5S$ – 624,38

Description – Po brown or light red.

Solubility Practically insoluble in water, soluble in ethanol and dilute solutions of alkali hydroxides.

BROMOTHYMOL Blue SI

Preparation – Heat 1 g of bromothymol blue with 3.2 mL of 0.05 M sodium hydroxide and 5 mL of ethanol. After dissolution, make up the volume to 250 mL with ethanol.

PH Range – 6.0 – 7.0

Color Change – Provides yellow coloration in weakly acidic solutions and blue coloration in weakly alkaline solutions. In neutral solution provides green coloring.

Sensitivity Test – A mixture of 0.3 mL of bromothymol blue SI and 100 mL of water free from carbon dioxide presents yellow coloration. The color changes to blue by addition of not more than 0.1 mL of sodium hydroxide solution 0.02 M.

Blue hidroxinaftol

(CAS 63451-35 -4)

Formula and molecular mass – $C_{20}H_{11}N_2Na_3O_{11}S_3$ – 620,47

Blue of hidroxinaftol SI

Preparation – Dissolve 0.1 g in ethanol to 100 mL. Color *Change* – In a pH range between 12.0 and 13.0, your solution has reddish-pink color in the presence of calcium ions. Before excess edetate disodium, presents intense blue color.

Oracet Blue B

(CAS 12769-16 -3)

Formula and molecular mass – $C_{21}H_{16}N_2O_2$ – 328,36

Specification – It is comprised of a mixture of 1-methylamino phosphine oxide-4-anilinantraquinona and 1-amino-4- anilinantraquinona.

Oracet Blue B SI

Preparation – Dissolve 0.5 g of glacial acetic acid anhydrous and make up the volume to 100 mL.

Color Change – When used in titrations in a non-aqueous medium changes from blue staining (basic means) to purple (middle) and for rosa (acidic environment).

Thymol Blue

(CAS 76-61 -9)

Formula and molecular mass – $C_{27}H_{30}O_5S$ – 466,60

Description – crystalline Powder green-brownish or greenish- blue.

Solubility – Slightly soluble in water, soluble in ethanol 96% (v/v) and in dilute solutions of alkali hydroxides.

Thymol Blue SI

Preparation – Heat 0.1 g of gauge with 4.3 mL of sodium hydroxide to 0.05% (p/v) and 5 mL of ethanol at 90% (v/v). After dissolution, make up the volume to 250 mL with ethanol 20% (v/v).

PH Range – 1.2 – 2.8 and 8.0 – 9.6

Color Change – Presents red coloration in strongly acidic solutions (pH range: 1.2 – 2.8), yellow coloration in weakly acidic and alkaline solutions and blue coloration in solutions more alkaline (pH range: 8.0 – 9.6).

Sensitivity Test – A mixture of 0.1 mL of thymol blue SI, 100 mL of carbon dioxide free water and 0.2 mL of sodium hydroxide 0.02 M presents blue color. The color changes to yellow by addition of not more than 0.1 mL of 0.2 M hydrochloric acid

Nile Blue (CI 51180)

(CAS 3625-57 -8)

Formula and molecular mass – $C_{40}H_{40}N_6O_6S$ – 732,85

Description – green crystalline Powder with brightness of bronze.

Solubility – Slightly soluble in ethanol, in glacial acetic acid and pyridine.

Nile Blue A SI

Preparation – Dissolve 1 g anhydrous in glacial acetic acid to 100 mL.

PH Range – 9.0 – 13.0

Color Change – Gives blue coloration to weakly alkaline solutions and red coloring to weakly alkaline solutions.

Sensitivity Test – A mixture of 0.25 mL of Nile blue ITSELF in 50 mL of glacial acetic acid anhydrous presents blue color. The coloration becomes blue-greenish by addition of no more than 0.1 mL of 0.1 *M perchloric acid* in glacial acetic acid.

Test of identification – The solution to 0.0005% (w/v) in ethanol 50% (v/v) presents absorption maximum (2.5.14) at 640 nm.

Shimon

(CAS 2538-85 -4)

Formula and molecular mass – $C_{20}H_{13}N_2NaO_5S$ – 416,4

Description – Po pardo-black with violet hints. – Solubility Very soluble in water and readily soluble in ethanol and acetone.

Shimon SI

Preparation – Dissolve 0.1 g in 100 mL of anhydrous methanol.

Color Change – Provides red-purple with calcium ions in alkaline medium. In the presence of excess edetate disodium, the solution acquires blue color.

Shimon, mixture composed

Preparation – Mix one part of shimon with 99 parts of sodium sulphate.

Sensitivity Test – Dissolve 0.2 g of mixture composed of shimon in 5 mL of water. Mix 1 mL of the dye solution, 50 mL of water, 10 mL of sodium hydroxide *M* and 1 mL of magnesium sulphate to 1% (p/v). The solution formed is blue, making it violet by addition of 0.1 mL of calcium chloride 0.15% (p/v). The addition of 0.1 mL of 0.01 *M disodium edetate* provides intense blue color.

Metilrosanilinio chloride (CI 42555)

(CAS 548-62 -9)

Synonymy – Crystal violet

Formula and molecular mass – $C_{25}H_{30}ClN_3$ – 408,00

Description – Powder or crystals dark-green.

Solubility – Soluble in water and in ethanol 96% (v/v).

Chloride metilrosanilinio SI

Preparation – Dissolve 0.5 g in 100 mL of glacial acetic acid anhydrous.

Change of color – In titrations in a non-aqueous medium the coloration changes from violet (basic means) to greenish blue- (middle) and for green-yellowish (acidic).

Sensitivity Test – A mixture of 0.1 mL of chloride of metilrosanilinio ITSELF with 50 mL of glacial acetic acid anhydrous shows purple coloration bluish. The addition of 0.1 mL of 0.1 *M perchloric acid* in acetic acid changes the color to green.

Ferric chloride

(CAS 10025-77 -1)

Synonymy – ferric chloride.

Formula and molecular mass – $FeCl_3.6H_2O$ – 270.30

Description – Mass crystallized orange yellow, deliquescent. – Solubility Very soluble in water and soluble in ethanol and ethyl ether. The salt and their solutions, exposed to light, suffer partial reduction.

Ferric chloride ITSELF (approximately 0.4 *M)*

Specification – Contains 10.5 g in water to 100 mL. *Conservation* – In well-closed containers. *Storage* – Protect from light.

Dye BVF

Preparation – Dissolve 0.1 g of bromothymol blue, 0.02g of methyl red and 0.2 g of phenolphthalein in ethanol. Complete with the same solvent to make 100 mL. Filter.

Diphenylcarbazide

(CAS 140-22 -7)

Formula and molecular mass – $C_{13}H_{14}N_4O$ – 242,29

Description – crystalline Powder white or almost white, gradually becomes rosa with exposure to air. *Solubility*: Very slightly soluble in water, soluble in acetone and in 96% ethanol (v/v) and glacial acetic acid.

Diphenylcarbazide SI

Preparation – Dissolve 1 g of diphenylcarbazide in 100 mL of hot ethanol. Store under the light.

Diphenyl Carbazone Indicator

(CAS 538-62 -5)

Formula and molecular mass – $C_{13}H_{12}N_4O$ – 240.27

Description – Crystals or crystalline powder orange-yellow. – Solubility Practically insoluble in water and readily soluble in ethanol 96% (v/v).

Diphenyl Carbazone Indicator SI

Preparation – Dissolve 0.1 g in 100 mL of ethanol. Store under the light.

Eosin Y (CI 45380)

(CAS 17372-87 -1)

Formula and molecular mass – $C_{20}H_6Br_4Na_2O_5$ – 691,91

Description – brown Powder.

Solubility – Soluble in water.

Eosin Y SI

Preparation – Dissolve 1 g in 100 mL water.

Color Change – The addition of 20 mL of sodium hydroxide at 40% (p/v) about 10 mL of eosin Y SI to 1% (p/v) form red precipitate.

Ethyl Ester of tetrabromofenolftaleina

(CAS 1176-74 -5)

Chemical Name – acid ethyl Ester of 2- (3,5-dibromo-4-Hydroxyphenyl) (3,5-dibromo-4-oxo-2,5-cicloexadien-1-Ylidene) methyl] -benzoic acid

Synonymy – Bromofenolftaleina magenta AND

Formula and molecular mass – $C_{22}H_{14}Br_4O_4$ – 661,96

Ethyl Ester of tetrabromofenolftaleina SI

Preparation – Dissolve 0.1 g of ethyl ester of tetrabromofenolftaleina in 90 mL of glacial acetic acid and make up the volume to 100 mL with the same solvent. Prepare immediately before use.

Phenolphthalein

(CAS 77-09 -8)

Formula and molecular mass – $C_{20}H_{14}O_4$ – 318,33

Description – amorphous, crystalline Powder or white or slightly yellowish. Odourless.

Solubility Insoluble in water and soluble in ethanol

Phenolphthalein SI

Preparation – Dissolve 0.1 g in 100 mL of 80% ethanol (v/v). *PH Range* – 8.3 – 10.0

Color Change – Provides colorless solutions in acid medium and weakly alkaline. Presents violet intense in alkaline solutions stronger.

Sensitivity Test – A mixture of 0.1 mL of phenolphthalein SI in 1000 mL in water free of carbon dioxide is colorless. Are required not more than 0.2 mL of sodium hydroxide 0.02 *M* for the appearance of pink coloration.

Phenolphthalein paper

Preparation – Immerse strips of filter paper common in phenolphthalein THEMSELVES for a few minutes and to air dry at ambient temperature.

Ferroina

(CAS 14634-91 -4)

Formula and molecular mass – C36H24FeN6O4S – 692,52

Ferroina SI

Preparation – Dissolve 0.7 g of ferrous sulphate heptahydrate and 1.49 g of 1,10-phenanthroline in 70 mL of water, and make up the volume to 100 mL with the same solvent.

Sensitivity Test – 165 mL of 1 M sulfuric acid add 0.15 mL of osmium tetroxide SR and 0.1 mL of ferroina ITSELF. After the addition of 0.1 mL of 0.1 *M ammonium sulfate cerico* SV, the color changes from red-orange to pale green.

Conservation – In well-closed containers.

Magneson

(CAS 74-39 -5)

Formula and molecular mass – $C_{12}H_9N_3O_4$ – 259.22

Description – Po reddish brown.

Magneson SI

Preparation – Dissolve 0.2 g in 100 mL of toluene.

Color Change – In titrations of a non-aqueous medium changes the color orange (acidic) to blue (basic means), passing by pink coloration.

Magneson, reagent

Preparation – Solubilizing 0.1 g of magneson in 100 mL of sodium hydroxide (1% w/v).

1-Naftolbenzeina

(CAS 6948-88 -5)

Synonymy – Fenilbis (4-hidroxinaftil) methanol.

Formula and molecular mass – $C_{27}H_{20}O_3$ – 392,50

Description – Po brown-reddish.

Solubility: Insoluble in water; soluble in benzene, ethyl ether and glacial acetic acid.

1-Naftolbenzeina SI

Preparation – Dissolve 0.2 g of 1-naftolbenzeina in 100 mL of glacial acetic acid.

Color Change – When used in non- aqueous titrations, changes the color blue or bluish-green (basic means) to orange (middle) and for dark-green (acidic).

Sensitivity Test – Add 0.25 mL of a solution of 1-naftolbenzeina SI 165 mL of glacial acetic acid anhydrous. Are required not more than 0.05 mL of 0.1 *M perchloric acid* in glacial acetic acid to make a change of the color yellow-brown to green.

1-Naftolftaleina

(CAS 596-01 -0)

Formula and molecular mass – $C_{28}H_{18}O_4$ – 418,42

Description – Powder colorless when pure, is usually gray red.

Solubility – Practically insoluble in water and soluble in ethanol.

Naftolftaleina SI

Preparation – Dissolve 0.5 g in 100 mL of 96% ethanol (v/v). *Color Change* – Provides solution colorless or pale red in acid media and neutral and blue coloration in moderately alkaline solutions.

Eriochrome black T (CI 14645)

(CAS 1787-61 -7)

Formula and molecular mass – $C_{20}H_{12}N_3$not 7S – 461.38

Description – Po dark brown.

Solubility – Soluble in water and in ethanol 96% (v/v). Eriochrome black T SI

Preparation – Dissolve 0.5 g of eriochrome black-T and

G of hydroxylamine hydrochloride in methanol 330 mL. Prepare at the time of use.

Color Change – In hydrochloric acid medium produces violet-brown precipitate; treated with sulfuric acid form precipitate dark blue that, diluted, changes to brownish color. In an aqueous solution of sodium hydroxide presents violet color.

Ammonium oxalate

(CAS 6009-70 -7)

Formula and molecular mass – $C_2H_{8\ N2\ O4}.\ H_{2\ O}$- 142.11

Description – transparent colorless crystals or white crystalline powder. Odourless.

Solubility – Soluble in water.

Ammonium oxalate SI

Specification – Contains 4 g of ammonium oxalate in water to 100 mL.

Bromocresol Purple

(CAS 115-40 -2)

Formula and molecular mass – $C_{21}H_{16}Br_2O_5S$ – 540,24

Description – white crystalline Powder for rosa.

Solubility Practically insoluble in water, soluble in ethanol 96% (v/v) and dilute solutions of alkali hydroxides.

Bromocresol Purple SI

Preparation – Heat 0.1 g of bromocresol purple with 5 mL of ethanol 90% (v/v) until dissolved. Add 3.7 mL of 0.05 *M sodium hydroxide* and ethanol 20% (v/v) to complete the volume of 250 mL.

PH Range – 5.2 – 6.8

Color Change – Provides yellow coloration in weakly acidic solutions and staining violet-blue in alkaline solutions, neutral and acidic very near neutrality. *Sensitivity Test* – Mix 0.2 mL of bromocresol purple SI and 100 mL of water free of carbon dioxide. Add 0.05 mL of sodium hydroxide 0.02 *M.* This solution has the violet blue color. To change the color to yellow are required not more than 0.2 mL of hydrochloric acid 0.02 *M.*

Bromocresol Purple, reagent

Solution – Dissolve 38 g of monobasic sodium phosphate and 2 g of anhydrous dibasic sodium phosphate in water and make up to 1000 mL. Adjust the pH to 5.3. *Solution B*: Dissolve 0.4 g of bromocresol purple in 30 mL of water, add 6.3 mL of 0.1 *M sodium hydroxide* and make up the volume to 500 mL with water.

Preparation – Mix equal volumes of Solution A, *Solution B* and chloroform. Shake for 5 minutes, decant and despise the chloroform layer.

Metacresol Purple

(CAS 2303-01 -7)

Formula and molecular mass – $C_{21}H_{16}O_5S$ – 382,44

Description – crystalline Powder olive green.

Solubility Slightly soluble in water, soluble in ethanol, glacial acetic acid and methanol.

Purple of metacresol SI

Preparation – Dissolve 0.1 g in 100 mL of sodium hydroxide 0.001 M.

PH Range – 0.5 – 2.5 and 7.5 – 9.2

Color Change – Presents red coloration in solutions are strongly acidic (pH range: 0.5 – 2.5), yellow coloration in solutions less acidic and neutral and violet coloration in moderately alkaline solutions (pH range: 7.5 – 9.2).

Resazurin

(CAS 550-82 -3)

Formula and molecular mass – $C_{12}H_7NO_4$ – 229,19

Description – Crystals small dark-red with greenish lustre.

Solubility Insoluble in water and ethyl ether, slightly soluble in ethanol and soluble in dilute solutions of alkali hydroxides.

Resazurin SI

Preparation – Dissolve 0.1 g in 100 mL of sodium hydroxide solution 0.02 M. This indicator must be recently prepared.

PH Range – 5.0 – 7.0

Color Change – Provides pink coloration in weakly acidic solutions and violet coloration in weakly alkaline solutions.

Resorcinol

(CAS 108-46 -3)

Synonymy – Resorcinol.

Formula and molecular mass – $C_6H_6O_2$ – 110,11

Description – Crystals or crystalline powder colorless or pale yellow; exposed to the light and air, acquires pink coloration. *Solubility* – Soluble in water and ethanol.

Resorcinol SI

Preparation – Dissolve 0.2 g of resorcinol in 100 mL of benzene. Decant.

Thymolphthalein

(CAS 125-20 -2)

Formula and molecular mass – $C_{28}H_{30}O_4$ – 430,54

Description – white Powder or clear yellow.

Solubility Practically insoluble in water, soluble in ethanol and in solutions of alkali hydroxides.

Thymolphthalein SI

Preparation – Dissolve 0.1 g in 100 mL of 96% ethanol (v/v). *PH Range* – 9.3 – 10.5

Color Change – is colorless in acidic medium and weakly alkaline. Provides blue coloration in alkaline solutions more intense.

Sensitivity Test – A mixture of 0.05 mL of thymolphthalein ITSELF with 100 mL of water free of carbon dioxide is colorless. Are required not more than 0.05 mL of sodium hydroxide 0.1 *M* to change the color to blue.

Ammonium thiocyanate

(CAS 1762-95 -4)

Formula and molecular mass – NH_4SCN – 76,12

Description – colorless crystals and deliquescent. – Solubility Very soluble in water and soluble in ethanol.

Ammonium thiocyanate SI

Preparation – Dissolve 7.6 g in 100 mL of water.

Litmus

(CAS 1393-92 -6)

Specification – is composed of pigment indigo blue prepared from several species of *Rocella, Lecanosa* or other lichens. The pigment has characteristic odour.

SI Litmus

Preparation – Boil under reflux for one hour, 25 g of litmus test, finely pulverized, with 100 mL of ethanol at 90% (v/v). Despise the ethanol and repeat the operation twice, using in each extract 75 mL of ethanol at 90% (v/v). Treat the litmus extracted with 250 mL of water. Filter.

PH Range – 5.0 – 8.0

Color Change – Provides red coloring with the acids and blue with the alkalis.

Blue Litmus paper,

Preparation – Boil 10 parts of litmus, finely pulverized, with 100 parts of 96% ethanol (v/v), under reflux for one hour. Decant and discard the ethanol, add to the residue mixture of 45 parts of ethanol and 15 parts water. Leave 'dying' for two days. Decant the supernatant and impregnate strips of filter paper common with the extract. Dry at room temperature.

Sensitivity Test – dive strip of litmus paper blue, measuring 10 mm x 60 mm, in 100 mL of the mixture of 10 mL of 0.02 *M hydrochloric acid* and 90 mL of water. Shake. The role acquires red color at the end of 45 seconds.

Red Litmus paper,

Preparation – Add 2 M hydrochloric acid to extract obtained in the process of preparing the blue paper, drop by drop, until the solution presents red coloring. Impregnate strips of filter paper with this solution and allow to dry at room temperature.

Sensitivity Test – dive strip of litmus paper red in 100 mL of sodium hydroxide 0.002 *M.* Shake. The paper should be blue at the end of 45 seconds.

Tropeolina (CI 14270)

(CAS 547-57 -9)

Formula and molecular mass – $C_{12}H_9N_2NaO_5S$ – 316,27

Description – brown Powder.

Solubility – Soluble in water and ethanol.

Tropeolina SI

Preparation – Dissolve 25 mg in 165 mL of methanol and water to complete 100 mL

PH Range – 11.0 – 12.7

Color Change – Provides solutions to yellow coloration in the midst moderately alkaline and orange coloring in strongly alkaline solutions.

Test of homogeneity – Apply 0.01 mL of tropeolina SI in cromatoplaca cellulose G. Develop the chromatogram with. mixture *n-propyl alcohol, ethyl acetate and water (5:1:4). The chromatogram should show a single spot with Rf approximately 0.9.*

Tropeolina OO (CI 13080)

(CAS 554-73 -4)

Formula and molecular mass – $C_{18}H_{14}N_3NaO_3S$ – 375,38

Description – Po yellow or orange yellow.

Solubility – Soluble in water.

PH Range – 1.0 – 2.8

Color Change – Provides red coloration in solutions are strongly acidic and yellow coloration in solutions less acidic.

Bromocresol Green

(CAS 76-60 -8)

Formula and molecular mass – $C_{21}H_{14}Br_4O_5S$ – 698,02

Description – white Powder brownish.

Solubility Slightly soluble in water, soluble in ethanol and dilute solutions of alkali hydroxides.

Bromocresol Green SI

Preparation – Heat 0.1 g of dye with 2.9 mL of 0.05 M sodium hydroxide and 5 mL of ethanol 90% (v/v). After dissolution, add ethanol 20% (v/v) until the volume of 250mL.

PH Range – 3.6 – 5.2

Color Change – Provides yellow coloration in moderate-mind acidic solutions and blue in weakly acidic and alkaline solutions.

Sensitivity Test – A mixture of 0.2 mL of bromocresol green SI and 100 mL of water free from carbon dioxide presents blue color. Are required not more than 0.2 mL of 0.02 M hydrochloric acid to change the color to yellow.

Malachite Green oxalate

(CAS 2437-29 -8)

Formula and molecular mass – $_{48}H_{50}N_4O_4.2HC_2O_4$ – 927,10

Description – crystalline Solid green.

Solubility Very soluble in water.

Malachite Green SI

Preparation – Dissolve 1 g of calcium oxalate malachite green in 100 mL of glacial acetic acid.

PH Range – 0.0-2.0

Color Change – Provides yellow coloration in acidic solutions and green in less acidic and alkaline solutions.

Methyl Green (CI 42590)

(CAS 14855-76 -6)

Formula and molecular mass – $C_{27}H_{35}BrClN_3$ – 516,94

Description – Powder green. Normally, it presents itself in the form of salt with $ZnCl_2$.

Solubility – Soluble in water.

Green Methyl SI

Preparation – Dissolve 0.1 g in 100 mL of water.

Color Change – In sulfuric acid solution color is yellow. By diluting returns the green coloring.

Cresol Red

(CAS 1733-12 -6)

Formula and molecular mass – $C_{21}H_{18}O_5S$ – 382,43

Description – reddish brown crystalline Powder.

Solubility Slightly soluble in water, soluble in ethanol and dilute solutions of alkali hydroxides.

Cresol Red SI

Preparation – Heat 50 mg of red cresol with 2.65 mL of 0.05 M sodium hydroxide and 5 mL of ethanol at 90 %. After dissolution, add ethanol at 20% until completing 250 mL.

PH Range – 0.2 – 1.8 and 7.2 – 8.8

Color Change – Provides, red coloration in strongly acidic solutions (pH range: 0.2 – 1.8), yellow coloration in solutions less acidic and neutral; in moderately alkaline solutions presents red color (pH range: 7.2 – 8.8).

Sensitivity Test – A mixture of 0.1 mL of cresol red SI and 1000 mL of water, free from carbon dioxide, added to 715 mL of sodium hydroxide 0.02 M presents coloring red purple. The color changes to yellow by addition of not more than 0.15 mL of hydrochloric acid 0.02 M.

Red of Congo (CI 22120)

(CAS 573-58 -0)

Formula and molecular mass – $C_{32}H_{22}N_6Na_2O_6S_2$ – 696,67

Description – Dust red brownish.

Solubility – Soluble in water.

Red Congo SI

Preparation – Dissolve 0.25 g of Congo red in 50 mL of ethanol at 90% (v/v) and water until completing 250 mL.

PH Range – 3.0 – 5.0

Color Change – Presents blue coloration in moderately acidic solutions and red coloration in weakly acidic and alkaline solutions.

Sensitivity Test – A mixture of 0.2 mL of red of Congo ITSELF, 100 mL of carbon dioxide free water and 0.3 mL of 0.1 M hydrochloric acid has blue coloration. Are required not more than 0.3 mL of sodium hydroxide 0.1 M to change the color to pink.

Red of Congo, paper

Preparation – Dip strips of filter paper common in red of Congo ITSELF and allow to dry at room temperature.

Phenol Red

(CAS 143-74 -8)

Formula and molecular mass – $C_{19}H_{14}O_5S$ – 354,38

Description – crystalline Powder red light or dark. Solubility: Very slightly soluble in water and slightly soluble in ethanol.

Phenol Red SI

Preparation – Heat 0.1 g of phenol red with 1.42 mL of 0.2 M sodium hydroxide and 5 mL of ethanol 90% (v/v). After dissolution, add ethanol 20% (v/v) to complete 250 mL.

PH Range – 6.8-8.4 -6.6 trade

Change of pH – Provides yellow coloration in the middle and red in weakly alkaline solution.

Sensitivity Test – A mixture of 0.1 mL of phenol red SI and 100 mL of carbon dioxide free water color is yellow. Are required not more than 0.1 mL of sodium hydroxide 0.02 M to change the color to reddish-violet.

Methyl Red (CI 13020)

(CAS 493-52 -7)

Formula and molecular mass – $C_{15}H_{15}N_3O_2$ – 269,30

Description – violet crystals or powder dark red. – Solubility Practically insoluble in water and soluble in ethanol.

Methyl Red SI

Preparation – Heat 0.1 g of methyl red with 1.85 mL of 0.2 M sodium hydroxide and 5 mL of ethanol 90% (v/v). After dissolution, make up the volume to 250 mL with ethanol 50% (v/v).

PH Range – 3.0 – 4.4

Color Change – Provides red coloration in weakly acidic solutions and yellow coloration in solutions very weakly acidic and alkaline.

Sensitivity Test – A mixture of 0.1 mL of methyl red SI, 100 mL of water free of carbon dioxide and 0.05 mL of 0.02 M hydrochloric acid presents red color. Are required not more than 0.1 mL of sodium hydroxide 0.02 M to change the color to yellow.

Using quinaldine Red

(CAS 117-92 -0)

Formula and molecular mass – $C_{21}H_{23}IN_2$ – 430,33

Description – Po dark blue.

Solubility Slightly soluble in water and readily soluble in ethanol.

Using quinaldine Red SI

Preparation – Dissolve 0.1 g in 100 mL of methanol. Color Change – change Occurs carmine staining for almost colorless. Used in titrations of bases with perchloric acid.

14.2 REAGENTS AND REAGENT SOLUTIONS

Reagents are substances used, either as such or as constituents of solutions, in carrying out the tests pharmacopoeial assays.

Acetal

(CAS 105-57 -7)

Formula and molecular mass – $C_6H_{14}O_2$ – 118,17

Description – colorless Liquid, limpid and volatile. Physical Characteristics – Density (20 ºC): approximately 0.824. Index of refraction (20 ºC): approximately 1.382. Boiling Temperature: around 103 ºC.

Miscibility – Miscible in water and in ethanol.

Acetaldehyde

(CAS 75-07 -0)

Synonymy – Ethanal.

Formula and molecular mass – C_2H_4O – 44.05

Description – colorless, clear Liquid.

Physical Characteristics – Density (20 ºC): approximately 0.788. Index of refraction (20 ºC): approximately 1.332. Boiling Temperature: about 21 ºC.

Miscibility – Miscible in water and in ethanol.

Safety – Flammable!

Acetanilide

(CAS 103-84 -4)

Chemical Name – N-Fenilacetamida

Formula and molecular mass – C_8H_9NO – 135,17

Description – white crystalline Powder, odourless.

Physical Characteristic – Range of fusion: 114 ºC to 116 ºC. – Solubility Slightly soluble in water, readily soluble in chloroform and ethanol, soluble in boiling water, ethyl ether and glycerin.

Conservation – In closed containers.

Ammonium Acetate

(CAS 631-61 -8)

Formula and molecular mass – C_2H_7NO 2 – 77.08

Specification – Contains, at a minimum, 98.0% (p/p).

Description – colorless crystals, very deliquescent, weak odor acetic acid.

Solubility Very soluble in water and ethanol. Conservation – In well-closed containers. Storage – Protect from moisture.

Ammonium Acetate SR

Specification – Contains 15 g of ammonium acetate in water to 100 mL.

Conservation – In well-closed containers.

Stability – Prepare for immediate use.

Bornila Acetate

(CAS 5655-61 -8)

Formula and molecular mass – $C_{12}H_{20}O_2$ – 196,29

Description – colorless crystals or colorless liquid.

Physical Characteristic – melting Temperature: about 28 ºC.

Solubility: Very slightly soluble in water and soluble in ethanol.

Butyl Acetate

(CAS 123-86 -4)

Formula and molecular mass – $C_6H_{12}O_2$ – 116,16

Description – colorless Liquid and flammable with sweetish odor of fruit.

Physical Characteristics – Density (20 ºC): approximately 0.88. Index of refraction (20 ºC): approximately 1.395. Boiling Range: 125 ºC to 126 ºC.

Miscibility – Slightly soluble in water. Miscible with ethanol and ethyl ether.

Conservation – In closed containers.

Cellulose Acetate

(CAS 9004-35 -7)

Specification – partially acetylated Cellulose, with varying degrees of acetylation.

Description – white amorphous Solid.

Category – Adsorbent in thin layer chromatography.

Lead Acetate, tri-hydrate

(CAS 6080-56 -4)

Synonymy – Acetate of lead (II) tri-hydrate.

Formula and molecular mass – $C_4H_6PbO_4.3H_20$ – 379,33

Specification – Contains, at a minimum, 99.0% (p/p).

Description – transparent, colorless crystals or white crystalline powder, odourless acetic acid weak. Efflorescent. Physical Characteristics – melting Temperature: 75 ºC (fast heating); it breaks down completely to 200 ºC. – Solubility Easily soluble in water and soluble in ethanol.

Conservation – In airtight containers.

Security – Toxic. Pollutant.

Lead Acetate, paper

Preparation – Impregnate appropriate role (usually in size 6 mm x 80 mm) with lead acetate solution SR. Dry the paper reagent at 100 ºC, avoiding contact with metal.

Conservation – In well-closed containers. Storage – Protect from light and moisture.

Lead Acetate SR (approximately 0.25 M)

Specification – Contains 9.5 g of lead acetate in 100 mL of water free of carbon dioxide. Conservation – In well-closed containers.

Security – Toxic. Pollutant.

Lead Acetate, saturated solution

Specification – Contains approximately 35 g of lead acetate in 50 mL of water free of carbon dioxide. Conservation – In well-closed containers.

Security – Toxic. Pollutant.

Chlorhexidine Acetate

(CAS 56-95 -1)

Formula and molecular mass – $C_{26}H_{38}Cl_2N_{10}O_4$ – 625,58

Description – Crystals or white crystalline powder to pale cream; odorless.

Physical Characteristic – Range of fusion: 154 ºC to 155 ºC. Conservation – In well-closed containers. Storage – Protect from light.

Security – Irritant.

Category – Bactericides.

The chlorhexidine Acetate 0.1% (p/v)

Specification – Contains 0.1 g of chlorhexidine acetate in 100 mL of water.

Conservation – In well-closed containers.

Security – Toxic.

Category – Bactericides.

Copper Acetate

(CAS 142-71 -2)

Formula and molecular mass – $C_4H_6CuO_4.H_2O$ – 199,65

Description – Powder or crystals bluish-green.

Solubility Easily soluble in boiling water, soluble in water and in ethanol slightly soluble in glycerol.

Cortisone Acetate

(CAS 50-04 -4)

Formula and molecular mass – $C_{23}H_{30}O_6$ – 402,49

Specification – Contains, at a minimum, 96.0% (p/p), calculated on the dried substance.

Description – weakly yellowish colorless crystals or white crystalline powder or almost white. Odourless tasteless; initially, then bitter.

Physical Characteristics – melting Temperature: approximately 240 ºC. Optical Rotation: + 209º to + 219º (determined in a solution of 1.0% (w/v) in dioxana). Conservation – In well-closed containers.

Storage – Protect from light.

Category – Steroids.

Cortisone Acetate, injectable

Description – It Consists of a suspension in aqueous medium suitable, with pH between 5.0 and 7.0.

Specification – Contains, at a minimum, 90.0% (p/p). Conservation – In ampoules of single dose.

Desoxicortona Acetate

(CAS 56-47 -3)

Synonymy – Acetate desoxicorticosterona.

Formula and molecular mass – $C_{23}H_{32}O_4$ – 372,50

Specification – Contains, at a minimum, 96.0% (p/p), calculated on the dried substance.

Description – colorless crystals or white crystalline powder. Odourless.

Physical Characteristics – Range of fusion: 157 ºC to 161 ºC. Optical Rotation: +171º to +179º (determined in a solution of 1.0% (w/v) in dioxana).

Conservation – In well-closed containers. Storage – Protect from light.

Category – Steroids.

Ethyl Acetate

(CAS 141-78 -6)

Formula and molecular mass – $C_4H_8O_2$ – 88,11

Specification – Contains, at a minimum, 99.9% (p/v)

Description – Liquid clear, colorless, volatile, characteristic odour.

Physical Characteristics – Density: approximately 0.90. Boiling Temperature: approximately 77 ºC. Index of refraction (20 ºC): 1.371 to 1.373.

Conservation – In well-closed containers.

Storage – Protect from heat.

Safety – Flammable.

Phenylmercury Acetate

(CAS 62-38 -4)

Formula and molecular mass – $C_8H_8HgO_2$ – 336,74

Specification – Contains, at a minimum, 98.0% (p/p).

Description – small crystals or white crystalline powder or cream color, gloss.

Physical Characteristic – Range of fusion: 149 ºC to 153 ºC. Conservation – In well-closed containers. Storage – Protect from light.

Security – Toxic. Pollutant.

Acetate of indophenol SR

Synonymy – 2.6-Diclorofenolindofenol in sodium acetate buffer.

Preparation – Dilute 12 mL of the standard solution of 2,6-dichlorophenol-indophenol sodium in 100 mL of water. TO this solution add 100 mL of acetate buffer pH 7.0. Conservation – In well-closed containers.

Stability – Use in more than two weeks. Storage – Keep under refrigeration.

Magnesium Acetate

(CAS 16674-78 -5)

Formula and molecular mass – $C_4H_6MgO_4.4H_2O$ – 214,45

Description – colorless crystals and deliquescent. – Solubility Easily soluble in water and in ethanol. Conservation – In well-closed containers.

Menthyl Acetate

(CAS 2623-23 -6)

Formula and molecular mass – $C_{12}H_{22}O_2$ – 198,30

Description – colorless Liquid.

Physical Characteristics – Density (20 ºC): approximately 0.92. Index of refraction (20 ºC): approximately 1.447. Boiling Temperature: about 228 ºC.

Miscibility – Slightly soluble in water and miscible with ethanol.

Mercury Acetate

(CAS 1600-27 -7)

Synonymy – mercuric Acetate.

Formula and molecular mass – $C_4H_6HgO_4$ – 318,68–318.68 Description – Crystals or white crystalline powder, or almost white, with weak odor acetic acid.

Physical Characteristic – Range of fusion: 178 ºC to 180 ºC (overheating results in decomposition). Conservation – In well-closed containers Storage – Protect from light.

Security – Toxic.

Mercury Acetate SR

Preparation – Dissolve 6 g of acetate of mercury in glacial acetic acid to 100 mL.

Conservation – In closed containers.

Storage – Protect from direct sunlight.

Methyl Acetate

(CAS 79-20 -9)

Formula and molecular mass – $C_3H_6O_2$ – 74,07

Description – colorless, clear Liquid.

Physical Characteristics – Density (20 ºC): approximately 0.933. Index of refraction (20 ºC): approximately 1.361. Boiling Range: 56 ºC to 58 ºC.

Miscibility – Soluble in water and miscible with ethanol.

Potassium Acetate

(CAS 127-08 -2)

Formula and molecular mass – $C_2H_3KO_2$ – 98,14

Specification – Contains, at a minimum, 99.0% (p/p), calculated on the dried substance.

Description – colorless crystals or white crystalline powder, odourless or weak acetic acid odor. Deliquescent. Physical Characteristic – melting Temperature: 292ºC. Conservation – In well-closed containers.

Potassium Acetate SR

Specification – Contains 10 g of potassium acetate in 100 mL of water.

Conservation – In well-closed containers.

Prednisolone Acetate

(CAS 52-21 -1)

Formula and molecular mass – $C_{23}H_{30}O_6$ – 402,49

Specification – Contains, at a minimum, 96.0% (p/p) calculated on the dried substance.

Description – white crystalline Powder or almost white. Odourless. Bitter.

Physical Characteristics – melting Temperature: approximately 247 ºC. Optical Rotation: +112° to + 119º (determined in solution at 1.0% (w/v) in dioxana). Conservation – In well-closed containers.

Category – Steroids.

Sodium Acetate

(CAS 6131-90 -4)

Formula and molecular mass – $C_2H_3NaO_2.3H_2O$ – 136,08 (if anhydrous – 82,03)

Specification – Contains, at a minimum, 99.0% (p/p). Description – colorless crystals or white crystalline powder, odourless or weak acetic acid odor. Efflorescent. Conservation – In well-closed containers.

Sodium Acetate SR (approximately 0.02 M)

Specification – Contains 0.272 g of sodium acetate tri-hydrated in water to 100 mL.

Conservation – In well-closed containers.

Uranyl Acetate

(CAS 6159-44 -0)

Formula and molecular mass – $C_4H_6O_6U.2H_2O$ – 424,15

Description – yellow, crystalline Powder odor acetic acid weak. Conservation – In well-closed containers.

Security – radioactive Substance.

Uranyl Acetate and zinc SR

Preparation – Dissolve 10 g of uranyl acetate in 50 mL of hot water and 5 mL of glacial acetic acid 30% (p/v). Dissolve 30 g of zinc acetate in 30 mL of water hot and 3 mL of glacial acetic acid 30% (p/v). Mix the previous preparations. Let cool. Filter.

Conservation – In well-closed containers. Storage – Protect from light.

Security – radioactive Substance.

Zinc Acetate

(CAS 5970-45 -6)

Formula and molecular mass – $C_4H_6O_4Zn.2H_2O$ – 219,50

Specification – Contains, at a minimum, 98.0% (p/p).

Description – colorless or white crystals or crystalline flakes or granules, odor weak acetic acid, metallic taste astringent. Efflorescent.

Physical Characteristic – melting Temperature: 237ºC. – Solubility Easily soluble in water and soluble in ethanol.

Conservation – In well-closed containers.

Security – Irritant.

Acetylacetone

(CAS 123-54 -6)

Formula and molecular mass – $C_5H_8.2$ – 100.11

Description – clear Liquid, colorless or yellowish, aromatic odour.

Physical Characteristics – boiling Temperature: approximately 139 ºC. Density: approximately 0.97. Index of refraction (20 ºC): 1.4505 to 1.4525.

Miscibility – Miscible in acetone and ethanol.

Conservation – In well-closed containers.

Security – Irritant. Flammable.

Acetone

(CAS 67-64 -1)

Formula and molecular mass – C_3H_60 – 58,08

Specification – Contains, at a minimum, 98.0% (p/v). Description – Liquid clear, colorless, volatile, characteristic odour.

Physical Characteristics – Density: 0.790 to 0.793. Index of refraction (20 ºC): 1.358 to 1.360. Boiling Temperature: approximately 56 ºC.

Conservation – In airtight containers.

Safety – Flammable. Irritating and toxic.

Acetone dehydrated

Specification – Acetone, dried on anhydrous sodium sulphate.

Conservation – Prepare at the time of use.

Acetone buffered SR

Preparation – Dissolve 8.15 g of sodium acetate tri-hydrated and 42 g of sodium chloride in water, add 68 mL of 0.1 M hydrochloric acid and 150 mL of acetone. Fill up to volume with water to 500 mL.

Acetonitrile

(CAS 75-05 -8)

Formula and molecular mass – C_2H_3N – 41,05

Description – Liquid clear and colorless. Odour similar to ether.

Physical Characteristics – Density (20ºC): approximately 0.78. Index of Refraction (20 ºC): approximately 1.344.

Miscibility – Miscible in water, acetone and methanol. Conservation – In airtight containers.

Security – Toxic. Flammable!

Acetic acid M

Specification – Contains 6 g of glacial acetic acid in water to 100 mL.

Conservation – In airtight containers.

Additional Information – To use, confirm the title.

Acetic acid 6 M

Specification – Contains 348 g of glacial acetic acid in water to 1000 mL.

Conservation – In well-closed containers. Storage – Protect from heat.

Safety – Corrosive and flammable.

Dilute acetic acid

Specification – Contains 12 g of glacial acetic acid in water to 100 mL.

Conservation – In airtight containers.

Acetic acid SR

Specification – Contains 30 g of glacial acetic acid in water to 100 mL. Corresponds to acetic acid 5 M. Description – clear, colorless Liquid, pungent odor. Conservation – In airtight containers.

Glacial acetic acid

(CAS 64-19 -7)

Formula and molecular mass – $C_2H_4O_2$ – 60,05

Specification – Contains, at a minimum, 98.0% (p/p).

Description – Liquid clear, colorless, volatile, pungent odor and characteristic. Glacial at low temperatures. Physical Characteristics – Density: approx.

Boiling Temperature: approximately 118 ºC. Freezing Temperature: approximately 14 ºC. Conservation – In airtight containers.

Safety – Corrosive. Flammable. Protect eyes, skin and mucous membranes.

Acid 7-aminodesacetoxicefalospornico

(CAS 22252-43 -3)

Synonymy – 7-ADCA')

Formula and molecular mass – $C_8H_{10}N_2O_3S$ – 214,24

Ascorbic Acid

(CAS 50-81 -7)

Formula and molecular mass – $C_6H_8O_6$ – 176,13

Specification – Contains, at a minimum, 99.0% (p/p).

Description – colorless crystals or white crystalline powder. Odourless.

Physical Characteristics – 5% Solution (w/v) has a pH of 2.2 to 2.5. Melting Temperature: approximately 190 ºC with decomposition. Optical Rotation specifies: between +20,5° and +21,5 °, determine in aqueous solution at 1% (p/v). Conservation – In well-closed containers, non metallic.

Storage – Protect from light.

Benzoic acid

(CAS 65-85 -0)

Formula and molecular mass – $C_7H_6O_2$ – 122,12

Specification – Contains, at a minimum, 99.0% (p/p).

Description – colorless crystals or white crystalline powder, characteristic odour.

Physical Characteristic – melting Temperature: approximately 122 ºC.

Solubility Slightly soluble in water, soluble in boiling water and readily soluble in ethanol.

Conservation – In well-closed containers.

Boric Acid

(CAS 10043-35 -3)

Formula and molecular mass – H_3BO_3 – 61.83

Specification – Contains, at a minimum, 99.5% (p/p). Description – brilliant colorless crystals or white crystalline powder fine, unctuous to the touch, taste weakly acid and bitter.

Solubility – Soluble in water and in ethanol, readily soluble in boiling water.

Conservation – In well-closed containers.

Boric Acid, saturated solution Preparation – Dissolve 5 g in 100 mL of water. Conservation – In well-closed containers.

Acid hydrobromic acid

(CAS 10035-10 -6)

Formula and molecular mass – HBr – 80.91 Specification – Contains 48.0% (p/v).

Description – Liquid colorless or faintly yellow, strong odor and irritant. Darkens slowly by exposure to air and light.

Conservation – In well-closed containers. Storage – Protect the air and light.

Security – Irritant. Corrosive.

Caffeic Acid

(CAS 331-39 -5)

Formula and molecular mass – $C_9H_8O_4$ – 180,16

Description – white crystals or almost white. Physical Characteristic – melting Temperature: approximately 225 °C with decomposition.

Solubility Easily soluble in hot water and ethanol, slightly soluble in cold water.

Acid calconcarboxilico

(CAS 3737-95 -9)

Formula and molecular mass – $C_{21}H_{14}N_2O_7S$ – 438,40

Description – Po brown-black.

Solubility Slightly soluble in water, very slightly soluble in acetone and ethanol, slightly soluble in dilute solutions of sodium hydroxide.

Conservation – In well-closed containers.

Acid ciclobutano-1,1-dicarboxylic acid

(CAS 5445-51 -2)

Formula and molecular mass – $C_6H_{10}O_4$ – 144,13

Description – white crystals.

Physical Characteristic – melting Temperature: about 160 ºC. Conservation – In closed containers.

Acid1,2-cicloexileno-pyridazinone-dicmiaocyclohexane tetra

(CAS 125572-95 -4)

Synonymy – Acid 1,2-cicloexileno-diamine-dicmiaocyclohexane tetra, CDTA.

Formula and molecular mass – $C_{14}H_{22}N_2O_8.H_2O$ – 364,35

Description – white Powder.

Conservation – well-closed Containers, protected from heat.

Security – Irritant.

Methoxycinnamic Acid

(CAS 140-10 -3)

Formula and molecular mass – C_9H_8O – 148.16

Description – colorless crystals.

Physical Characteristic – melting Temperature: 133ºC.

Solubility: Very slightly soluble in water and readily soluble in ethanol.

Citric acid, monohydrate

(CAS 5949-29 -1)

Formula and molecular mass – $C_6H_8O_7.H_2O$ – 210,14

Description – Crystals or granules, colorless or white crystalline powder or almost white. Efflorescent.

Solubility Very soluble in water and readily soluble in ethanol.

Conservation – In well-closed containers.

Hydrochloric acid

(CAS 7647-01 -0)

Synonymy – hydrogen chloride and concentrated hydrochloric acid.

Formula and molecular mass – HCl – 36.46 Specification – Contains, at a minimum, 35.0% (p/p) consisting of gaseous HCl solution in water.

Description – clear, colorless Liquid, steamy, pungent odor.

Physical Characteristics – Density: approximately 1.18.

Conservation – In airtight containers, inert to the reagent.

Storage – Protect from heat (keep in temperatures less than 20 ºC).

Safety – Corrosive. Avoid external contact, eyes and skin, inhalation and ingestion.

Hydrochloric acid brominated SR

Preparation – Add 1 mL of bromine SR in 100 mL of hydrochloric acid.

Conservation – In well-closed containers.

Dilute hydrochloric acid

Specification – Use hydrochloric acid SR.

Hydrochloric acid M

Specification – Contains 103 g of hydrochloric acid in 1000 mL of water.

Conservation – In well-closed containers.

Stability – Protect from heat.

Safety – Corrosive.

Additional Information – To use, confirm the title.

Hydrochloric acid SR

Specification – Contains 27.4 g of concentrated hydrochloric acid in 100 mL of water.

Physical Characteristics – Density: approx. 1.05.

Conservation – In well-closed containers.

Stability – Protect from heat.

Safety – Corrosive.

0.01 M hydrochloric acid did not produce any signals

Preparation – Transfer 0.85 mL of hydrochloric acid to 1000 mL volumetric flask and Fill up to volume with methanol.

Hydrochloric acid-tin SR

Preparation – Mix 1 mL of stannous chloride SR1 with 100 mL of hydrochloric acid.

Acid chlorogenic acid

(CAS 327-97 -9)

Formula and molecular mass – $C_{16}H_{18}O_9$ – 354,34

Description – Needles or crystalline powder white or nearly white.

Physical Characteristic – melting Temperature: around 208 ºC. – Solubility Easily soluble in boiling water, in acetone and ethanol.

Acid cloroplatinico

(CAS 18497-13 -7)

Synonymy – platinico chloride, platinum chloride, acid cloroplatinico (IV).

Formula and molecular mass – $H_2PtCl_6.6H_2O$ – 517,90

Specification – Contains, at a minimum, 37.0% (p/p) of platinum.

Description – Earth-yellow crystalline samples presented brown color, very deliquescent.

Physical Characteristics – Density: 2.431. Melting Temperature: 60 ºC.

Solubility Easily soluble in water and soluble in ethanol.

Conservation – In closed containers.

Storage – Protect from light.

Security – Toxic.

Chromic Acid

Where appear, use chromium trioxide (CrO_3).

Acid 3,5-dinitrobenzoic acid ethylester

(CAS 99-34 -3)

Formula and molecular mass – $C_7H_4N_2O_6$ – 212,12

Description – almost colorless crystals.

Physical Characteristic – melting Temperature: around 206 ºC.

Acid edetico

(CAS 60-00 -4)

Synonymy – ethylenediaminetetraacetic acid, EDTA.

Formula and molecular mass – $C_{10}H_{16}N_{2\ O8}$ – 292.24 Specification – Contains, at a minimum, 98.0% (p/p). Description – colorless crystals.

Physical Characteristic – It breaks down around 220ºC, and may descarboxilar to 150 ºC.

Conservation – In well-closed containers.

Acid fenoldissulfonico SR

(CAS 96-77 -5)

Formula and molecular mass – $C_6H_6O_7S_2$ – 254,24

Description – clear Liquid to light brown.

Preparation – Dissolve 2.5 g of phenol in 15.0 mL of sulfuric acid. Add 7.5 mL of dilute sulfuric acid steaming. Warm up to 100 ºC for two hours. Transfer the product fluid to suitable container. For use, liquidise in water bath.

Conservation – glass Container with lid flipped. Security – Irritant and corrosive.

Phenoxyacetic acid, Acid

(CAS 122-59 -8)

Formula and molecular mass – $C_8H_8O_3$ – 152,15

Description – almost white crystals.

Physical Characteristic – melting Temperature: about 98 ºC.

Solubility Slightly soluble in water and readily soluble in ethanol and glacial acetic acid.

Hydrofluoric acid

(CAS 7664-39 -3)

Formula and molecular mass – HF – 20.01 Specification – Contains, at least, 40% (p/p) of HF. Description – colorless, clear Liquid.

Conservation – In polyethylene containers well closed.

Formic acid

(CAS 64-18 -6)

Synonymy – Acid Methanoic acid.

Formula and molecular mass – CH_2O_2 – 46,03

Specification – The anhydrous form contains, at a minimum, 98.0% (p/p). The commercial contains around 90.0% (p/p). Description – colorless Liquid, highly caustic, pungent odor.

Physical Characteristics – boiling Temperature: 100.5 ºC. Density: approximately 1.22. Index of refraction (20 ºC): 1.3714. Solidifies at 70 ºC.

Conservation – In well-closed containers.

Safety – Caustic.

Acid phosphomolybdic acid

(CAS 51429-74 -4)

Synonymy – Acid molybdophosphoric acid reagent.

Molecular Formula – Approximately $2MoO_3.H_3PO_4.xH_2O$.

Description – weakly yellowish crystals.

Conservation – In well-closed containers.

Acid phosphomolybdic acid SR

Preparation – Dissolve 4 g of phosphomolybdic acid in 40 mL of water under heating. After cooling add 60 mL of sulfuric acid.

Phosphoric acid

(CAS 7664-38 -2)

Synonymy – orthophosphoric acid

Formula and molecular mass – H_3PO_4 – 98,00

Specification – Contains, at a minimum, 85.0% (p/p). Description – Liquid clear, colorless, odourless. In Hygroscopic Equilibrium; a syrupy consistency.

Physical Characteristic – Density: approximately 1.7. Conservation – In airtight containers.

Safety – Corrosive! Avoid contact with skin, mucous membranes.

Phosphoric acid SR

Preparation – Mix quantity corresponding to 15 g of concentrated phosphoric acid with water to 100 mL. Physical Characteristic – Density: approximately 1.15.

Acid phosphotungstic acid SR

Preparation – Heat under reflux for 3 hours, the mixture of 10 g of sodium tungstate with 8 mL of phosphoric acid and 75 mL of water. Let cool and dilute to 100 mL with water.

Phthalic acid

(CAS 88-99 -3)

Formula and molecular mass – $C_8H_6O_4$ – 166,14

Description – white crystalline Powder or almost white. Solubility – Soluble in hot water and ethanol.

Gallic acid

(CAS 5995-86 -8)

Formula and molecular mass – $C_7H_6O_5.H_2O$ – 188,14

Description – long Needles or crystalline powder colorless or light yellow.

Physical Characteristics – Loses water of crystallisation temperature of 120 ºC and melts at about 206 ºC with decomposition.

Solubility – Soluble in water, readily soluble in hot water, ethanol and glycerol.

Hydroxybenzoic Acid

(CAS 99-96 -7)

Formula and molecular mass – $C_7H_6O_3$ – 138,13

Description – colorless crystals.

Physical Characteristic – melting Range: 213-214 ºC. Conservation – In well-closed containers.

Acid hipofosforoso

(CAS 6303-21 -5)

Synonymy – hipofosforoso Acid diluted.

Formula and molecular mass – H_3PO_2 – 66,00

Specification – Contains, at least, 48% (w/v) of H_3PO_2.

Description – Liquid colorless or slightly yellow. Miscibility – Miscible in water and ethanol.

Hydriodic acid Acid

(CAS 10034-85 -2)

Formula and molecular mass – HI – 127.91

Description – aqueous Solution of hydriodic acid. When freshly prepared, is colorless, but with exposure to air and light, presents color yellowish to brown.

Conservation – In well-closed containers.

Storage – Protect the light and contact with the air. Keep in temperatures less than 30ºC.

LACTIC acid

(CAS 50-21 -5)

Synonymy – Acid 2-hydroxypropanoic acid

Formula and molecular mass – $C_3H_6O_3$ – 90,08

Specification – Mixture of 2-hydroxypropanoic acid and its condensation products. The balance between the lactic acid and the acid polilaticos is dependent upon the concentration and temperature. The lactic acid is normally a crystallized (R,S-lactic acid).

Description – viscous Liquid colorless or slightly yellow. Miscibility – Miscible in water and in ethanol. Conservation – In closed containers.

Metaphosphoric Acid

(CAS 10343-62 -1)

Formula and molecular mass – $(HPO_3)n$, monomer – 79.98.

Specification – Contains a certain proportion of sodium metaphosphate.

Description – Solid mass or vitreous, colorless. Hygroscopic. In aqueous solution, it turns slowly in phosphoric acid (H_3PO_4).

Physical Characteristic – Volatilization under intensive heat. Conservation – In airtight containers.

Metaphosphoric Acid-acetic acid SR

Specification – Contains 3 g of metaphosphoric acid and 8 mL of glacial acetic acid in water to 100 mL.

Conservation – In well-closed containers.

Stability – Limited to two days.

Storage – Keep under refrigeration.

Methanesulfonic Acid

(CAS 75-75 -2)

Formula and molecular mass – CH_4O_3S – 96,11

Description – Liquid clear and colorless (solidifies at 20 ºC). Physical Characteristics – Density (20 ºC): approximately 1.48. Index of refraction: approximately 1.430. Melting Temperature: 20 ºC.

Miscibility – Miscible in water, little miscible in toluene and practically immiscible in hexane.

Conservation – In well-closed containers.

Safety – Annoying!

Nitric Acid

(CAS 7697-37 -2)

Formula and molecular mass – HNO_3 – 63.01

Specification – Contains, at a minimum, 63.0% (p/p). Description – clear Solution, almost colorless, characteristic odour.

Physical Characteristic – Density: 1.384 to 1.416.

Conservation – In airtight containers, under the light.

Safety – Corrosive.

Nitric Acid steaming

Specification – Contains, at a minimum, 95.0% (p/p). Description – clear Liquid, slightly yellowish, steaming in the air.

Nitric Acid SR

Specification – Contains about 12.5% (w/v) of HNO_3. Physical Characteristic – Density: approximately 1.5.

Oxalic acid

(CAS 6153-56 -6)

Synonymy – Acid etanodioico.

Formula and molecular mass – $C_2H_2O_4.2H_2O$ – 126,07

Specification – Contains, at least, 99% (p/p).

Description – colorless crystals or white crystalline powder. Physical Characteristic – melting Temperature: approximately 101 ºC.

Safety – Poison!

Oxalic acid SR

Specification – Solution of oxalic acid to 6.3% (p/v).

Perchloric acid

(CAS 7601-90 -3)

Formula and molecular mass – $HClO_4$ – 100,46

Specification – Contains, at a minimum, 70.0% (p/p) and, at most, 72.0% of HClO.

Description – Liquid clear, colorless, volatile and pungent odor. Hygroscopic.

Physical Characteristic – Density: approximately 1.7.

Conservation – Decomposes spontaneously and can explode especially in contact with oxidisable substances.

Security – Irritant. Corrosive!

Perchloric acid M

Specification – Contains 8.5 mL of HClO 4 in water, totaling 100 mL.

Stability – Use solution freshly prepared.

Perchloric acid SR

Use perchloric acid M.

Performic Acid

(CAS 107-32 -4)

Synonymy – Acid peroxiformico.

Formula and molecular mass – CH_2O_3 – 62,03

Preparation – Mix 1 mL of hydrogen peroxide to 30.0% (v/v), or 9.0% (p/p), with 90 mL of formic acid. Conservation – Prepare at the time of use. Storage – Protect from heat.

Security – Irritant. You can explode in contact with metals and their oxides, reducing substances, or in distillation.

Periodic Acid

(CAS 10450-60 -9)

Formula and molecular mass – H_5IO_6 – 227,94

Description – white crystals to colorless.

Physical Characteristics – melting Temperature: 122 degrees C. Decomposes between 130 ºC and 140ºC, forming $_2O_5$, H_2O e O_2.. – Solubility Easily soluble in water and soluble in ethanol.

Picric Acid

(CAS 88-89 -1)

Synonymy – 2,4,6-Trinitrophenol.

Formula and molecular mass – $C_6H_3N_3O_7$ – 229,10

Specification – yellow crystals or plates moistened with water.

Conservation – In well-closed containers, mixed with an equal weight of water.

Storage – At ambient temperature.

Safety – Explodes when heated rapidly or subjected to shock. For safe transport, 10% to 20% of water are usually added.

Picric Acid SR

Preparation – Add 0.25 mL of 10 M sodium hydroxide in 100 mL of saturated solution of picric acid in water.

Picric Acid SR1

Preparation – Dissolve the equivalent to 1 g of picric acid in 100 mL of hot water. Cool and filter, if necessary.

Acid rosmarinico

(CAS 20283-92 -5)

Formula and molecular mass – $C_{18}H_{16}O_8$ – 360,31

Description – Dust red orange.

Physical Characteristic – Range of fusion: 170 ºC to 174 ºC.

Salicylic acid

(CAS 69-72 -7)

Synonymy – 2-hydroxybenzoic Acid.

Formula and molecular mass – $C_7H_6O_3$ – 138,12

Specification – Contains, at a minimum, 99.0% (p/p), calculated on dry matter basis.

Description – white crystalline Powder or colorless crystalline needles. Odourless and acid taste sweet and annoying.

Physical Characteristic – melting Range: 156-160 ºC.

Solubility Slightly soluble in water, readily soluble in ethanol 96% (v/v), slightly soluble in methylene chloride.

Conservation – In well-closed containers.

Acid selenioso

(CAS 7783-00 -8)

Formula and molecular mass – H_2SeO_3 – 128,97

Specification – Contains, at least, 93% (p/p) of H_2SeO_3.

Description – white crystals or colorless. Efflorescent to dry air and hygroscopic to moist air.

Solubility – Soluble in water and in ethanol.

Conservation – In well-closed containers.

Sulphamic Acid

(CAS 5329-14 -6)

Formula and molecular mass – H_3NO_3S – 97,09

Synonymy – Acid amidossulfonico.

Specification – white crystals or crystalline powder. Physical Characteristic – melting Temperature: around 205 °C with decomposition.

Solubility Easily soluble in water, slightly soluble in acetone, ethanol and methanol.

Conservation – In well-closed containers of amber glass.

Safety – Moderately irritating to skin and mucous membranes.

Sulphanilic acid

(CAS 6101-32 -2)

Synonymy – Acid 4-aminobenzenossulfonico.

Formula and molecular mass – C6H7NO3S.H2O – 191,20 - Anhydrous – 173.84.

Specification – Contains, at a minimum, 99.0% (p/p).

Description – colorless crystals or white powder. Physical Characteristic – The acid monohydrate decomposes without fusing to approximately 288 ºC.

Solubility Slightly soluble in water, practically insoluble in ethanol.

Sulphanilic acid diazotized and coupled with 2-aminoethyl-1SR

Preparation – Dissolve, carefully, 0.2 g of sulfanilic acid in 20 mL of dilute hydrochloric acid M, cool in an ice bath and add, drop by drop, with continuous stirring, 2.2 mL of sodium nitrite solution at 4% (p/v). Leave on ice for 10 minutes and add 1 mL of sulphamic acid solution at 5% (p/v).

Sulphanilic acid SR

Preparation – Dissolve 0.5 g of sulfanilic acid finely pulverized, in water. Add 6 mL of hydrochloric acid M. Make up to 100 mL with water.

Sulfuric acid

(CAS 7664-93 -9)

Formula and molecular mass – H_2SO_4 – 98,07

Specification – Contains at least 95.0% (p/p).

Description – colorless Liquid, caustic, oily consistency, very hygroscopic.

Physical Characteristic – Density: 1.834 to 1.839. Conservation – In well-closed containers.

Security – Irritant. Corrosive!

Dilute sulfuric acid

Use sulfuric acid SR.

Free sulfuric Acid nitrogen

Specification – Meets the following test: in 5 mL of water, add, carefully, 45 mL of sulfuric acid, wait until it cools to 40 ºC and add 8 mg of difenilbenzidina. The resulting solution is slightly pink or a pale blue.

Sulfuric acid did not produce any signals 0.1 M

Preparation – Dilute 5.4 mL of sulfuric acid with 20 mL of methanol. Make up to 1000 mL with the same solvent.

Additional Information – Prepare 24 hours before use.

Sulfuric acid/methanol SR

Preparation – slowly Add 10 mL of dilute sulfuric acid in 90 mL of methanol.

Note – Keep the system cool.

Sulfuric acid did not produce any signals SR

Preparation – To 30 mL of anhydrous methanol cooled in an ice bath, carefully add sulfuric acid in small quantities, under agitation. Cool to room temperature and add to 100 mL with sulfuric acid. Mix Well.

Sulfuric acid, ethanolic solution

Preparation – Carefully and with constant cooling, add 20 mL of dilute sulfuric acid in 60 mL of ethanol. Continue the cooling and dilute to 100 mL with ethanol. Prepare immediately before use.

Sulfuric acid SR

Specification – Contains sulfuric acid diluted to 10% (p/v) in water.

Preparation – Add, carefully, 57 mL of sulfuric acid in 100 mL of water, cool and dilute to 1000 mL with water.

Conservation – In well-closed containers.

Sulfurous Acid

(CAS 7782-99 -2)

Formula and molecular mass – H_2SO_3 – 82,07

Specification – Contains 5.0 to 6.0% (p/p) of sulfur dioxide pure. Prepare according to consumption. Description – Liquid acid, clear, colorless, suffocating odor of sulfur dioxide. The air oxidizes gradually to sulfuric acid.

Conservation – In containers almost full, well-sealed, in a cool place.

Tartaric acid

(CAS 87-69 -4)

Synonymy – Acid L- (+) -tartaric acid.

Formula and molecular mass – $C_4H_6O_6$ – 150,09

Description – Crystals or white crystalline powder. Physical Characteristics – Range of fusion: 168 ºC to 170 ºC. Density (20 ºC): 1.756.

Solubility Very soluble in water and readily soluble in ethanol.

Conservation – In well-closed containers.

Thioglycollic acid

(CAS 68-11 -1)

Synonymy – Acid Mercaptoacetic acid.

Formula and molecular mass – $C_2H_4.2S$ – 92.11 Specification – Contains, at a minimum, 79.0% (p/p).

Description – Liquid colorless or near colorless, strong unpleasant odor.

Physical Characteristic – Density: approximately 1.33. Miscibility – Miscible in water and in ethanol. Conservation – Protect the air.

Safety – Can cause severe skin burns. Additional Information – Its decomposition frees hydrosulphuric gas.

Acid-toluenossulfonico

(CAS 6192-52 -5)

Formula and molecular mass – $C_2H_4O_2S$ – 92,11

Specification – Contains, at least, 87% of C_7H_83S.

Description – Crystals or white crystalline powder or almost white.

Solubility Easily soluble in water and soluble in ethanol.

Trichloroacetic Acid

(CAS 76-03 -9)

Formula and molecular mass – $C_2HC1_3O_2$ – 163,39

Specification – Contains, at a minimum, 98.0% (p/p).

Description – colorless crystals or crystalline mass, deliquescent, characteristic odour weakly pungent, irritating.

Physical Characteristic – melting Range: 55 to 61 ºC. Conservation – In airtight containers.

Storage – Protect from heat and moisture. Security – very corrosive Acid.

Trichloroacetic Acid-chloramine-T SR

Solution – Chloramine-T to 3% (p/v).

Solution B – trichloroacetic Acid at 25% (v/v) in absolute ethanol.

Preparation – Mix 10 mL of the Solution with 40 mL of Solution B.

Acid Trifluoroacetic acid

(CAS 76-05 -1)

Synonymy – Acid trifluoroacetic acid.

Formula and molecular mass – $C_2HF_3O_2$ – 114,02

Description – colorless Liquid, volatile, pungent odor and characteristic.

Physical Characteristics – boiling Temperature: 72.4 ºC. Density: 1.535.

Miscibility – Miscible in acetone, benzene, ethanol, ethyl ether, hexane and carbon tetrachloride.

Conservation – In well-closed containers.

Safety – Corrosive. Flammable. Protect eyes, skin and mucous membranes.

Acrylamide

(CAS 79-06 -1)

Synonymy – 2-Propenamide.

Formula and molecular mass – C_3H5NO – 71.08 Specification – Quality suitable for electrophoresis.

Description – white crystalline Powder, or almost white, or colorless or white scales.

Physical Characteristic – melting Temperature: about 84 ºC. – Solubility Very soluble in water and methanol, easily soluble in ethanol.

Conservation – In well-closed containers.

Safety – Highly toxic and irritating. Cause paralysis of the central nervous system. Can be absorbed through the skin.

Acrylamide/Bisacrylamide (29:1) to 30% (p/v) Mr.

Preparation – Prepare a solution containing 290 g of acrylamide and 10 g of methylenebisacrylamide per 1000 mL of hot water. Filter.

Conservation – In well-closed containers.

Agar

(CAS 9002-18 -0)

Synonymy – Agar-agar, agar.

Specification – Polysaccharide extracted from Gelidium cartilagineum (L) Gaillon (Gelidiaceae), Gracilaria confervoides (L) Greville (Sphaerococcaceae) and red algae affines (Rhodophyceae).

Description – fine Dust, colorless or slightly yellowish, dry, hydrophilic.

Conservation – In airtight containers.

Agarose gel

(CAS 9012-36 -6)

Specification – linear Polysaccharide, neutral component of agar.

Description – white or almost white Powder.

Solubility Practically insoluble in cold water and very slightly soluble in hot water.

Use – Electrophoresis.

DEAE-Agarose for ion exchange chromatography

Specification – Agarose containing reticulated dietilaminoetilo groupings. It Presents in the form of spheres.

Bromine Water SR

Preparation – Mix 3 mL of bromine with 100 mL of water until saturation. Shake before use. After decanting, use the supernatant solution clear.

Conservation – In airtight containers.

Storage – Keep with excess bromine and under the light.

Security – Toxic.

Water chlorine SR

Specification – saturated Solution of chlorine in water. Conservation – In well-closed containers. Storage – Protect from light and air. Keep in a cool place and dark.

Water free of carbon dioxide

Specification – Water boiled vigorously for 5 minutes or more and protected from the atmosphere, during cooling, and conservation.

Conservation – Protect the air (absorption of CO2).

Water free of ammonia

Preparation – Transfer 0.1 mL of sulfuric acid 96% (p/p) to 100 mL of distilled water and employing equipment with walls free of ammonia.

Water free of nitrate

Preparation – Transfer 5 mg of potassium permanganate and 5 mg of barium hydroxide to 100 mL of distilled water and employing equipment with walls free of nitrate.

Free Water of particles

Specification – Water obtained by filtration through a membrane with a porosity of 0.22 ^m.

Bovine Albumin

(CAS 9048-46 -8)

Synonymy – serum Albumin of bovine origin.

Description – white Powder or light yellowish brown.

Specification – Contains, at least, 96% of proteins. Water (5.2.20.3) – Determine in 0.8 g of the sample. No more than 30 %.

Storage – At temperatures between 2 C and 8 º C.

Human Albumin

Synonymy – human serum Albumin.

Specification – Contains, at least, 96% of albumin.

Human Albumin solution, reagent

Preparation – Dilute human albumin solution with 15% to 25% (p/v) solution of sodium chloride 0.9% (p/v) up to a concentration of 0,1% (w/v) in proteins. Adjust the pH to 3.5-4.5 with glacial acetic acid.

Isoamyl Alcohol

(CAS 123-51 -3)

Synonymy – 3-Metilbutan-1-ol.

Formula and molecular mass – $C_5H_{12}O$ – 88,15

Description – colorless Liquid.

Physical Characteristic – boiling Temperature: about 130 ºC.

Miscibility – Slightly soluble in water and miscible with ethanol.

Undenatured iso-Butyl alcohol

(CAS 78-83 -1)

Synonymy – 2--propanol, 2-methyl-1-propanol, isobutanol.

Formula and molecular mass – $C_4H_{10}O$ – 74,12

Description – colorless, clear Liquid.

Physical Characteristics – Density (20 ºC): approximately 0.80. Index of refraction (15 ºC): 1.397 to 1.399. Boiling Temperature: around 107 ºC.

Conservation – In well-closed containers.

Safety – Flammable.

Isopropyl Alcohol

(CAS 67-63 -0)

Synonymy – Isopropanol, 2-propanol.

Formula and molecular mass – C_3H_8O – 60,10

Specification – Contains, at a minimum, 99.0 %.

Description – colorless Liquid, characteristic odor. Physical Characteristics – boiling Temperature: approximately 82 ºC. Density: approximately 0.785. Index of refraction (20 ºC): 1.376 to 1.378.

Miscibility – Miscible in water and in ethanol. Conservation – In well-closed containers.

Safety – Flammable.

W-amyl Alcohol

(CAS 71-41 -0)

Synonymy – 1-Pentanol, pentan-1-ol

Formula and molecular mass – $C_5H_{12}O$ – 88,15

Description – colorless Liquid.

Physical Characteristics – Index of refraction (20 ºC): approximately 1.41. Boiling Temperature: approximately 137 ºC. Melting Temperature: approximately -79 ºC.

Miscibility – Slightly soluble in water and miscible with ethanol.

Conservation – In well-closed containers Safety – Annoying!

W-propyl Alcohol

(CAS 71-23 -8)

Synonymy – 1-Propanol, 2-propanol.

Formula and molecular mass – C_3H_8O – 60.10

Description – clear, colorless Liquid, weak odor alcoholic.

Physical Characteristics – boiling Temperature: approximately 97 ºC Density: 0.803 to 0.805. Miscibility – Miscible with water and ethanol. Conservation – In well-closed containers.

Safety – Flammable.

Polyvinyl Alcohol

(CAS 9002-89 -5)

Molecular Formula – $(C_2H_4O)_n$

Description – white Powder.

Solubility – Soluble in water and insoluble in organic solvents.

Tert-amyl Alcohol

(CAS 75-85 -4)

Synonymy – 2-Methyl-2-butanol.

Formula and molecular mass – $C_5H_{12}O$ – 88,15

Description – Liquid clear and colorless. Volatile. Physical Characteristics – relative Density (20 ºC): approximately 0.81. Melting Temperature: approximately -8 °C. Boiling Temperature: 102ºC.

Miscibility – Easily miscible in water. Miscible with ethanol and glycerol.

Conservation – In well-closed containers. Storage – Protect from light.

Safety – Flammable.

Tert-butyl Alcohol

(CAS 75-65 -0)

Synonymy – 2-Methyl-2-propanol.

Formula and molecular mass – $C_4H_{10}O$ – 74,12

Description – colorless, clear Liquid, or crystalline mass of odor CMCP.

Physical Characteristics – Density (25 ºC): 0.778 to 0.782. Melting Temperature: 25.7 ºC. Boiling Temperature: °C to 83.5 ºC.

Miscibility – Soluble in water and miscible with ethanol and ethyl ether.

Aluminum, metallic

(CAS 7429-90 -5)

Element and atomic mass – Al – 26.98

Description – Metal white or almost white to bluish, malleable, flexible. Available in barra, dust, strips or wires.

Aluminon

(CAS 569-58 -4)

Formula and molecular mass – $C_{22}H_{23}N_3O_9$ – 473,43

Description – reddish brown crystals.

Solubility Easily soluble in water.

Amaranth (CI 16185)

(CAS 915-67 -3)

Formula and molecular mass – $C_{20}H_{11}N_2Na_3O_{10}S_3$ – 604,06

Description – fine Powder, easily soluble in water, practically insoluble in ethanol, acetone, ethyl ether and chloroform.

Conservation – In well-closed containers.

Starch iodetado SR

Use starch iodetado ITSELF.

Starch iodetado SR1

Preparation – Dissolve 0.75 g of potassium iodide in 100 mL of water. Heat to boiling and add, stirring constantly, a solution containing 0.5 g of soluble starch in 35 mL of water. Boil for 2 minutes and cool.

Starch iodide-free SR

Use starch iodide-free THEMSELVES.

Soluble Starch

Synonymy – Amilodextrina, amilogenio.

Description – white, thin Powder, odorless, tasteless. Conservation – In well-closed containers. Storage – Protect from moisture.

Starch SR

Use starch ITSELF.

Starches

Description – Extracted from mature caryopsis of Zea mays L., Triticum aestivum L. or Oryza sativa L. (Jobless households Graminae). white, thin Powder, odourless and tasteless that produces slight crackling when compressed. Conservation – In well-closed containers. Storage – Protect from moisture.

Additional Information – The labelling should indicate the botanical origin.

4-Aminoantipyrine

(CAS 83-07 -8)

Synonymy – one Formula and molecular mass – $C_{11}H_{13}N_3O$ – 203,24

Description – Crystals or crystalline powder, yellow-clear. Physical Characteristic – melting Temperature: approximately 109 ºC.

Conservation – In well-closed containers.

Aminobutanol

(CAS 96-20 -8)

Chemical Name – 2-Amino-1-butanol

Formula and molecular mass – $C_4H_{11}NO$ – 89,14

Description – oily Liquid.

Physical Characteristic – boiling Temperature: around 180 ºC.

Miscibility – Miscible with water, soluble in alcohol.

2-Aminoeptano

(CAS 123-82 -0)

Synonymy – 2-Heptanamina; 2-heptylamine;

Metilexanamina

Formula and molecular mass – $C_7H_{17}N$ – 115,22

Description – volatile Fluid.

Physical Characteristic – boiling Temperature: around 143 ºC.

Miscibility – Little miscible in water, easily miscible in chloroform, ethanol and ethyl ether.

4-Aminofenol

(CAS 123-30 -8)

Formula and molecular mass – C_6H_7NO – 109,13

Description – white crystalline Powder or a little color due to exposure to air and light.

Physical Characteristic – melting Temperature: approximately 186 °C with decomposition.

Solubility Slightly soluble in water and soluble in ethanol.

Conservation – In closed containers.

Storage – Protect from light.

Aminopyridine

(CAS 504-29 -0)

Synonymy --Aminopyridine, 2-piridinamina.

Description – large crystals or flyers.

Physical Characteristic – melting Temperature: around 58 ºC.

Ammonia SR

Description – Contains 37.5 mL of concentrated ammonium hydroxide solution in 100 mL of aqueous solution. This contains, at least, 10% (p/v) of ammonium hydroxide (approximately 6 M).

Ammonia 6 M

Use ammonia SR.

Ammonia 10 M

Preparation – Dilute 56 mL of ammonia to 100 mL with water.

Ammonia, concentrated solution

Synonymy – ammonium hydroxide.

Formula and molecular mass – NH_3 – 17.03

Specification – Contains, at a minimum, 28.0% (p/p) and, at most, 30.0% (p/p).

Description – clear, colorless Liquid, characteristic odor and suffocating.

Conservation – In airtight containers, not completely filled.

Storage – Protect the air and light.

Safety – Caustic.

Anethole

(CAS 4180-23 -8)

Synonymy – trans-Anethole.

Description – Earth crystalline white or nearly white in temperature between 20ºC and 21 ºC, liquid at temperatures above 23 ºC.

Physical Characteristics – Index of refraction (25 ºC): approximately 1.56. Boiling Temperature: about 230 ºC. – Solubility Practically insoluble in water, soluble in ethanol, and easily soluble in ethyl acetate and petroleum ether.

Acetic anhydride

(CAS 108-24 -7)

Formula and molecular mass –$C_4H_6O_3$ – 102,09

Specification – Contains, at a minimum, 97.0% (p/p).

Description – colorless, mobile Liquid odour acetic acid intense and irritating.

Physical Characteristics – Density: approximately 1.075. Boiling Range: 136 to 142 ºC.

Conservation – In airtight containers.

Safety – Easily combustible. Strong Irritant.

Acetic anhydride-pyridine SR

Synonymy – Mixing acetic anhydride-pyridine SR

Description – Mix cautiously, and under cooling, 25 g (or 23 mL) of acetic anhydride in 50 mL of anhydrous pyridine.

Conservation – In airtight containers.

Storage – Protect the air and light.

Stability – Prepare at the time of use.

Security – Toxic.

Phthalic anhydride

(CAS 85-44 -9)

Formula and molecular mass – $C_8H_4O_3$ – 148,12

Description – white flakes or almost white. *Physical Characteristic* – Range of fusion: 130 ºC to 132 ºC. – Solubility Slightly soluble in water and soluble in ethanol. *Conservation* – In closed containers.

Propionic Anhydride

(CAS 123-62 -6)

Formula and molecular mass – $C_6H_{10}O_3$ – 130,14

Description – colorless Liquid of pungent smell. *Physical Characteristics* – Density: 1.01. Boiling Temperature: around 167 ºC.

Solubility – Soluble in ethanol.

Anisaldeido

(CAS 123-11 -5)

Synonymy – Aldehyde anisico and p-methoxybenzaldehyde.
Formula and molecular mass – $C_8H_8O_2$ – 136,14

Description – oily Liquid, colorless and yellowish, aromatic odour.

Physical Characteristics – Density: approx.

Boiling Temperature: approximately 248 ºC. Miscibility – Slightly soluble in water and miscible with ethanol.

Conservation – In well-closed containers. *Storage* – Protect from light.

Anisaldeido, solution

Preparation – Mix, in order, 0.5 mL of anisaldeido, 10 mL of glacial acetic acid, 85 mL of methanol and 5 mL of sulfuric acid.

Anisaldeido SR

Preparation – The 10 mL of anisaldeido add 90 mL of ethanol, mix, add 10 mL of sulfuric acid and mix.

Anisaldeido SR1

Preparation – Mix 25 mL of glacial acetic acid with 25 mL of ethanol, add 0.5 mL of anisaldeido and 1 mL of sulfuric acid.

Antithrombin III

(CAS 90170-80 -2)

Specification – The antithrombin III is purified from human plasma by chromatography on agarose-heparin and must have specific activity of at least 6 IU/mg.

Antithrombin III SR

Preparation – Reconstitute the antithrombin III according to the manufacturer's instructions and dilute with buffer tris- sodium chloride, pH 7.5, to obtain solution to 1 IU/mL.

Aprotinin

(CAS 9087-70 -1)

Description – almost white Powder.

Solubility – Soluble in water and isotonic solutions, practically insoluble in organic solvents.

Asiaticosideo

(CAS 16830-15 -2)

Formula and molecular mass – $C_{48}H_{78}O_{19}$ – 958,51

Description – white or almost white Powder. Hygroscopic. *Physical Characteristic* – melting Temperature: approximately 232 °C with decomposition.

Solubility – Soluble in methanol, slightly soluble in ethanol and insoluble in acetonitrile.

Asparagine

(CAS 5794-13 -8)

Formula and molecular mass – $C_4H_8N_2O_3.H_2O$ – 150,13

Description – colorless, odourless crystals.

Physical Characteristics – isomer L: melting Temperature: 234-235 ºC. Isomer D: melting Temperature: 215ºC. – Solubility Slightly soluble in water, practically insoluble in ethanol and in methylene chloride.

Sodium azide

(CAS 26628-22 -8)

Formula and molecular mass – – NaN_3 – 65,01

Description – Crystals or white crystalline powder or almost white.

Solubility Easily soluble in water and slightly soluble in ethanol.

Acid Blue 83

(CAS 6104-59 -2)

Synonymy – brilliant Blue.

Formula and molecular mass – $C_{45}H_{44}N_3NaO_7S_2$ – 825,99.

Description – Po brown.

Solubility Insoluble in cold water, slightly soluble in boiling water and ethanol, soluble in sulfuric acid and glacial acetic acid, soluble in dilute solutions of hydroxides of alkali metals.

Acid Blue 90

(CAS 6104-58 -1)

Formula and molecular mass – $C_{47}H_{48}N_3NaO_7S_2$ – 854,04

Description – Po dark brown, with reflexes violaceous and particles with metallic reflections.

Solubility – Soluble in water and ethanol.

Astra Blue

(CAS 82864-57 -1)

Formula and molecular mass – $C_{47}H_{52}CuN_{14}O_6S_3$ – 1068,75

Coomassie Blue SR

Preparation – Prepare a solution of acid blue 83 to 0.125% (p/v) in a mixture of glacial acetic acid, methanol and water (1:4:5) and filter.

Blue sulfano (CI 42045)

(CAS 129-17 -9)

Synonymy – Blue glacial I.

Formula and molecular mass – $C_{27}H_{31}N_2NaO_6S_2$ – 566,68

Description – Po violet. In dilute solutions, presents blue coloration. After the addition of concentrated hydrochloric acid, there is no change in color to yellow.

Solubility – Soluble in water.

Blue tetrazolium

(CAS 1871-22 -3)

Formula and molecular mass – $C_{40}H_{32}N_8O_2Cl_2$ – 727,65

Description – yellow crystals.

Physical Characteristic – melting Temperature: around 245 °C with decomposition.

Solubility Slightly soluble in water, readily soluble in chloroform, ethanol and methanol, insoluble in acetone and ethyl ether.

Canada Balsam

(CAS 8007-47 -4)

Description – oily Liquid yellow or greenish, extracted from *Abies balsames* L., Pinaceae. With pleasant aroma of pine. If exposed to air, will solidify gradually in non-crystalline mass.

Physical Characteristics – Density: 0.987 to 0.994. Index of refraction: 1.53.

Miscibility – Miscible in water, benzene, chloroform and xylene.

Additional Information – Used to attach blades to microscope.

Barbaloina

(CAS 1415-73 -2)

Synonymy – Add Aloin.

Description – yellow Needles or crystalline powder yellow to dark yellow. Darkens with exposure to air and light. – Solubility Slightly soluble in water and in ethanol, soluble in acetone, in ammonia and hydroxy- alkaline solutions.

Barbitol Buffer

(CAS 57-44 -3)

Formula and molecular mass – $C_8H_{12}N_2O_3$ – 184,19

Specification – Contains, at a minimum, 99.0% (p/p), calculated in relation to the substance dried.

Description – colorless crystals or white crystalline powder, odourless, weakly bitter taste.

Physical Characteristic – melting Temperature: approximately 190 ºC.

Solubility Slightly soluble in water, soluble in boiling water and ethanol.

Nedocromil sodium Barbitol Buffer

(CAS 144-02 -5)

Formula and molecular mass – $C_8H_{11}N_2NaO_3$ – 206,18

Specification – Contains, at a minimum, 99.0% (p/p), calculated in relation to the substance dried.

Description – colorless crystals or white crystalline powder, odourless, bitter taste and weakly caustic. – Solubility Easily soluble in water and slightly soluble in ethanol.

Conservation – In well-closed containers.

Barium Ms. – 1 mg/mL

Specification – Contains 1.775 g of barium chloride in water to 1000 mL

Conservation – In well-closed containers, inert (polyethylene type).

Benzene

(CAS 71-34 -2)

Synonymy – Benzol.

Formula and molecular mass – C_6H_6 – 78,11

Description – Liquid clear, colorless, volatile, refractive, characteristic odour.

Physical Characteristics – boiling Range: 79 ºC to 81 ºC. Density: 0.878 to 0.880. Index of refraction: 1.5016. *Miscibility* – Practically insoluble in water and miscible with ethanol.

Conservation – In well-closed containers. *Storage* – Protect from heat.

Safety – Highly flammable. Carcinogenic. *Additional Information* – Whenever possible using toluene.

Benzenossulfonamida

(CAS 98-10 -2)

Formula and molecular mass – $C_6H_5SO_2NH_2$ – 157,19

Description – white crystals or beige-pale. *Physical Characteristic* – Range of fusion: 150 ºC to 153 ºC.

Benzyl

(CAS 134-81 -6)

Chemical Name – Difeniletanodiona

Formula and molecular mass – $C_{14}H_{10}O_2$ – 210,23

Description – yellow crystalline Powder.

Physical Characteristic – melting Temperature: around 95 ºC.

Solubility Practically insoluble in water and soluble in ethanol, ethyl acetate and toluene.

Benzyl benzoate

(CAS 120-51 -4)

Description – oily Liquid, transparent and colorless. By cooling, form colorless crystals.

Physical Characteristics – freezing Temperature: approximately 17 ºC. Boiling Temperature: approximately 324 ºC. *Miscibility* – Practically insoluble in water and glycerol, miscible with ethanol, ethyl ether and chloroform.

Sodium benzoate colesterila

(CAS 604-32 -0)

Formula and molecular mass – $C_{34}H_{50}O_2$ – 490,76

Description – white Solid.

Solubility Insoluble in water.

Methyl benzoate

(CAS 93-58 -3)

Formula and molecular mass – $C_8H_8O_2$ – 136,15

Description – colorless Liquid.

Physical Characteristics – Density (20 ºC): 1.088. Boiling Temperature: about 200 ºC.

Benzophenone

(CAS 119-61 -9)

Formula and molecular mass – $C_{13}H_{10}O$ – 182,22

Description – white crystalline Powder.

Characteristics – melting Temperature: around 48ºC.

Solubility Practically insoluble in water, readily soluble in ethanol.

Benzoin

(CAS 119-53 -9)

Chemical Name – 2-Hydroxy-1,2-difeniletanona

Formula and molecular mass – $C_{14}H_{12}O_2$ – 212,25

Description – slightly yellow crystals.

Solubility: Very slightly soluble and water, readily soluble in acetone, soluble in ethanol heated

Sodium bicarbonate

(CAS 144-55 -8)

Synonymy – acid sodium carbonate, sodium hydrogencarbonate.

Formula and molecular mass – $NaHCO_3$ – 84,01

Specification – Contains, at a minimum, 99.0% and at most 101,0% (p/p), calculated on a dry basis.

Description – white crystalline Powder, odourless, salty flavor and weakly alkaline. By heating, it transforms into sodium carbonate.

Solubility – Soluble in water, practically insoluble and ethanol.

Disodium Bicinconinato

(CAS 979-88 -4)

Formula and molecular mass – $C_{20}H_{10}N_2Na_2O_4$ – 388,28

Potassium Biftalato

(CAS 877-24 -7)

Synonymy – potassium Hydrogen phthalate, potassium hydrogen phthalate, diftalato of potassium.

Formula and molecular mass – $C_8H_5KO_4$ – 204,22

Specification – Contains, at a minimum, 99.9% and, at most, 100.3% (p/p), calculated in relation to the substance dried at 120 ºC for two hours.

Description – colorless crystals or white crystalline powder. *Solubility* – Soluble in water and slightly soluble in ethanol.

Conservation – In well-closed containers.

Potassium Biftalato 0.05 *M*

Preparation – Dissolve 10.21 g in water to 1000 mL *Conservation* – In well-closed containers.

Potassium Bisulphate

(CAS 7646-93 -7)

Synonymy – potassium hydrogen sulphate; acid sulphate of potash.

Formula and molecular mass – $KHSO_4$ – 136,16

Specification – Contains, at a minimum, 98.0% (p/p), calculated on the dried substance.

Description – colorless crystals or white grease; hygroscopic.

Physical Characteristics – aqueous Solution with character strongly acidic. Melting Temperature of 197 ºC. – Solubility Easily soluble in water, resulting in a very acidic solution.

Conservation – In well-closed containers.

Sodium Bisulfite

(CAS 7681-38 -1)

Synonymy – acid sulphate sodium hydrogen sulphate, sodium, sodium pirossulfato.

Formula and molecular mass – $NaHSO_4$ – 120,06

Physical Characteristic – melting Temperature: around 315 ºC.

Solubility Easily soluble in water, very soluble in boiling water. Decomposes in ethanol, forming sodium sulphate and sulfuric acid free.

Sodium Bisulphite

(CAS 7631-90 -5)

Synonymy – Hidrogenossulfito sodium, acid sulphite of sodium.

Formula and molecular mass – $NaHSO_3$ – 104,06

Description – white crystalline Powder or almost white. Exposure to air, can cause loss of sulfur dioxide and the substance is gradually oxidized to sulfate. – Solubility Easily soluble in water and slightly soluble in ethanol.

Sodium Bitartrate

(CAS 6131-98 -2)

Synonymy – Acid sodium tartrate.

Formula and molecular mass – $C_4H_5NaO_6.H_2O$ – 190,08

Description – Crystals or white crystalline powder.

Solubility – Soluble in water.

Sodium Bitartrate SR

Preparation – Dissolve 1 g of sodium bitartrate in water and make up the volume to 10 mL. Prepare the solution for immediate use.

Biuret

(CAS 108-19 -0)

Formula and molecular mass – $C_2H_5N_3O_2$ – 103,08

Description – white crystals, or nearly white. Hygroscopic.

Physical Characteristic – Range of fusion: 188 ºC to 190ºC with decomposition.

Solubility – Soluble in water, slightly soluble in ethanol, very little soluble in ethyl ether.

Conservation – In closed container.

Biuret reagent,

Preparation – Dissolve 1.5 g of cupric sulfate penta-hydrated and 6 g of potassium sodium tartrate in 500 mL of water. Add 300 mL of sodium hydroxide solution to 10% (p/v) free of carbonate, complete 1000 mL with the same solution and mix.

Boldina

(CAS 476-70 -0)

Formula and molecular mass – $C_{19}H_{21}NO_4$ – 327,37 – Description white crystalline Powder, or nearly white. *Physical Characteristic* – melting Temperature: around 163 ºC.

Solubility: Very slightly soluble in water, soluble in ethanol and diluted in acidic solutions.

Conservation – In closed containers.

Borneol

(CAS 507-70 -0)

Formula and molecular mass – $C_{10}H_{18}O$ – 154,25

Description – colorless crystals, sublime quickly. *Physical Characteristic* – melting Temperature: around 208 ºC. – Solubility Practically insoluble in water, readily soluble in ethanol and in petroleum ether.

Potassium Bromate

(CAS 7758-01 -2)

Formula and molecular mass – $KBrO_3$ – 167.00

Description – Crystals or powder granular white or nearly white.

Solubility – Soluble in water and slightly soluble in ethanol.

Bromelain

(CAS 37189-34 -7)

Specification – Concentrate of proteolytic enzymes derived from *Ananas comosus* Merr.

Description – yellowish Powder.

Bromelain SR

Preparation – Solubilize 1 g of bromelain in 100 mL of a mixture of phosphate buffer pH 5.5 and solution of sodium chloride 0.9% (p/v) (1:9).

Hemibody bromide

(CAS 518-67 -2)

Formula and molecular mass – $C_{20}H_{18}BrN_3$ – 380,28

Description – red Crystal-dark.

Solubility Slightly soluble in water at 20ºC, slightly soluble in water at 60 ºC and in ethanol.

Hemibody bromide-blue sulfano SR

Preparation – Dissolve separately, 0.5 g of metameres bromide and 0.25 g of blue sulfano in 30 mL of a mixture of hot water and ethanol (1:9) (v/v) and shake. Mix the two solutions, and make up to 250 mL with the same mixture of solvents. Mix 20 mL of this solution with 20 mL of a solution of sulfuric acid at 14 % (v/v), previously diluted with about 250 mL of water and dilute to 500 mL with water.

Conservation – In closed containers.

Storage – Protect from exposure to light.

Hexadimetrina bromide

(CAS 28728-55 -4)

Molecular Formula – $(C_{13}H_{30}Br_2N_2)_n$

Description – white Powder, or nearly white. Hygroscopic. Amorphous Polymer.

Solubility – Soluble in water.

Conservation – In closed containers.

Iodine bromide

(CAS 7789-33 -5)

Formula and molecular mass – IBr – 206,81

Description – Crystals dark brown or dark blue. *Physical Characteristics* – boiling Temperature: approximately 116 ºC. Melting Temperature: around 40 ºC.

Solubility Easily soluble in water, ethanol and glacial acetic acid.

Conservation – In closed containers.

Storage – Protect from light.

Iodine bromide SR

Preparation – Dissolve 13.2 g of iodine in glacial acetic acid to 1000 mL. Determine the content of iodine in 20 mL of this solution, upon titration with sodium thiosulphate 0,1 *M* SV. The rest of the iodine solution (980 mL), add amount of bromine equivalent to iodine determined.

Conservation – In glass containers as well closed. *Storage* – Protect from light.

Potassium bromide

(CAS 7758-02 -3)

Formula and molecular mass – KBr – 119.00 *Specification* – Contains, at a minimum, 98.0% (p/p), calculated in relation to the substance dried.

Description – colorless crystals or white crystalline powder, markedly salty flavor.

Solubility Easily soluble in water and glycerol, slightly soluble in ethanol.

Conservation – In well-closed containers.

Tetrabutylammonium bromide

(CAS 1643-19 -2)

Formula and molecular mass – $C_{16}H_{36}BrN$ – 322,37

Description – white crystalline Powder.

Physical Characteristic – Range of fusion: between 103 ºC and 105ºC.

Tetraeptilamonio bromide

(CAS 4368-51 -8)

Formula and molecular mass – $C_{28}H_{60}BrN$ – 490,69

Description – white Powder, squamous cell carcinoma.

Physical Characteristic – Range of fusion: between 89 ºC and 91 ºC.

Mercuric bromide

(CAS 7789-47 -1)

Synonymy – bromide of mercury (II)

Formula and molecular mass – Br_2Hg – 360.39

Description – white crystals or crystalline powder, sensitive to light. *Physical Characteristics* – melting Temperature: around 237 ºC.

Solubility Slightly soluble in water and soluble in ethanol. Conservation – In well-closed containers. *Storage* – Protect from light.

Safety – Poison!

Bromine

(CAS 7726-95 -6)

Formula and molecular mass – Br_2 – 159.80

Description – red-brown Liquid, irritant, suffocating

And steamy.

Physical Characteristic – Density (20 ºC): approximately 3.1. *Miscibility* – Slightly soluble in water and soluble in ethanol. *Conservation* – In airtight containers or ampoules.

Security – Toxic.

Bromine 0.2 *M* acetic acid glacial

Preparation – Add 15 g of potassium bromide and 5.5 mL of bromine in glacial acetic acid to 1000 mL. Stir and leave

to rest for 24 hours. Holder before use. *Conservation* – In airtight containers.

Storage – Protect from heat.

Security – Toxic.

Bromine SR

Preparation – Dissolve 30 g of bromine and 30 g of potassium bromide in sufficient quantity of water to make 100 mL.

1-Butanol

(CAS 71-36 -3)

Synonymy – normal butyl Alcohol or primary, n-butanol, *n-butyl alcohol.*

Formula and molecular mass – $C_4H_{10}O$ – 74,12

Description – clear, colorless Liquid, retrativo, characteristic odour.

Physical Characteristics – boiling Range: 117 ºC to 118 ºC. Density (20 ºC): 0.810. Index of refraction (20 ºC): 1.3993.

Conservation – In well-closed containers.

Security – Irritant. Flammable.

Sodium Butanossulfonato

(CAS 2386-54 -1)

Formula and molecular mass – $C_3H_9NaO_3S$ – 160,17– Description white crystalline Powder or almost white. *Physical Characteristic* – melting Temperature: greater than 300ºC.

Solubility – Soluble in water.

Butyl hidroxianisol

(CAS 25013-16 -5)

Synonymy – BHA.

Formula and molecular mass – $C_{11}H_{16}O_2$ – 180,24

Specification – a mixture of two isomers: 2-tert-butyl-4-hidroxianisol and 3-tert-butyl-4-hidroxianisol

Description – Solid waxy appearance.

Physical Characteristic – Range of fusion: 48ºC to 55 ºC. – Solubility Practically insoluble in water and soluble in petroleum ether.

Conservation – In closed containers.

Butilamina

(CAS 109-73 -9)

Synonymy – n-Butilamina

Formula and molecular mass – $C_4H_{11}N$ – 73,14

Description – colorless Liquid, ammoniacal odor. *Physical Characteristic* – boiling Temperature: around 78 ºC.

Miscibility – Miscible in water and ethanol.

Additional Information – Distil and use in, at most, 30 days.

Butylparaben

(CAS 94-26 -8)

Formula and molecular mass – $C_{11}H_{14}O_3$ – 194,23

Description – white crystalline Powder.

Physical Characteristic – Range of fusion: 68 ºC to 69 ºC.

Solubility: Very slightly soluble in water and readily soluble in acetone, ethyl ether and chloroform. Conservation – In closed containers.

Calciferol

(CAS 50-14 -6)

Synonymy – Ergocalciferol, vitamin D_2.

Formula and molecular mass – $C_{28}H_{44}O$ – 396.65 *Specification* – A gram corresponds in activity anti- sickly 40 million Ul.

Description – colorless crystals or white crystalline powder. – Solubility Practically insoluble in water, soluble in ethanol, easily soluble in oil fatty acids.

Conservation – In airtight containers, under inert gas. *Storage* – Protect from heat and light.

Calcium Ms. – 400 |ag/mL

Specification – Contains 1.001 g of calcium carbonate in 25 mL of dilute hydrochloric acid *M.* Boil. Complete with water to 1000 mL.

Conservation – In well-closed containers, inert (polyethylene type).

Camphene

(CAS 79-92 -5)

Formula and molecular mass – $C_{10}H_{16}$ – 136,23

Camphor

(CAS 76-22 -2)

Formula and molecular mass – $C_{10}H_{16}O$ – 152,23

Kaolin lightweight

(CAS 1332-58 -7)

Specification – natural aluminum Silicate, hydrated, purified. Contains an agent of dispersant appropriate.

Description – white Powder, little dense, particle free granulous, unctuous to the touch.

Solubility Practically insoluble in water and inorganic acids.

Coarse particulates – Add 5 g of the sample in a beaker with stopper (160 mm in length and 35 mm in internal diameter) and add 60 mL of solution of sodium pyrophosphate to 1% (p/v). Shake vigorously and leave to rest for 5 minutes. Using a pipette, remove Ml of the supernatant liquid, from a position approximately 5 cm below the surface of the preparation. The remaining liquid add 50 mL of water, shake, allow to rest for 5 minutes and

remove 50 mL of liquid under the conditions prescribed above. Repeat the process to remove a total of 400 mL. Transfer the suspension to a porcelain dish, evaporate to dryness on a water-bath and dessecar to 100-105 °C until constant weight. The mass of the residue is not greater than 25 mg (0.5 %).

Fine Particles – Disperse 5 g of sample in 250 mL of water, shake vigorously for 2 minutes and immediately transfer to a glass test tube (50 mm internal diameter). Using a pipette, transfer 20 mL of the liquid to a watch glass. Evaporate to dryness on a water-bath and dessecar to 100-105 °C until constant mass *(m)*. Leave at rest to 20 ºC for 4 hours the suspension remaining. Remove 20 mL of liquid, from a position approximately 5 cm below the surface of the preparation, avoiding disperse the pellet. Transfer to a watch glass, evaporate to dryness in bath-mary and dessecar to 100-105 °C until constant mass (m2). The value of m2 is not less than 70% of the value of m1.

Ammonium carbonate

(CAS 506-87 -6)

Formula and molecular mass – $(NH_4)_2CO_3$ – 96,09

Specification – Mixture in varying proportions of ammonium bicarbonate (NH_4HCO_3 – 79,06) and ammonium carbamate ($H_2NCOONH_4$ --leveraged). Contains, at a minimum, 30.0% of NH_3 (MM – 17.3) (p/p).

Description – white crystalline Masses, translucent, ammoniacal odor strong.

Solubility – Soluble in water. Decomposes in boiling water.

Conservation – In well-closed containers. *Storage* – Protect from heat and light.

Ammonium carbonate SR

Specification – Contains 15.8 g of ammonium carbonate in water to 100 mL.

Conservation – In well-closed containers. *Storage* – Protect from heat and light.

Calcium carbonate

(CAS 471-34 -1)

Formula and molecular mass – $CaCO_3$ – 100.09 *Specification* – Contains, at a minimum, 98.5% (p/p), calculated in dry substance.

Description – white Powder, odourless and tasteless.

Solubility Practically insoluble in water. Conservation – In well-closed containers.

Strontium carbonate

(CAS 1633-05 -2)

Formula and molecular mass – $SrCO_3$ – 147.64

Description – white Powder, odourless and tasteless.

Conservation – In well-closed containers.

Lithium carbonate

(CAS 554-13 -2)

Formula and molecular mass – Li_2CO_3 – 73,89

Specification – Contains, at a minimum, 98.5 %, calculated on a dry basis.

Description – white Powder, mild, odorless.

Solubility Slightly soluble in water and very slightly soluble in ethanol.

Conservation – In well-closed containers.

Potassium carbonate, anhydrous

(CAS 584-08 -7)

Formula and molecular mass – K_2CO_3 – 138,21

Description – granular Powder or granules white or nearly white. Hygroscopic.

Physical Characteristic – melting Temperature: 891 ºC. – Solubility Very soluble in water and practically insoluble in ethanol.

Conservation – In well-closed containers.

Potassium carbonate, sesqui-hydrated

(CAS 6381-79 -9)

Formula and molecular mass – $K_2CO_3.1½H_2O$ – 165,23

Description – small granular crystals.

Conservation – In well-closed containers.

Safety – Annoying! Caustic!

Sodium carbonate, anhydrous

(CAS 497-19 -8)

Formula and molecular mass – Na_2CO_3 – 105,99

Specification – Contains, at a minimum, 99.0% (p/p), calculated on a dry basis.

Description – white, hygroscopic Powder.

Solubility Easily soluble in water.

Conservation – In airtight containers.

Storage – Protect from moisture.

Sodium carbonate, deca-hydrated

(CAS 6132-02 -1)

Formula and molecular mass – $Na_2CO_3.10H_2O$ – 286,09

Specification – Contains, at a minimum, 36.7% (p/p).

Description – transparent, colorless crystals, eflorescentes, or white crystalline powder; odourless, alkaline and salty flavor.

Solubility Easily soluble in water and practically insoluble in ethanol.

Conservation – In well-closed containers.

Sodium carbonate monohydrate

(CAS 5968-11 -6)

Formula and molecular mass – $Na_2CO_3.H_2O$ – 124,00

Specification – Contains, at a minimum, 83.0% (p/p)

Description – colorless crystals or white crystalline powder; odourless, alkaline and salty flavor.

Solubility Easily soluble in water and practically insoluble in ethanol.

Conservation – In well-closed containers.

Additional Information – When prescribed sodium carbonate for powder mixture, using $Na_2CO_3.H_2O$.

Sodium carbonate SR

Specification – Contains 10.6 g of anhydrous sodium carbonate in 100 mL of water.

Conservation – In well-closed containers.

Carvone

(CAS 2244-16 -8)

Formula and molecular mass – $C_{10}H_{14}O$ – 150,24

Description – colorless Liquid.

Physical Characteristics – Density (20 ºC): approximately 0.965. Index of refraction (20 ºC): approximately 1.500. Boiling Temperature: about 230 ºC. ROTATING Power (20 ºC): approximately +61º.

Miscibility – Practically insoluble in water, miscible with ethanol.

Catechin

(CAS 154-23 -4)

Formula and molecular mass – $C_{15}H_{14}O_6.xH_2O$ – 290,27

(For the anhydrous substance)

Physical Characteristics – Range of fusion: 93 ºC to 96 ºC or 175 ºC to 177 ºC when in anhydrous form.

Cephaeline

Specification – consists of acid esters glicerofosforico with long-chain fatty acids, being the group phosphate esferificado with ethanolamine.

Description – amorphous Substance yellowed, characteristic odour and flavor.

Category – local Hemostatic and laboratory reagent in liver function tests.

Cephaeline SR

Preparation – Add 20 mL of acetone in a quantity of 0.5 to 1 g of powder from the brain of an ox, leave to rest for 2 hours. Centrifuge for 2 minutes and decant the supernatant liquid. Dry the residue under reduced pressure. Add 20 mL of chloroform to the dry material. Leave to rest for 2 hours, stirring frequently. After eliminating the solid material, by filtration or centrifugation, evaporate the chloroform at reduced pressure. Place the residue suspended in 5 to 10 mL of sodium chloride solution to 0.9 % (p/v). The solvents used to prepare this reagent contains an appropriate antioxidant, such as butyl hidroxianisol 0,002% (p/v).

Conservation – Use up to 3 months, after freezing or lyophilization.

Chromatographic Cellulose

(CAS 9004-34 -6)

Synonymy – Cellulose chromatography.

Description – fine, white Powder, homogeneous. Average Size of particles is not less than 30 ^m.

Category – Support for chromatography.

Lead Ms. – 100 |ag/mL

Specification – Contains 0.16 g of lead nitrate (II) in 5 mL of nitric acid. Complete with water to 1000 mL. *Conservation* – In well-closed containers, inert (polyethylene type).

Potassium Cyanide

(CAS 151-50 -8);]

Formula and molecular mass – KCN – 65.12 *Specification* – Contains, at a minimum, 96.0% (p/p), calculated on the dried substance.

Description – crystalline Powder, pasta or granules; white deliquescent.

Physical Characteristic – melting Temperature: 634ºC. *Conservation* – In airtight containers.

Storage – Protect from light.

Stability – It breaks down gradually by exposure to air, carbon dioxide and moisture.

Safety – Poison!

Potassium Cyanide SR

Preparation – Dissolve 50 g of potassium cyanide in distilled water, make up the volume to 100 mL. Remove the lead of this solution by extraction with successive portions of stripper solution of dithizone. Remove the dithizone remaining in cyanide solution stirring with chloroform. Dilute the solution of cyanide with enough distilled water to which, each 100 mL, containing 10 g of potassium cyanide Conservation – In airtight containers.

Safety – Poison!

Cyanide-ammonia SR

Preparation – Dissolve 2 g of potassium cyanide in 15 mL of ammonium hydroxide solution and dilute to 100 mL with distilled water.

Ethyl Cianoacetato

(CAS 105-56 -6)

Formula and molecular mass – $C_5H_7NO_2$ – 113,11

Description – Liquid colorless or pale yellow. *Physical Characteristics* – Density (25 ºC): 1.056. Boiling Range: 205 ºC to 209ºC with decomposition. *Miscibility* – Slightly soluble in water and miscible with ethanol and ethyl ether.

Conservation – In well-closed containers.

Cyclohexane

(CAS 110-82 -7)

Formula and molecular mass – C_6H_{12} – 84,16

Description – Liquid clear, colorless, volatile, characteristic odor (similar to that of gasoline).

Physical Characteristics – boiling Temperature: approximately 80 ºC. Density: approximately 0.78. Index of refraction (20 ºC): 1.426 to 1.427.

Miscibility – Practically insoluble in water. Miscible organic solvents.

Conservation – In well-closed containers.

Safety – Flammable.

Benzyl Cinnamate

(CAS 103-41 -3)

Formula and molecular mass – $C_{16}H_{14}O_2$ – 238,28

Description – colorless crystals or yellowish. *Physical Characteristic* – melting Temperature: about 39 ºC.

Methyl Cinnamate

(CAS 103-26 -4)

Formula and molecular mass – $C_{10}H_{10}O_2$ – 162,19

Description – colorless crystals.

Physical Characteristics – Range of fusion: 34 ºC to 36 ºC. Boiling Temperature: about 260 ºC. Index of refraction (20 ºC): approximately 1.56.

Solubility Practically insoluble in water and soluble in ethanol.

Cinchonina

(CAS 118-10 -5)

Formula and molecular mass – $C_{19}H_{22}N_2O$ – 294,39

Description white crystalline Powder, or nearly white. Physical Characteristics – specific optical rotation (20 ºC): +225º to +230º, determined in a 5% solution (w/v) in 96% ethanol (v/v). Melting Temperature: approximately 263 ºC.

Conservation – In closed containers.

Storage – Protect from exposure to light.

1,8-Cineole

(CAS 470-82 -6)

Synonymy – Eucalyptol.

Formula and molecular mass – $C_{10}H_{18}O$ – 154,25

Description – colorless Liquid.

Physical Characteristics – Density (20 ºC): 0.922 to 0.927. Index of refraction (20 ºC): 1.456 to 1.459.

Miscibility – Practically insoluble in water and miscible with ethanol.

Citral

(CAS 5392-40 -5)

Formula and molecular mass – $C_{10}H_{16}O$ – 152,24

Description – light yellow Liquid.

Miscibility – Practically insoluble in water and miscible with ethanol and glycerol.

Ammonium Citrate SR

Preparation – Dissolve 40 g of citric acid in 90 mL of distilled water. Add two or three drops of phenol red to 0.1% (p/v) in ethanol. Add, carefully, ammonium hydroxide until the solution becomes reddish. Remove any lead this by extraction with portions of 20 mL of solution of dithizone puller until the green color-orangey in dithizone solution is maintained.

Alkaline cupric Citrate SR

Preparation – Under heating, dissolve 173 g of sodium citrate and 177 g of sodium carbonate monohydrate in 700 mL of water. Filter if necessary to obtain a clear solution. In a separate flask, dissolve 17.3 G of cupric sulfate pentahydrate in 100 mL of water. Add (slowly and under constant agitation) about this solution, the first solution prepared. Supplement to the volume to 1000 mL with water.

Sodium Citrate

(CAS 6132-04 -3)

Synonymy – trisodium Citrate.

Formula and molecular mass – $C_6H_5Na_3O_7.2H_2O$ – 294,10

Specification – Contains, at a minimum, 99.0% (p/p), calculated on the dried substance.

Description – Crystals or white crystalline powder, odourless, salty flavor, refreshing. Deliquescent.

Solubility Easily soluble in water and practically insoluble in ethanol.

Conservation – In well-closed containers.

Citronellal

(CAS 106-23 -0)

Formula and molecular mass – $C_{10}H_{18}O$ – 154,25

Description – colorless Liquid or clear yellow. *Physical Characteristics* – Density (20 ºC): 0.848 to 0.856. Index of refraction (20 ºC): approximately 1.446.

Miscibility – Very slightly soluble in water and soluble in ethanol.

Citronellol

(CAS 106-22 -9)

Formula and molecular mass – $C_{10}H_{20}O$ – 156,26

Description – colorless, clear Liquid.

Physical Characteristics – Density (20 ºC): 0.857. Index of refraction (20 ºC): 1.456. Boiling Range: 220ºC to 222 ºC.

Miscibility – Practically insoluble in water and miscible with ethanol.

Chloramine-T

(CAS 7080-50 -4)

Synonymy – sodium Salt hydrate of tri-N-chloro-p-toluenossulfonamida.

Formula and molecular mass – $C_7H_7ClNNaO_2S.3H_2O$ – 281,69

Description – Crystals eflorescentes white or slightly yellowish or crystalline powder.

Physical Characteristic – Range of fusion: 167 ºC to 170 ºC. – Solubility Easily soluble in water, soluble in ethanol with decomposition, insoluble in benzene, chloroform and ethyl ether. *Conservation* – In containers perfectly closed, protected from light, in cooler.

Potassium Chlorate

(CAS 3811-04 -9)

Formula and molecular mass – $KClO_3$ – 122,55

Description – Crystals or granules, or white or almost white powder.

Physical Characteristic – melting Temperature: 368ºC.

Solubility – Soluble in water.

Conservation – In well-closed containers.

Safety – Avoid contact with organic materials or other oxidisable substances.

Chloride Cobaltso

(CAS 7791-13 -1)

Synonymy – cobalt chloride (II).

Formula and molecular mass – $CoCl_2.6H_2O$ – 237,93

Specification – Contains, at a minimum, 99.0% (p/p).

Description – crystalline Powder or crystals red-violet. – Solubility Very soluble in water and soluble in ethanol. *Conservation* – In well-closed containers.

Chloride Cobaltso SR

Specification – Contains 6.5 g, added to 70 mL of hydrochloric acid SR, in water to 100 mL.

Conservation – In well-closed containers.

Chloride acetylates

(CAS 75-36 -5)

Formula and molecular mass – C_2H_3ClO – 78,50

Description – Liquid clear and colorless. Flammable. Decomposes on contact with water and ethanol. *Physical Characteristics* – Density (20 ºC): approximately 1.10. Boiling Temperature: 52 ºC.

Miscibility – Miscible in ethylene chloride, ethyl ether and glacial acetic acid.

Conservation – In well-closed containers.

Safety – Irritating to eyes!

Aluminum chloride hexahydrate

(CAS 7784-13 -6)

Formula and molecular mass – $AlCl_3.6H_2O$ – 241,43

Description – white or slightly yellowish Powder or colorless crystals, deliquescent.

Solubility Very soluble in water, readily soluble in ethanol, soluble in glycerol.

Conservation – In airtight containers.

Aluminum chloride SR

Preparation – Dissolve 2 parts of aluminum chloride hexahydrate in enough water to 3 parts. Treat the solution with activated charcoal, filtered and, if necessary, adjust the pH to 1.5 with sodium hydroxide (1% w/v).

Ammonium chloride

(CAS 12125-02 -9)

Formula and molecular mass – NH_4Cl – 53,49

Specification – Contains at least 99.5% (p/p), calculated on the dried substance.

Description – colorless crystals or white crystalline powder, odourless, salty flavor. Hygroscopic.

Physical Characteristic – Sublimates without fusing to 338 ºC. – Solubility Easily soluble in water.

Conservation – In airtight containers.

Storage – Protect from moisture.

Ammonium chloride SR

Specification – Contains 10.7 g in 330 mL water (approximately 2 *M).*

Conservation – In well-closed containers.

Ammonium chloride-ammonium hydroxide SR

Preparation – Mix equal volumes of ammonium hydroxide and water and saturate with ammonium chloride.

Barium chloride

(CAS 10326-27 -9)

Formula and molecular mass – $BaCl_2.2H_2O$ – 244,27

Specification – Contains, at a minimum, 99.0% (p/p).

Description – colorless crystals or white crystalline powder. – Solubility Easily soluble in water and slightly soluble in ethanol.

Conservation – In well-closed containers.

Safety – Toxic!

Barium chloride SR

Specification – Contains 10 g in 330 mL water. *Conservation* – In well-closed containers.

Benzalkonium chloride

(CAS 8001-54 -5)

Formula and molecular mass – $[C_6H_5CH_2N(CH_3)_2R]^+ Cl^-$ - 360,00 (average)

Chemical Composition – Mixture of chlorides of alquildimetilbenzilamonio, in which R represents alkyl, from *n*-C_8H_{17} and homologous higher: *n*-$C_{12}H_{25}$, *n*-$C_{14}H_{29}$, *n*-$C_{16}H_{33}$, in greater proportion.

Specification – Contains, at a minimum, 95.0% in relation to the mixing desiccated. Content of alkylated counterparts present, in relation to the total calculated on dry basis: n-C 12.25: at least 40.0% (p/p); n-$C_{14}H_{29}$: at least 10,0% (p/p); the sum of the two counterparts above: at least 70.0% (p/p).

Description – amorphous Powder or gelatinous mass white or yellowish-white, aromatic odour and taste bitter.

Solubility Very soluble in water and ethanol. In aqueous solution, foaming under agitation.

Conservation – In well-closed containers. *Storage* – Protect from light and air.

Category – disinfectant. Detergent. Preservative.

Benzethonium chloride

(CAS 121-54 -0)

Formula and molecular mass – $C_{27}H_{42}ClNO_2.H_2O$ – 466,09

Description – colorless crystals, or fine powder white, or nearly white.

Physical Characteristic – melting Temperature: around 163 ºC. *Solubility* – Soluble in water and ethanol.

Conservation – In closed containers.

Storage – Protect from exposure to light.

Benzyl chloride

(CAS 100-44 -7)

Synonymy – Clorometilbenzeno

Formula and molecular mass – C_7H_7Cl – 126,58

Description – colorless Liquid.

Physical Characteristics – Density (20 ºC): 1.100. Boiling Temperature: 179ºC. Melting Range: -48ºC to -43 ºC.

Miscibility – Insoluble in water. Miscible with ethanol, chloroform and ethyl ether.

Conservation – In airtight containers. *Storage* – Protect from heat.

Calcium chloride

(CAS 10035-04 -8)

Formula and molecular mass – $CaCl_2.2H_2O$ – 147,02

Specification – Contains, at a minimum, 96.0% (p/p).

Description – white crystalline Powder or granules, odourless, salty flavor and strongly bitter. Hygroscopic. – Solubility Easily soluble in water and soluble in ethanol.

Conservation – In well-closed containers. *Storage* – Protect from moisture.

Calcium chloride, anhydrous

(CAS 10043-52 -4)

Formula and molecular mass – $CaCl_2$ –110,99

Specification – Contains, at a minimum, 98.0 % (p/p), calculated on the dried substance.

Description – Granules white and dry. Deliquescent. – Solubility Very soluble in water, readily soluble in ethanol and methanol.

Conservation – In airtight containers *Storage* – Protect from moisture *Category* – Desiccant

Calcium chloride SR

Specification – Contains 7.35 g of calcium chloride in water to 100 mL (approximately 0.5 *M)*.

Conservation – In well-closed containers.

Caesium chloride

(CAS 7647-17 -8)

Formula and molecular mass – CsCl – 168,36

Description – white or almost white Powder.

Solubility Very soluble in water, readily soluble in methanol and practically soluble in acetone.

Chloride of tin (II) Mr.

Preparation – Heat 20 g of tin with 85 mL of dilute hydrochloric acid until no longer occurs release of hydrogen.

Magnesium chloride

(CAS 7791-18 -6)

Formula and molecular mass –$MgCl_2 6H_2O$ – 203,30

Specification – Contains, at a minimum, 98.0 % (p/p).

Description – colorless crystals, bitter taste. Hygroscopic. Solubility Very soluble in water and readily soluble in ethanol.

Conservation – In well-closed containers. *Storage* – Protect from moisture.

Mercury chloride (II)

(CAS 7487-94 -7)

Synonymy – mercuric chloride.

Formula and molecular mass – $HgCl_2$ – 271,50

Specification – Contains, at a minimum, 99.0 % (p/p), calculated on the dried substance.

Description – colorless crystals or white crystalline powder or almost white, or mass crystallized; odorless. *Physical Characteristic* – melting Temperature: 277ºC (volatilization temperature of approximately 300 ºC). *Solubility* – Soluble in water and glycerol, readily soluble in ethanol. *Conservation* – In well-closed containers. *Storage* – Protect from light. *Security* – Irritant. Caustic. Toxic. Pollutant. *Additional Information* – Antidote: toxicity.

Methylene chloride

(CAS 75-09 -2)

Synonymy – Dichloromethane.

Formula and molecular mass – CH_2Cl_2 – 84,94

Description – Liquid clear, colorless, volatile, odor similar to chloroform.

Physical Characteristics – boiling Temperature: approximately 40 ºC. Density: approximately 1.32. Index of refraction (20 ºC):1,424.

Miscibility – Slightly soluble in water and miscible with ethanol.

Conservation – In well-closed containers. *Storage* – Protect from light.

Security – Irritant. Toxic.

Methylene chloride saturated with ammonia

Preparation – Mix 100 mL of methylene chloride with 30 mL of concentrated ammonium hydroxide solution in separating funnel. Allow to separate the phases and use the lower layer.

Methylthioninium chloride

(CAS 7220-79 -3)

Synonymy – Methylthioninium chloride tri-hydrate, methylene blue.

Formula and molecular mass – $C_{16}H_{18}ClN_3.3H_2O$ – 373,90

Description – crystalline Powder dark green or bronze. Can be found in different form hydrated.

Solubility Easily soluble in water and in ethanol.

Methylthioninium chloride SR

Synonymy – methylene Blue SR

Preparation – Dissolve 23 mg of Methylthioninium chloride in sufficient quantity of water to make 100 mL.

Methylthioninium chloride SR1

Synonymy – methylene Blue SR1

Preparation – Dissolve 125 mg of Methylthioninium chloride

In 100 mL of ethanol and diluted in ethanol to make 250 mL.

Chloride of nickel (II)

(CAS 7791-20 -0)

Formula and molecular mass – $NiCl_2.6H_2O$ – 237,71

Description – green, hygroscopic crystalline Powder.

Chloride nitrobenzoila

(CAS 122-04 -3)

Formula and molecular mass – $C_7H_4ClNO_3$ – 185,57

Description – yellow crystals, pungent odour. *Physical Characteristic* – melting Temperature: around 73 ºC.

Gold chloride

(CAS 16961-25 -4)

Formula and molecular mass – $HAuCl_4.3H_2O$ – 393,83

Description – Crystals monoclinicos yellow-reddish to yellow-gold. In hygroscopic equilibrium and Very deliquescent.

Conservation – In well-closed containers. *Storage* – Protect from light.

Chloride of gold SR

Preparation – Dissolve 1 g of chloride of gold in 35 mL of water.

Conservation – In well-closed containers. *Storage* – Protect from light.

Palladium chloride

(CAS 7647-10 -1)

Formula and molecular mass – $PdCl_2$ – 177,31

Specification – Contains, at a minimum, 59.0% (p/p) in palladium.

Description – reddish brown crystalline Powder. *Physical Characteristic* – In high temperatures decomposes to palladium and chlorine.

Conservation – In well-closed containers.

Safety – Toxic!

Potassium chloride

(CAS 7447-40 -7)

Formula and molecular mass – KCl – 74,55

Specification – Contains, at a minimum, 99.0 % (p/p), calculated on the dried substance.

Description – colorless crystals or white crystalline powder, odourless salt flavor, weakly bitter.

Conservation – In well-closed containers.

Potassium chloride, saturated solution

Specification – Contains 17 g in 165 mL water. *Conservation* – In well-closed containers.

Sodium chloride

(CAS 7647-14 -5)

Formula and molecular mass – NaCI – 58,44.

Specification – Contains, at a minimum, 99.0% (p/p) calculated on the dried substance.

Description – colorless crystals or white crystalline powder, odourless salt taste.

Solubility Easily soluble in water and practically insoluble in ethanol anhydrous.

Conservation – In well-closed containers.

Additional Information – Salt additive-free.

Sodium chloride 0.9% (p/v)

Synonymy – sodium chloride approximately 0.15 M solution of sodium chloride, isotonic saline solution, saline solution.

Description – Contains 9 g of sodium chloride in water to 1000 mL.

Conservation – In closed containers.

Stannous chloride

(CAS 10025-69 -1)

Formula and molecular mass – $SnCl_2.2H_2O$ – 225,63 Specification – Contains, at a minimum, 97.0% (p/p).

Description – colorless or almost colorless crystals. – Solubility Very soluble in water, readily soluble in ethanol, in glacial acetic acid, and in dilute hydrochloric acid and concentrated.

Conservation – In well-closed containers. *Storage* – Protect the air and heat.

Stannous chloride SR

Specification – Contains 10 g of stannous chloride in hydrochloric acid to 100 mL.

Conservation – Prepare at the time of use. *Storage* – Protect from light.

Stannous chloride SR1

Alternate Name – Chloride of tin (II) SR.

Preparation – Heat 20 g of tin with 85 mL of dilute hydrochloric acid until no longer occurs release of hydrogen.

Ferric chloride

(CAS 10025-77 -1)

Synonymy – ferric chloride hexahydrate.

Formula and molecular mass – $FeCl_3.6H_2O$ – 270,30

Specification – Contains, 99.0% (p/p), calculated on the dried substance.

Description – Mass crystallized, yellow-orange or brown. Deliquescent.

Physical Characteristic – melting Temperature: approximately 37 ºC.

Conservation – In well-closed containers. *Storage* – Protect from light.

Ferric chloride SR (approximately 0.4 *M)*

Use ferric chloride ITSELF.

Ferric chloride acid SR

Preparation – Dissolve 15 mg of ferric chloride hexahydrate -hydrate in 20 mL of glacial acetic acid and sulfuric acid (1: 1).

Ferric chloride did not produce any signals

Preparation – Dissolve 1 g of ferric chloride in 100 mL of methanol.

Mercuric chloride SR (approximately 0.2 *M)*

Specification – Contains 5.4 g of chloride of mercury (II) in water to 100 mL.

Conservation – In well-closed containers.

Security – Toxic. Pollutant.

Chloride platinico SR

Synonymy – platinum chloride SR

Preparation – Dissolve 2.6 g of glacial cloroplatinico in water to 20 mL.

Conservation – In well-closed containers. *Storage* – Protect from light.

Benzoyl hydrochloride

(CAS 98-88 -4)

Formula and molecular mass – C_7H_5ClO – 140,57

Description – colorless Liquid. Decomposes in water and ethanol. *Physical Characteristics* – Density (20 ºC): approximately 1.21. Boiling Temperature: approximately 197 ºC.

Hydrochloride (2-chloroethyl) diethylamine

(CAS 869-24 -9)

Formula and molecular mass – $C_6H_{14}ClN.HCl$ – 172,10

Description – white, crystalline Powder, very soluble in water and methanol, readily soluble in methylene chloride, practically insoluble in n-hexane.

Physical Characteristic – melting Temperature: around 211 ºC.

Hydrochloride of dimethyl-p-phenylenediamine

(CAS 536-46 -9)

Synonymy – Dihydrochloride of N,N-dimethyl-p-phenylenediamine.

Formula and molecular mass – $C_8H_{12}N_2.2HCl$ – 209,12. Description white crystalline Powder or almost white. Hygroscopic.

Solubility Easily soluble in water and soluble in ethanol.

Conservation – In well-closed containers.

O-phenylenediamine hydrochloride

(CAS 615-28 -1)

Synonymy – Dihydrochloride 1,2-benzenediamine.

Formula and molecular mass – $C_6H_8N_2.2HCl$ – 181,14

Description – white Powder or slightly pinkish.

P-phenylenediamine hydrochloride

(CAS 624-18 -0)

Synonymy – Dihydrochloride 1,4-benzenediamine.

Formula and molecular mass – $C_6H_8N_2.2HCl$ – 181,144

Description – white crystalline Powder, it becomes reddish in contact with the air.

Solubility Easily soluble in water, slightly soluble in ethanol and ethyl ether.

Hydrochloride fenilidrazina

CAS – (*59-88-1*)

Formula and molecular mass – $C_6H_8N_2.HCl$ – 144,60

Description – crystalline Powder white or almost white, becoming brown by exposure to air.

Physical Characteristic – melting Temperature: around 245 °C with decomposition.

Solubility – Soluble in water and in ethanol.

Conservation – In well-closed containers. *Storage* – Protected from light.

Fenilidrazina hydrochloride SR

Preparation – Dissolve 0.9 g of hydrochloride of fenilidrazina in 165 mL of water. Decolourize with activated charcoal and filtered. Collect the filtrate in 250 mL volumetric flask, add 30 mL of hydrochloric acid and supplement to volume with water.

Hydrastine hydrochloride

(CAS 5936-28 -7)

Formula and molecular mass – $C_{21}H_{22}ClNO_6$ – 419,86

Description – white Powder, or nearly white. Hygroscopic. *Physical Characteristics* – rotating Power (17 ºC): approximately +127 °. Melting Temperature: approximately to 116 ºC.

Solubility Very soluble in water and ethanol.

Hydroxylamine hydrochloride

(CAS 5470-11 -1)

Formula and molecular mass – NH_4ClO – 69,49

Specification – Contains, at a minimum, 96.0% (p/p).

Description – colorless crystals or white crystalline powder. *Physical Characteristic* – melting Temperature: approximately 151 ºC.

Solubility Very soluble in water and soluble in ethanol. Conservation – In well-closed containers. *Storage* – Protect from moisture.

Hydroxylamine hydrochloride SR

Preparation – Dissolve 5 g in 5 mL of hot water. Make up to 100 mL with ethanol.

Conservation – In well-closed containers.

Safety – Flammable.

Hydroxylamine hydrochloride SR1

Preparation – Dissolve 20.0 g of hydroxylamine hydrochloride in distilled water to obtain approximately 65 mL. Transfer to separating funnel. Add five drops of thymol blue SI and add ammonia until the solution becomes yellow color. Add 10 mL of an aqueous solution of sodium diethyldithiocarbamate to 4% (p/v), shake, and leave to rest for 5 minutes. Remove this solution with successive portions of 10 to 15 mL of chloroform until a 5 mL portion of the chloroform extract not purchase yellow coloration when agitated with cupric sulfate to 12.5% (p/v). Add hydrochloric acid 3 *M* up to get pink coloration (if necessary, add one or two drops of thymol blue SI) and dilute to 100 mL with distilled water.

Chlorine SR

Specification – saturated Solution of chlorine in water. *Conservation* – In bottles completely filled and closed.

Storage – In a cool place, protected from light and air.

Comments – The solution tends to deteriorate even if protected from light and air.

Stability – Use freshly prepared solution.

p-Cloroacetanilida

(CAS 539-03 -7)

Formula and molecular mass – C_8H_8ClNO – 169,61

Description – crystalline Powder.

Physical Characteristic – melting Temperature: about 178 ºC.

Solubility Practically insoluble in water and soluble in ethanol.

Chlorobenzene

(CAS 108-90 -7)

Formula and molecular mass – C_6H_5Cl – 112,56

Description – colorless Liquid, hyperechoic, characteristic odour.

Physical Characteristics – boiling Temperature: approximately 132 ºC. Density: approximately 1.11. Index of refraction (20 ºC): 1.5251.

Conservation – In well-closed containers.

Security – Toxic. Flammable.

1-Chloro-2,4-dinitrobenzene.

(CAS 97-00 -7)

Formula and molecular mass – $C_6H_3ClN_2O_4$ – 202,55

Description – pale-yellow crystals or crystalline powder. *Physical Characteristic* – melting Temperature: around ºC.

Chloroform

Synonymy – Trichloromethane *Formula and molecular mass* – $CHCl_3$ – 119,40

Specification – Contains, at a minimum, 99.9 % (p/p).

Description – mobile Liquid, colorless, sweetish odor. *Physical Characteristics – Density*: approximately 1.48. Boiling Temperature: approximately 62 ºC. *Conservation* – In well-closed containers.

Security – Toxic.

Free Chloroform alcohol

Preparation – Prepare immediately before use. Shake carefully 20 mL of chloroform with 20 mL of water for 3 minutes. Carefully Remove the organic phase and wash with two portions of 20 mL of water. Filter the chloroform through dry paper. Add 5 g of anhydrous sodium sulphate, shake for 5 minutes and leave to rest for 2 hours. Decant or filter.

Clorotiazida

(CAS 58-94 -6)

Formula and molecular mass – $C_7H_6ClN_3O_4S_2$ – 295,73

Description – white crystalline Powder, odourless or almost white. *Physical Characteristic* – melting Temperature: around 340 °C with decomposition.

Solubility: Very slightly soluble in water, slightly soluble in acetone, slightly soluble in ethanol. Dissolve in dilute solutions of hydroxy-alkaline soil.

Sodium Cobaltinitrito

(CAS 13600-98 -1)

Formula and molecular mass – $Na_3CoN_6O_{12}$ – 403,94

Description – an orange-yellow crystalline Powder. Solubility Easily soluble in water and slightly soluble in ethanol.

Conservation – In well-closed containers

Sodium Cobaltinitrito SR

Specification – Contains 10 g in 330 mL water.

Conservation – Prepare at the time of use.

Copper

(CAS 7440-50 -8)

Element and atomic mass – Cu – 63,546.

Description – Blade, wire, powder or fragment, reddish in color and metallic lustre.

Conservation – In non-metallic containers.

Copper Ms. – 1 mg/mL

Specification – Contains 1 g of dissolved copper in the lowest possible volume of nitric acid in 50% (v/v). Complete with nitric acid at 1% (v/v) to 1000 mL.

Conservation – In well-closed containers, inert (polyethylene type).

o-Cresol

(CAS 95-48 -7)

Synonymy – 2-Methylphenol.

Formula and molecular mass – C_7H_8O – 108.14.

Description – Liquid or solid, colorless to yellow-brown, that if cora by light and in the presence of oxygen; phenolic odor. Deliquescent.

Physical Characteristics – melting Temperature: approximately 30 ºC. Boiling Temperature: approximately 191 ºC. Density: approximately 1.03. Index of refraction (20 ºC): 1.540 -1.550.

Miscibility – Miscible with anhydrous ethanol, slightly soluble in water and soluble in solutions hydroxy-alkaline. *Conservation* – In airtight containers.

Storage – Protect from light, moisture and oxygen. *Security* – Irritant. Caustic. Toxic.

Category – disinfectant.

OF potassium Chromate

(CAS 7789-00 -6)

Formula and molecular mass – K_2CrO_4 – 194,19

Specification – Contains, at a minimum, 99.0% (p/p), calculated on the dried substance.

Description – yellow crystals or crystalline powder.

Solubility Easily soluble in water.

Conservation – In well-closed containers.

Safety – Oxidant. Pollutant.

OF potassium Chromate SR

Specification – Contains 10 g in 330 mL water. *Conservation* – In well-closed containers.

Safety – Oxidant. Pollutant.

Disodium Cromotropato

(CAS 5808-22 -0)

Synonymy – disodium Salt of chromotropic acid di-hydrated.

Formula and molecular mass – $C_{10}H_6Na_2O_8S_2.H_2O$ – 400,29

Description – Po yellowish-white.

Solubility – Soluble in water and practically insoluble in ethanol.

Sodium Desoxycholate

(CAS 302-95 -4)

Formula and molecular mass – $C_{24}H_{39}NaO_4$ – 414,55

Description – white crystalline Powder or almost white.

Dextrose

See glucose.

Dextrose 0.1% (p/v)

See glucose 0.1% (w/v) in pyridine.

Chlorhexidine diacetate

Use chlorhexidine acetate.

1.8-Diaminonaftaleno

(CAS 479-27 -6)

Synonymy – 1.8-Naftalenodiamina.

Formula and molecular mass – $C_{10}H_{10}N_2$ – 158,20.

Description – sublimaveis crystals.

Physical Characteristic – Range of fusion: 63 ºC to 67 ºC.

Diaveridina

(CAS 5355-16 -8)

Chemical Name: 5- (3,4-Dimethoxyphenyl) methyl -2,4-pirimidinodiamina

Formula and molecular mass – $C_{13}H_{16}N_4O_2$ – 260,30

Physical Characteristic – melting Temperature: around 233 ºC.

2.6-Dibromoquinona-4-chlorimide

(CAS 537-45 -1)

Formula and molecular mass – $C_6H_2Br_2ClNO$ – 299,35

Description – yellow crystalline Powder.

Physical Characteristic – Range of fusion: between 82 ºC and 84ºC. – Solubility Insoluble in water and soluble in ethanol and in alkaline solutions hydroxy-diluted.

Conservation – In closed containers.

Dibutilamina

(CAS 111-92 -2)

Formula and molecular mass – $C_8H_{19}N$ – 129,24

Description – colorless Liquid.

Physical Characteristics – boiling Temperature: approximately

ºC. Index of refraction (20 ºC): approximately 1.417. Miscibility – Soluble in water and ethanol.

Conservation – In well-closed containers.

Ethylene dichloride

(CAS 107-06 -2)

Synonymy – 1,2-Dichloroethane

Formula and molecular mass – $C_2H_4Cl_2$ – 98,96

Description – colorless, clear Liquid, odor similar to chloroform.

Physical Characteristics – boiling Temperature: around 83 ºC. Density (20 ºC): at around 1.25. Index of refraction (20 ºC): 1.444.

Solubility Slightly soluble in water and readily soluble in ethanol.

Conservation – In well-closed containers.

Security – Irritant. Toxic. Flammable.

Dihydrochloride of N- (1-naphthyl) etuenodiamma

(CAS 1465-25 -4)

Synonymy – Dihydrochloride JV-1-naftalenil-1,2 &

Etanodiamina.

Formula and molecular mass – $C_{12}H_{14}N_2.2HCl$ – 259,18

Description – white Powder or white to yellowish. *Characterizes physics* – Range of fusion: 188 ºC to 190 ºC. *Solubility* – Soluble in water and slightly soluble in Ethanol

Dihydrochloride of N- (1-naphthyl) etuenodiamma SR

Synonymy – Bratton-Marshall reagent

Preparation – Dissolve 71 g of N- (1 -naphthyl) ethylenediamine in 100 mL of water.

Conservation – In well-closed containers.

Dicloroquinona-4-chlorimide

(CAS 101-38 -2)

Synonymy – Reagent of Gibbs, 2,6-dichloro-4 (chloroimino) -2,5-cicloexadien-1-ona.

Formula and molecular mass – $C_6H_2Cl_3NO$ – 210,45

Description – crystalline Powder yellow or orange. *Physical Characteristic* – melting Temperature: around d 66 ºC.

Solubility Practically insoluble in water, soluble ei ethanol and diluted in alkaline solutions.

1- (2,6-Didorofeml) -1,3-dihydro-2fl-mdol-2-ona

(Impurity of diclofenac)

(CAS 15362-40 -0)

Synonymy – 1- (2,6-diclorofenil) indolin-2-ona

Formula and molecular mass – $C_{14}H_9Cl_2NO$ – 278,14

Description white crystalline Powder.

Conservation – In well-closed containers. *Storage* – Protect from exposure to light.

2,6 - Dicloroindofenol

Nedocromil sodium

(CAS 620-45 -1)

Synonymy – 2.6-Diclorofenolindofenol nedocromil sodium.

Formula and molecular mass – $C_{12}H_6Cl_2NNaO_2$ – 290,08.

Description – Po dark green.

Solubility Easily soluble in water and in etanc anhydrous. The aqueous solution presents dark blue staining when acidified becomes pink.

Conservation – In well-closed containers.

Potassium dichromate

(CAS 7778-50 -9)

Formula and molecular mass – $K_2Cr_2O_7$ – 294,18

Specification – Contains, at a minimum, 99.8 % (p/p), calculated on the dried substance.

Description – orange red crystals, and odourless. *Solubility* – Soluble in water and practically insoluble in ethanol.

Conservation – In well-closed containers.

Safety – Caustic. Oxidant. Pollutant.

Potassium dichromate SR

Specification – Contains 5 g in 330 mL water. *Conservation* – In well-closed containers.

Safety – Caustic. Oxidant. Pollutant.

Diethylamine

(CAS 109-89 -7)

Formula and molecular mass – $C_4H_{11}N$ – 73,14

Description – Liquid clear, colorless, volatile, ammoniacal odor. Strongly alkaline Reaction.

Physical Characteristics – boiling Range: 55 ºC to 58 ºC. Index of refraction (20 ºC): 1.386. Density: approximately 0.707.

Miscibility – Miscible in water and in ethanol. *Conservation* – In well-closed containers.

Security – Irritant. Flammable.

Dietilaminoetildextrano

(CAS 9015-73 -0)

Formula and molecular mass – $C_{12}H_{28}N_2O$ – 216,36

Description – Po.

Solubility – Soluble in water.

Silver Diethyldithiocarbamate

(CAS 1470-61 -7)

Formula and molecular mass – $C_5H_{10}AgNS_2$ – 256,13

Description – pale yellow Powder to grayish yellow. – Solubility Practically insoluble in water and soluble in pyridine.

Conservation – In well-closed containers.

Silver Diethyldithiocarbamate SR

Specification – Contains 0.25 g in 50 mL pyridine. *Stability* – Prepare for immediate use.

Security – Toxic.

Sodium Diethyldithiocarbamate

(CAS 20624-25 -3)

Formula and molecular mass – $C_5H_{10}NNaS_2.3H_2O$ – 225,33

Description – white crystals or almost white or inolores. – Solubility Easily soluble in water and soluble in ethanol.

N,N-dietiletilenodiamina

(CAS 100-36 -7)

Chemical Name – N,N-Diethyl-1,2-diaminoetano

Formula and molecular mass – $C_6H_{16}N_2$ – 116,21

Description – Liquid appearance slightly oily, colorless or slightly yellowish, with strong odor ammoniacal, irritating to skin, eyes and mucous membranes. *Physical Characteristics* – Density (20 ºC): 0.827. Boiling Range: 145 ºC to 147 ºC.

Water (5.2.20.1) – Determine at 0.5 g. Up 1.0 %.

Diethyl Phthalate

(CAS 84-66 -2)

Formula and molecular mass – $C_{12}H_{14}O_4$ – 222,24

Description – oily Liquid colorless and virtually odourless. *Specification* – Contains, at a minimum, 99.0% (p/p). *Physical Characteristics*: density 1.118. Boiling Temperature: 295ºC.

Miscibility – Miscible in water, ethanol, ethyl ether and other organic solvents.

Conservation – In well-closed containers.

Safety – Annoying!

Diphenylamine

(CAS No 122-39 -4)

Formula and molecular mass – $C_{12}H_{11}N$ – 169,22

Description – white crystals or almost white. *Physical Characteristics* – melting Temperature: about 55 ºC. Boiling Temperature: 302ºC. Lose the color in the presence of light.

Solubility Slightly soluble in water and soluble in ethanol. Form salt in solution with strong acids.

Conservation – In well-closed containers. *Storage* – Protect from light.

Diphenylamine SR

Preparation – Dissolve 1 g of diphenylamine in 100 mL of sulfuric acid.

Conservation – In well-closed containers. *Storage* – Protect from exposure to light.

Difenilbenzidina

(CAS 531-91 -9)

Synonymy – N,N' -Difenilbenzidina.

Formula and molecular mass – $C_{24}H_{20}N_2$ – 336,43

Description white crystalline Powder or slightly gray. Physical Characteristic – melting Temperature: approximately 248 ºC. – Solubility Practically insoluble in water, slightly soluble in acetone and ethanol.

Conservation – In closed containers.

Storage – Protect from exposure to light.

Difenilborato of aminoethanol.

(CAS 524-95 -8)

Formula and molecular mass – $C_{14}H_{16}BNO$ – 225,09

Description – white crystalline Powder or yellowish. *Physical Characteristic* – melting Temperature: about 193 ºC. – Solubility Practically insoluble in water and soluble in ethanol.

Difenilborato of aminoethanol SR

Preparation – Dissolve 1 g of difenilborato of aminoethanol in methanol and make up the volume to 100 mL with the same solvent.

Diphenylcarbazide

(CAS 140-22 -7)

Formula and molecular mass – $C_{13}H_{14}N_4O$ – 242,28

Description – white crystalline Powder; it becomes pinkish by exposure to air.

Physical Characteristic – Range of fusion: 168 ºC to 171 ºC.

Solubility: Very slightly soluble in water, soluble in acetone, ethanol and glacial acetic acid.

Conservation – In airtight containers.

Storage – Protect from light and air.

Diphenylcarbazide SR

Specification – Contains 1 g of diphenylcarbazide in ethanol to 100 mL.

Conservation – In well-closed containers. *Storage* – Protect from light.

Safety – Flammable.

Diphenyl Carbazone Indicator

(CAS 538-62 -5)

Formula and molecular mass – $C_{13}H_{12}N_4O$ – 240,26

Description – Crystals of color reddish-orange. *Physical Characteristic* – melting Temperature: approximately 157 °C with decomposition. – Solubility Practically insoluble in water and readily soluble in ethanol.

Conservation – In well-closed containers.

Diphenyl Carbazone Indicator-bromophenol blue SR

Preparation – In volumetric flask of 25 mL, dissolve 12 mg of diphenyl carbazone indicator and 12.5 mg of bromophenol blue in 15 mL of ethanol. Fill up to volume with ethanol.

Conservation – Wrapping the solution in container amber glass at a temperature of 4ºC to 8 ºC.

Diphenyl Carbazone Indicator mercurica SR

The Solution – Dissolve 0.1 g of diphenyl carbazone indicator in ethanol and make up the volume to 50 mL with the same solvent.

Solution B: Dissolve 1 g of sodium chloride of mercury (II) in ethanol and supplement the volume to 50 mL with the same solvent. Preparation – Mix equal volumes of *Solutions A and B* at the time of use.

N,N' -Diisopropiletilenodiamina

(CAS 4013-94 -9)

Formula and molecular mass – $C_8H_{20}N_2$ – 144,26

Description – Liquid colorless or yellowish. Corrosive, flammable and hygroscopic.

Physical Characteristics – Density (20 ºC): approximately 0.798. Index of refraction (20 ºC): approximately 1.429. Boiling Temperature: about 170 ºC.

Dimethylacetamide

(CAS 127-19 -5)

Formula and molecular mass – C_4H_9NO – 87,12

Description – colorless, clear Liquid.

Physical Characteristics – boiling Temperature: approximately 165 ºC. Index of refraction (20 ºC): approximately 1.437. Density (20 ºC): approximately 0.94.

Miscibility – Miscible in water and in most organic solvents.

Conservation – In closed containers.

p-Dimethylaminobenzaldehyde

(CAS 100-10 -7)

Synonymy – 4-Dimethylaminobenzaldehyde and Ehrlich's reagent.

Formula and molecular mass – $C_9H_{11}NO$ – 149,19.

Description – crystalline Powder, white to faintly yellowish. *Physical Characteristic* – melting Temperature: approximately 74 ºC.

Solubility – Soluble in ethanol and diluted in acidic solutions.

Conservation – In well-closed containers. *Storage* – Protect from light.

p-Dimethylaminobenzaldehyde SR

Preparation – Dissolve, without heating, 0.2 g of *p-dimethylaminobenzaldehyde in mixture of 4.5 mL of water and 5.5 mL of hydrochloric acid. Prepare at the time of use.*

p-Dimethylaminobenzaldehyde SR1

Preparation – Dissolve 0.2 g dep-dimethylaminobenzaldehyde in 20 mL of ethanol and add 0.5 mL of hydrochloric acid. Stir the solution with activated charcoal and filtered. The color of the solution is less intense than a solution of iodine to 0.0001 *M* recently prepared. Use immediately after preparation.

p-Dimethylaminobenzaldehyde SR2

Synonymy – Wasicky reagent.

Preparation – Dissolve 0.5 g of p-dimethylaminobenzaldehyde in 8.5 mL of sulfuric acid and add, carefully,

Ml of water.

4-Dimetilaminocinamaldeido

(CAS 6203-18 -5)

Formula and molecular mass – $C_{11}H_{13}NO$ – 175,22

Description – Crystals orange or brown-orange or dust.

Physical Characteristic – melting Temperature: around 138 ºC.

Solubility – Soluble in ethanol, acetone and benzene.

2,6-Dimethylaniline

(CAS 87-62 -7)

Synonymy – 2.6-Xilidina.

Formula and molecular mass – $C_8H_{11}N$ – 121,18 Description – colorless Liquid.

Physical Characteristic – Density (20 ºC): approximately 0.98

N,N-Dimethylaniline

(CAS 121-69 -7)

Synonymy – N,N-Dimetilbenzenamina.

Formula and molecular mass – $C_8H_{11}N$ – 121,18

Description – oily Liquid, clear, almost colorless, darkens during storage.

Physical Characteristic -Range of distillation: 192ºC to 194 ºC. Miscibility – Practically insoluble in water, readily soluble in ethanol and ethyl ether.

1,1-Dimetiletilamina

(CAS 75-64 -9)

Synonymy – tert-Butilamina.

Formula and molecular mass – $C_4H_{11}N$ – 73,14

Description – colorless Liquid.

Physical Characteristics – Density (20 ºC): approximately 0.694. Index of refraction (20 ºC): approximately 1.378. Boiling Temperature: about 46 ºC.

2,5-Dimethylphenol

(CAS 95-87 -4)

Synonymy – p-Xylenol.

Formula and molecular mass – $C_8H_{10}O$ – 122,16.

Description – white crystals or almost white. *Physical Characteristic* – melting Temperature: around 46 ºC.

Dimethylformamide

(CAS 68-12 -2)

Formula and molecular mass – C_3H_7NO – 73,09

Description – clear, colorless Liquid with odor similar to amines.

Physical Characteristics – boiling Temperature: approximately 153 ºC. Density: approximately 0.95. Index of refraction (20 ºC): 1.428.

Miscibility – Miscible in water and in ethanol. *Conservation* – In well-closed containers.

Security – Irritant. Toxic.

Dimethyl Sulfoxide

CAS – [:67,68 -5)

Synonymy – DMSO.

Formula and molecular mass – C_2H_6OS – 78,13

Description – Liquid colorless and odourless. Hygroscopic. *Physical Characteristics* – boiling Temperature: approximately 189 ºC. Density: approx.

Index of refraction (20 ºC): 1.479.

Miscibility – Miscible in water and in ethanol. *Conservation* – In well-closed containers. *Storage* – Protect from moisture and exposure to light. *Security* – Irritant.

Dinitrobenzene

(CAS 99-65 -0)

Formula and molecular mass – $C_6H_4N_2O_4$ – 168,11

Description – yellowish crystals.

Physical Characteristic – melting Temperature: around 89 ºC.

Solubility Practically insoluble in water and slightly soluble in ethanol.

Conservation – In well-closed containers.

Dinitrobenzene Mr.

Specification – Contains 1 g of 1,3-dinitrobenzene in ethanol to 100 mL.

Conservation – Container tightly closed.

Dioxana

(CAS 123-91 -1)

Synonymy – 1,4-dioxide Dioxana, ethylene, dioxane. *Formula and molecular mass* – $C_4H_8O_2$ – 88,11

Description – clear, colorless Liquid with odor similar to that of ethyl ether.

Physical Characteristics – boiling Temperature: around 101 ºC. Density: at around 1.03. Index of refraction (20 ºC): 1.421 to 1.424.

Miscibility – Miscible in water and in most organic solvents.

Conservation – In well-closed containers.

Security – Irritant. Toxic. Flammable.

Sulfur Dioxide

(CAS 7446-09 -5)

Synonymy – Sulfur dioxide.

Formula and molecular mass – SO_2 – 64,06.

Specification – Contains, at a minimum, 97.0 % (v/v)

Description – colorless Gas, characteristic odour, suffocating. *Conservation* – In pressurized cylinders.

Security – Irritant. Toxic.

Manganese Dioxide

(CAS 1313-13 -9)

Formula and molecular mass – MnO_2 – 86,94

Description – fine Dust black or dark brown.

Conservation – In well-closed containers. *Storage* – Protect from heat.

Safety – Oxidant energetic.

Dipropilenoglicol

(CAS 25365-71 -8)

Synonymy – 1,1 ‘ -Oxide-2-propanol

Formula and molecular mass – $C_6H_{14}O_3$ – 134,17

Description – colorless Liquid, virtually no odor. *Physical Characteristics – Density*: approximately 1.02. Boiling Temperature: approximately 230 ºC. *Conservation* – In well-closed containers. *Storage* – In well-ventilated areas.

Carbon Disulfide

(CAS 75-15 -0)

Formula and molecular mass – CS_2 – 76.14

Description – Liquid colorless or yellowish. Flammable. *Physical Characteristics* – Density (20 ºC): approximately 1.26. Boiling Range: 46 ºC to 47 ºC.

Miscibility – Practically insoluble in water and miscible with ethanol.

Conservation – In well-closed containers.

Safety – Poisonous!

Ditiol

(CAS 496-74 -2)

Synonymy – 1,2-Dimercapto-4-metilbenzeno; toluene -3.4-ditiol.

Formula and molecular mass – $C_7H_8S_2$ – 156,27

Description – white crystals or almost white. *Physical Characteristic* – melting Temperature: 31 ºC. *Solubility* – Soluble in methanol and hydroxy- alkaline solutions.

Ditiol SR

Specification – Contains 0.5 g in 100 mL of ethanol. *Stability* – Prepare at the time of use.

Safety – Flammable.

Dithiothreitol

(CAS 3483-12 -3)

Formula and molecular mass – $C_4H_{10}O_2S_2$ – 154,26

Description – white crystals.

Solubility Easily soluble in water, acetone and ethanol.

Conservation – In well-closed containers.

Dithizone

CAS – (60-10 -6)

Synonymy – Difeniltiocarbazona.

Formula and molecular mass – $C_{13}H_{12}N_4S$ – 256,32

Specification – Contains, at a minimum, 98.0% (p/p).

Description – dark brown crystalline Powder.

Physical Characteristic – melting Temperature: 168ºC with decomposition.

Solubility Practically insoluble in water and soluble in ethanol.

Conservation – In well-closed containers. *Storage* – Protect from exposure to light.

Dithizone SR

Specification – Contains 0.05 g in 100 mL of carbon tetrachloride.

Conservation – In airtight containers.

Storage – Protect from heat.

Safety – Poison!

Dithizone, concentrated solution

Preparation – Dissolve 35 mg of dithizone in 80 mL of chloroform (for analysis with dithizone). Transfer to 500 mL volumetric flask complete the volume with chloroform.

Conservation – In closed containers and amber. *Storage* – *Protect from* exposure to light and maintain a temperature of 4º to 8 ºC.

Stability – This solution is stable for 5 months.

Dithizone, diluted solution

Preparation – Dilute the concentrated solution of dithizone in chloroform (1:7).

Dithizone, forcing solution

Preparation – Dissolve 30 mg of dithizone in 1000 mL of chloroform and add 5 mL of ethanol. Before use, shake a suitable volume of solution of dithizone extractor with half of its volume of nitric acid 1% (v/v) discarding the acid phase.

Storage – In cooler.

Edetate disodium

(CAS 6381-92 -6)

Synonymy – disodium EDTA; dihydrated disodium Salt of acetic acid (etilenodinitrila)

Formula and molecular mass – $C_{10}H_{14}N_2Na_2O_8.2H_2O$ – 372,24

Specification – Contains, at a minimum, 97.0% (p/p), calculated on the dried substance.

Description – white crystalline Powder, salt to taste weak. *Solubility* – Soluble in water and practically insoluble in ethanol.

Conservation – In well-closed containers.

Category – Chelator.

Edetate disodium, solution 0.05 *M*

Specification – Contains 1.861 g, added to 10 mL of sodium hydroxide M, and dilute with water to 100 mL. *Conservation* – In well-closed containers.

Emodina

(CAS 518-82 -1)

Formula and molecular mass – $C_{15}H_{10}O_5$ – 270,25

Description – Needles red-orange.

Solubility Practically insoluble in water, soluble in ethanol and in hydroxy-alkaline solutions.

Sulfur

(CAS 7704-34 -9)

Element and atomic mass – S – 32,1

Description – light Powder, yellow, gray or yellow *Greenish.*

Escina

(CAS 11072-93 -8)

Specification – Mixture of saponins obtained from seeds *OF Aesculus hippocastanum L.*

Description – amorphous Powder, thin, almost white or reddish or yellowish.

Metallic Tin

(CAS 7440-31 -5)

Element and atomic mass – Sn – 118.71

Specification – Purity of at least 99.5 %.

Description – ash Granules.

Characteristic Physics - Temperature OF Fusion:

Approximately 231.9 ºC.

Conservation – In well-closed containers. *Storage* – Protect from exposure to light and heat. *Security* – Irritant.

Methyl stearate

(CAS 112-61 -8)

Formula and molecular mass – $C_{19}H_{38}O_2$ – 298,50

Description – white crystals or crystalline mass white or pale yellow.

Characteristic Physics –Temperature OF Fusion:

Approximately 38 ºC.

Solubility – Soluble in ethanol and petroleum ether. *Conservation* – In well-closed containers.

Ethyl Ester of tetrabromofenolftaleina

(CAS 1176-74 -5)

Synonymy – Bromofenolftaleina magenta AND

Formula and molecular mass – $C_{22}H_{14}Br_4O_4$ – 661,96

For Estolate erythromycin

(CAS 3521-62 -8)

Formula and molecular mass – $C_{52}H_{97}NO_{18}S$ – 1056,43

Physical Characteristic – Range of fusion:135 ºC to 138 ºC. – Solubility Practically insoluble in water, readily soluble in ethanol, soluble in acetone. It is practically insoluble in dilute hydrochloric acid.

Conservation – In airtight containers.

Storage – Protect from heat and light.

Category – antibiotic.

Strontium Ms. – 1 mg/mL

Specification – Contains 1.685 g of strontium carbonate in 10.0 mL of hydrochloric acid in 50% (v/v). Complete with water to 1000 mL.

Conservation – In well-closed containers, inert (polyethylene type).

Ethanol

(CAS 64-17 -5)

Synonymy – ethyl Alcohol.

Formula and molecular mass – C_2H_6O – 46,07

Specification – Contains, at a minimum, 96.0% (v/v).

Description – Liquid clear, colorless, volatile, characteristic odour.

Physical Characteristics – boiling Temperature: approximately 78 ºC. Density: 0.803 to 0.808. *Miscibility* – Miscible in water and in methylene chloride. *Conservation* – In well-closed containers. *Storage* – Protect from heat.

Security – Toxic. Flammable.

Absolute Ethanol

(CAS 64-17 -5)

Synonymy – anhydrous Alcohol.

Formula and molecular mass – C_2H_6O – 46,07

Specification – Contains at least 99.5% (v/v).

Description – Liquid clear, colorless, volatile, characteristic odour. Hygroscopic.

Physical Characteristics – boiling Temperature: 78-79 °C. Density: 0.790 to 0.794. Index of refraction: (20 ºC): 1.361.

Conservation – In airtight containers.

Storage – Protect from heat and moisture. *Security* – Toxic. Flammable.

Ethanol with glycerin

Preparation – Mix 20 mL of glycerin and 80 mL of ethanol 70% (v/v).

Petroleum Ether

(CAS 8032-32 -4)

Synonymy – benzine.

Description – Liquid clear, colorless, volatile, characteristic odour. Non-fluorescent.

Physical Characteristics – boiling Range: 40 ºC to 60 ºC. Density: 0.630 to 0.656.

Miscibility – Practically insoluble in water and miscible with ethanol.

Conservation – In well-closed containers. *Storage* – Protect from heat.

Safety – Flammable.

Ethyl Ether

(CAS 60-29 -7)

Formula and molecular mass – $C_4H_{10}O$ – 74,12

Specification – Contains, at a minimum, 96.0% (v/v).

Description – clear, colorless Liquid, very volatile, characteristic odour, pungent. Hygroscopic. *Physical Characteristics* – boiling Temperature: approximately 35 ºC. Density: approximately 0.715. Index of refraction (20 ºC): 1.355.

Miscibility – Miscible in water and in ethanol.

Conservation – In well-closed containers.

Storage – Protect from light and heat (do not exceed 15 ºC).

Category – Local Anesthetic.

Safety – Flammable. Risk of explosion!

Isopropylether

(CAS 108-20 -3)

Synonymy – di-isopropyl Ether.

Formula and molecular mass – $C_6H_{14}O$ – 102,17

Description – colorless, clear Liquid.

Physical Characteristics – boiling Range: 67 ºC to 69 ºC. Density (20 ºC): 0.723 to 0.728.

Miscibility – Very little miscible in water and miscible with ethanol.

Conservation – In closed containers.

Storage – Protect from light.

Safety – Flammable

Ethylene Glycol

(CAS 107-21 -1)

Chemical Name – 1,2-Ethanediol

Formula and molecular mass – $C_2H_6O_2$ – 62,07262

Description – viscous Liquid, colorless.

Physical Characteristics – boiling Temperature: around 196 ºC. Density: 1.113 to 1.115.

Miscibility – Miscible in water and in ethanol.

Ethylparaben

(CAS 120-47 -8)

Formula and molecular mass – $C_9H_{10}O_3$ – 166,17

Description – small and colorless crystals or white powder. *Physical Characteristics* – melting Temperature: 116ºC. Boiling Range: 297 ºC to 298 ºC with decomposition. – Solubility Slightly soluble in water. Easily soluble in acetone, ethanol and ethyl ether.

Conservation – In closed containers.

Category – Preservative.

Eugenol

(CAS 97-53 -0)

Formula and molecular mass – $C_{10}H_{12}O_2$ – 164,20

Description – oily Liquid, colorless or slightly yellowish. Darkens, and becomes more viscous, with exposure to light and with the contact with the air.

Physical Characteristics – Density (20 ºC): approximately 1.07. Boiling Temperature: about 250 ºC.

Miscibility – Practically insoluble in water miscible with ethanol, in fatty oils and essential oils.

Fast green (CI 42053)

(CAS 2353-45 -9)

Formula and molecular mass – $C_{37}H_{34}N_2Na_2O_{10}S_3$ – 808,86

Description – Powder or granule dark green, with metallic sheen.

Solubility – Soluble in water and slightly soluble in ethanol.

Conservation – In closed containers.

The blood coagulation Factor Xa beef

Specification – Enzyme that enables the conversion of prothrombin into thrombin. The semi-purified substance is obtained from bovine plasma liquid and is prepared by zimogenio activation of Factor X by means of an appropriate agent, such as the Russell viper venom. *Storage* – The lyophilized preparation should be stored at a temperature of -20ºC. The preparation frozen should be stored at a temperature below -20 ºC.

Factor Xa beef, solution

Preparation – Reconstitute, according to the manufacturer's instructions, and dilute with the buffer solution of tromethamine- sodium chloride, pH 7.4.

Absorbance (2.5.14) – Any modification of the absorbance of the solution at 405 nm, using a buffer of ketorolac tromethamine-sodium chloride pH 7.4, such as white, is not greater than 0.15-0.20 per minute.

1,10-Phenanthroline

(CAS 5144-89 -8)

Synonymy – Orthophenanthroline.

Formula and molecular mass – $C_{12}H_{8\ N2}.\ H_{2\ O}$- 198.22

Description – white crystalline Powder.

Physical Characteristic – Range of fusion: 100 ºC to 104 ºC.

Solubility Slightly soluble in water, soluble in acetone and ethanol.

Category – Indicator for systems of oxireduction and proteins all; reagent for colorimetry.

DL-phenylalanine

(CAS 150-30 -1)

Formula and molecular mass – $C_9H_{11}NO_2$ – 165,19

Specification – Contains, at a minimum, 99.0 %.

Description – monoclinicos crystals.

Physical Characteristic – Sublimated in a vacuum.

Phenol

(CAS 108-95 -2)

Formula and molecular mass – C_6H_6O – 94,11

Specification – Contains, at a minimum, 98.0% (p/p).

Description – Earth crystalline or colorless crystals or faintly pinkish or yellowish, characteristic odour. Deliquescent.

Physical Characteristics – melting Temperature: approximately 43 ºC. Boiling Temperature: approximately 180 ºC.

Solubility – Soluble in water, very soluble in ethanol, glycerol and in methylene chloride.

Conservation – In airtight containers.

Storage – Protect from heat and light.

Labelling – shall indicate the name and quantity of stabilizer.

Safety – Caustic. Toxic.

Category – disinfectant.

Phenolphthalein

(CAS 77-09 -8)

Formula and molecular mass – $C_{20}H_{14}O_4$ – 318,33

Specification – Contains, at a minimum, 97.0% (p/p), calculated on the dried substance.

Description – amorphous, crystalline Powder or white or slightly yellowish. Odourless.

Physical Characteristic – melting Temperature: approximately 258 ºC.

Solubility Practically insoluble in water and soluble in ethanol.

Conservation – In well-closed containers.

Category – acid-base Indicator.

Phenolphthalein to 0.1% (p/v)

Specification – Contains 0.1 g in ethanol 80% (v/v) to 100 mL. *Conservation* – In well-closed containers.

Safety – Flammable.

Additional Information – For preparation of indicator paper.

2-Phenoxyethanol

(CAS 122-99 -6)

Formula and molecular mass – $C_8H_{10}O_2$ – 138,17

Description – colorless Liquid, weakly viscous, aromatic odour weak and fiery flavor.

Physical Characteristics – Density: approx.

Boiling Temperature: approximately 245 ºC. Index of refraction (20 ºC): 1.534.

Misibilidade – Slightly soluble in water, readily soluble in ethanol.

Conservation – In well-closed containers.

Category – Preservative.

Potassium Ferricyanide

(CAS 13746-66 -2)

Formula and molecular mass – $K_3Fe(CN)_6$ – 329,25

Specification – Contains, at a minimum, 99.9% (p/p), calculated on the dried substance.

Description – red crystals.

Solubility Easily soluble in water.

Conservation – In well-closed containers. *Storage* – Protect from light.

Potassium Ferricyanide SR

Specification – Contains 5 g in 330 mL water. *Conservation* – Prepare at the time of use. *Storage* – Protect from light.

Potassium Ferricyanide ammoniacal

Preparation – Dissolve 2 g of potassium ferricyanide in 75 mL of water. Add 25 mL of ammonium hydroxide solution and mix thoroughly.

Potassium ferrocyanide

(CAS 14459-95 -1)

Formula and molecular mass – $K_4Fe(CN)_6.3H_2O$ – 422,39

Specification – Contains, at a minimum, 99.0% (p/p), calculated on the dried substance.

Description – transparent crystals or crystalline powder, yellow. Efflorescent. It Becomes anhydrous at 100 ºC. – Solubility Easily soluble in water and practically insoluble in ethanol.

Conservation – In well-closed containers.

Potassium ferrocyanide SR

Specification – Contains 5.3 g in water 330 mL (approximately 0.125 *M).*

Conservation – Prepare at the time of use.

Fibrinogen

(CAS 9001-32 -5)

See monograph lyophilized human Fibrinogen. Acid Phloroglucin SR

Preparation – Dissolve 1 g of phloroglucinol in ethanol and dilute to 100 mL with the same solvent.

Conservation – In well-closed containers. *Storage* – Protect from light.

Phloroglucinol

(CAS 6099-90 -7)

Formula and molecular mass – $C_6H_6O_3.2H_2O$ – 162,14

Description – Crystals or white crystalline powder, or clear yellow.

Solubility Slightly soluble in water and soluble in ethanol and ethyl ether.

Simulated gastric Fluid

Preparation – Dissolve 2 g of sodium chloride and 3.2 g of pepsin purified in 7 mL of hydrochloric acid and supplement the volume to 1000 mL with water. Has a pH of about 1,2.

Simulated gastric Fluid (without enzyme)

Preparation – Dissolve 2 g of sodium chloride in 100 mL of water. Add 7 mL hydrochloric acid and dilute to 1000 mL with water. Adjust the pH to 1.2 ±0.1 with hydrochloric acid or sodium hydroxide 10 *M.*

Simulated intestinal Fluid without pancreatin pH 7.5

Preparation – Dissolve 6.8 g of monobasic potassium phosphate in 900 mL of water, add 77 mL of sodium hydroxide 0.2 *M* and adjust the pH to 7.5 ±0.1 with sodium hydroxide 0.2 *M. Make up to 1000 mL with water and mix.*

Ammonium Fluoride

(CAS 12125-01 -8)

Formula and molecular mass – NH_4F – 37,04

Description – colorless crystals.

Physical Characteristic – melting Temperature: approximately 100 ºC.

Conservation – Protect from light, heat and humidity. *Security* – Irritant.

Calcium Fluoride

(CAS 7789-75 -5)

Formula and molecular mass – CaF_2 – 78,08

Description – Crystals or white powder.

Conservation – In well-closed containers.

Sodium Fluoride

(CAS 7681-49 -4)

Formula and molecular mass – NaF – 41,99

Description – colorless crystals or white powder or almost white.

Physical Characteristics – Density: 2.78. Melting Temperature: 993 ºC.

Solubility – Soluble in water and practically insoluble in ethanol.

Conservation – In well-closed containers.

Safety – Poisonous!

Sodium Fluoride SR

Preparation – Dry approximately 0.5 g of sodium fluoride to 200 ºC for 4 hours. Weigh exactly 0.222 g of dry material and dissolve water, supplementing the volume to 100 mL. Pipette 10 mL of this solution to 1000 mL volumetric flask and Fill up to volume with water. Each mL of this solution is equivalent to 10 µg of fluorine.

Conservation – In well-closed containers.

Formaldehyde solution

Synonymy – Formaldehyde, formalin.

Formula and molecular mass – CH_2O – 30,03

Specification – Contains, at a minimum, 34.0% (p/v) and, at most, 37.0% (p/v).

Description – colorless Liquid, limpid; irritant vapors. *Physical Characteristics* – Density: approx.

Index of refraction (20 ºC): 1.374.

Conservation – In well-closed containers. *Storage* – Protect from light, air and temperature below 9ºC.

Stability – May contain methanol as a stabilizer. *Security* – Irritant. Toxic.

Category – disinfectant.

Formamide

(CAS 75-12 -7)

Formula and molecular mass – CH_3NO – 45,04

Description – Liquid clear, colorless, viscous, ammoniacal odor weak.

Physical Characteristics – boiling Temperature: approximately 210 ºC. Density: approx.

Index of refraction (20 ºC) 1.447.

Conservation – In airtight containers.

Storage – Protect from moisture.

Security – Irritant.

Ammonium Format

(CAS 540-69 -2)

Formula and molecular mass – CH_5NO_2 – 63,06

Description – Granules and deliquescent crystals. *Physical Characteristic* – Range of fusion: between 119 ºC to 121 ºC. – Solubility Very soluble in water and soluble in ethanol. *Conservation* – In well-closed containers.

Alkaline Phosphatase, solution

Solution – Dissolve 3.1 g of boric acid in 500 mL of water. Add 21 mL of sodium hydroxide *and* 10 mL of magnesium chloride 0.1 M. Dilute with water to 1000 mL. *Preparation* – Dissolve 95 mg of the enzyme alkaline phosphatase in *Solution A*. Dilute to 50 mL with the same solvent.

Ammonium Phosphate dibasic

(CAS 7783-28 -0)

Formula and molecular mass – $(NH_4)_2HPO_4$ – 132,06

Description – Granules or crystals white or nearly white. Hygroscopic.

Physical Characteristic – Has a pH of about 8.0 in aqueous solution at 20 % (p/v).

Solubility Very soluble in water and practically insoluble in ethanol.

Conservation – In well-closed containers.

Ammonium Phosphate monobasic

(CAS 7722-76 -1)

Synonymy – Intermixtures of ammonium phosphate.

Formula and molecular mass – $(NH_4)H_2PO_4$ – 115,03

Description – white crystals or crystalline powder. *Physical Characteristic* – The pH of the solution 0.2 *M* is approximately 4.0.

Solubility Easily soluble in water, slightly soluble in ethanol, insoluble in acetone.

Codeine Phosphate

(CAS 41444-62 -6)

Synonymy – Phosphate of codeine hemi-hydrate.

Formula and molecular mass – $C_{18}H_{21}NO_3.H_3PO_4.1/2H_2O$ – 406,37

Description – crystalline Powder white or almost white, or small and colorless crystals.

Solubility Easily soluble in water. Little or very slightly soluble in ethanol.

Conservation – In well-closed containers.

Potassium Phosphate

(CAS 7778-53 -2)

Formula and molecular mass – K_3PO_4 – 212,27

Synonymy – potassium Phosphate (tribasic).

Description – Crystals or white crystalline powder, deliquescent. *Solubility* – Soluble in water and insoluble in ethanol. *Conservation* – In well-closed containers.

Potassium Phosphate monobasic

(CAS 7778-77 -0)

Synonymy – Dicalcium Phosphate, potassium dihydrogen phosphate, potassium acid phosphate, potassium dihydrogen phosphate, potassium phosphate Sorensen.

Formula and molecular mass – KH_2PO_4 – 136,09

Specification – Contains, at a minimum, 98.0 %, calculated on the dried substance.

Description – colorless crystals or white crystalline powder. *Conservation* – In well-closed containers.

Potassium Phosphate dibasic

(CAS 7758-11 -4)

Synonymy – potassium Phosphate monoacido.

Formula and molecular mass – K_2HPO_4 – 174,18

Description – colorless crystals or white powder or almost white. Very hygroscopic.

Solubility Very soluble in water, very slightly soluble in ethanol.

Conservation – In well-closed containers.

Sodium Phosphate dibasic, anhydrous

(CAS 7558-79 -4)

Formula and molecular mass – Na_2HPO_4 – 141,96

Description – white hygroscopic Powder.

Conservation – In well-closed containers. *Storage* – Protect from moisture.

Dibasic sodium Phosphate, di-hydrate

(CAS 10028-24 -7)

Formula and molecular mass – $Na_2HPO_4.2H_2O$ – 178,00

Specification – Contains, In Minimum, 99.5% (P/p). Calculated on the dried substance.

Description – colorless crystals.

Solubility – Soluble in water and practically insoluble in ethanol.

Conservation – In well-closed containers. *Storage* – Protect from heat and moisture.

Sodium Phosphate, dibasic dodecahydrate

(CAS 10039-32 -4)

Formula and molecular mass – $Na_2HPO_4.12H_2O$ – 358,08

Specification – Contains, In Minimum, 98.5% (P/p). Calculated on the dried substance.

Description – Crystals or granules colorless, transparent, odourless, taste salt, weakly alkaline. Efflorescent. *Conservation* – In well-closed containers. *Storage* – Protect from heat.

Dibasic sodium Phosphate dodecahydrate SR

Specification – Contains 9 g in 330 mL water. *Conservation* – In well-closed containers.

Sodium Phosphate dibasic, heptahydrate

(CAS 7782-85 -6)

Formula and molecular mass – $Na_2HPO_4.7H_2O$ – 268,07

Description – granular Powder or crystal colorless or white. It is stable in air. The aqueous solution is alkaline.

Conservation – In well-closed containers.

Sodium Phosphate dibasic heptahydrate SR

Specification – Contains 12 g of sodium phosphate dibasic heptahydrate in 100 mL of water.

Conservation – In well-closed containers.

Monobasic sodium Phosphate

(CAS 7558-80 -7)

Synonymy – Di-sodium hydrogen orthophosphate.

Formula and molecular mass – NaH_2PO_4 – 119,98

Description – white or almost white Powder. In Hygroscopic Equilibrium. *Conservation* – In airtight containers.

Sodium Phosphate monobasic, monohydrate

(CAS 10049-21 -5)

Formula and mass molecule – $NaH_2PO_4.H_2O$ – 137,99

Description – Crystals or granules white or almost white, a little deliquescent.

Solubility Easily soluble in water and practically insoluble in ethanol. The aqueous solution is acidic.

Conservation – In well-closed containers.

Monobasic sodium Phosphate, di-hydrate

(CAS 13472-35 -0)

Formula and mass molecule –$NaH_2PO_4.2H_2O$ – 156,01

Description – colorless crystals or white powder or almost white.

Physical Characteristic – melting Temperature: 60 ºC.

Solubility Very soluble in water and very slightly soluble in ethanol.

Conservation – In closed containers.

Sodium Phosphate (tribasic), dodecahydrate

(CAS 10101-89 -0)

Synonymy – nedocromil sodium, Phosphate (tribasic) trisodium phosphate.

Formula and molecular mass – $Na_3PO_4.12H_2O$ – 380,12

Description – colorless or white crystals. Efflorescent. *Physical Characteristic* – Melts at 75 ºC for rapid heating.

Solubility Easily soluble in water.

Conservation – In well-closed containers. *Storage* – Protect from heat.

Tetrabutylammonium Phosphate

(CAS 5574-97 -0)

Formula and molecular mass – $C_{16}H_{38}NO_4P$ – 339,46

Description – white or almost white Powder. Hygroscopic. *Solubility* – Soluble in water.

Conservation – In closed container.

Tributyl Phosphate

(CAS 126-73 -8)

Formula and molecular mass – $C_{12}H_{27}O_4P$ – 266,31

Description – colorless Liquid, or slightly yellowish, and odourless.

Miscibility – Little miscible in water.

Equimolar Phosphate 0.05 *M*

Specification – Contains 3.53 g of dibasic sodium phosphate and 3.39 g of potassium phosphate monobasic phosphates in water to 1000 mL.

Conservation – In closed containers.

Phosphate-bromocresol purple SR

Solution – Dissolve 38 g of monobasic sodium phosphate and 2 g of anhydrous dibasic sodium phosphate in water and dilute to 1000 mL with the same solvent. Adjust the pH, if necessary, in 5.3 ±0.1 using sodium hydroxide 5 *M* or phosphoric acid.

Solution B: Dissolve 400 mg of bromocresol purple in 30 mL of water, add 6.3 mL of 0.1 M sodium hydroxide and dilute with water to 500 mL.

Preparation – On the day of use, mix the Solutions *A and B* and chloroform (1:1:1) in separating funnel. Shake and despise the organic phase. Repeat the extraction with equal portions of chloroform until the organic layer if present colorless. Use the aqueous phase.

Red Phosphorus

(CAS 7723-14 -0)

Description – Po dark-red.

Solubility Insoluble in water and dilute acids. Safety – Flammable!

Fructose

(CAS 57-48 -7)

Synonymy – b-D-Fructose, levulose.

Formula and molecular mass – $C_6H_{12}O_6$ – 180,16

Specification – Contains, at a minimum, 98.0 %, calculated on the dried substance.

Description – white crystalline Powder, odourless, strong flavor adocidado.

Physical Characteristic – melting Temperature with decomposition: approximately 103 ºC.

Solubility Very soluble in water and soluble ethanol. Conservation – In well-closed containers.

Fructose 0,1 % (p/v)

Specification – Contains 0.1 g in 330 mL pyridine. *Conservation* – In well-closed containers.

Security – Toxic.

Phthalaldehyde

(CAS 643-79 -8)

Formula and molecular mass – $C_8H_6O_2$ – 134,14 *Description* – yellow crystalline Powder.

Physical Characteristic – melting Temperature: about 55 ºC.

Conservation – In closed containers.

Storage – Protect from exposure to light and the contact with the air.

Phthalate dibutyl phosphates

(CAS 84-74 -2)

Formula and molecular mass – $C_{16}H_{22}O_4$ – 278,3

Dibutylphthalate Synonymy – Ester of phthalic acid, hydrogen phthalate di- n-butyl and dibutyl phthalate.

Description – oily Liquid, clear, colorless or slightly stained.

Physical Characteristics – boiling Temperature: 340ºC. Density: 1.043 to 1.048.

Miscibility – Very slightly soluble in water, very soluble in acetone, benzene, ethanol and ethyl ether.

Ftalazina

(CAS 253-52 -1)

Formula and molecular mass – $C_8H_6N_2$ – 130,15

Description – pale yellow crystals.

Physical Characteristic – Range of fusion: between 90 ºC and 91 ºC. – Solubility Easily soluble in water and soluble in anhydrous ethanol, ethyl acetate and methanol.

Basic Fuchsin (CI 42510)

(CAS 632-99 -5)

Synonymy – Magenta I, hydrochloride of rosalina.

Formula and molecular mass – $C_{20}H_{20}ClN_3$ – 337,85

Description – Glossy crystals of metallic green color. *Physical Characteristic* – Decomposes at temperatures above 200 ºC.

Solubility – Soluble in water and in ethanol.

Conservation – In well-closed containers. *Storage* – Protect from light.

Category – Dye. Antifungal.

Fuchsin pale (Mr.

Synonymy – Schiff's reagent.

Preparation – Dissolve 1 g of basic fuchsin in 600 mL of water, add 100 mL of sodium sulphite anhydrous a 10% (p/v). Cool externally with ice, under agitation. Slowly Add 10 mL of hydrochloric acid, dilute with water to 1000 mL and filter. If the solution darken, shake with 0.2 to 0.3 g of activated charcoal until discoloration, filtering out immediately. If still remain a pinkish color, add 2 to 3 mL of hydrochloric acid and stir. *Conservation* – Leave for 1 hour before use, keep under the light.

Galactose

(CAS 59-23 -4)

Formula and molecular mass – $C_6H_{12}O_6$ – 180,16 Description – white crystalline Powder.

Physical Characteristic – melting Temperature: 167ºC. – Solubility Easily soluble in water.

Conservation – In well-closed containers.

Galactose to 0.1% (p/v) in pyridine

Specification – Contains 0.1 g in 330 mL pyridine. *Conservation* – In well-closed containers. *Storage* – Protect from heat.

Security – Toxic.

Gelatine

(CAS 9000-70 -8)

Specification – is mixture of water soluble proteins obtained by extraction of material containing collagen.

Description – Powder, granules, flakes or transparent sheets, shiny, colorless or slightly yellowish. Hygroscopic, characteristic odour and taste slightly pronounced.

Conservation – In well-closed containers. *Storage* – Protect from heat and moisture.

Glycerin Gelatin

Preparation – Dissolve 1 g of gelatin in 100 mL of water heated to a temperature not exceeding 30 ºC. Add 1 mL of sodium salicylate to 2% (p/v) and 15 mL of glycerol; shake well and filter the mixture heated on glass wool.

Gelatine SR

Preparation – Dissolve 2.5 g of gelatin in 100 mL of hot water. Use after cooling to ambient temperature.

Glycerol

(CAS 56-81 -5)

Synonymy – Glycerine.

Formula and molecular mass – $C_3H_8O_3$ – 92,09

Specification – Contains, at a minimum, 97.0% (p/p).

Description – viscous Liquid, clear, colorless, odorless, hygroscopic, sweetish flavor.

Physical Characteristics – Density: 1.255 to 1.263. Index of refraction (20 ºC): 1.470 to 1.474.

Miscibility – Miscible in water and ethanol, slightly soluble in acetone and practically insoluble in fatty oils and essential oils.

Conservation – In airtight containers.

Storage – Protect from oxidants.

Glycine

(CAS 56-40 -6)

Formula and molecular mass – $C_2H_5NO_2$ – 75,07 – Description white crystalline Powder, odourless.

Physical Characteristic – Range of fusion: 232 ºC to 236 ºC with decomposition.

Solubility Easily soluble in water, slightly soluble in ethanol and very slightly soluble in diethyl ether.

Glucose

(CAS 50-99 -7)

Synonymy – Dextrose.

Formula and molecular mass – $C_6H_{12}O_6$ – 180,16

Description – white crystalline Powder, odourless, sweetish flavor. *Physical Characteristic* – specific optical rotation (20 ºC): + 52.5° to + 53.0º (dissolve 10 g glucose in 100 mL of water and add 0.2 mL of ammonia). Solubility Easily soluble in water and slightly soluble in ethanol.

Conservation – In well-closed containers.

Glucose at 0.1% (w/v) in pyridine

Specification – Contains 0.1 g in 330 mL pyridine. *Conservation* – In well-closed containers. *Storage* – Protect from heat.

Security – Toxic.

Glutaraldehyde

(CAS 111-30 -8)

Formula of molecular mass – $C_5H_8O_2$ – 100,12

Description – oily Liquid.

Physical Characteristics – Index of refraction (25 ºC): approximately 1.434. Boiling Temperature: approximately 188 ºC. *Miscibility* – Miscible in water.

Guaiacol

(CAS 95-05 -1)

Synonymy – 2-methoxyphenol, metilcatecol.

Formula and molecular mass – $C_7H_8O_2$ – 124,14

Description – white or slightly yellowish crystals, or colorless liquid or slightly yellowish. Hygroscopic. *Physical Characteristics* – melting Temperature: about 28 ºC. Boiling Temperature: about 205 ºC. Slightly soluble in water, very soluble in methylene chloride and readily soluble in ethanol.

Conservation – In well-closed containers. *Storage* – Protect from light.

Guanine

(CAS 73-40 -5)

Formula and molecular mass – $C_5H_5N_5O$ – 151,13 *Description* – Powder white or almost white, amorphous. – Solubility Practically insoluble in water, slightly soluble in ethanol. Dissolve in alkaline solutions hydroxy-diluted.

Heparin sodium

(CAS 9041-08 -1)

Description – Consists in mixture of active principles, possessing the property of prolong the clotting time of blood. Obtained, normally, intestinal mucosa, lungs or other tissue suitable for domestic mammals used for food of man.

Solubility Easily soluble in water.

Conservation – In airtight containers.

Labelling – The labelling should indicate the component and the species of origin. The power must be indicated in IU.

Category – anticoagulant.

Heptane

Specification – Contains usually mixture of hydrocarbons – fraction of petroleum – with predominance of *n* -heptane.

Description – Liquid clear, colorless, volatile, highly flammable, characteristic odour.

Physical Characteristics – boiling Range: 95 to 99 ºC. Density: approximately 0.69.

Miscibility – Practically insoluble in water and soluble in absolute ethanol. Miscible in ethyl ether, chloroform, benzene and in most oils volatile and non-volatile.

Conservation – In airtight containers.

Storage – Protect from heat. Keep away from flame/spark.

Safety – Irritating to respiratory tract. Flammable.

n-Heptane

(CAS 142-82 -5)

Formula and molecular mass – C_7H_{16} – 100,20

Specification – Main component of heptane.

Description – clear Liquid and flammable.

Physical Characteristics – boiling Temperature: 98.4 ºC. Density: 0.684. Index of refraction (20 ºC): 1.3855. *Miscibility* – Practically insoluble in water and miscible in anhydrous ethanol.

Sodium Heptanossulfonato

(CAS 22767-50 -6)

Formula and molecular mass – $C_7H_{15}NaO_3S$ – 202,25

Description – Earth crystalline white or nearly white. – Solubility Easily soluble in water and soluble in methanol.

Conservation – In airtight containers.

Hexane

Specification – Contains usually mixture of isomers of C_6H_{14}, predominantly n-hexane and metilciclopentano *(C_6H_{12})*.

Description – Liquid clear, colorless, volatile, highly flammable, characteristic odour.

Physical Characteristics – boiling Range: 67 to 70 ºC. Density: 0.66.

Conservation – In airtight containers.

Storage – Protect from heat. Keep away from flame/spark.

Safety – Irritating to respiratory tract. Flammable.

W-Hexane

(CAS 110-54 -3)

Formula and molecular mass – C_6H_{14} – 86,18

Specification – Main component of petroleum ether and hexane.

Description – clear Liquid, volatile, odour similar to that of petroleum.

Physical Characteristics – boiling Temperature: 69 ºC. Density: 0.66. Index of refraction (20 ºC): 1.375. *Miscibility* – Practically insoluble in water and miscible in anhydrous ethanol.

Conservation – In airtight containers.

Storage – Protect from heat. Keep away from flame/spark.

Safety – Flammable.

Sodium Hexanossulfonato

(CAS 2832-45 -3)

Formula and molecular mass – $C_6H_{13}NaO_3S$ – 188,22

Description – white or almost white Powder.

Hexilamina

(CAS 111-26 -2)

Synonymy – Hexanamina.

Formula and molar mass – $C_6H_{15}N$ – 101,19

Description – colorless Liquid.

Physical Characteristics – Density (20 ºC): approximately 0.766. Index of refraction (20 ºC): approximately 1.418. Boiling Temperature: 127ºC to 131 ºC.

Miscibility – Slightly soluble in water and soluble in ethanol.

Chloral Hydrate

(CAS 302-17 -0)

Synonymy – Chloral hydrate.

Formula and molecular mass – $C_2H_3Cl_3O_2$ – 165,40

Specification – Contains, at a minimum, 98.5% (p/p).

Description – Crystals transparent, colorless, pungent odour characteristic and fieriness and weakly bitter. Deliquescent.

Physical Characteristic – melting Temperature: 57 ºC. – Solubility Very soluble in water and readily soluble in ethanol.

Conservation – In well-closed containers. *Storage* – Protect from heat and light.

Safety – Irritating to skin.

Category – Sedative, hypnotic.

Hydrazine hydrate,

(CAS 7803-57 -8)

Formula and molecular mass – $N_2H_4.H_2O$ – 50,06

Description – colorless, clear Liquid.

Miscibility – Miscible in water.

Conservation – In well-closed containers.

Ammonium hydroxide

Use ammonia, concentrated solution.

Barium hydroxide

(CAS 12230-71 -6)

Formula and molecular mass – $Ba(OH)_2.8H_2O$ – 315,46

Description – colorless crystals.

Physical Characteristic – melting Temperature: 78 ºC. *Solubility* – Soluble in water.

Conservation – In well-closed containers.

Calcium hydroxide

(CAS 1305-62 -0)

Formula and molecular mass – $Ca(OH)_2$ – 74,09 *Specification* – Contains, at a minimum, 93.0% (p/p).

Description – soft white Powder or granules, odourless. – Solubility Practically insoluble in water. *Conservation* – In well-closed containers. – Protect Storage of carbon dioxide.

Calcium hydroxide, saturated solution

Use calcium hydroxide SR.

Calcium hydroxide SR

Specification – Contains 0.15 g in carbon dioxide free water 330 mL (saturated solution).

Conservation – In well-closed containers.

Stability – Prepare at the time of use. – Protect Storage of carbon dioxide. *Category* – Astringent.

Lithium hydroxide

(CAS 1310-66 -3)

Formula and molecular mass – $LiOH.H_2O$ – 41,96 Description – granular Powder white, or nearly white. *Solubility* – Soluble in water, forming a strongly alkaline solution. Slightly soluble in ethanol. *Conservation* – In well-closed containers.

Safety – Corrosive.

Potassium hydroxide

(CAS 1310-58 -3)

Formula and molecular mass – KOH – 56,11 *Specification* – Contains, at a minimum, 85.0% (p/p), calculated as KOH, and, at most, 3.5% of K_2CO_3.

Description – white Grease, hard, dry, crystalline structure, odorless, very hygroscopic and avid for CO_2. Liquefies – if the air. Presented in the forms of lentils, cylinders or scales.

Solubility Very soluble in water and readily soluble in ethanol.

Conservation – In airtight containers, inert.

Storage – Protect from moisture and carbon dioxide.

Safety – Very caustic.

Ethanolic potassium hydroxide SR (approximately 0.5 M)

Preparation – Dissolve 34.04 g of potassium hydroxide in 20 mL of water; completing the 1000 mL with ethanol-free (aldehyde). 24 Hours Rest In airtight containers. Decant. Use the supernatant clear. *Conservation* – In airtight containers.

Storage – Protect from light.

Ethanolic potassium hydroxide 2 M

Preparation – Dissolve 6.6 g of potassium hydroxide in 5 mL of water, cool and make up the volume to 50 mL with ethanol. Decant for 24 hours and use the supernatant clear.

Sodium hydroxide

(CAS 1310-73 -2)

Synonymy – Caustic soda.

Formula and molecular mass – NaOH – 40,00.

Specification – Contains, at a minimum, 95.0% (p/p) of total alkali, calculated as NaOH, and, at most, 3.0% (p/p) of Na_2CO_3.

Description – Earth lasts, crystalline structure, white in the form of lumps, lentils and sticks. Deliquescent and absorb carbon dioxide.

Solubility Very soluble in water and readily soluble in ethanol.

Conservation – In airtight containers.

Storage – Protect from moisture and carbon dioxide.

Safety – Caustic, corrosive.

Sodium hydroxide SR

Specification – Contains 8% (p/v) of NaOH in water. *Conservation* – See sodium hydroxide *M.*

M sodium hydroxide

Specification – Contains 40 g in carbon dioxide free water to 1000 mL.

Conservation – In glass containers or alkali-resistant polyethylene.

Storage – Protect from moisture and carbon dioxide.

Sodium hydroxide, concentrated solution SR (approximately 10 *M)*

Specification – Contains 20.0 g of sodium hydroxide in water to 165 mL.

Conservation – In well-closed containers. – Protect Storage of carbon dioxide. *Safety* – Caustic.

Tetrabutylammonium hydroxide

(CAS 2052-49 -5)

Formula and molecular mass – $(C_4H_9)_4NOH$ – 259,47

Description – white crystals or almost white. *Solubility* – Soluble in water.

Tetramethylammonium hydroxide

(CAS 75-59 -2)

Formula and molecular mass – $C_4H_{13}NO$ – 91,15 Description – is a stronger base than ammonia and quickly absorb carbon dioxide from the air. A preparation in aqueous medium at 25% (p/v), is clear and colorless. *Physical Characteristic* – melting Temperature: 63 ºC. *Conservation* – In well-closed containers.

D- " -4-hidroxifenilglicina

(CAS 22818-40 -2)

Formula and molecular mass – $C_8H_9NO_3$ – 167,16

Description – Leaflets shiny.

Physical Characteristic – Range of decomposition: between 220ºC and 247ºC.

Solubility Slightly soluble in water, ethanol, diethyl ether and acetone. Soluble in mineral acids and alkali.

Hydroxyquinoline

(CAS 148-24 -3)

Synonymy – 8-hydroxyquinoline *Formula and molecular mass* – C_9H_7NO – 145,16 –

Description white crystalline Powder, or slightly yellowish. Physical Characteristic – melting Temperature: around 75ºC.

Solubility Slightly soluble in water, readily soluble in acetone, ethanol and in dilute solutions of mineral acids.

Butylated Hydroxytoluene sets

(CAS 128-37 -0)

Synonymy – BHT.

Formula and molecular mass – $C_{15}H_{24}O$ – 220,34

Specification – Contains, at a minimum, 99.0% (p/p).

Description – crystalline Powder white or yellowish. *Physical Characteristics* – freezing Temperature: not less than 69.2 ºC. Boiling Temperature: 265ºC. Density: 1.048.

Solubility Practically insoluble in water, freely soluble in acetone, readily soluble in ethanol and in vegetable oils.

Safety – Can cause contact dermatitis.

Hiperosideo

(CAS 482-36 -0)

Formula and molecular mass – $C_{21}H_{20}O_{12}$ – 464,38

Description – pale yellow Needles.

Physical Characteristic – melting Temperature: approximately 240 °C with decomposition.

Solubility – Soluble in methanol.

Sodium Hypochlorite

(CAS 7681-52 -9)

Formula and molecular mass – NaClO – 74,44

Description – white crystals. It is Usually obtained in the form penta-hydrated, being that its anhydrous form is explosive.

Physical Characteristic – melting Temperature: 18 ºC (form penta-hydrated)

Solubility Very soluble in water.

Conservation – In well-closed containers.

Safety – Annoying!

Sodium Hypochlorite SR

See monograph sodium Hypochlorite diluted solution.

Sodium Hipofosfito

(CAS 10039-56 -2)

Formula and molecular mass – $NaH_2PO_2\,H_2O$ – 105,99

Specification – Contains, at a minimum, 99.0% (p/p), calculated on the dried substance.

Description – granular Powder or colorless crystals or white crystalline powder, odourless, taste salt. Hygroscopic. – Solubility Easily soluble in water and soluble in ethanol.

Conservation – In well-closed containers. *Storage* – Protect from heat.

Sodium Hipofosfito SR

Specification – Contains 5 g of hipofosfito sodium in 10 mL of water, plus 165 mL with hydrochloric acid. Separate any crystals formed. The solution should be clear and colorless.

Imidazole Solution

(CAS 288-32 -4)

Synonymy – Glioxalina.

Formula and molecular mass – $C_3H_4N_2$ – 68,08

Description white crystalline Powder.

Physical Characteristic – melting Range: 90 to 91 ºC. *Solubility* – Soluble in water and in ethanol.

Iminodibenzila

(CAS 494-19 -9)

Formula and molecular mass – $C_{14}H_{13}N$ – 195,26

Description – pale yellow crystalline Powder.

Physical Characteristic – melting Temperature: around 106 ºC. – Solubility Practically insoluble in water and readily soluble in acetone.

Conservation – In well-closed containers.

Potassium Iodate

(CAS 7758-05 -6)

Formula and molecular mass – KIO_3 – 214,00

Description – white crystals or crystalline powder, odourless. *Physical Characteristic* – melting Temperature: around 560 ºC, with partial decomposition.

Solubility – Soluble in water, insoluble in ethanol. *Category* – oxidizing Agent.

Mercury iodide (II)

(CAS 7774-29 -0)

Synonymy – Bi-iodide of mercury, mercury iodide red.

Formula and molecular mass – HgI_2 – 454,40

Description – crystalline Powder, scarlet red, dense, almost odourless and tasteless.

Physical Characteristic – melting Temperature of 259 ºC. – Solubility Slightly soluble in water, slightly soluble in acetone and ethanol, soluble in a solution of potassium iodide in excess.

Conservation – In well-closed containers. *Storage* – Protect from light.

Category – Poison!

Potassium iodide

(CAS 7681-11 -0)

Formula and molecular mass – KI – 166,00

Specification – Contains, at a minimum, 99.0% (p/p), calculated on the dried substance.

Description – colorless crystals or white crystalline powder, odourless, salty flavor and bitter. Weakly deliquescent.

Physical Characteristic – melting Temperature: 680ºC. – Solubility Very soluble in water, readily soluble in glycerol, soluble in ethanol.

Conservation – In well-closed containers. *Storage* – Protect from light and moisture.

Potassium iodide approximately *M*

Use potassium iodide SR.

Potassium iodide SR

Specification – Contains 16.5 g of potassium iodide in 330 mL water.

Conservation – In opaque containers well closed. *Storage* – Protect from light.

Potassium iodide mercuric alkaline SR

Synonymy – Nessler reagent, alkaline solution of tetraiodomercurato (II) of potassium, potassium iodide-mercury chloride SR.

Preparation – Dissolve 5 g of potassium iodide in 5 mL of water, add little to the little solution of chloride of mercury (II) to 25% (p/v), controlling-if the addition, for that

The PRECIPITATE formed at the outset is completely dissolved. Let cool. Then, add potassium hydroxide solution at 50% (p/v), dilute with water to make the volume of 100 mL and add 0.5 mL of the solution of chloride of mercury (II) to 25% (p/v). Decant and use the supernatant.

Potassium iodide mercuric alkaline SR1

Alternate Name – Tetraiodomercurato potassium alkali SR.

Preparation – Dissolve in water, 11 g of potassium iodide and 15 g of sodium iodide of mercury (II) and make up the volume to 100 mL with the same solvent. Immediately before use, mix the previous solution with an equal volume of sodium hydroxide at 25% (p/v).

Potassium iodide mercury SR

Synonymy – Reagent for Mayer.

Solution – Dissolve 13.5 g of chloride of mercury (II) in 600 mL of water.

Solution B: Dissolve 50 g of potassium iodide in 100 mL of water.

Preparation – Mix the Solutions *A and B* and complete the volume to 1000 mL with water.

Sodium iodide

(CAS 7681-82 -5)

Formula and molecular mass – NaI – 149.89

Specification – Contains, at a minimum, 99.0% (p/p), calculated in relation to the substance dried.

Description – white crystalline Powder or colorless crystals, hygroscopic, odorless.

Solubility Very soluble in water and readily soluble in ethanol.

Conservation – In airtight containers.

Sodium iodide in acetic acid

Specification – Contains 10 g of glacial acetic acid to 165 mL.

Conservation – In well-closed containers. *Storage* – Protect from light.

Tetrabutylammonium iodide

(CAS 311-28 -4)

Synonymy – iodide of tetra-n-butilamonio.

Formula and molecular mass – $C_{16}H_{36}IN$ – 369,38

Description – Crystals or white crystalline powder or slightly colored.

Solubility Slightly soluble in water and soluble in ethanol.

Indigo carmine

(CAS 860-22 -0)

Formula and molecular mass – $C_{16}H_8N_2NaO_8S_2$ – 466,36

Description – blue Granules with brightness of copper, or powder blue or violet-blue.

Solubility Slightly soluble in water, practically insoluble in ethanol. Precipitates in aqueous solutions of sodium chloride.

Indigo carmine SR

Preparation – In a mixture of 10 mL of hydrochloric acid and 990 mL of sulfuric acid to 20% (p/v), add 0.2 g of indigo carmine.

Iodine

(CAS 7553-56 -2)

Formula and molecular mass – I_2 – 253,80

Description – Scales, plates or small crystals, black bluish or violet grayish; metallic sheen, pungent odor.

Physical Characteristics – Sublimates slowly to ambient temperature; heated, releases vapors violet. Melting Temperature: 113.6 °C

Solubility: Very slightly soluble in water, soluble in ethanol, and slightly soluble in glycerol.

Conservation – In airtight containers of glass. *Safety* – corrosive Vapors!

Iodine SR

Synonymy – aqueous Solution of iodine – Iodinated, reactive lugol's iodine.

Specification – Contains 1 g of iodine and 2 g of potassium iodide in 330 mL water.

Preparation – Dissolve 1 g of iodine in 100 mL of water, add 2 g of potassium iodide, shake, allow to rest for a few hours and filter on glass wool. *Conservation* – In glass containers amber well closed.

Storage – Protect from light.

Iodine 0.05 *M*

Preparation – Dissolve 20 g of potassium iodide in minimal amount of water, add 13 g of iodine, then add water to produce 1000 mL.

Iodine 0.5 % (w/v) in chloroform

Specification – Contains 0.5 g of iodine in 330 mL chloroform.

Conservation – In well-closed containers. *Storage* – Protect from light.

Security – Toxic.

Iodine 1 % (p/v) in ethanol

Synonymy – alcoholic Solution of iodine, ethanolic solution of iodine.

Specification – Contains 1% (w/v) of iodine in ethanol. *Conservation* – In glass containers as well closed. *Storage* – Protect from light.

Safety – Flammable.

Potassium iodobismuthate

Use iodobismutato potassium aqueous-acetic acid.

Potassium iodobismuthate aqueous-acetic acid

Preparation – Mix 58 mL of water, 1.21 g of bismuth subnitrate, 14 mL of glacial acetic acid and 28 mL of potassium iodide solution to 40% (p/v).

Potassium iodobismuthate diluted SR

Preparation – Dissolve 100 g of tartaric acid in 500 mL of water. Separately, dissolve 100 g of tartaric acid in 400 mL of water and add 8.5 g of bismuth subnitrate. Stir for one hour, add 200 mL of potassium iodide solution to 40% (p/v) and shake well. Leave to rest for 24 hours and filtered. Mix the first solution with 50 mL of the second.

Potassium iodide and bismuth subnitrate SR

Synonymy – Dragendorff reagent

Preparation – Mix equal volumes of potassium iodide solution to 40% (p/v) in water and the solution prepared by

dissolving 0.85 g of bismuth subnitrate in a mixture of 10 mL of glacial acetic acid and 40 mL of water. Dilute

Volume of the mixture with 2 volumes of glacial acetic acid and 10 volumes of water immediately before use. Storage – Protect from light.

Potassium iodobismuthate SR

Preparation – Dissolve 16.6 g of tartaric acid in 67 mL of water and add 1.41 g of bismuth subnitrate. Shake for one hour, add 33 mL of potassium iodide solution to 40% (p/v). Shake for one more hour. Leave to rest for 24 hours. Filter.

Conservation – In well-closed containers. *Storage* – Protect from light.

Potassium iodobismuthate SR1

Preparation – Dissolve 10 g of tartaric acid in 40 mL of water and add 0.85 g of bismuth subnitrate. Stir for one hour. Add 20 mL of potassium iodide solution to 40% (p/v) and mix thoroughly. Leave to rest for 24 hours and filtered.

Potassium iodobismuthate SR2

Preparation – Suspend 1.7 g of bismuth subnitrate and 20.0 g of tartaric acid in 40 mL of water. Add, suspension, 40 mL of potassium iodide solution to 40% (p/v). Stir for one hour and filtered. Protect the solution from light exposure. Immediately before use, mix 5 mL of previous solution with 15 mL of water.

Conservation – In well-closed containers. *Storage* – Protect from exposure to light.

Iodinessulfuroso SR

Preparation – Use round flask 3000 mL to 4000 mL, with three necks, fitted with a stirrer, a thermometer and a drying tube. The balloon must be dry and closed during the preparation. Mix 700 mL OF anhydrous pyridine with 700 mL of methoxyethanol; join, with stirring, 220 g of iodine, finely pulverized and dry previously, under phosphorus pentoxide. Agitation must be maintained until complete dissolution (for about 30 minutes.). Cool Down to -10ºC and in agitation, quickly enter 190 g of sulfur dioxide liquid. The temperature should not exceed 30 ºC. Cool.

Determination – Determine the title at the time of use, always working under the moisture. Enter in an erlenmeyer flask approximately 20 mL of anhydrous methanol and proceed to Determination *of water* by the method semi-micro (5.2.20.3), with the sample, up to the end point of the titration. Enter into a quantity of water exactly measured and log a new titration. Calculate the water equivalent of the sample, in milligrams per millilitre. Each milliliter of iodossulfuroso SR corresponds, at the very least, the 3.5 mg H_2O.

Conservation – In dry container.

Irganox 1010

CAS – [*6683-19-8*)

Formula and molecular mass – $C_{73}H_{108}O_{12}$ – 1177,81

Description – Powder white to slightly yellowish. Odorless, tasteless.

Physical Characteristics – Range of fusion: 110 ºC to 125 ºC. Crystallizes in two forms: alpha form, melting range 120 ºC to 125 ºC; and form beta, melting range 110 ºC to 115ºC The melting range varies according to the ratio of the crystalline forms in the mixture; this proportion does not influence the efficiency of the product.

Additional Information – Stabilizer for organic substances, such as polyethylene and polypropylene, protecting them against thermo-oxidative degradation.

Irganox 1076

(CAS 2082-79 -3)

Formula and molecular mass – $C_{35}H_{62}O_3$ – 530,97

Description – Powder white to slightly yellowish. Odourless, stable to light.

Physical Characteristic – Range of fusion: 49 ºC to 54 ºC *additional Information* – Antioxidant for organic substrates, such as polyethylene and polypropylene, protecting them from thermal-oxidative degradation.

Irganox PS 800

(CAS 123-28 -4)

Formula and molecular mass – $C_{30}H_{58}O_4S$ – 514,94

Description – white crystals.

Physical Characteristic – Range of fusion: 38 ºC to 40 ºC *additional Information* – Stabilizer of polyolefins, especially polypropylene and high density polyethylene.

Iso-octane

(CAS 540-84 -1)

Synonymy – 2,2,4-Trimethylpentane.

Formula and molecular mass – C_8H_{18} – 114,23

Description – colorless Liquid and flammable.

Physical Characteristics – Density (20 ºC): 0.691 to 0.696. Index of refraction (20 ºC): 1.391 to 1.393.

Miscibility – Practically insoluble in water and soluble in ethanol.

Conservation – In closed containers.

Fluorescein isothiocyanate

(CAS 27072-45 -3)

Formula and molecular mass – $C_{21}H_{11}NO_5S$ – 389,38 *Specification* – Mixture of isomers: 5-isothiocyanate and 6-isothiocyanate.

Description – Solid orange, decomposes with heating.

Lactose

(CAS 5989-81 -1)

Synonymy – Lactose monohydrate.

Formula and molecular mass – $C_{12}H_{22}O_{11}.H_2O$ – 360,31

Description – white crystalline Powder or granules. Odourless, weak sweetish flavor.

Physical Characteristics – specific optical Rotation (20 ºC): +52,2° to + 52.8º (determined in solution of anhydrous lactose 0.1 g/mL). Melting Temperature: 202ºC *Conservation* – In well-closed containers.

Additional Information – Adsorbs strange odors.

Lactose to 0.1% (p/v) in pyridine

Specification – Contains 0.1% (w/v) in pyridine. *Conservation* – In well-closed containers.

Security – Toxic

Methyl Laurate

(CAS 111-82 -0)

Formula and molecular mass – $C_{13}H_{26}O_2$ – 214,40

Specification – Contains, at a minimum, 98.0% (p/v).

Description – Liquid colorless or yellowish.

Physical Characteristics – Density: approximately 0.870. Index of refraction (20 ºC): approximately 1.431. Melting Temperature: approximately 5 °C *Conservation* – In well-closed containers.

Sodium lauryl sulphate

(CAS 151-21 -3)

Synonymy – nedocromil sodium dodecyl Sulfate, sodium dodecylsulphate was also sodium.

Formula and molecular mass – $C_{12}H_{25}NaO_4S$ – 288,38

Specification – Mixture of, at a minimum, 85.0% (p/p), alquilsulfatos sodium, consisting mainly of sodium lauryl sulphate $[CH_3(CH_2)_{10},H_2SO_4,Na]$.. The combined content of NaCl e Na_2SO_4 is, at most, 8,0% (p/p).

Description – Po, scales or white crystals or clear yellow; weak and characteristic odor.

Solubility Easily soluble in water, and partially soluble in ethanol.

Conservation – In well-closed containers.

Sodium lauryl sulphate SR

Description – Contains 1 g in 100 mL of water.

Conservation – In well-closed containers.

Lecithin

Specification – Mixture of diglicerideos, mainly of stearic acid, palmitic acid and oleic acid, phosphoric ester linked to the hill. Structure and composition variables according to the source of production.

Description – fatty Mass brownish yellow to brown, weak characteristic odor.

Conservation – In well-closed containers.

Labelling – Specify origin.

Nickel-aluminum Alloy

Description – fine Powder gray.

Solubility Practically insoluble in water, soluble in mineral acids with formation of salt.

Linalool

(CAS 78-70 -6)

Formula and molecular mass – $C_{10}H_{18}O$ – 154,25

Description – Liquid. Mixture of two stereoisomers (licareol and coriandrol).

Physical Characteristics – Density (20 ºC): approximately 0.860. Boiling Temperature: about 200 ºC. Index of refraction (20 ºC): approximately 1.462.

Solubility Practically insoluble in water.

Lithium

(CAS 7439-93 -2)

Element and atomic mass – Li – 6.94 *Solubility* – Reacts violently with water. Soluble in methanol, forming metoxido of lithium. Practically insoluble in petroleum ether.

Lithium Ms. – 2 mg/mL

Specification – Contains 1.064 g of lithium carbonate in 5 mL of hydrochloric acid. Complete with water to 100 mL. *Conservation* – In well-closed containers, inert (polyethylene type).

Macrogol 300

(CAS 25322-68 -3)

Synonymy – PEG 300, polyethylene glycol 300.

Formula and molecular mass – $H(OCH_2CH_2)_nOH$ – molecular Weight not less than 95% of the nominal value labeled. Displays the average number of oxyethylene groups: n = 6 or 7. *Specification* – Mixture of polycondensation products of ethylene oxide and water.

Description – viscous Liquid, clear, colorless or almost, weak and characteristic odor, hygroscopic.

Physical Characteristics – Density: approximately 1.125. Index of refraction (20 ºC): approximately 1.465. Viscosity: approximately 80 cP.

Conservation – In airtight containers.

Labelling – Must contain the average molecular mass. *Storage* – Protect from moisture.

Macrogol 1000

(CAS 25322-68 -3)

Synonymy – PEG 1000, polyethylene glycol 1000.

Formula and molecular mass – $H(OCH_2CH_2)_nOH$ – molecular Weight not less than 95% of the nominal value labeled. Description – Solid white or almost white with a waxy appearance. Hygroscopic.

Physical Characteristics – Density: approximately 1.080. Freeze Range: between 35 ºC and 40 ºC. – Solubility Very soluble in water, readily soluble in ethanol and in methylene chloride. Practically insoluble in fatty oils and mineral oils.

Conservation – In airtight containers.

Labelling – Must contain the average molecular mass. *Storage* – Protect from moisture.

Magnesium Ms. – 1 mg/mL

Specification – Contains 9 g of magnesium chloride in water to 500 mL.

Standardization – In 25 mL of this solution, add 25 mL of water, 10 mL of ammonium chloride buffer pH 10.7 and 0.1 g of eriochrome black-t indicator T. Holder with edetate disodium 0.05 *M* SV Each mL of titrant corresponds to 0.001215 g of Mg. For use dilute the concentration of 1 mg/mL. *Conservation* – In well-closed containers, inert (polyethylene type).

Magneson

(CAS 74-39 -5)

Formula and molecular mass – $C_{12}H_9N_3O_4$ – 259,22

Description – Po reddish brown.

Category – Indicator for magnesium and molybdenum.

Melamine

(CAS 108-78 -1)

Formula and molecular mass – $C_3H_6N_6$ – 126,12

Description – amorphous Powder, white or almost white. *Solubility*: Very slightly soluble in water and ethanol.

2-Mercaptoethanol

(CAS 60-24 -2)

Formula and molecular mass – C_2H_6OS – 78,14

Description – Liquid clear and colorless.

Physical Characteristics – Density (20 ºC): approximately 1.116. Boiling Temperature: about 157 ºC. *Miscibility* – Miscible in water.

Mercury

(CAS 7439-97 -6)

Element and atomic mass – Hg – 200,59

Specification – liquid Metal, mobile, dense, silver, mirrored surface.

Physical Characteristics – Density: approximately 13.5. Boiling Temperature: approximately 357 ºC. *Conservation* – In well-closed containers.

Safety – Poison! Volatile at ambient temperature.

Mercury Ms. – 1 mg/mL

Specification – Contains 1.080 g of mercuric oxide dissolved into the smallest possible volume of hydrochloric acid 2 *M*. Complete with water to 1000 mL.

Conservation – In well-closed containers, inert (polyethylene type).

Nedocromil sodium Metabisulphite

(CAS 7681-57 -4)

Synonymy – sodium Disulphite, sodium pyrosulphite. *Formula and molecular mass* – $Na_2S_2O_5$ – 190,10

Specification – Contains, at least, 95% (p/p). Contains sodium metabisulphite quantity equivalent to, at the very least, 65.0% and, at most, 67.4% of $ONLY_2$.

Description – colorless crystals or white crystalline powder or white-cream, smell sulfurous acids and acidic flavor and salt. – Solubility Easily soluble in water and slightly soluble in ethanol.

Conservation – In well-closed containers, well filled.

Storage – Protect from excessive heat, air and moisture.

Stability – Oxidizes slowly to sulfate, on exposure to air and moisture, with disintegration of the crystals.

Methanol

(CAS 67-56 -1)

Synonymy – methyl Alcohol.

Formula and molecular mass – CH_4O – 32,04

Specification – Contains at least 99.5% (p/v).

Description – Liquid clear, colorless, flammable, characteristic odour.

Physical Characteristics – boiling Temperature: 64 ºC to 65 ºC. Density: 0.790 to 0.793. Index of refraction (20 ºC): 1.328 to 1.330.

Conservation – In airtight containers.

Security – Toxic. Flammable.

Methenamine

(CAS 100-97 -0)

Synonymy – Hexamethylenetetramine.

Formula and molecular mass – $C_6H_{12}N_4$ – 140,19

Specification – Contains, at a minimum, 99.0% (p/p), after drying under phosphorus pentoxide during 4 hours.

Description – colorless crystalline Powder.

Physical Characteristics – Sublimates without fusing and with partial decomposition to approximately 263 ºC. The pH of the solution to 0.2 *M: 8.4.*

Solubility Very soluble in water.

Conservation – In well-closed containers.

Category – urinary Antiseptic.

Methylcellulose 450

(CAS 9004-67 -5)

Specification – Cellulose partially O-methylated with a viscosity of 450 mPa/second.

Description – Granule or powder white, or white to yellowish or grayish white. Hygroscopic.

Solubility Practically insoluble in hot water, acetone, ethanol and toluene.

Methylenebis-N,N-dimethylaniline

(CAS 101-61 -1)

Synonymy – Tetrametildiaminodifenilmetano.

Formula and molecular mass – $C_{17}H_{22}N_2$ – 254,37

Description – white crystals or leaflets, or white- glasses.

Physical Characteristic – Range of fusion: 90 ºC to 91 ºC. – Solubility Practically insoluble in water, slightly soluble in ethanol and soluble in mineral acids. *Conservation* – In closed containers.

Methylenebisacrylamide

(CAS 110-26 -9)

Synonymy – N,N' - methylenebisacrylamide, metilenobispropenamida.

Formula and molecular mass – $C_7H_{10}N_2O_2$ – 154,19

Description – fine Powder white or nearly white. *Physical Characteristic* – melting Temperature: above 300 ºC with decomposition.

Methyl-ethyl-ketone

(CAS 78-93 -3)

Synonymy – Ethyl-methyl ketone; 2-butanone.

Formula and molecular mass – C_4H_8O – 72,11

Description – Liquid clear and colorless. Characteristic Odor of acetone.

Physical Characteristics – Density (20 ºC): approximately 0.81. Boiling Temperature: 79.6 ºC.

Conservation – In airtight containers.

Security – Toxic. Flammable.

Methylisobutylketone

(CAS 108-10 -1)

Synonymy – 4-Methyl-2-pentanona, isopropilacetona.

Formula and molecular mass – $C_6H_{12}O$ – 100,16

Description – colorless Liquid odor ketotic and CMCP. *Physical Characteristics* – boiling Temperature: around 115 °C

Methylparaben

(CAS 99-76 -3)

Chemical Name – methyl Ester of 4-hydroxybenzoic acid

Formula and molecular mass – $C_8H_8O_3$ – 152,15

Description – white crystals, slightly soluble in water, readily soluble in acetone, ethanol and ethyl ether. *Solubility*: Very slightly soluble in water and readily soluble in ethanol and methanol.

Category – Preservative.

4-Metilpentan-2-ol

(CAS 108-11 -2)

Formula and molecular mass – $C_6H_{14}O$ – 102,17

Description – colorless Liquid, limpid and volatile. *Physical Characteristics* – Density (20 ºC): approximately 0.802. Index of refraction (20 ºC): approximately 1.411. Boiling Temperature: about 132 ºC.

3-Methyl-2-pentanona

(CAS 565-61 -7)

Formula and molecular mass – $C_6H_{12}O$ – 100,16

Description – colorless Liquid and flammable.

Physical Characteristics – boiling Temperature: approximately 118 ºC. Density (20 ºC): approximately 0.815. Index of Refraction (20 ºC): approximately 1.400.

Conservation – In closed containers.

Metoxiazobenzeno

(CAS 2396-60 -3)

Formula and molecular mass – $C_{13}H_{12}N_2O$ – 212,3

Description – Blades orange.

Solubility Practically insoluble in water, soluble in ethanol, in petroleum ether and other organic solvents. Thin layer chromatography – Apply, in silica gel plate G, solution of 5 mg of metoxiazobenzeno in benzene and develop chromatogram with the same solvent. Appears a single spot with Rf at around 0.6.

Metoxiazobenzeno SR

Specification – Solution to 0.2% (w/v) in a mixture of 1 volume of benzene and 4 volumes of petroleum ether.

Potassium Metoxido

(CAS 865-33 -8)

Formula and molecular mass – CH_3OK – 70,13 Use – extemporaneous Preparation.

Sodium Metoxido

(CAS 124-41 -4)

Formula and molecular mass – CH_3ONa – 54,02

Description – fine white Powder. Reacts violently with water with formation of heat. Sensitive to air. It may be in the form of: CH_3ONa. $2CH_3OH$, white powder. In solution can be prepared *in situ.*

Solubility – Soluble in ethanol and methanol. *Conservation* – In airtight containers.

Storage – Protect from moisture.

Methoxyethanol

(CAS 109-86 -4)

Synonymy – 2-Methoxyethanol, ethylene glycol monomethyl ether.

Formula and molecular mass – $C_3H_8O_2$ – 76,09

Description – colorless, clear Liquid.

Physical Characteristics – Density (20 ºC): approximately 0.9663. Index of refraction (20 ºC): approximately 1.4028. Boiling Temperature: about 125 ºC.

Miscibility – Miscible in water, acetone and ethanol. *Conservation* – In well-closed containers.

Safety – Poisonous! Use in environments with adequate ventilation.

Methyl Myristate

(CAS 124-10 -7)

Formula and molecular mass – $C_{15}H_{30}O_2$ – 242,40

Specification – Contains, at a minimum, 98.0% (p/v).

Description – colorless Liquid or weakly yellowish. *Physical Characteristics* – Density: approximately 0.868. Index of refraction (20 ºC): approximately 1.437. Melting Temperature: approximately 20 ºC. *Miscibility* – Miscible in ethanol and petroleum ether. *Conservation* – In well-closed containers.

Mixture of eriochrome black-T

Preparation – Mix 0.2 parts of eriochrome black-T with 100 parts of sodium chloride.

Conservation – In well-closed containers.

Category – Indicator for calcium and magnesium.

Reducing mixture

Preparation – Spray the substances, which are added in the following order, so as to obtain a homogeneous mixture: 20 mg of potassium bromide, 0.5 g of hydrazine sulfate and 5 g of sodium chloride.

Chromo

Preparation – Dissolve 50 g of potassium dichromate in about 50 mL of water and add 1000 mL of sulfuric acid.

Conservation – In well-closed containers.

Ammonium Molybdate

(CAS 12054-85 -2)

Formula and molecular mass – $(NH_4)_6Mo_7O_{24}.4H_2O$ – 1235,86

Specification – Contains, at a minimum, 99.0% (p/p).

Description – colorless crystals until slightly yellow or green glasses, bright.

Solubility – Soluble in water and practically insoluble in ethanol.

Physical Characteristics – By heating loses water and ammonia.

Conservation – In well-closed containers.

Ammonium Molybdate SR

Specification – Contains 10 g of ammonium molybdate in water to 100 mL.

Conservation – In well-closed containers.

Ammonium Molybdate SR1

Preparation – Dissolve 6.5 g of glacial molibdinico, finely crushed, in a mixture of 14 mL of water and 14.5 mL of ammonium hydroxide. Cool the solution and adds- la, slowly and with stirring, to a cooled mixture of 32 mL of nitric acid and 40 mL of water. Leave in

Rest for 48 hours and filter through a sintered crucible with background porosity thin. This solution deteriorates under storage and is inadequate for the use if, after addition of 2 mL of sodium phosphate dibasic dodecahydrate SR for 5 mL of solution, a yellow precipitate abundant does not form immediately or after mild heating. If occur formation of precipitate during storage, employ only the supernatant solution clear. Storage – Protect from light.

Ammonium Molybdate, acid solution

Preparation – Dilute 25 mL of ammonium molybdate to 7% (p/v) to 200 mL with water. Slowly Add 25 mL of sulfuric acid 3.75 *M* and mix.

Ammonium Molybdate to 1% (w/v) in 1 M sulfuric acid

Preparation – Weigh out 1 g of ammonium molybdate R and dissolve in 50 mL of sulfuric acid solution *M. Dilute to 100 mL with the same solvent.*

Sodium Molybdate

(CAS 10102-40 -6)

Formula and molecular mass – $Na_2MoO_4.2H_2O$ – 241,95

Description – colorless crystals or white crystalline powder or almost white.

Solubility Easily soluble in water.

Molibdovanadio SR

Synonymy – Reagent, reagent molibdatovanadato molibdovanadio.

Preparation – Using substances finely pulverized, prepare suspension of 4 g of ammonium molybdate and 0.1 g of ammonium vanadate in 70 mL of water. Add 20 mL of nitric acid. Make up the volume to 100 mL with water. *Conservation* – In well-closed containers. *Storage* – Protect from light.

Morpholine

(CAS 110-91 -8)

Synonymy – Tetrahydro-2H-1,4-oxazina; diethylene oximida

Formula and molecular mass – C_4H_9NO – 87,12

Description – colorless Liquid. Hygroscopic.

Physical Characteristic – boiling Temperature: around 128 ºC.

Miscibility – Miscible in water and in ethanol. *Conservation* – In airtight containers.

Morina

(CAS 6472-38 -4)

Formula and molecular mass – $C_{15}H_{10\,O7}.2H_{2\,O}$- 338.27

Naftaleno

(CAS 91-20 -3)

Formula and molecular mass – $C_{10}H_8$ – 128,17

Description – white crystals or almost white. *Physical Characteristics* – melting Temperature: about 80 ºC. Boiling Range: between 217 ºC and 219ºC.

Solubility Practically insoluble in water, soluble in ethanol and readily soluble in benzene and chloroform. Conservation – Container tightly closed.

1,3-Naftalenodiol

(CAS 132-86 -5)

Formula and molecular mass – $C_{10}H_8O_2$ – 160,17

Description – crystalline Powder, generally, Violet-

Brownish.

Physical Characteristic – melting Temperature: about 125 ºC. – Solubility Easily soluble in water and in ethanol.

2.7-Naftalenodiol

(CAS 582-17 -2)

Formula and molecular mass – $C_{10}H_8O_2$ – 160,17

Description – Powder or crystalline solid yellow to almost white. *Physical Characteristics* – Range of fusion: 187 ºC and 191ºC. *Solubility* – Soluble in water and in ethanol.

Naftalenodiol, reagent

Preparation – Dissolve 20 mg of 1,3-naftalenodiol in 10 mL of ethanol containing 0.2 mL of sulfuric acid.

1-Naphthylamine

(CAS 134-32 -7)

Synonymy – a-Naphthylamine.

Formula and molecular mass – $C_{10}H_9N$ – 143,12

Description – colorless crystals or white crystalline powder. By exposure to air and light, it becomes reddish. Unpleasant Smell.

Physical Characteristic – Range of fusion: 49 ºC to 51 ºC.

Solubility Slightly soluble in water and readily soluble in ethanol.

Conservation – In well-closed containers. *Storage* – Protect from light and air.

Safety – Vapor and dust harmful.

1-Naphthol

(CAS 90-15 -3)

Synonymy – Alpha-Naphthol, a-naphthol.

Formula and molecular mass – $C_{10}H_8O$ – 144,17

Description – colorless crystals or white or almost white crystalline powder; or white or nearly white. Darken with exposure to light.

Physical Characteristic – melting Temperature: about 95 ºC.

Solubility Slightly soluble in water and readily soluble in ethanol.

Conservation – In closed containers.

Storage – Protect from light.

Naphthol Mr.

Specification – Contains 20% (w/v) in ethanol. *Conservation* – In well-closed containers.

Stability – Prepare for immediate use.

Storage – Protect from light.

Naphthol

(CAS 135-19 -3)

Synonymy – Betanaftol, b-naphthol

Formula and molecular mass – $C_{10}H_8O$ – 144,17

Description – white crystalline Powder slightly pinkish, phenolic odor weak.

Physical Characteristic – melting Temperature: approximately 122 ºC

Solubility: Very slightly soluble in water and very soluble in ethanol.

Conservation – In well-closed containers. *Storage* – Protect from light.

Naphthol SR

Synonymy – Betanaftol SR, b-naphthol SR.

Specification – Contains 1 g in 100 mL of sodium hydroxide to 1% (p/v).

Conservation – In well-closed containers.

Stability – Prepare for immediate use.

Storage – Protect from light.

Naphthol SR1

Synonymy – Betanaftol SR1, SR1 b-naphthol solution *Preparation* – Dissolve 5 g of 2-naphthol, recently recristalizado, in 40 mL of 2 M sodium hydroxide and supplement to 100 mL with water.

Conservation – In well-closed containers.

Stability – Prepare for immediate use.

Storage – Protect from light.

Naringina

(CAS 10236-47 -2)

Formula and molecular mass – $C_{27}H_{32}O_{14}$ – 580,54

Description – white crystalline Powder or almost white.

Physical Characteristic – melting Temperature: about 171 ºC.

Solubility Slightly soluble in water, soluble in methanol and in dimethylformamide.

OF starch 10B

(CAS 1064-48 -8)

Formula and molecular mass – $C_{22}H_{14}N_6Na_2O_9S_2$ – 616,50

Description – Po dark brown to black.

Solubility Slightly soluble in water, soluble in ethanol.

OF starch 10B SR

Specification – starch Solution of AMIDO black 10B to 0.5% (w/v) in a mixture of acetic acid and methanol (10:90).

Ninhydrin

(CAS 485-47 -2)

Synonymy – Ninhidrina.

Formula and molecular mass – $C_9H_4O_3.H_2O$ – 178,14

Specification – Contains, at a minimum, 96.0% (p/p).

Description – white crystalline Powder to weakly pale yellow.

Solubility – Soluble in water and in ethanol.

Conservation – In well-closed containers. *Storage* – Protect from light.

Ninhydrin acetous ethanolic SR

Preparation – Dissolve 1 g ninhydrin in 50 mL ethanol and add 10 mL of glacial acetic acid.

Ninhydrin SR

Synonymy – Ninhidrina SR.

Specification – Contains 0.2% (w/v) in a mixture of

1-Butanol and 2 M acetic acid (95:5).

Conservation – In well-closed containers. *Storage* – Protect from light.

Safety – Flammable.

Ammoniacal Ammonium nitrate cerico

(CAS 16774-21 -3)

Formula and molecular mass – $(NH_4)_2[Ce(NO_3)_6]$ – 548,22

Description – yellow-orange crystalline Powder or crystals orange transparent.

Solubility – Soluble in water.

Aluminum nitrate, ninth-hydrated

(CAS 7784-27 -2)

Formula and molecular mass – $Al(NO_3)_3.9H_2O$ – 375,14

Description – deliquescent crystals.

Solubility Very soluble in water and ethanol, very little soluble in acetone.

Conservation – In hermetically sealed containers

AN

(CAS 6484-52 -2)

Formula and molecular mass – NH_4NO_3 – 80,04

Description – colorless crystals, deliquescent, or white powder, salty flavor.

Physical Characteristics – melting Temperature: approximately 155 ºC, it breaks down around 210 ºC in water and oxides of nitrogen.

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

Conservation – In well-closed containers.

AN SR

Specification – Contains 5 g of ammonium nitrate in water to 100 mL.

Ammonium nitrate, saturated solution

Specification – Contains 20.1 g in 10 mL of water. *Conservation* – In well-closed containers.

Barium nitrate

(CAS 10022-31 -8)

Formula and molecular mass – BaN_2O_6 – 261,34

Description – Crystals or crystalline powder.

Physical Characteristic – melting Temperature: approximately 590 ºC.

Solubility Easily soluble in water, very slightly soluble in ethanol and acetone.

Conservation – In well-closed containers.

Safety – Poison!

Cadmium nitrate

(CAS 10022-68 -1)

Formula and molecular mass – $Cd(NO_3)_2.4H_2O$ – 308,47

Description – colorless crystals. Hygroscopic. – Solubility Very soluble in water and soluble in acetone and ethanol.

Lead nitrate

(CAS 10099-74 -8)

Synonymy phenylmercury nitrate of lead (II).

Formula and molecular mass – $Pb(NO_3)_2$ – 331,21

Specification – Contains, at a minimum, 99.0% (p/p).

Description – translucent, colorless crystals or white crystalline powder.

Solubility Easily soluble in water.

Conservation – In well-closed containers.

Safety – Poison!

OF cobalt (II)

(CAS 10026-22 -9)

Synonymy phenylmercury nitrate Cobaltso.

Formula and molecular mass – $CoN_2O_6.6H_2O$ – 291,03

Specification – Contains, at a minimum, 99.0% (p/p).

Description – small, red crystals, hygroscopic. *Physical Characteristic* – melting Temperature: approximately 55ºC.

Solubility – Soluble in water.

Conservation – In well-closed containers. *Storage* – Protect from heat.

OF cobalt (II) Mr.

Description – Contains 1.0% (w/v) in methanol.

Conservation – In well-closed containers.

Safety – Flammable. Toxic.

Nitrate

(CAS 10277-43 -7)

Formula and molecular mass – $LaN_3O_9.6H_2O$ – 433,01

Description – colorless crystals, deliquescent. – Solubility Easily soluble in water.

Conservation – In well-closed containers.

Nitrate SR

Specification – Contains 5% (w/v) in water.

Conservation – In well-closed containers.

Magnesium nitrate

(CAS 13446-18 -9)

Formula and molecular mass – $Mg(NO_3)_2.6H_2O$ – 256,41

Description – colorless crystals and deliquescent. – Solubility Very soluble in water and readily soluble in ethanol.

Conservation – In well-closed containers.

Nitrate (I)

(CAS 14836-60 -3)

Synonymy phenylmercury nitrate Mercurous chloride.

Formula and molecular mass – $Hg_2N_2O_6.2H_2O$ – 561,22

Description – colorless crystals, usually with weak odor of nitric acid.

Physical Characteristic – melting Temperature: approximately 70 ºC with decomposition.

Conservation – In well-closed containers. *Storage* – Protect from light.

Safety – Poison!

Nitrate (I) SR

Synonymy phenylmercury nitrate Mercurous chloride SR.

Specification – Contains 15 g in a mixture of 90 mL of water and 10 mL of nitric acid in 10% (v/v)

Conservation – In closed containers of amber glass. *Stability* – Add a small corpuscle of metallic mercury.

Storage – Protect from light.

Nitrate (II)

(CAS 7783-34 -8)

Synonymy phenylmercury nitrate mercuric.

Formula and molecular mass – $HgN_2O_6.H_2O$ – 342,62

Description – colorless crystals or weakly stained. Hygroscopic.

Solubility – Soluble in water in the presence of small amount of nitric acid.

Conservation – In airtight containers.

Storage – Protect from light and moisture.

Safety – Poison!

Potassium nitrate

(CAS 7757-79 -1)

Formula and molecular mass – KNO_3 – 101,10

Specification – Contains, at a minimum, 99.5% (p/p).

Description – colorless crystals and transparent or white powder, granular or crystalline.

Solubility Very soluble in water.

Conservation – In well-closed containers.

Silver nitrate

(CAS 7761-88 -8)

Formula and molecular mass – $AgNO_3$ – 169,87

Specification – Contains, at a minimum, 99.0% (p/p).

Description – transparent, colorless crystals or white crystalline powder. Odourless

Physical Characteristic – melting Temperature: 212ºC. – Solubility Very soluble in water and soluble in ethanol. *Conservation* – In non-metallic containers closed. *Storage* – Protect from light.

Safety – Caustic. Poison!

Silver nitrate 0.1 *M*

Specification – Contains 17 g in water to 1000 mL. *Conservation* – In well-closed containers. *Storage* – Protect from light.

Silver nitrate SR

Specification – Contains 4.25 % (p/v) in water. *Conservation* – In well-closed containers. *Storage* – Protect from light.

Silver nitrate SR1

Alternate Name – silver nitrate reagent.

Preparation – Mix 3 mL of concentrated ammonium hydroxide solution and 40 mL of sodium hydroxide *M, add dropwise, with stirring,* 8 mL of silver nitrate solution to 20% (p/v). Dilute to 200 mL with water.

Sodium Azide

(CAS 7631-99 -4)

Formula and molecular mass – $NaNO_3$ – 84,99

Description – colorless crystals and transparent or, granule or powder white or nearly white. Deliquescent. *Physical Characteristic* – melting Temperature: 308ºC.

Solubility Easily soluble in water and slightly soluble in ethanol.

Conservation – In well-closed containers.

Sodium Azide SR

Specification – Contains 10 g in water to 100 mL. *Stability* – Prepare immediately before use.

Nitrate,

(CAS 13470-07 -0)

Formula and molecular mass – $ThN_4O_{12}.4H_2O$ – 552,12

Description – Crystals or white crystalline powder, slightly deliquescent.

Solubility Very soluble in water and ethanol. Conservation – In well-closed containers. *Storage* – Protect from moisture.

Nitrate zirconila

(CAS 14985-18 -3)

Synonymy – Nitrate, zirconium.

Molecular Formula – approximately, $ZrO(NO_3)_2.xH_2O$

Description – Crystals or white powder, or nearly white. *Conservation* – In well-closed containers.

Nitrate zirconila SR

Preparation – Dissolve 71 g of ammonium nitrate zirconila in a mixture of 60 mL of hydrochloric acid and 40 mL of water. *Conservation* – In well-closed containers.

Ammonium nitrate fenilmercurico

(CAS 55-68 -5)

Synonymy – Nitrate and nitrate phenylmercury phenylmercury.

Formula and molecular mass – $C_6H_5HgNO_3$ – 339,70

Specification – Consists in a mixture of ammonium nitrate and hydroxide ion phenylmercury ($C_6H_5Hg^+$).. Contains, at a minimum,

87.9% OF ion fenilmercurico (p/p) and, not least, of 62,75% of mercury (Hg) (p/p).

Description – white crystalline Powder, or bright white scales. Odourless.

Physical Characteristic – Range of fusion: between 175ºC and 190ºC with decomposition.

Solubility Very soluble in water and ethanol, slightly soluble in hot water. Dissolved in glycerol and fatty oils.

Conservation – In airtight containers.

Storage – Protect from light.

Nitrazepam

(CAS 146-22 -5)

Formula and molecular mass – $C_{15}H_{11}N_3O_3$ – 281,27

Description – yellow crystalline Powder.

Physical Characteristic – Range of fusion: 226 ºC to 230ºC. – Solubility Practically insoluble in water, slightly soluble in ethanol.

Conservation – In closed containers.

Storage – Protect from exposure to light.

Sodium nitrite

(CAS 7632-00 -0)

Formula and molecular mass – $NaNO_2$ – 69,00

Specification – Contains, at a minimum, 97.0% (p/p).

Description – colorless crystals or white powder, granular or slightly yellowish. Hygroscopic.

Physical Characteristics – melting Temperature: 271ºC. Decomposes above 320 ºC.

Solubility Easily soluble in water.

Conservation – In well-closed containers.

Stability – it Oxidizes in air very slowly to no3.

Sodium nitrite SR

Specification – Contains 10 g of sodium nitrite in water to 100 mL.

Conservation – Prepare for immediate consumption.

p-Nitroanilina

(CAS 100-01 -6)

Formula and molecular mass – $C_6H_6N_2O_2$ – 138,12

Description – clear crystalline Powder.

Physical Characteristic – Range of fusion: 146ºC to 148ºC.

Solubility Insoluble in water and soluble in ethanol and ethyl ether. Form a salt soluble in aqueous solution with strong mineral acid.

Conservation – In well-closed containers.

p-Nitroanilina and sodium nitrite SR

Solution – Dissolve 0.3 g of *p-nitroanilina in 100 mL of hydrochloric acid 10 M.*

Solution B: Dissolve 2.5 g of sodium nitrite in 50 mL of water.

Preparation – Mix 90 mL of Solution A and 10 mL of Solution B at the time of use.

2-Nitrobenzaldeido

(CAS 552-89 -6)

Formula and molecular mass – $C_7H_5NO_3$ – 151,12

Description – yellow crystals, odor similar to almond oil.

Physical Characteristic – melting Temperature: around 42ºC.

Solubility Slightly soluble in water and readily soluble in ethanol.

Nitrobenzene

(CAS 98-95 -3)

Synonymy – Nitrobenzol.

Formula and molecular mass –$C_6H_5NO_2$ – 123,11

Description – Liquid colorless to pale yellow, odor similar to almond oil.

Physical Characteristics – boiling Temperature: approximately 211ºC. Density: approximately 1.20. *Miscibility* – Practically insoluble in water and miscible with ethanol.

Conservation – In well-closed containers.

Safety – Poison!

Nitromethane

(CAS 75-52 -5)

Formula and molecular mass – CH_3NO_2 – 61,04

Description – colorless oily Liquid, characteristic odor. *Physical Characteristic* – boiling Temperature: around 102 ºC.

Miscibility – Little miscible in water and miscible with ethanol.

Sodium Nitroprusseto[a]

(CAS 13755-38 -9)

Synonymy – Pentacianonitrosilferrato (III) dihydrated disodium, sodium nitroprusside, nitroferrocianeto sodium.

Formula and molecular mass – $Na_2[Fe(CN)_5(NO)].2H_2O$ – 297,95

Description – Powder or crystals transparent, dark red.

Solubility Easily soluble in water and slightly soluble in ethanol.

Nitroprusseto sodium and piperazine SR

Specification – Contains 0.1 g of sodium nitroprusseto and 0.25 g of piperazine in 5 mL of water.

Conservation – In well-closed containers.

1-Octanossulfonato sodium

(CAS 5324-84 -5)

Molecular Formula and mass – $C_8H_{17}NaO_3S$ – 216,27

Specification – Contains, at a minimum,$C_8H_{17}NaO_3S$.

Description – Flakes or powders crystalline white or nearly white.

Sodium Octilsulfato

(CAS 142-31 -4)

Formula and molecular mass – $C_8H_{17}NaO_4S$ – 232,27

Description – Flakes or crystalline powder white or nearly white.

Solubility Easily soluble in water and soluble in methanol.

Octoxinol 10

(CAS 9002-93 -1)

Formula and molecular mass – $((C_2H_4O)_{10}C_{14}H_{22}O$ – 647,00 Description – viscous Liquid, limpid, clear yellow. *Miscibility* – Miscible in water, acetone and ethanol. Soluble in toluene.

Conservation – In tightly closed container.

Olive Oil

(CAS 8001-25 -0)

Specification – fixed Oil obtained from the ripe fruit of *Olea europaea* L. – Oleaceae).

Description – Oil pale yellow or greenish yellow. *Physical Characteristic* – Density: 0.910 to 0.915. *Miscibility* – Practically insoluble in ethanol, miscible in chloroform, ethyl ether and petroleum ether.

Ammonium oxalate

(CAS 6009-70 -7)

Formula and molecular mass – $C_2H_8N_2O_4.H_2O$ – 142,11

Specification – Contains, at a minimum, 99.0% (p/p).

Description – transparent colorless crystals or white crystalline powder. Odourless.

Physical Characteristic – melting Temperature: 212ºC. *Solubility* – Soluble in water.

Conservation – In well-closed containers.

Safety – Caustic. Corrosive. Poison!

Ammonium oxalate SR

Use ammonium oxalate ITSELF.

Potassium oxalate

(CAS 6487-48 -5)

Formula and molecular mass –$K_2C_2O_4.H_2O$ – 184,23, se anidro – 166,22

Description – colorless crystals, odorless, eflorescentes to air dry and hot.

Physical Characteristic – Loses its water to approximately *160 ºC*

Conservation – In airtight containers.

Storage – Protect from moisture.

Safety – Poison!

Sodium oxalate

(CAS 62-76 -0)

Formula and molecular mass – $Na_2C_2O_4$ – 134,00

– Description white crystalline Powder or almost white. *Solubility* – Soluble in water and practically insoluble in ethanol.

Malachite green oxalate

(CAS 633-03 -4)

Synonymy – brilliant Green

Formula and molecular mass – $C_{27}H_{34}N_2O_4S$ – 428,64

Description – bright golden-yellow crystals. *Conservation* – In well-closed containers.

Aluminum Oxide

(CAS 1344-28 -1)

Synonymy – Alumina.

Formula and molecular mass – Al_2O_3 – 101,96

Description – fine, white Powder granulated.

Physical Characteristic – The pH of the suspension to 10.0% (p/v): between 9.0 and 10.0.

Conservation – In airtight containers.

Holmium Oxide

(CAS 12055-62 -8)

Formula and molecular mass – Ho_2O_3 – 377,85

Specification – Contains, at a minimum, 99.9% (p/p).

Description – yellowish Powder.

Solubility Practically insoluble in water. Conservation – In well-closed containers.

Magnesium Oxide

(CAS 1309-48 -4)

Synonymy – magnesium Oxide light or heavy.

Formula and molecular mass – MgO – 40.30

Specification – Contains, at a minimum, 95.0% (p/p).

Description – amorphous Powder fine, white, odourless, alkaline taste weak.

Conservation – In well-closed containers.

Storage – Protect from contact with the air and with the humidity.

Silver Oxide

(CAS 20667-12 -3)

Formula and molecular mass – Ag_2O – 231,74

Description – dark gray Powder. Solubility Practically insoluble in water and in ethanol, readily soluble in dilute nitric acid and ammonium hydroxide.

Conservation – In closed containers.

Storage – Protect from light.

Mercuric Oxide

(CAS 21908-53 -2)

Synonymy – yellow Oxide of mercury, mercury oxide (II).

Formula and molecular mass – for HgO Urgents – 216.59 *Specification* – Contains at least 99.5% (p/p).

Description – Po yellow-orange, dense, odorless. – Solubility Practically insoluble in water and in ethanol. *Storage* – Protect from light.

Safety – Poison!

Palladium Ms. – 1 mg/mL

Specification – Contains l. 67 g of palladium chloride in 200 mL of hydrochloric acid to 50% (v/v). Heat until complete dissolution. Cool and dilute with water to 1000 mL. *Conservation* – In well-closed containers, inert (polyethylene type).

Methyl palmitate

(CAS 112-39 -0)

Formula and molecular mass – $C_{17}H_{34}O_2$ – 270,50

Description – white or yellow crystalline Mass. *Physical Characteristics* – Density (30 ºC):

Approximately 0.86. Melting Temperature: about 30 ºC.

Solubility – Soluble in ethanol and in petroleum ether. *Conservation* – In well-closed containers.

Role of silver-manganese

Preparation – The mixture of equal volumes of silver nitrate 0.1 *M* and manganese sulphate (1.5% (w/v) add, drop by drop, sodium hydroxide 0.1 *M* until that form precipitate persistent. Filter. Next, dip strips of filter paper (for example, Whatman No 1) in the solution for 15 minutes. Dry at room temperature, under the light and acid vapors or alkaline soil. The role of silver-manganese should be colorless.

Sensitivity Test – In beaker of approximately 40 mL capacity introduce 1 mL of ammonium chloride to 1% (p/v). Add 9 mL of water and 1 g of oxide

Magnesium. Close the container immediately with polyethylene cover, under which puts the role of silver-manganese. Stir the solution, taking care to ensure that the particles of magnesium do not come in contact with

The Paper. Keep the beaker to 50 ºC to 60 ºC for 1 hour. Appears in gray color reagent.

Liquid Paraffin

Specification – Mixture of purified liquid saturated hydrocarbons obtained from petroleum.

Description – oily Liquid colorless and transparent. *Physical Characteristics* – relative Density: 0.827 to 0.890. Viscosity: 110 mPa to 230 mPa.

Miscibility – Practically insoluble in water and slightly soluble in ethanol. Miscible in hydrocarbons. *Conservation* – In well-closed containers. *Storage* – Protect from light.

Pentanossulfonato sodium, monohydrate

(CAS 207605-40 -1)

Formula and molecular mass – $C_5H_{11}NaO_3S.H_2O$ – 192,21

Description – crystalline Solid white or nearly white. *Solubility* – Soluble in water.

Conservation – In well-closed containers.

Phosphorus Pentoxide

(CAS 1314-56 -3)

Synonymy – phosphoric Anhydride.

Formula and molecular mass – P_2O_5 – 141,94

Description – white Powder, amorphous, very deliquescent. *Physical Characteristics* – melting Temperature: 340ºC Temperature of sublimation: 360 ºC.

Conservation – In airtight containers.

Storage – Protect from moisture.

Security – Irritant. Corrosive to the skin, mucous membranes and eyes.

Vanadium Pentoxide

(CAS 1314-62 -1)

Formula and molecular mass – V_2O_5 – 181,88

Specification – Contains at least 99.5% (p/p).

Description – fine Dust yellow to orange yellow.

Physical Characteristic – melting Temperature: 690ºC.

Solubility Slightly soluble in water and soluble in strong mineral acids and hydroxy-alkaline solutions with formation of salts.

Conservation – In well-closed containers.

Purified Pepsin

Specification – Derived from the stomach mucosa of pork, with activity of 800 to 2500 units/mg of protein.

Description – amorphous, crystalline Powder or white or slightly yellow. In Hygroscopic Equilibrium.

Solubility – Soluble in water, practically insoluble in ethanol. The solution in water can become a little opalescent glass with a small amount of acid.

Conservation – In closed container.

Storage – Protected from light and temperature of 2ºC to 8ºC.

Labelling – should express the pepsin activity.

Peptone

Specification – Mixing products of nature polypeptide derived from animal protein (meat, casein). The origin determines the physical characteristics, composition and production process.

Description – Po clear yellow to brown. Odour and flavor characteristics. Minimum Content in nitrogen: 12,0% (p/p) of casein and 14.2% (p/p) of meat.

Conservation – In well-closed containers. *Storage* – Protect from moisture.

Labelling – should express origin and nitrogen content.

Sodium Perchlorate

(CAS 7791-07 -3)

Chemical Name – sodium Salt monohydrate of perchloric acid

Formula and molecular mass – $NaClO_4.H_2O$ – 140,46

Specification – Contains, at a minimum, 99.0% (p/p).

Description – colorless crystals, deliquescent. – Solubility Very soluble in water, soluble in ethanol. *Conservation* – In well-closed containers.

Potassium Periodate

(CAS 7790-21 -8)

Synonymy – Metaperiodato potassium *Formula and molecular mass* – KIO – 230.00 – Description white crystalline Powder or colorless crystals. *Physical Characteristic* – melting Temperature: 582ºC. *Safety* – Highly irritating to skin, eyes and mucous membranes.

Ferric potassium Periodate SR

Preparation – Dissolve 1 g of potassium periodate in 5 mL of the potassium hydroxide solution 12% (p/v), recently prepared. Add 20 mL of water and 1.5 mL of ferric chloride SR. Dilute to 50 mL with potassium hydroxide solution 12% (p/v) recently prepared.

Sodium Periodate

(CAS 7790-28 -5)

Synonymy – Metaperiodato sodium.

Formula and molecular mass – Did not Reoccur, – 213.89 *Specification* – Contains, at a minimum, 99.0% of sodium periodate.

Description – white crystals tetragonais.

Physical Characteristic – melting Temperature: approximately 300 °C with decomposition. *Solubility* – Soluble in water, acetic acid, nitric acid and sulfuric acid.

Conservation – In well-closed containers. *Storage* – In ventilated places.

Security – strong Oxidant.

Potassium permanganate

(CAS 7722-64 -7)

Formula and molecular mass – $KMnO_4$ – 158,03

Specification – Contains, at a minimum, 99.0% (p/p), calculated on the dried substance.

Description – dark violet crystals, with metallic sheen, odourless, taste sweetish, astringent.

Solubility – Soluble in cold water and readily soluble in boiling water.

Conservation – In well-closed containers. *Storage* – Protect from light.

Safety – The substance and its solutions present risk of explosion when in contact with oxidizable materials. *Category* – Oxidant energetic.

Potassium permanganate SR (approximately 0.2 *M)*

Specification – Contains 3% (w/v) in water.

Stability – Prepare for immediate consumption. *Conservation* – In well-closed containers. *Storage* – Protect from light.

Security – Irritant. Caustic.

Carbamide peroxide

(CAS 124-43 -6)

Synonymy – hydrogen peroxide and urea.

Formula and molecular mass – $CH_6N_2O_3$ – 94,07

Description – Crystals or white crystalline powder.

Decomposes on contact with the air in urea, oxygen and water.

Solubility – Soluble in water.

Conservation – In well-closed containers.

Additional Information – oxidizing Agent.

Concentrated hydrogen peroxide

(CAS 7722-84 -1)

Synonymy – Peridrol.

Formula and molecular mass – H_2O_2 – 34,01

Specification – Contains, at a minimum, 29.0% (p/p) of H_2O_2. . Corresponds to approximately 100 parts by volume. May contain stabilizer.

Description – colorless Liquid, annoying, weak odor. *Physical Characteristic* – Density: 1.11.

Conservation – In containers filled partially, provided with closure of relief.

Storage – Protect from heat and light.

Security – strong Oxidant.

Hydrogen peroxide, 30 volumes, Mr.

Formula and molecular mass – H_2O_2 – 34,01..

Specification – Contains, at a minimum, 9.7% (w/v) and, at most, 10.7% (p/v) H 2O2, corresponding to approximately 30 parts by volume. May contain stabilizer.

Description – Dilute hydrogen peroxide, concentrate. *Conservation* – In closed containers.

Stability – Avoid long periods of storage. *Storage* – Protect from heat and light.

Hydrogen peroxide at 3% (p/v)

Formula and molecular mass – H_2O_2 – 34,01

Specification – Contains, at a minimum, 2.5% (w/v) and, at most, 3.5% (w/v) H 2O2, corresponding to approximately 10 parts by volume. May contain stabilizer.

Description – clear, colorless Liquid.

Conservation – In closed containers. Avoid long periods of storage.

Storage – Protect from heat and light.

Hydrogen peroxide did not produce any signals

Preparation – On the day of use, dilute 2 mL of concentrated hydrogen peroxide to 100 mL with methanol and store in the refrigerator. Immediately before use, dilute 2 mL of this solution to 100 mL with methanol.

Sodium peroxide

(CAS 1313-60 -6)

Formula and molecular mass – Na_2O_2 – 77,98

Description – granular Powder yellowish-white.

Solubility Easily soluble in water, forming sodium hydroxide and hydrogen peroxide, which decomposes to oxygen gas and water.

Conservation – In well-closed containers, protected from organic compounds and oxidisable substances.

Ammonium Persulphate

(CAS 7727-54 -0)

Synonymy -Peroxidissulfato of ammonium.

Formula and molecular mass – $H_8N_2O_8S_2$ – 228,10

Specification – Contains, at a minimum, 95.0% (p/p).

Description – granulated white crystals or powder. Odourless. Stable during months when pure and dry; it breaks down in the presence of moisture.

Solubility Easily soluble in water.

Conservation – In airtight containers.

Storage – Protect from moisture, heat and organic matter.

Additional Information – strongly oxidizing Agent.

Potassium Persulphate

(CAS 7727-21 -1)

Formula and molecular mass – $K_2S_2O_8$ – 270,32

Description – colorless crystals or white crystalline powder or almost white.

Solubility Slightly soluble in water, practically insoluble in ethanol. In aqueous solution decomposes to ambient temperature, and increases speed of decomposition with the increase of temperature.

Conservation – In well-closed containers.

Storage – In a ventilated area.

Sodium Persulphate

(CAS 7775-27 -1)

Formula and molecular mass – $Na_2O_8S_2$ – 238,13

Description – white crystalline Powder. It breaks down slowly with moisture and heat.

Conservation – In airtight containers.

Storage – Protect from moisture and heat. *Security* – Irritant.

Alkaline sodium Picrate SR

Preparation – Mix 20 mL of picric acid to 1% (p/v) with 10 mL of sodium hydroxide to 5% (p/v) and dilute to 100 mL with water.

Stability – Use within, at most, two days.

Piperazine

(CAS 110-85 -0)

Formula and molecular mass – $C_3H_{10}N_2$ – 86,14

Description – Lumps or flakes white or nearly white. Ammoniacal Odor.

Solubility – Soluble in water and in ethanol, insoluble in ethyl ether.

Pyridine

(CAS 110-86 -1)

Formula and molecular mass – C_5H_5N – 79,10

Description – colorless Liquid, characteristic odor and unpleasant.

Physical Characteristics – boiling Range: 115 ºC to 116 ºC Density (25 ºC): approximately 0.980. Index of refraction (20 ºC): 1.5092.

Miscibility – Miscible in water and in ethanol. *Conservation* – In well-closed containers. *Storage* – Protect from moisture.

Safety – Flammable. Toxic.

Anhydrous pyridine

Specification – Contains, at most, 0.01% (w/w) of water.

Preparation – Dry pyridine with anhydrous sodium carbonate. Filter and distil.

Conservation – In well-closed containers. *Storage* – Protect from moisture.

Safety – Flammable. Toxic.

Sodium pyrophosphate

(CAS 13472-36 -1)

Formula and molecular mass – $Na_4P_2O_7.10H_2O$ – 265,90

Description – colorless crystals little eflorescentes. *Physical Characteristic* – melting Temperature: 79.5 ºC. – Solubility Easily soluble in water.

Conservation – In well-closed containers.

Pyrogallol

(CAS 87-66 -1)

Formula and molecular mass – $C_6H_6O_3$ – 126,11

Description – Crystals white or nearly white. Becoming brown on exposure to air and light.

Physical Characteristic – melting Temperature: around 131 ºC. – Solubility Very soluble in water and ethanol. Aqueous Solutions become brown on exposure to air. *Conservation* – In closed containers.

Storage – Protect from light.

Polyacrylamide

(CAS 9003-05 -8)

Synonymy – Polymer of acrylamide.

Formula and molecular mass – (C_3H_5NO) n; monomer - 71,08.

Specification – Polymer of various forms, soluble and insoluble in water, obtained by heating with various polymerization catalysts.

Conservation – In well-closed containers.

Safety – Highly toxic and irritating. Cause paralysis of the central nervous system. Can be absorbed through the skin.

Polysorbate 20

See monograph Polysorbate 20.

Polysorbate 80

Specification – Mixture of oleatos of sorbitol and its anhydrides copolimerizados with approximately 20 *M* of ethylene oxide for each mol of sorbitol anhydrous and anhydride.

Description – clear Liquid, yellowish or dark yellow. Oily. Weak characteristic odour.

Physical Characteristics – Density: at around 1.08. Viscosity: approximately 400 cP.

Conservation – In well-closed containers.

Category – Tenside.

Potassium Ms. – 600 |ag/mL

Specification – Contains 1.144 g of potassium chloride in water to 1000 mL.

Conservation – In well-closed containers, inert (polyethylene type).

Prednisolone

(CAS 50-24 -8)

Formula and molecular mass – $C_{21}H_{28}O_5$ – 360,45

Specification – Contains, at a minimum, 97.0% (p/p), calculated on the dried substance.

Description – white crystalline Powder or almost white. Hygroscopic. Presented in the anhydrous form or containing one or half a molecule of water of hydration.

Physical Characteristic – melting Temperature: 240-241 °C with decomposition.

Solubility: Very slightly soluble in water, soluble in ethanol and methanol, slightly soluble in acetone and slightly soluble in methylene chloride.

Conservation – In well-closed containers.

Category – Steroids.

Prednisone

(CAS 53-03 -2)

Formula and molecular mass – $C_{21}H_{26}O_5$ – 358,43.

Specification – Contains, at a minimum, 97.0% (p/p), $C_{21}H_{26}O_5$" calculated on the dried substance.

Description – white crystalline Powder or almost white. *Physical Characteristic* – melting Temperature: approximately 233ºC with decomposition.

Solubility Practically insoluble in water and slightly soluble in ethanol and in methylene chloride.

Conservation – In well-closed containers.

Category – Steroids.

Brilliant Black BN

(CAS 2519-30 -4)

Formula and molecular mass – $C_{28}H_{17}N_5Na_4O_{14}S_4$ – 867,69

Description – fine crystals, powder blue violet or gray black. Ti oxide reduction. Oxidized Form: violet blue. Reduced Form: yellow-brown. *Physical Characteristic* – The (1 %, 1 cm) is greater than 0.390 at 570 nm.

Conservation – In well-closed containers.

Propylene glycol

(CAS 57-55 -6)

Synonymy – 1,2-Propanediol.

Formula and molecular mass – $C_3H_8O_2$– 76,09

Description – colorless Liquid, viscous, hygroscopic. *Physical Characteristics* – Density (25 ºC): 1.035 to 1.037. Boiling Range: 187 ºC to 189 ºC.

Miscibility – Miscible in water and in ethanol. *Conservation* – In well-closed containers. *Storage* – Protect from moisture.

Propylparaben

(CAS 94-13 -3)

Chemical Name – propyl Ester acid 4-hidroxibenzico

Formula and molecular mass – $C_{10}H_{12}O_3$ – 180,20

Description – white crystals.

Solubility – Very slightly soluble in water, readily soluble in ethanol and in ethyl ether.

Category – Preservative.

Henoch-Sch ftaleina

(CAS 2411-89 -4)

Synonymy – Metalftaleina.

Formula and molecular mass – $C_{32}H_{32}N_2O_{12}$ – 636,61

Description – Po clear yellow to brown. Can be found in the form of sodium salt: pale yellow powder pink. – Solubility Practically insoluble in water and soluble in ethanol. In the form of sodium salt is soluble in water and practically insoluble in ethanol.

Sensitivity Test – Dissolve 10 mg in 1 mL of concentrated ammonium hydroxide solution and dilute to 100 mL with water. TO 5 mL of solution, add 95 mL of water, 4 mL of concentrated ammonium hydroxide solution, 50 mL of ethanol and 0.1 mL of barium chloride 0.1 *M* SV. The solution presents coloring blue violet. Add 715 mL of disodium edetate 0.1 *M* SV. The solution becomes colorless.

Quinalizarina (CI 58500)

(CAS 81-61 -8)

Synonymy – Mordant violet 26

Formula and molecular mass – $C_{14}H_8O_6$ – 272,20.

Description – Po dark red.

Conservation – In well-closed containers.

Quinidine

(CAS 56-54 -2)

Formula and molecular mass – $C_{20}H_{24}N_2O_2$ – 324,42

Description – white crystals, or nearly white. Physical Characteristics – specific optical rotation (20 ºC): approximately +260 °, determined and a 1 % solution (w/v) in ethanol. Melting Temperature: around 172 ºC. Solubility: Very slightly soluble in water, slightly soluble in ethanol, and slightly soluble in methanol. Conservation – In closed containers.

Storage – Protect from exposure to light

Quinhydrone

(CAS 106-34 -3)

Formula and molecular mass – $C_{12}H_{10}O_4$ – 218,21

Description – glossy crystals or crystalline powder dark green.

Physical Characteristic – melting Temperature: 170ºC, can sublimes and decompose partially.

Solubility Slightly soluble in cold water, soluble in hot water, ammonia and ethyl ether.

Conservation – In closed containers.

Quinine

(CAS 130-95 -0)

Formula and molecular mass – $C_{20}H_{24}N_2O_2$ – 324,42

Description – micro-crystalline Powder white, or nearly white. Physical Characteristics – specific optical rotation (20 ºC): approximately with °, determined and a 1% solution (w/v) in ethanol. Melting Temperature: about 175 ºC. Solubility: Very slightly soluble in water, slightly soluble in boiling water and very soluble in ethanol. Conservation – In well-closed containers. Storage – Protect from light.

Raponticina

(CAS 155-58 -8)

Formula and molecular mass – $C_{21}H_{24}O_9$ – 420,41

Description – gray-yellowish crystalline Powder.

Solubility – Soluble in ethanol and methanol.

Aluminon reagent

Solution – Dissolve 250 g of ammonium acetate in 500 mL of distilled water. Add 40 mL of glacial acetic acid, 0.5 g of aluminon dissolved in 50 mL of distilled water, 1 g of benzoic acid dissolved in 150 mL of isopropyl alcohol and 225 mL of isopropyl alcohol. Make the volume up to 1000 mL with distilled water. Solution B: Dissolve 5 g of gelatin in 125 mL of hot distilled water and mix with 250 mL of cold distilled water. Filter and make up to 500 mL with distilled water.

Preparation – Mix with agitation solutions A and B. The mixture must be completely clear when cold. Store in polyethylene bottle, protected from light.

Coloring reagent

Preparation – Mix 50 mL of glacial acetic acid and 50 mL of sulfuric acid. Leave to rest for 2 hours before use. Store in the refrigerator for a maximum of 24 hours.

Erlich reagent modified

Preparation–Dissolve0.1gofp-dimethylaminobenzaldehyde in 1 mL of hydrochloric acid and dilute to 100 mL with ethanol.

Content – Folin-Denis reagent reagent

Preparation – The 75 mL of water add l0 g of sodium tungstate, 2 g of phosphomolybdic acid and 5 mL of phosphoric acid. Keep the mixture at reflux for 2 hours, cool and dilute to 100 mL with water. The solution presents greenish coloration.

Reagent Hantzach

Preparation – Dissolve 150 g of ammonium acetate in 500 mL of distilled water containing 3 mL of glacial acetic acid and Ml of acetylacetone. Make the volume up to 1000 mL. Conservation – In closed container amber glass.

Reagent of Jones

Preparation – The 40 mL of water add 5.3 g of chromium trioxide and 24 mL of the mixture of water and sulfuric acid (1:1).

Reagent of Marquis

Preparation – Mix 4 mL of formaldehyde solution with 100 mL of sulfuric acid.

Reagent of xanthydrol

Preparation – Dissolve 0.125 g of xanthydrol in 100 mL of glacial acetic acid. Add 1 mL of hydrochloric acid before use.

Reagent fosfomolibdotungstico

Preparation – Dissolve 100 g of sodium tungstate and 25 g sodium molybdate in 700 mL of water. Add 100 mL of hydrochloric acid and 50 mL of phosphoric acid. Heat the mixture under reflux in gadgets glass, during 10 hours. Add 150 g of lithium sulphate, 50 mL of water and a few drops of bromine. Boil to remove the excess of bromine (for about 15 minutes), let cool and dilute to 1000 mL with water. Filter. The reagent presents yellow coloration. If the solution present greenish coloration, should not be used, it must be regenerated with the addition of a few drops of bromine reagent to boiling. Then boil the reagent to eliminate the excess of bromine.

Storage – Keep at a temperature of 2ºC to 8 ºC.

Reagent iodoplatinado

Preparation – Mix equal volumes of glacial cloroplatinico to 0.3% (p/v) and of potassium iodide to 6% (p/v).

Reagent sulfomolibdico

Preparation – Dissolve, with heating, 2.5 g of ammonium molybdate in 20 mL of water. Dilute 28 mL of sulfuric acid in 50 mL of water and cool. Mix the two solutions and dilute to 100 mL with water.

Ammonium Reineckato

(CAS 13573-16 -5)

Synonymy – Tetratiocianatodiaminocromato of ammonium.

Formula and molecular mass – $C_4H_{10}CrN_7S_4.H_2O$

Description – dark-red crystals or crystalline powder red.

Solubility Slightly soluble in water, soluble in hot water and ethanol. Decomposes slowly and solution.

Ammonium Reineckato SR

Preparation – Shake, constantly, about 0.5 g of ammonium reineckato in 20 mL of water for one hour and filtered.

Stability – Use in, at most, two days.

Resazurin

(CAS 550-82 -3)

Synonymy – Diazorresorcinol *Formula and molecular mass* – $C_{12}H_7NO_4$ – 229,18.

Description – Crystals or crystalline powder, dark red. *Conservation* – In well-closed containers.

Resorcinol

(CAS 108-46 -3)

Synonymy – Resorcinol.

Formula and molecular mass – $C_6H_6O_2$ – 110,11

Specification – Contains, at a minimum, 99.0% (p/p).

Description – Crystals or crystalline powder colorless or pale yellow. Exposed to the light and air, acquires pink coloration. *Physical Characteristic* – Range of fusion: 109 ºC to 111 ºC.

Solubility – Soluble in water and ethanol.

Conservation – In well-closed containers. *Storage* – Protect from light and air.

Ristocetin

(CAS 1404-55 -3)

Synonymy – Ristocetin A.

Formula and molecular mass – $C_{94}H_{108}N_8O_{44}$ – 2053,89

Description – white Solid. Also Found, as ristocetin sulfated.

Rhodamine B

(CAS 81-88 -9)

Synonymy – Tetraetilrodamina, basic Violet 10.

Formula and molecular mass – $C_{28}H_{31}ClN_2O_3$ – 479,01

Description – Crystals, green or reddish dust. – Solubility Very soluble in water and ethanol. *Conservation* – well-closed Containers.

Storage – Protect from exposure to light and heat. *Safety* – Irritating

Rutin

(CAS 153-18 -4)

Formula and molecular mass – $C_{27}H_{30}O_{16}$ – 610,52

Description – needle-shaped crystals yellow-pale. Darken in the presence of light.

Physical Characteristic – melting Temperature: approximately 210 °C with decomposition.

Solubility: Very slightly soluble in water and soluble in pyridine.

Conservation – In closed containers.

Storage – Protect from exposure to light.

Sucrose

(CAS 57-50 -1)

Formula and molecular mass – C_{12}.22OR – 342.30 *Specification* – is obtained from *Saccharum officinarum* Linne (Family Gramineae), *Beta vulgaris* Linne (Family Chenopodiaceae.) and other sources.

Description – white crystals or crystalline powder or colorless; mass white crystalline or blocks. Odourless. Sweetish Flavor. Stable in air. Finely divided is hygroscopic and absorbs up to 1 % of moisture. Does not contain additives. *Caracteristicafisica* – Decomposition: between 160ºC and 186ºC.

Solubility Very soluble in water, slightly soluble in ethanol, and practically insoluble in ethanol anhydrous. Conservation – In well-closed containers.

Sucrose 0.1% (w/v) in pyridine

Specification – Contains 0.1 g of sucrose in pyridine at 100 mL.

Conservation – In well-closed containers.

Security – Toxic.

Safranin O

(CAS 477-73 -6)

Description – Po dark red. Consists of chloride mixture of 3,7-diamine 2.8-dimethyl-5-fenilfenazinio ($C_{20}H_{19}ClN_4$ – 350,85) and chloride of 3,7-diamine 2.8-Dimethyl-5,o-tolilfenazinio ($C_{21}H_{21}ClN_4$ – 364,88). Ti oxide reduction. Oxidized Form: acid pH, violet- bluish; alkaline pH, brown reduced Form: colorless in both acidity and alkalinity.

Physical Characteristic – maximum Absorption: 530-533 nm.

Conservation – In well-closed containers.

Sodium salicylate

(CAS 54-21 -7)

Formula and molecular mass –$C_7H_5NaO_3$ – 160,10

Description – small colorless crystals or white crystalline powder or flakes shiny.

Physical Characteristic – melting Temperature: 440ºC. – Solubility Easily soluble in water and slightly soluble in ethanol.

Conservation – In closed containers.

Storage – Protect from light.

Santonina

(CAS 481-06 -1)

Formula and molecular mass – $C_{15}H_{18}O_3$ – 246,30

Description – colorless crystals. If exposed to light, can acquire yellow coloration.

Physical Characteristic – Range of fusion: 174 ºC to 176 ºC. *Solubility*: Very slightly soluble in water, readily soluble in ethanol to hot and slightly soluble in ethanol.

Saponins

(CAS 8047-15 -2)

Description – pale yellow Powder.

Solubility – Soluble in water, and under agitation, foaming.

Conservation – In closed containers.

Silica, desiccated

(CAS 7631-86 -9)

Formula and molecular mass – SiO_2 – 60,08

Specification – colloidal silicic Acid, polymerized, previously dehydrated; contains cobalt chloride as an indicator.

Description – Granules glassy, amorphous, particle size variable, with granules impregnated with TI adsorption capacity by the color blue to pink.

Conservation – In airtight containers.

Storage – Protect from moisture.

Category – Desiccant.

Silica-gel "G"

(CAS 112926-00 -8)

Synonymy – silica Gel "G".

Specification – Contains approximately 13.0% (p/p) of calcium sulphate hemi-hydrate.

Description – white fine Powder of particle size varies between 10 and 40 |am, homogeneous.

Physical Characteristic – The pH of the suspension to 10% (p/v) in water free of carbon dioxide, obtained by stirring for 15 minutes; potentiometric determination: approximately 7.0.

Conservation – In well-closed containers.

Category – Support for chromatography.

Silica-gel "GF_{254}"

Synonymy – silica Gel "GF_{254} ".

Specification – Contains approximately 13.0% (p/p) of calcium sulphate hemi-hydrate and approximately 1.5% (p/p) of fluorescence indicator of maximum intensity at 254 nm.

Description – white fine Powder of particle size varies between 10 and 40 ^m, homogeneous.

Physical Characteristic – See silica-gel "G".

Conservation – In well-closed containers.

Category – Support for chromatography.

Silica-gel "HF_{254}"

Synonymy – silica Gel "HF_{254} ".

Specification – Contains approximately 1.5% (w/v) of fluorescence indicator of maximum intensity at 254 nm.

Description – white fine Powder of particle size varies between 10 and 40 |am, homogeneous.

Physical Characteristic – See silica-gel "G".

Conservation – In well-closed containers.

Category – Support for chromatography.

Silica kieselguhr

Description – white Powder or clear yellow.

Solubility Practically insoluble in water, dilute acid solutions and organic solvents.

Silica kieselguhr "G"

Specification – kieselghur Silica treated with hydrochloric acid and calcined, in which adds approximately 15% (p/p) of calcium sulphate hemi-hydrate.

Description – fine Dust grayish white. With an average size of particles of 10 ^m to 40 ^m.

Sodium Ms. – 200 µmL

Specification – Contains 0.5084 g of sodium chloride in water to 1000 mL.

Conservation – In well-closed containers, inert (polyethylene type).

Solution of stannous chloride and ninhydrin

Preparation – Dissolve 0.2 g ninhydrin in 4 mL of hot water. Add 5 mL of stannous chloride to 0.16% (p/v) and leave to rest for 30 minutes. Filter and store in the refrigerator. At the time of use, dilute 2.5 mL with 5 mL of water and 45 mL of isopropyl alcohol.

Solution of Jeffrey

Preparation – Mix equal parts of nitric acid to 10% (p/v) and chromic acid to 10% (p/v).

Conservation – In well-closed containers.

Solution of Karl-Fischer -titrations Scope

Synonymy – Reagent iodine-sulphide.

Specification – Consisting of two solutions. *Solution 1: a mixture of 70 mL of methanol and 35 mL of pyridine, free water, add, under refrigeration and absence of moisture, sulfur dioxide dry until you get an increase in weight of 9 g. Mix. Solution 2: Contains 12.6 g of iodine in methanol 330 mL.*

Conservation – In airtight containers.

Stability – It breaks down continuously.

Storage – Protect from moisture and light. Keep under refrigeration.

Security – Toxic. Flammable.

Additional Information – For determination of water content.

Cleaning Solution of chromic acid

Preparation – In 100 mL of sulfuric acid, add gradually and under constant agitation, 3 g of potassium dichromate. Stirring up dissolving of salt and let cool until 40 ºC and store in glass container.

Standard ethanolic Solution of calcium (100 ppm Ca)

Preparation – Dissolve 2.5 g of calcium carbonate, previously dried, in 12 mL of 5 M acetic acid and dilute with water to 1000 mL. Dilute 1 volume of this solution in 10 volumes of ethanol, immediately, before use.

Standard Solution of acetaldehyde (100 ppm C_2H_4O)

Preparation – Dissolve 1 g of acetaldehyde in isopropyl alcohol and complete for 100 mL. For use dilute 1:100, with the same solvent.

Information – extemporaneous Preparation.

Standard Solution of ammonium (1 ppm NH_4)

Preparation – Dissolve 0.4444 g of ammonium nitrate in 1000 mL of distilled water, corresponds to 100 mg/mL of ammonium. For use dilute 1:100.

Standard Solution of barium (10 ppm Ba)

Specification – Contains 1.779 g of BaCl2.2H2O in water 1000 mL. For use, dilute 1:100.

Conservation – In well-closed containers and inert (polyethylene type).

Standard Solution of cadmium (0.1% Cd)

Preparation – Dissolve quantity of ammonium nitrate cadmium containing 0.1 g of cadmium in minimum quantity of mixture of water and hydrochloric acid (1:1) and dilute to 100 mL with hydrochloric acid at 1% (v/v).

Standard Solution of cadmium (5 ppm Cd)

Specification – Contains 0.229 g of cadmium sulphate in water 330 mL, corresponds to 1000 µg/mL of cadmium. For use, dilute 1:200.

Conservation – In well-closed containers and inert (polyethylene type).

Standard Solution of 10 ppm calcium (Ca)

Preparation – Dissolve 0.624 g of calcium carbonate previously dried in distilled water containing 3 mL of glacial acetic acid 5 *M. Dilute to 250 mL with water. Dilute Volume of this solution in 100 volumes of distilled water immediately before use.*

Standard Solution of 0.1% lead (Pb)

Preparation – Dissolve 0.4 g of lead nitrate (II) in water and dilute to 250 mL with the same solvent.

Standard Solution of copper (10 ppm Cu)

Preparation – Dissolve 392.9 mg of cupric sulfate penta-hydrated in 100 mL of water. Dilute 1 mL of this solution with water to 100 mL at the time of use.

Standard Solution of chloride (Cl) 8 ppm

Specification – Contains 1.318 g of sodium chloride in water 1000mL. For use dilute 1:100.

Conservation – In well-closed containers.

Standard Solution of chloride (Cl) 5 ppm

Specification – Contains 0.824 g of sodium chloride in water to 1000 mL. For use dilute 1:100.

Conservation – In well-closed containers.

Standard Solution of dithizone

Preparation – Dissolve 10 mg of dithizone in chloroform and supplement the volume to 1000 mL with chloroform. *Conservation* – Stow in container lead-free, fitted with glass lid, and properly, packaged to protect them from light.

Storage – In cooler.

Standard Solution of tin (Sn) 5 ppm

Specification – Contains 1.225 g of acetate of tin hemi-hydrate in 25 mL of hydrochloric acid in water to 1000 mL. For use, dilute 1:100 in hydrochloric acid 2.5% (p/v).

Conservation – In well-closed containers.

Standard Solution of magnesium (10 ppm Mg)

Preparation – Dissolve 1.010 g of magnesium sulphate heptahydrate in water and make up the volume to 100 mL with the same solvent. Dilute 10 mL of this solution to 1000 mL with water.

Standard Solution of nitrate (100 ppm NO_3)

Preparation – Dissolve 163.1 mg of potassium nitrate in 100 mL of water. Dilute 10 mL of this solution with water to 100 mL at the time of use.

Nitrate standard Solution (2 ppm NO_3)

Preparation – Dissolve 1.2903 g of ammonium nitrate in 1000 mL of water, corresponds to 1000 mg/mL of nitrate. For use, dilute 1:500.

Standard Solution of silver (5 ppm Ag)

Preparation – Dissolve 79 mg of silver nitrate in 100 mL of water. Dilute 1 mL of this solution with water to 100 mL at the time of use.

Standard Solution of selenium (100 ppm Se)

Preparation – Dissolve 71 g of selenium in nitric acid, evaporate to dryness, dissolve the residue in 2 mL of water and evaporate to dryness. Repeat the procedure three times. Dissolve the residue with 2 M hydrochloric acid and supplement the volume to 1000 mL with the same solvent.

Standard Solution of sodium (200 ppm In)

Preparation: Dissolve 0.509 g of sodium chloride in 100 mL of water. At the time of use, dilute 1:10.

Standard Solution of sulphate (10 ppm SO_4)

Preparation: Dissolve 0.182 g of potassium sulfate in 100 mL of water. Dilute 1 mL of this solution to 100 mL of water at the time of use.

Standard Solution of zinc (100 ppm Zn)

Preparation – Dissolve 0.440 g of zinc sulphate in water containing 1 mL of 5 M acetic acid and dilute to 100 mL with water. Immediately before use, dilute 1 volume to 10 volumes with water.

Standard Solution of zinc (10 ppm Zn)

Preparation – Dilute 1 volume of standard solution of zinc (100 ppm Zn) for 10 volumes with water immediately before use.

Standard Solution brown

Preparation – Make a solution consisting of 30 mL of *base Solution of ferric chloride* (2.5.12), 30 mL of *base Solution of chloride Cobaltso* (2.5.12), 24 mL of *base Solution of cupric sulfate* (2.5.12) and 16 mL of hydrochloric acid (1% w/v).

Reducing Solution

Preparation – Dissolve 5 g of tetraidroborato sodium in 500 mL of sodium hydroxide (1% w/v).

Bismuth Subnitrate

(CAS 1304-85 -4)

Synonymy – Oxinitrato of bismuth.

Formula and molecular mass – Bi5O (OH) 9 (NO3.4 – 1461,99. *Specification* – is basic salt that contains, at a minimum, the equivalent to 79.0% of bismuth trioxide (Bi2O3) (p/p). Description – white Powder, dense, hygroscopic, odorless and without taste. Presents alkaline reaction before the litmus test.

Solubility Practically insoluble in water. Conservation – In well-closed containers. *Storage* – Protect from light.

Category – Antacid.

Replacement of platelets

Preparation – a quantity between 0.5 g and 1 g of phospholipids, add 20 mL of acetone, and shake the mixture, often, during 2 hours. Centrifugue for 2 minutes and discard the supernatant. Dry the residue with the aid of a water-jet pump, add 20 mL of chloroform and shake during 2 hours. Filter the reduced pressure and suspend the residue obtained in 5 mL to 10 mL of sodium chloride solution at 0.9% (w/v).

Determination of the activity of Factor IX – Prepare a dilution solution of sodium chloride 0.9% (p/v), such that the difference between the times of coagulation of successive dilutions of the preparation of reference is approximately 10 seconds.

Conservation – The suspensions diluted can be used during the 6 weeks that follow the preparation, if kept at -30 ºC.

7503 Plasma

Preparation – Separating the plasma from human blood, or beef harvested at 1/9 of its volume of sodium citrate solution to 3.8% (p/v), or in 2/7 of its volume of a solution containing 2% (w/v) sodium citrate acid and 2.5% (w/v) glucose. In the first case the substrate is prepared on the day of blood collection; in the latter case, the substrate of plasma can be prepared in 2 days that follow the collection.

Conservation – In plastic tubes, in small quantities, at a temperature less than or equal to -20ºC.

7503 Plasma1

Preparation – Use equipment manufactured in hydrophobic material appropriate plastic or glass siliconized for collection and handling of blood. An appropriate number (5 at least) of rams, live or at the time of slaughter, a collection of blood volume appropriate to each one (it is considered as an appropriate volume of 285 mL of blood collected on 15 mL of anticoagulant solution). The collection is done by means of a needle adapted to a cannula with a sufficient length to reach the bottom of the container. Reject the first millilitres and harvest, only the blood that flow freely. Mix the blood with a sufficient quantity of anticoagulant solution containing 8.7 g of sodium citrate and 4 mg of aprotinin in 100 mL of water. To obtain a final ratio of 19 volumes of blood to 1 volume of anticoagulant solution. During and immediately after the collection print to the container a rotatory movement so that the mixture if drill without foaming. As soon As they finish the collection close the flask and leave to cool to 10

7503 Plasma, thawed can be slightly, centrifuged, if necessary (do not use filtration processes).

7503 Plasma2

Preparation – Prepare from the human blood that has a content in Factor IX less than 1 % of the normal content. Collecting the blood in 1/9 of its volume of a solution of sodium citrate to 3.8% (p/v).

Conservation – In plastic tubes, in small quantities, at a temperature less than or equal to -30 ºC.

7503 Plasma deficient in Factor V

Specification – Use, preferably, a plasma congenitally deficient or prepared as follows: to separate the plasma from human blood which has been harvested at 1:10 of its volume of a solution of sodium oxalate to 1.34% (p/v). Incubate at 37 ºC for 24-36 hours. The plasma presents a coagulation time 70

Conservation: in small quantities, the temperature less than or equal to -20ºC.

Sudan III

(CAS 85-86 -9)

Formula and molecular mass- $C_{22}H_{16}N_4O$ – 352,40

Description – Dust red-brown.

Conservation – In well-closed containers.

Sudan III SR

Preparation – Dissolve 0.5 g of Sudan III in 100 mL of 80% ethanol (v/v), heated to 60ºC, cool and filter.

Sudan IV

(CAS 85-83 -6)

Formula and molecular mass – $C_{24}H_{20}N_4O$ – 380,44

Description – Po brown or reddish brown. *Physical Characteristics* – Range of fusion: 181 ºC to 188 ºC. Decomposes completely to 260 ºC.

Solubility Practically insoluble in water. Slightly soluble in acetone, ethanol and benzene. Soluble in paraffin and phenol

Conservation – In well-closed containers.

Sudan IV SR

Preparation – Dissolve 2 g of Sudan IV in 100 mL of ethanol 92% (v/v), heated to 60 ºC, cool, filter and add 5 mL of glycerin.

Ammonium Sulphamate

(CAS 7773-06 -0)

Formula and molecular mass – $NH_4SO_3NH_2$ – 114,13

Description – white, crystalline Powder or colorless crystals. *Physical Characteristic* – melting Temperature: around 131 ºC.

Solubility Very soluble in water and slightly soluble in ethanol.

Conservation – In containers perfectly closed.

Sulphanilamide

(CAS 63-74 -1)

Synonymy – 4-Aminobenzenossulfonamida.

Formula and molecular mass – $C_6H_8N_2O_2S$ – 172,20

Description – Crystals or fine powder- white or yellowish.

Physical Characteristic – melting Temperature: approximately 165 ºC.

Solubility – Soluble in glycerol and practically insoluble in chloroform, ethyl ether and benzene.

Conservation – In well-closed containers.

Category – Antibacterial.

Sulphate cerico

(CAS 13590-82 -4)

Synonymy – Disulfate are cerico.

Formula and molecular mass – $Ce(SO_4)_2$ – 332,24

Description – Crystal or powder yellow-orange. *Physical Characteristic* – melting Temperature: approximately 350 ºC.

Conservation – Protect from light, heat and humidity. *Security* – Toxic and oxidizing.

Ammoniacal sulphate cerico

(CAS 10378-47 -9)

Formula and molecular mass – $((NH_4)_4Ce(SO_4)_4.2H_2O$ – 632,58

Description – yellow-orange crystals.

Physical Characteristic – melting Temperature: around 130 ºC.

Solubility – Soluble in water.

Conservation – In well-closed containers.

Category – Standard for volumetry oxidation-reduction.

Cupric Sulfate, pentahydrate

(CAS 7758-99 -8)

Synonymy – copper sulphate pentahydrate.

Formula and molecular mass – $CCuSO_4.5H_2O$ – 249,68

Specification – Contains at least 98.5% (p/p) calculated on the dried substance to 250 ºC.

Description – Crystals, granules or powder blue. In contact with the air efloresce slowly.

Physical Characteristic – Heated to 250 ºC until constant weight, loses between 33.0 to 36.5% of its weight. – Solubility Very soluble in water and slightly soluble in ethanol.

Conservation – In well-closed containers. *Storage* – Protect the air.

Security – Irritant.

Cupric Sulfate SR

Specification – Contains 12.5 g cupric sulfate penta-hydrated in water to 100 mL.

Conservation – In well-closed containers.

Ammoniacal cupric Sulfate SR

Synonymy – copper sulphate ammoniacal SR and reagent of Schweitzer.

Preparation – Dissolve 10 g of cupric sulfate in 100 mL of water, add sufficient amount of sodium hydroxide solution (1:5) to precipitate the copper hydroxide. Filter and collect the precipitate. Wash with cold water. Dissolve the precipitate, which must be kept moist during the process, in the smallest quantity of ammonia SR needed to complete the solution.

Aluminum potassium sulphate, dodecahydrate

(CAS 7784-24 -9)

Synonymy – Alumen potassium

Formula and molecular mass – $AlK(SO_4)_2.12H_2O$ – 474,38

Description – Powder, granular mass or colorless, transparent.

Solubility Very soluble in boiling water, soluble in glycerin, practically insoluble in ethanol.

Conservation – In well-closed containers

Ammonium sulphate

(CAS 7783-20 -2)

Formula and molecular mass – $((NH_4)_2SO_4$ – 132,13

Specification – Contains, at a minimum, 99.0% (p/p).

Description – colorless, odorless crystals.

Physical Characteristic – Decomposes at temperatures above 280 ºC.

Solubility Very soluble in water, practically insoluble in acetone and ethanol.

Conservation – In well-closed containers.

Barium sulphate

(CAS 7727-43 -7)

Formula and molecular mass – $BaSO_4$ – 233,39

Specification – Contains, at a minimum, 97.5% (p/p).

Description – white Powder, fine and dense. Odourless and tasteless.

Solubility Practically insoluble in water and organic solvents, very slightly soluble in acids and hydroxy-alkaline solutions.

Conservation – In well-closed containers.

Category – radiological Contrast to the gastrointestinal tract.

Cadmium sulphate

(CAS 7790-84 -3)

Formula and molecular mass – $3CdSO_4.8H_2O$ – 769,49

Specification – Contains, at a minimum, 99.0% (p/p).

Description – crystalline Powder, colorless and odourless.

Conservation – In well-closed containers.

Calcium sulphate hemi-hydrate

(CAS 10034-76 -1)

Formula and molecular mass – $CaSO_4.1/2H_2O$ – 145,14

Specification – Contains, at a minimum, 98.0 % (p/p), calculated on dry matter basis.

Description – white Powder, thin; contains approximately 7.0% of water.

Solubility: Very slightly soluble in water, practically insoluble in ethanol. When mixed with half of its mass in water, is quickly solidified into a porous mass and lasts.

Conservation – In well-closed containers.

Calcium sulphate SR

Preparation – Shake 5 g of calcium sulphate hemi- hydrated with 100 mL of water, during one hour. Filter before use.

Conservation – In well-closed containers.

Sulphate of N,N-dimethyl-p-phenylenediamine

(CAS 536-47 -0)

Synonymy – Sulphate of N,N-dimethyl-1,4-benzenediamine.

Formula and molecular mass – $C_8H_{12}N_2.H_2SO_4$ – 234,28

Physical Characteristic – Range of fusion: 200 ºC to 205 ºC with decomposition.

Storage – Protect from light.

Security – Toxic.

Sulphate trifluorated

(CAS 77-78 -1)

Synonymy – Dimethyl sulphate, DMS.

Formula and molecular mass – $(CH_3)_2SO_4$ – 126,13

Description – colorless Liquid.

Physical Characteristics – boiling Temperature: approximately 188 °C with decomposition. Index of refraction (20 ºC): 1.3874.

Miscibility – Miscible with water (hydrolysis) and in ethyl ether and acetone.

Conservation – In closed containers.

Safety – Corrosive! Poisonous!

Hydrazine sulphate

(CAS 10034-93 -2)

Formula and molecular mass – $H_6N_2O_4S$ – 130,12

Description – colorless crystals.

Solubility Slightly soluble in cold water, soluble in hot water (50ºC) and readily soluble in boiling water. Practically insoluble in ethanol.

Lithium sulphate

(CAS 10102-25 -7)

Formula and molecular mass – $Smooth_4.H_2O$ – 127,95

Description – colorless crystals.

Solubility Easily soluble in water and practically insoluble in ethanol.

Magnesium Sulfate, heptahydrate

(CAS 10034-99 -8)

Formula and molecular mass – $MgSO_4.7H_2O$ – 246,48 – Description white crystalline Powder or colorless crystals shiny, salt taste, soluble in water, very soluble in boiling water, practically insoluble in ethanol. *Conservation* – In well-closed containers.

Manganese sulphate

(CAS 10101-68 -5)

Formula and molecular mass – $MnSO_4.4H_2O$ – 223,14

Specification – Contains, at a minimum, 98.0% (p/p) of $MnSO_4$, calculated on the substance dried at 450 ºC – 500 °C

Description – Crystals or crystalline powder pink. Odourless. Efloresce slowly.

Physical Characteristic – Loses water to approximately 450 ºC.

Solubility Easily soluble in water, very soluble in boiling water and practically insoluble in ethanol. Conservation – In well-closed containers.

Additional Information – The commercial product is normally mixture of manganese sulphate tetra and penta-hydrate.

Sulphate 4-metilaminofenol

(CAS 55-55 -0)

Formula and molecular mass – $C_{14}H_{20}N_2O_6S$ – 344,38

Description – colorless crystals.

Physical Characteristic – melting Temperature: approximately 260 °C with decomposition.

Solubility Very soluble in water and slightly soluble in ethanol.

Conservation – In container tightly closed. *Storage* – Protect from light.

Sulphate 4-metilaminofenol SR

Preparation – Dissolve 735 g of ammonium sulfate Metilaminofenol in 165 mL of water. Add 20 g of sodium bisulphite and mix. Dilute to 100 mL with water.

Potassium sulphate

(CAS 7778-80 -5)

Formula and molecular mass – K2SO4 – 174.25 *Specification* – Contains, at a minimum, 99.0% (p/p), calculated on the dried substance.

Description – colorless crystals or white crystalline powder, bitter taste.

Physical Characteristics – aqueous Solution with neutral character. Melting Temperature: 1067 ºC.

Conservation – In closed containers.

Protamine Sulfate

(CAS 9009-65 -8)

Specification – Consists in a mixture of simple proteins, obtained from sperm and testes of appropriate species of fish. Has the property to neutralize heparin.

Description – fine, white crystalline Powder or amorphous weakly stained.

Conservation – In well-closed containers, under refrigeration.

Storage – Protect from heat.

Sodium sulphate, anhydrous

(CAS 7757-82 -6)

Earth and molecular formula: Na_2SO_4 – 142.0

Specification – Prepared from Na2SO4.10H2O by heating to approximately 100 ºC. Contains, at a minimum, 99.0% (p/p), calculated on the dried substance.

Description – fine, white Powder, odourless, salty taste faintly bitter. Hygroscopic.

Physical Characteristic – melting Temperature: approximately 800 ºC.

Solubility Easily soluble in water.

Conservation – In well-closed containers. *Storage* – Protect from moisture.

Sodium sulphate, deca-hydrated

(CAS 7727-73 -3)

Synonymy – Salt Glauber.

Formula and molecular mass – $Na_2SO_4.10H_2O$ – 322,19

Specification – Contains at least 99.0 % (p/p) of Na_2SO_4, calculated in relation to the substance dried. Description – transparent colorless crystals or white crystalline powder, odourless, efflorescent salty flavor weakly bitter.

Physical Characteristic – melting Temperature: 32.5 ºC. Dissolves in its own water of crystallization, at a temperature of approximately 33 ºC.

Solubility Easily soluble in water, practically insoluble in ethanol.

Conservation – In well-closed containers *Storage* – Protect from heat.

Tetrabutylammonium Sulfate

(CAS 32503-27 -8)

Chemical Name – Sulfate N,N,N-tributyl-1-butanaminio, tetrabutylammonium hydrogen sulphate

Formula and molecular mass – $C_{16}H_{36}N.HSO_4$ – 339,53

Description – white crystalline Powder.

Physical Characteristic – Range of fusion: 169 ºC to 173 ºC. – Solubility Easily soluble in water and methanol.

Zinc sulphate heptahydrate

(CAS 7446-20 -0)

Formula and molecular mass – $ZnSO_4.7H_{2\,O}$- 287.58

Specification – Contains, at a minimum, 99.0% (p/p) of $ZnSO_4.7H_2O$, or at the very least, 55.6 % (p/p) of $ZnSO_4$.

Description – white crystalline Powder or colorless crystals transparent. Odourless, astringent taste. Efflorescent. *Physical Characteristic* – At temperature of 280 ºC, it becomes anhydrous.

Solubility Very soluble in water and practically insoluble in ethanol.

Conservation – In non-metallic containers well closed.

Storage – Protect from moisture.

Zinc sulphate 0.1 *M*

Description – Contains 28.75 g of zinc sulphate hepta-hydrated in water to 1000 mL.

Conservation – In non-metallic containers well closed.

Ferric sulphate

(CAS 10028-22 -5)

Synonymy – ferric Ammonium Persulfate.

Formula and molecular mass – $Fe_2(SO_4)_3.xH_2O$

Specification – The commercial product typically contains approximately 20% (p/p) of water.

Description – white Powder to yellow, very hygroscopic; it breaks down in the presence of air.

Solubility Slightly soluble in water and ethanol. Conservation – In well-closed containers. *Storage* – Protect from light and air.

Ferric sulphate ammoniacal

(CAS 7783-83 -7)

Formula and molecular mass – $FeNH_4(SO_4)_2.12H_2O$ – 482,18.

Description – Crystals transparent colorless to pale violet-. Odourless. Efflorescent.

Physical Characteristic – melting Temperature: approximately 37 ºC.

Solubility Very soluble in water and practically insoluble in ethanol.

Conservation – In well-closed containers.

Ammoniacal ferric sulphate acid SR

Preparation – Dissolve 20 g of ferric ammonium sulfate in 70 mL of water, add 10 mL of sulfuric acid 0.05 *M* and Fill up to volume with water to 100 mL.

Ferric sulphate ammoniacal SR

Specification – Contains 10 g in 330 mL water. *Conservation* – In well-closed containers.

Ferric sulphate ammoniacal SR1

Preparation – Dissolve 30 g of ferric ammonium sulfate in 40 mL of nitric acid and make up the volume to 100 mL with water.

Conservation – In well-closed containers. *Storage* – Protect from light.

Ferric sulphate ammoniacal SR2

Preparation – Dissolve 0.2 g of ferric ammonium sulfate in 50 mL of water, add 5 mL of nitric acid and dilute to 100 mL with water.

Ferric sulphate-potassium ferricyanide SR

Preparation – Mix equal volumes of ferric sulphate solution at 0.5% (w/v) in 0.5 *M sulfuric acid* and the solution of potassium ferricyanide to 0.2% (p/v). *Stability* – Prepare at the time of use.

Ferrous sulphate acidilicado SR

Preparation – Dissolve 0.45 g of ferrous sulphate hepta-hydrated in 50 mL of 0.1 *M hydrochloric acid* and complete the volume to 100 mL with water free of carbon dioxide.

Ammonium ferrous Sulfate

(CAS 7783-85 -9)

Formula and molecular mass – $FFe(NH_4)_2(SO_4)_2.6H_2O$ – 392,14

Description – crystalline Powder or crystals pale bluish-green. It Oxidizes slowly in the air, becoming efflorescent. *Physical Characteristic* – melting Temperature: around 100 °C with decomposition.

Solubility – Soluble in water, practically insoluble in ethanol.

Conservation – In well-closed containers. *Storage* – *Protect from* light and air.

Ferrous sulphate heptahydrate

(CAS 7782-63 -0)

Synonymy – iron sulphate, heptahydrate.

Formula and molecular mass – $FeSO_4.7H_2O$ – 278,01

Specification – Contains, at a minimum, 98.0% (p/p) of $FeSO_4.7H_2O$.

Description – blue-green hues; crystals or granules, or crystalline powder green. Odourless. Efflorescent. It Oxidizes by light and humidity the basic sulphate of iron (III) in the brown color.

Physical Characteristic – from the temperature of 65 ºC, it transforms into monohydrate.

Solubility Easily soluble in water, very soluble in boiling water and practically insoluble in ethanol. Conservation – In well-closed containers. *Storage* – Protect the air and moisture.

Additional Information – Do not use when you brown color.

Ferrous Sulfate SR

Specification – Contains 8 g of ferrous sulphate hepta-hydrated in cold water, freshly boiled, 330 mL. Prepare at the time of use.

Conservation – In well-closed containers. *Storage* – Protect from light, air and heat.

Ammonium sulfide SR

Preparation – Saturate 60 mL of ammonia SR with hydrogen sulfide and add 40 mL of ammonia SR. Use freshly prepared solution.

Conservation – In small container, well filled and closed.

Storage – Protect from heat and light.

Stability – In the face of abundant precipitation of sulfur, despise the solution.

Hydrogen sulfide

(CAS 7783-06 -4)

Synonymy – hydrosulphuric Acid

Formula and molecular mass – H_2S – 34,08

Specification – Produced by treatment of ferrous sulfide (or other sulfides) with sulfuric acid or hydrochloric acid diluted.

Description – colorless Gas of characteristic odour and taste sweet; more dense than air.

Physical Characteristics – relative Density to air: 1.19. Ignition Temperature: 260ºC.

Security – Toxic. Venom. Flammable.

Hydrogen sulfide SR

Specification – The saturated aqueous solution at 20ºC, contains around 0.4 to 0.5% (p/v). Prepared by the passage of H2S in cold water.

Physical Characteristic – The pH of the aqueous solution freshly prepared: 4.5.

Stability – Prepare for immediate use.

Security – Toxic. Venom. Flammable.

Sodium sulfide

(CAS 1313-84 -4)

Formula and molecular mass – $Na_2S.9H_2O$ – 240,18

Description – colorless deliquescent crystals, that if amarelam by exposure to air, or by the action of light. Odour similar to that of hydrogen sulfide.

Physical Characteristic – melting Temperature: approximately 50 ºC.

Conservation – Container tightly closed, in the cold. *Storage* – Protect from air, light and heat.

Sodium sulfide SR

Specification – Contains 1 g in water to 10 mL. *Stability* – Prepare for immediate consumption.

Sodium sulfide SR1

Preparation – Dissolve, with heating, 12 g of sodium sulfide in 45 mL of the mixture of water and glycerol to 85% (v/v) (10:29). Cool and dilute to 100 mL volumetric flask with the same solvent. The solution should be colorless. Prepare for immediate consumption.

Sodium Sulphite

(CAS 7757-83 -7)

Formula and molecular mass – Na_2SO_3 – 126,04

Description – white Powder, or almost white, odourless. – Solubility Easily soluble in water and very slightly soluble in ethanol.

Conservation – In well-closed containers.

Tannin

(CAS 1401-55 -4)

Synonymy – tannic Acid.

Specification – Obtained from barks of various plants, consisting of, especially, mixing of polyphenolic substances.

Description – Po yellow to brown. Characteristic Odor faintly and astringent taste.

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

Conservation – In well-closed containers. *Storage* – Protect from light.

Labelling – The labelling should indicate the source botany.

Acid tartrate epinephrine

(CAS 51-42 -3)

Synonymy – epinephrine Bitartrate.

Formula and molecular mass – $C_{13}H_{19}NO_9$ – 333,29

Specification – Contains, at a minimum, 97.0% (p/p), calculated on the dried substance.

Description – Crystals or white crystalline powder, or light gray. Odourless.

Physical Characteristic – melting Temperature: approximately 150 °C with decomposition. – Solubility Easily soluble in water and slightly soluble in ethanol.

Conservation – In airtight containers.

Storage – Protect the air and light.

Stability – Darkens slowly by exposure to air and light.

Category – adrenergic Agonist.

Alkaline cupric tartrate SR

Synonymy – Fehling's Solution

Solution – Dissolve 34.6 g of cupric sulfate penta- hydrated in 500 mL of water.

Solution B – Dissolve 173 g of potassium sodium tartrate and 50 g of sodium hydroxide in 400 mL of water, and heat to boiling. Cool and make up the volume to 500 mL with water free of carbon dioxide.

Preparation – Mix equal volumes of *Solutions A and B* immediately before use.

Tartrate of antimony and potassium

(CAS 28300-74 -5)

Synonymy – antimony Salt and potassium.

Formula and molecular mass – $C_8H_4K_2O_{12}Sb_2.3H_2O$ – 667,85

Description – Crystals Colorless OR po White.

Physical Characteristic – Range of fusion: 332 ºC to 335 ºC. *Conservation* – well-closed Containers.

Security – Toxic.

Sodium tartrate

(CAS 6106-24 -7)

Formula and molecular mass – $C_4H_4O_6In_2.2H_2O$ – 230,08

Specification – Contains 84,34% of $C_4H_4O_6In_2$ And 15.66% H_2O. Heated to 150 ºC, loses, at least 15.6% and, at most, 15.7% of its weight.

Description – white crystals or almost white. – Solubility Very soluble in water and practically insoluble in ethanol.

Conservation – In well-closed containers.

Potassium sodium tartrate

(CAS 6381-59 -5)

Synonymy – Rochelle Salt or Rochelle tartrate, potassium and sodium, emetic tartar.

Formula and molecular mass – $C_4H_4KNaO_6.4H_2O$ –

Anhydrous – 210.16

Specification – Contains, at a minimum, 99.0% (p/p), calculated on a dry basis of $C_4H_4KNaO_6$

Description – colorless crystals or white crystalline powder, odourless, salty flavor. Efflorescent in hot air. – Solubility Very soluble in water and practically insoluble in ethanol.

Conservation – In airtight containers.

Storage – Protect from heat.

Potassium sodium tartrate SR

Specification – Contains 20% (w/v).

Conservation – In well-closed containers.

Ferrous tartrate SR

Preparation – Dissolve 1 g of ferrous sulphate hepta-hydrated, 2 g of potassium sodium tartrate and 71 g of sodium bisulphite in water. Make up the volume to 100 mL with water. Prepare at the time of use.

Nedocromil sodium tetraborate

(CAS 1303-96 -4)

Synonymy – nedocromil sodium borate, sodium borate, borax. *Formula and molecular mass*- $In_2B_4O_7.10H_2O$ – 381,37

Specification – Contains, at a minimum, 99.0% (p/p).

Description – colorless crystals or white crystalline powder, odourless, taste caustic. Efflorescent.

Solubility – Soluble in water, very soluble in boiling water and readily soluble in glycerol.

Conservation – In well-closed containers; efloresce to dry air.

Storage – Protect the air.

Carbon tetrachloride

(CAS 56-23-5)

Formula and molecular mass – CCl_4 – 153,82

Specification – Contains, at a minimum, 99.0% (p/p).

Description – colorless Liquid, limpid, dense and characteristic odor.

Physical Characteristics – boiling Range: 76 ºC to 77 ºC. Density: 1.588 to 1.590. Index of refraction (20 ºC): 1.4607.

Miscibility – Practically insoluble in water and miscible with ethanol.

Conservation – In airtight containers.

Storage – Protect from heat and light.

Safety – Poison (in liquid and gaseous forms) !

Additional Information – is not flammable, but frees phosgene (toxic) in the presence of a flame.

Degraded Tetradecane

(CAS 629-59 -4)

Formula and molecular mass – $C_{14}H_{30}$ – 198,39

Specification – Contains at least 99.5% (p/p).

Description – Liquid clear and colorless.

Physical Characteristics – Density (20 ºC): approximately 0.76. Index of refraction (20 ºC): approximately 1.429. Melting Temperature: approximately -5 °C. Boiling Temperature: about 252 ºC.

Conservation – In closed containers.

Sodium Tetraphenylborate

(CAS 143-66 -8)

Formula and molecular mass – $NaB(C_6H_5)_4$ – 342,22

Description – Powder or white crystals or almost white. – Solubility Easily soluble in water and acetone. *Conservation* – In well-closed containers.

Sodium Tetrahydroborate

(CAS 16940-66 -2)

Formula and molecular mass – $NaBH_4$ – 37,83

Description – colorless crystals and hygroscopic. – Solubility Easily soluble in water, soluble in absolute ethanol.

Storage – In well-closed containers.

3,3'-diaminobenzidine tetrachloride Tetrahydrochloride

(CAS 7411-49 -6)

Formula and molecular mass – $C_{12}H_{18}Cl_4N_4$ – 360,12

Description - White crystals or Yellowish,

Occasionally purple.

Physical Characteristic – melting Temperature: approximately 280 °C with decomposition.

Solubility – Soluble in water.

Conservation - In Containers as well Closed, Under

Cooling.

Security – Irritant.

3,3' - diaminobenzidine tetrachloride Tetraidrocloreto SR

Specification – Contains 1 g of 3,3 ' -diaminobenzidine tetrachloride tetraidrocloreto in 200 mL of water.

Conservation - In Containers as well Closed, Under

Cooling.

Security – Irritant.

Tetrahydrofuran

(CAS 109-99 -9)

Formula and molecular mass – C_4H_8O – 72,11

Specification – The product is added of stabilizers (p-cresol, hydroquinone) in proportion 0.05 *% to* 0.1 % (p/v), to avoid the excessive formation of peroxides.

Description – colorless Liquid. Strong Smelling and similar to that of ethyl ether.

Physical Characteristics – boiling Temperature: 65 ºC to 66 ºC. Density: approximately 0.889. Index of refraction (20 ºC): 1.4070.

Miscibility – Miscible in water and in ethanol.

Conservation – In well-closed containers: small and filled.

Storage – Protect from contact with the light.

Safety – Irritating to skin, eyes and mucous membranes.

1,1,3,3-Tetramethylbuthylamine

(CAS 107-45 -9)

Formula and molecular mass – $C_8H_{19}N$ – 129,24

Description – colorless, clear Liquid.

Physical Characteristics – Density (20 ºC): approximately 0.805. Index of refraction (20 ºC): approximately 1.424. Boiling Temperature: about 140 ºC.

Tetramethylethylenediamine

(CAS 110-18 -9)

Synonymy - N,N,N',N'-Tetramethylethylenediamine, Temed.

Formula and molecular mass – $C_6H_{16}N_2$. –116,21

Specification – Quality suitable for electrophoresis.

Description – colorless Liquid.

Physical Characteristics – Density (20 ºC):

Approximately 1.418. Boiling Temperature: approximately 121 ºC.

Miscibility – Miscible with water, ethanol and with ethyl ether.

Potassium Tetraoxalate

(CAS 6100-20 -5)

Formula and molecular mass– $C_4H_3KO_8.2H_2O$ – 254,20

Description – white crystalline Powder or colorless crystals or white.

Solubility Slightly soluble in water and soluble in boiling water, slightly soluble in ethanol.

Conservation – In well-closed containers.

Osmium Tetroxide

(CAS 20816-12 -0)

Formula and molecular mass – OsO_4 – 254,20

Description – yellow crystalline Mass, or needles clear yellow, hygroscopic, sensitive to light.

Solubility – Soluble in water, ethanol and ethyl ether. *Conservation* – In airtight containers.

Safety – toxic Vapors.

Osmium Tetroxide SR

Specification – Contains 0.25 g of osmium tetroxide in 0.05 *M sulfuric acid* to make 100 mL.

Conservation – In well-closed containers.

Thimerosal

(CAS 54-64 -8)

Formula and molecular mass – $C_9H_9HgNaO_2S$ – 404,81

Description – light yellow crystalline Powder.

Solubility Very soluble in water and readily soluble in ethanol.

Thymidine

(CAS 50-89 -5)

Synonymy– 1-(2-Desoxi-*β*-D-ribofuranosil)-5-metiluracila.

Formula and molecular mass – $C_{10}H_{14}N_2O_5$ – 242,23

Description – needle-shaped crystals or white powder. *Solubility* – Soluble in water, ethanol and hot in glacial acetic acid.

Thymine

(CAS 65-71 -4)

Synonymy – 5-Methyl-2,4- (1*H*,3*H* -pirimidinodiona.

Formula and molecular mass – $C_5H_6N_2O_2$ – 126,11 proposal for PM.

Description – Plaques or needle-shaped crystals small.

Solubility Slightly soluble in cold water, soluble in hot water. Dissolve in dilute solutions of hydroxy- alkaline soil.

Thymol

(CAS 89-83 -8)

Synonymy – 5-Methyl-2- (1-methylethyl) phenol

Formula and molecular mass – $C_{10.0014}$.150.22

Description – colorless crystals, aromatic odour. *Physical Characteristic* – melting Temperature: around 50 ºC.

Solubility: Very slightly soluble in water, very soluble in ethanol, readily soluble in essential oils and fatty acids in oils, slightly soluble in glycerol. Dissolve in hydroxy-alkaline solutions.

Thioacetamide

(CAS 62-55 -5)

Formula and molecular mass – C_2H_5NS -75.13.

Description – Crystals or white crystalline powder, or nearly white. Weak odor of mercapto.

Physical Characteristic – melting Temperature: 113ºC to 114 ºC. – Solubility Easily soluble in water and in ethanol. *Conservation* – In well-closed containers.

Thioacetamide SR

Preparation – Mix 0.2 mL of thioacetamide solution at 4% (p/v) and 1 mL of the following mixture: 1.5 mL of sodium hydroxide *M,* 0.5 mL of water and 2 mL of glycerol to 85% (p/v). Heat in a water bath for 20 seconds

Stability – Prepare at the time of use.

Ammonium thiocyanate

(CAS 1762-95 -4)

Synonymy – Sulfocianato of ammonium.

Formula and molecular mass – NH_4SCN – 76,12

Description – colorless crystals and deliquescent.

Physical Characteristic – melting Temperature: approximately 149 ºC.

Solubility Very soluble in water and soluble in ethanol. Conservation – In airtight containers.

Storage – Protect from moisture.

Ammonium thiocyanate SR

Specification – Contains 8 g in 330 mL water. *Conservation* – In well-closed containers.

Mercury thiocyanate

(CAS 592-85 -5)

Formula and molecular mass – $Hg(SCN)_2$ – 316,76

Description white crystalline Powder or almost white. – Solubility Very soluble in water, slightly soluble in ethanol, soluble in solutions of sodium chloride. *Conservation* – In well-closed containers. *Storage* – Protect from light.

Mercury thiocyanate SR

Preparation – Dissolve 0.3 g of mercury thiocyanate in ethanol. Make up the volume to 100 mL with the same solvent.

Conservation – In well-closed containers.

Stability – Limited to one week.

Potassium thiocyanate

(CAS 333-20 -0)

Synonymy – Sulfocianato of potassium.

Formula and molecular mass– KSCN -97,18

Specification – Contains, at a minimum, 99.0% (p/p). *Physical Characteristic* – melting Temperature: approximately 173 ºC.

Solubility Very soluble in water and ethanol.

Conservation – In well-closed containers.

Security – May cause skin rashes!

Sodium Thioglycollate

(CAS 367-51 -1)

Formula and molecular mass – $C_2H_3NaO_2S$ – 114,09

Specification – Contains, at a minimum, 95.0% (p/p).

Description – white crystalline Powder, hygroscopic, weak characteristic odor. It Oxidizes into contact with the air. – Solubility Easily soluble in water and methanol, slightly soluble in ethanol.

Conservation – In airtight containers.

Storage – Protect from light and air.

Thionin (CI 52000)

(CAS 135-59 -1)

Formula and molecular mass – $C_{12}H_{10}ClN_3S$ – 263,75

Description – dark green Needles, with brightness. – Solubility Easily soluble in hot water.

Thionin SR

Preparation – Add 1 g of thionin to 2.5 g of phenol and make up the volume to 100 mL with water.

Conservation – In closed containers.

Sodium thiosulphate

(CAS 10102-17 -7)

Synonymy – sodium Thiosulphate R.

Formula and molecular mass – $Na_2S_2O_3.5H_2O$ – 248,17

Specification – Contains, at a minimum, 99.0% (p/p), calculated on the dried substance.

Description – colorless crystals or white crystalline powder, easily eflorescentes, weakly bitter taste. *Physical Characteristics* – melting Temperature: approximately 48 ºC. Dissolves in its own water of crystallization temperature approximately 49 ºC. – Solubility Very soluble in water, practically insoluble in ethanol.

Conservation – In well-closed containers.

Sodium thiosulphate 0,1 ***M***

Preparation – Dissolve 2.5 g of sodium thiosulphate and 0.02 g of sodium carbonate in water free of carbon dioxide to 100 mL.

Conservation – In well-closed containers.

Thiourea

(CAS 62-56 -6)

Formula and molecular mass – CH_4N_2S – 76.12

Description – Crystals or white crystalline powder, or nearly white.

Physical Characteristic – Range of fusion: 176 ºC to 178 ºC. *Solubility* – Soluble in water and in ethanol.

Conservation – In closed containers.

Tyrosine

(CAS 60-18 -4)

Formula and molecular mass – $C_9H_{11}NO_3$ – 181,19

Description – colorless crystals or white, or almost white, crystalline powder or white, or nearly white. – Solubility Slightly soluble in water, practically insoluble in acetone and ethanol, soluble in dilute hydrochloric acid and hydroxy-alkaline solutions.

Tolualdehyde

(CAS 104-87 -0)

Formula and molecular mass – C_8H_8O – 120,15

Description – clear Liquid, colorless or yellowish. *Physical Characteristic* – Index of refraction (20 ºC): between 1.544 and 1.546.

Toluene

(CAS 108-88 -3)

Synonymy – Metilbenzeno, toluol.

Formula and molecular mass – C_7H_8 – 92,14

Description – colorless Liquid of characteristic odour.

Physical Characteristics – boiling Temperature:110 ºC to 111 ºC. Density of approximately 0.87. Index of refraction (20 ºC): 1.4967.

Miscibility – Very slightly soluble in water and miscible with ethanol.

Safety – Toxic! Flammable!

P-Toluidine

(CAS 106-49 -0)

Synonymy – 4-Metilanilina.

Formula and molecular mass – C_7H_9N – 107.15

Description – Crystals or flakes white or slightly yellowish.

Physical Characteristics – melting Temperature: about 44 ºC. Density (20 ºC): 1.046.

Solubility Easily soluble in ethanol, methanol, acetone and in dilute acids, and slightly soluble in water. Conservation – In well-closed containers.

Torina

(CAS 3688-92 -4)

Synonymy – Naftarson.

Formula and molecular mass – $C_{16}H_{11}AsN_2Na_2O_{10}S_2$ –576,30

Description – red Dust.

Solubility – Soluble in water.

Torina SR

Preparation – Dissolve 0.2 g of torina in water to 100 mL. *Conservation* – In closed container.

Storage – Protect from light.

Stability – Use in no more than one week after the preparation.

Tricin

(CAS 5704-04 -1)

Formula and molecular mass – $C_6H_{13}NO_5$ – 179,17

Specification – Quality suitable for electrophoresis. *Physical Characteristic* – melting Temperature: around 183 ºC.

1,1,1-Trichloroethane

(CAS 71-55 -6)

Formula and molecular mass – $C_2H_3Cl_3$ – 133,40

Description – non-flammable Liquid.

Physical Characteristics – Density (20 ºC): approximately 1.34. Boiling Temperature: about 74 ºC.

Miscibility – Practically insoluble in water, soluble in acetone and in methanol.

Trichloroethylene

(CAS 79-01 -6)

Synonymy – Trichlorethene.

Formula and molecular mass – C_2HCl_3 – 131.39

Specification – Contains, at a minimum, 99.5 % (p/p).

Description – colorless Liquid, characteristic odor. *Physical Characteristics – Density* (20ºC): approximately 1.46. Index of refraction (20 ºC): approximately 1.477. Boiling Temperature: approximately 87 ºC.

Miscibility – Practically insoluble in water, soluble in acetone and in methanol.

Conservation – In well-closed containers.

Security – Toxic.

With Triethanolamine.

(CAS 102-71 -6)

Synonymy – 2,2 ‘,2 “ -nitrilotriethanol

Formula and molecular mass – $C_6H_{15}NO_3$ – 149,19

Description – colorless Liquid, viscous, very hygroscopic, it becomes brown by exposure to air.

Physical Characteristic – Density: approximately 1.13.

Miscibility – Miscible with water, acetone, ethanol and methanol.

Conservation – In well-closed containers under the light.

Triethylamine

(CAS 121-44 -8)

Formula and molecular mass – $C_6H_{15}N$ – 101,20

Description – colorless Liquid, strongly ammoniacal odor. *Physical Characteristics* – Density: approximately 0.726. Boiling Range: 89 ºC to 90 ºC.

Conservation – In well-closed containers.

Security – Irritant. Flammable.

Triphenylmethanol

(CAS 76-84 -6)

Formula and molecular mass – $C_{19}H_{16}O$ - 260,33

Description – colorless crystals or white powder, or nearly white.

Solubility Practically insoluble in water and readily soluble in ethanol.

Conservation – In well-closed containers

Boron Trifluoride

(CAS 7637-07 -2)

Formula and molecular mass – BF_3 – 67,81

Description – colorless Gas, pungent odour and suffocating.

Boron Trifluoride, methanolic solution

Specification – commercial Solution containing about 14% (w/v) of BF_3 in methanol.

Trinitrophenol SR

Use picric acid SR1.

Arsenic trioxide

(CAS 1327-53 -3)

Synonymy – Oxide arsenous anhydride.

Formula and molecular mass – $The_{2\,O3}$ – 197.84

Description – white crystalline Powder or transparent, or amorphous mass.

Solubility Slightly soluble in water and soluble in boiling water.

Conservation – In well-closed containers.

Safety – Poison!

Chromium trioxide

(CAS 1333-82 -0)

Synonymy – chromic acid Anhydride.

Formula and molecular mass – CrO_3 – 99.99

Description – granulated crystals or powder or flakes-reddish brown, deliquescent.

Physical Characteristic – melting Temperature: approximately 197 ºC.

Solubility Very soluble in water.

Conservation – In glass containers airtight. *Storage* – Avoid proximity with flammable. *Safety* – Oxidant energetic. Irritant.

Bovine Thrombin

(CAS 9002-04 -4)

Specification – biological preparation obtained from bovine plasma, containing enzyme that converts fibrinogen to fibrin.

Description – Po yellowish-white.

Conservation – In closed containers.

Storage – In temperatures below 0 ºC.

Human Thrombin

(CAS 9002-04 -4)

Specification – biological preparation obtained from human plasma for fractionation techniques appropriate.

Description – amorphous Powder of cream color.

Conservation – In well-closed containers, under refrigeration, specifying date of preparation and power. *Storage* – Protect from light, moisture and oxygen. *Category* – Enzyme. Local Hemostasis.

Thromboplastin

(CAS 9035-58 -9)

Synonymy – Factor III (coagulation). *Specification* – biological preparation of animal origin, obtained by extraction of certain organs: brain, lung.

Description – Powder or suspension of a yellowish color, characteristic odour.

Physical Characteristic – In the presence of appropriate concentrations of calcium ions, presents tromboquinase activity on blood coagulation.

Conservation – In airtight containers.

Labelling – Specify in composition: ions and antimicrobial agents, their concentrations, as well as origin, date of preparation, activity.

Storage – Protect from heat and moisture. Keep under refrigeration.

Category – Preparation with enzymatic activity. Local Hemostasis.

Thromboplastin reagent

Preparation – Shake 1.5 g of powder of brain of boi, dry with acetone, with 60 mL of water at 50 ºC, during 10 to 15 minutes. Centrifuge at 1500 rpm for 2 minutes and decant the supernatant liquid.

Conservation – The extract, stored at temperature lower than 0 ºC, retains the activity for several days. Can be added cresol, in the amount of 3 g/L, as antimicrobial.

Tromethamine

(CAS 77-86 -1)

Formula and molecular mass – $C_4H_{11}NO_3$ – 121,14

Synonymy – Trometamol, tris (hydroxymethyl) aminomethane. *Specification* – Contains, at a minimum, 99.0 %, calculated on the dried substance.

Description – Crystals or white crystalline powder or almost white.

Physical Characteristics – Range of fusion: 168 ºC to 172 ºC. The pH of the solution 0.1 *M:* 10.4.

Solubility Easily soluble in water, slightly soluble in ethanol and very slightly soluble in ethyl acetate.

Conservation – In well-closed containers.

Sodium Tungstate

(CAS 10213-10 -2)

Formula and molecular mass – $In_2WO_4.2H_{2O}$- 329.87

Description – colorless crystals or white crystalline powder or almost white.

Solubility Easily soluble in water, forming a clear solution, and practically insoluble in ethanol.

Urea

(CAS 57-13 -6)

Synonymy – Carbamide.

Formula and molecular mass – $(NH_2)_2CO$ – 60,06

Description – Crystals or white powder, strong odor. *Physical Characteristic* – melting Temperature: approximately 132.7 ºC.

Solubility Very soluble in water, soluble in ethanol, and practically insoluble in methylene chloride. Conservation – In well-closed containers, in ventilated places.

Safety – Can cause damage if ingested or inhaled.

Ammonium Vanadate

(CAS 7803-55 -6)

Formula and molecular mass – NH_4VO_3 – 116,98

Description – white crystalline Powder or clear yellow. – Solubility Slightly soluble in water.

Vanillin

(CAS 121-33 -5)

Formula and molecular mass – $C_8H_8.3$ – 152.15

Description – needle-shaped crystals or white crystalline powder, or yellowish.

Physical Characteristic – Range of fusion: between 81 ºC and 84 ºC.

Solubility Slightly soluble in water, readily soluble in ethanol and methanol.

Conservation – In well-closed containers.

Vanillin SR

Preparation – Dissolve 1 g of vanillin in ethanol and supplement the volume to 100 mL with the same solvent. Carefully Add 2 mL of sulfuric acid and mix. Use the solution in 48 hours.

Vanillin sulfuric SR

Preparation – Dissolve 1 g of vanillin in 100 mL of methanol. Add 4 mL of hydrochloric acid and 5 mL of sulfuric acid.

Warfarin sodium

(CAS 129-06 -6)

Formula and molecular mass – $C_{19}H_{15}NaO_4$ – 330,31

Specification – Contains, at a minimum, 97.0% (p/p), calculated in relation to the substance dried.

Description – crystalline Powder or amorphous, weakly bitter taste.

Solubility Very soluble in water and ethanol, soluble in acetone, very soluble in methylene chloride. Conservation – In well-closed containers. *Storage* – Protect from light.

Category – anticoagulant.

Bromocresol Green SR

Solution – Dissolve 0.2 g of bromocresol green in 30 mL of water and 6.5 mL of 0.1 M sodium hydroxide *Solution B* – Dissolve 38 g of monobasic sodium phosphate and 2 g of anhydrous dibasic sodium phosphate in water and make up the volume to 1000 mL with the same solvent. *Preparation* – Dilute the Solution to 500 mL using the Solution *B* as diluent and mix. If necessary, adjust the pH to 4.6 ±0.1 with hydrochloric acid 0.1 M.

Phenol Red SR

Solution – Dissolve 33 mg of phenol red in 1.5 mL of 2 M sodium hydroxide and dilute to 100 mL with water.

Solution B: Dissolve 25 mg of ammonium sulfate in 235 mL of water. Add 105 mL of 2 M sodium hydroxide and 135 mL of glacial acetic acid 2 M.

Preparation – Add 25 mL of *Solution A into Solution B. If necessary, adjust the pH to 4.7.*

Conservation – In small containers and resistant to alkalis.

Vitexin

(CAS 3681-93 -4)

Formula and molecular mass – $C_{21}H_{20}O_{10}$ – 448,41

Description – yellow Powder.

Conservation – In well-closed containers. *Storage* – Protect from exposure to light.

Xanthydrol

(CAS 90-46 -0)

Formula and molecular mass – $C_{13}H_{10}O_2$ – 198,22

Specification – Contains, at least, 90% of $C_{13}H_{10}O_2$.

Description – white Powder or clear yellow.

Solubility Very soluble in water, soluble in ethanol and m glacial acetic acid.

Storage – Protect from light.

Xylene

(CAS 1330-20 -7)

Synonymy – Xylol.

Formula and molecular mass – C_8H_{10} – 106,17

Specification – Mixture of isomers: *o-, p-* and *m-xylene, with the predominance of m-xylene.*

Description – Liquid clear and colorless.

Physical Characteristics – Density (20ºC): approximately 0.867. Index of refraction (20 ºC): approximately 1.497. Boiling Temperature: approximately 138ºC.

Conservation – In airtight containers.

Security – Toxic. Flammable.

Zinc, enabled

Preparation – Cover a quantity of zinc granulated with solution of acid cloroplatinico 165 |ag/mL. Leave for 10 minutes. After washing, drain and dry immediately.

Conservation – In well-closed containers.

Zinc, granulated

(CAS 7440-66 -6)

Element and atomic mass – Zn – 65.38

Description – Metal lustrous bluish-white. Stable in air

Dry. It Turns into basic carbonate when exposed to

Moisture.

Physical Characteristics – Becomes malleable between 100ºC and 150ºC. Burning in the presence of air featuring green-bluish flame.

Conservation – In well-closed containers. *Storage* – Protect from moisture.

Safety – Toxic!

Zinc Ms. – 5 mg/mL

Specification – Contains 2.5 g of zinc pellets in 20 mL of 5 M hydrochloric acid supplement with water to 500 mL.

Conservation – In well-closed containers, inert (polyethylene type).

14.3 SOLUÇÕES VOLUMÉTRICAS

The volumetric solutions (SV) are accompanied by method of standardization, although there may be others that lead to the same degree of accuracy.

The values obtained in standardization are valid for all uses pharmacopoeial assays.

The reagents employees must possess degree chemically pure and, when necessary, be subjected to desiccation. The volumetric solutions are standardized and used at temperatures around 25ºC. Before significant variations of temperature, volumetric solution must have title confirmed at the same temperature or be gauged by correction factor.

Hydrochloric acid M SV

Specification – Contains 85 mL of hydrochloric acid in water to 1000 mL.

Standardization – Weigh exactly approximately 1.5 g of anhydrous sodium carbonate. Add 100 mL of water and two drops of methyl red ITSELF. Add the acid slowly, from the buret, until pinky color weak. Heat the solution to boiling; cool and continue the titration. Repeat this sequence of operations until the grille does not affect the pink coloration. Calculate the molarity. Each 52.99 mg of anhydrous sodium carbonate is equivalent to 1 mL of hydrochloric acid *M.*

Conservation – airtight Containers.

Storage – Protect from heat.

Oxalic acid 0.05 *M* SV

Specification – Contains 6.45 g of oxalic acid in water to 1000 mL.

Standardization – Transfer 15 mL of the sample to 250 mL erlenmeyer flask. Add 100 mL of water and 7 mL of sulfuric acid. Warm up to around 70 ºC and titrate with potassium permanganate 0.02 *M* SV recently standardized, adding slowly, with constant stirring, until appearance of pale pink color that persists for 15 seconds. The temperature at the end of the titration should not be less than 60ºC.

Conservation – glass Containers as well closed.

Storage – Protect from light.

Perchloric acid 0.1 *M* SV

Specification – Contains 10 g of perchloric acid in acetic acid to 1000 mL.

Standardization – Dissolve, under stirring, 8.5 mL of perchloric acid in 200 to 300 mL of glacial acetic acid. Add 20 mL of acetic anhydride, dilute the mixture to Ml with glacial acetic acid and leave to rest for 24 hours. Determine the water content, which must be between 0.02% and 0.05 %. Weigh exactly about 700 mg of potassium biftalato previously sprayed and dried at 120 ºC for 2 hours and dissolve it in 50 mL of glacial acetic acid in erlenmeyer flask of 250 mL capacity. Add 2 drops of sodium chloride metilrosanilinio and titrate with the solution of perchloric acid. until violet coloration change to emerald-green. Each 20.422 mg of potassium biftalato is equivalent to 1 mL of perchloric acid 0.1 *M.*

Sulfuric acid *M* SV

Specification – Contains 98.07 g of sulfuric acid in water to 1000 mL.

Standardization – Add slowly, with stirring, 60 mL of sulfuric acid about 1020 mL of water. Cool Down to ambient temperature. Determine the molarity by titration with sodium carbonate, as described for hydrochloric acid *M,* but weighing exactly about 3 g of anhydrous sodium

carbonate. Each 105.98 mg of anhydrous sodium carbonate is equivalent to 1 mL of sulfuric acid *M*.

Potassium Bromate 0.1 *M* SV

Specification – Contains 16.704 g of potassium bromate in water to 1000 mL.

Standardization – Measure exactly volume of around 40 mL of potassium bromate solution to 1.67% (p/v). Add 3 g of potassium iodide and 3 mL of hydrochloric acid SR. Wait 5 minutes and titrate the iodine released with sodium thiosulphate 0,1 *M* SV, using 3 mL of starch ITSELF as an indicator. Prepare a white. Correct and calculate the molarity. Each mL of potassium bromate corresponds to 6 mL of sodium thiosulphate 0,1 *M* SV.

Conservation – well-closed Containers.

Storage – Protect from light.

Bromine 0.05 *M* SV

Preparation – Dissolve 3 g of potassium bromate and 15 g of potassium bromide in water and dilute to 1000 mL with the same solvent.

Standardization – Transfer 25 mL of the solution to 500 mL erlenmeyer flask with cover and add 120 mL of water. Add 5 mL of hydrochloric acid, cap and shake gently. Add 5 mL of potassium iodide SR, recap, stir and leave to rest for 5 minutes under the light. Titrate the iodine released with sodium thiosulphate 0,1 *M* SV, adding 3 mL of starch SI Near the end point. Calculate the molarity. Each mL bromine 0.05 *M* SV is equivalent to 1 mL of sodium thiosulphate 0,1 *M* SV.

Conservation – amber glass Containers well closed. *Storage* – Protect from light.

Barium chloride 0.1 *M* SV

Preparation – Dissolve 24.4 g of barium chloride in water and dilute to 1000 mL with the same solvent. *Standardization* – In 10 mL of barium chloride solution, add 60 mL of water, 3 mL of concentrated solution of ammonia and 1 mg of Henoch-Sch ftaleina. Titrate with 0.1 *M disodium edetate* SV. When the solution discolor, add 50 mL of ethanol and continue the titration until the blue-violet coloration disappears.

Benzethonium chloride 0.004 *M* SV

Preparation – After dessecar 330 – 105ºC until constant mass, dissolve 1.792 g of benzethonium chloride in water and make up to 1000 mL with the same solvent. *Standardization* – Dissolve 0.350 g of benzethonium chloride, then dried at 100-105ºC until constant mass, in 30 mL of anhydrous acetic acid and add 6 mL of a solution of acetate of mercury SR. Titrate with 0.1 *M perchloric acid* SV in the presence of 0.05 mL of chloride metilrosanilinio ITSELF. Perform blank test. Each mL of perchloric acid 0.1 *M* SV corresponds to 44.81 mg of C27H42ClNO2.

Dichlorophenol-indophenol, standard solution

Preparation – Dissolve 165 mg of 2,6-dicloroindofenol nedocromil sodium in 165 mL of water with 42 mg of sodium bicarbonate. Shake vigorously. After dissolution, complete with water to 200 mL. Filter.

Standardization – Weigh exactly 165 mg of ascorbic acid and dilute with metaphosphoric acid-acetic acid SR 165 mL. For flask of 165 mL, immediately transfer 2 mL of the ascorbic acid solution and add 5 mL of metaphosphoric acid-acetic acid SR. Titrate rapidly with the solution of dichlorophenol-indophenol until pink color persists for at least 5 seconds. Do blank determination, by titrating 7 mL metaphosphoric acid-acetic acid SR, added amount of water equal to the solution of dichlorophenol-indophenol solution used in the titration of ascorbic acid. Express the concentration of the standard solution in terms of its equivalent in mg of ascorbic acid. *Conservation* – Containers of amber glass, well closed. *Stability* – Use in maximum 3 days and standardize immediately before use.

Edetate disodium 0.05 *M* SV

Synonymy – disodium EDTA disodium tetrasodium ethylenediaminetetraacetate (na 0.05 M, 0.05 *M*.

Specification – Contains 18.6 g of edetate disodium dihydrate (in water to 1000 mL.

Standardization – Weigh exactly about 200 mg of calcium carbonate. Transfer to glass of becher of 400 mL and add 10 mL of water. Stir and cover the beaker with a watch glass. Add 2 mL of dilute hydrochloric acid and shake until dissolution of calcium carbonate. Wash the Walls of glass of becher and the watch glass with water up to about 100 mL. Continue stirring, magnetically. Add 30 mL of the solution of disodium edetate from buret of 50.0 mL. Add 15 mL of sodium hydroxide SR and 300 mg of blue indicator hidroxinaftol. Continue the titration of edetate disodium solution until blue color. Calculate the molarity.

Conservation – well-closed Containers.

Edetate disodium 0.1 M SV

Preparation – Dissolve 37.5 g in 500 mL of water, add 100 mL of sodium hydroxide *M* and supplement to 1000 mL with water.

Standardization – Dissolve 0.12 g of zinc powder (with a degree of purity of 99.9 %) in 10 mL of dilute hydrochloric acid M. Add 0.1 mL of bromine water SR. Eliminate the excess of bromine by ebulindo the solution. Add sodium hydroxide solution to 8.5% (p/v) until reaction weakly acidic or neutral, and proceed as described in *titrations complexometricas* (point 5.3.3.4) for Zinc. Each mL of disodium edetate 0.1 *M* SV is equivalent to 6.536 mg of zinc. *Conservation* – well-closed Containers.

Ethanolic potassium hydroxide 0.5 *M* SV

Alternate Name – alcoholic potassium hydroxide 0.5 *M* SV

Preparation – Dissolve 3 g of potassium hydroxide in 5 mL of water and add ethanol to 100 mL. Leave the solution to

stand for approximately 24 hours. Decant the clear liquid, and transfer to containers of inert material and protected from light.

Standardization – Titrate 20 mL of hydrochloric acid solution with 0.5 *M* SV using 0.5 mL of phenolphthalein ITSELF as an indicator. Each mL of 0.5 *M hydrochloric acid* SV equals 28.060 mg of potassium hydroxide.

Potassium hydroxide *M* SV

Preparation – Dissolve 60 g of potassium hydroxide in water to 1000 mL. Add saturated solution of barium hydroxide, recently prepared, even that is not form more precipitate. Stir and leave to stand for approximately 12 hours. Decant the clear liquid, or filter, and transfer to containers of inert material (polyethylene type).

Standardization – Use the same procedure adopted for the sodium hydroxide *M* SV.

Conservation – In well-closed containers, inert (polyethylene type).

Safety – Caustic.

Sodium hydroxide *M* SV

Preparation – Prepare sodium hydroxide solution at 50% (p/v) with water free of carbon dioxide. Cool to room temperature and allow to settle. Take 82 mL of the supernatant solution and dilute with water to 1000 mL. *Standardization* – exactly Weigh approximately 5 g of potassium biftalato desiccated and dissolve in 75 mL of water free of carbon dioxide. Add two drops of phenolphthalein SI and titrate with the sodium hydroxide solution to permanent formation of pink color. Each mL of sodium hydroxide *M* SV equals 204.220 mg biftalato of potassium.

Conservation – well-closed Containers, inert (polyethylene type). Stoppers fitted with tube containing a mixture of sodium hydroxide and calcium oxide.

Protect Storage of carbon dioxide. Safety – Caustic.

Additional Information – confer the title with frequency.

Ethanolic sodium hydroxide 0.1 *M* SV

Preparation – Prepare sodium hydroxide solution at 50% (w/v) in water free of carbon dioxide. Cool to room temperature and allow to settle. Transfer 2 mL of the supernatant to volumetric flask of 250 mL and Fill up to volume with ethanol.

Standardization – Weigh, exactly, about 0.2 g of benzoic acid and dissolve in a mixture of 10 mL of ethanol and 2 mL of water. Add two drops of phenolphthalein SI and titrate with the sodium hydroxide solution ethanolic until pinky color permanent. Each mL of 0,1 M sodium hydroxide VS is equivalent to 122.120 mg of benzoic acid.

Conservation – In well-closed containers, inert (polyethylene type).

Storage – Protect from exposure to carbon dioxide.

Safety – Caustic.

Tetrabutylammonium hydroxide 0.1 *M* SV

Preparation – Dissolve 40 g of tetrabutylammonium iodide in 900 mL of anhydrous methanol, in erlenmeyer flask fitted with stopper lapped. Place in an ice bath, add 20 g of silver oxide sprayed, cap the bottle and shake vigorously for 60 minutes. Remove some mL and centrifuge. Check presence of iodide on the supernatant fraction. If the test is positive, add more G of silver oxide and leave to rest for 30 minutes, stirring occasionally. Filter through a funnel of porous plate, rinse the erlenmeyer flask and funnel with three portions of 50 mL of toluene and join the toluene from the filtrate. Make up the volume to 1000 mL with a mixture of 3 volumes of anhydrous toluene and a volume of anhydrous methanol. Pass on the solution for 10 minutes, current of nitrogen free of carbon dioxide. Store in container protected from carbon dioxide and moisture. Consume within 60 days. Determine the molarity on use, by dissolving approximately 400 mg of benzoic acid exactly weighed, in 80 mL of dimethylformamide. Add to this solution three drops of solution of thymol blue in 1% (w/v) in dimethylformamide and titrate with solution of tetrabutylammonium hydroxide until blue coloration. Protect the solution from contact with the air during titration. Use buret provida of carbon dioxide absorption tube. Log blank test. Each mL of tetrabutylammonium hydroxide is equivalent to 12.212 mg of benzoic acid.

Indigo carmine SV

Preparation – Crush 4 g of indigo carmine dye with successive portions of water until dissolved, without exceeding 900 mL. Transfer to 1000 mL volumetric flask, add Ml of sulfuric acid and Fill up to volume with water. *Standardization* – 10 mL of standard solution of nitrate (100 ppm NO3) add 10 mL of water, 0.05 mL of indigo carmine dye Sv and, carefully, 30 mL of sulfuric acid. Titrate immediately with indigo carmine SV until turning to blue coloration stable. The total volume, in mL, of indigo carmine SV required is equivalent to 1 mg of NO3.

Potassium Iodate 0.02 *M* SV

Specification – Contains 4.28 g of potassium iodate in water to 1000 mL.

Standardization – Dilute 50 mL of this solution to 100 mL with water. 25 Ml of this solution, add 2 g of potassium iodide and 10 mL of sulfuric acid *M. Titrate with sodium thiosulphate 0,1 M* SV using starch ITSELF, added near the end point, as indicator. Each mL of sodium thiosulphate 0,1 *M* SV is equivalent to 3.566 mg of KIO3.

Potassium Iodate 0.1 *M* SV

Preparation – Weigh exactly, 21.4 g of potassium iodate previously dried at 110 ºC, until constant weight, and dissolve in water and make up the volume to 1000 mL with the same solvent. It is not necessary to standardization, because this reagent is primary standard.

Iodine 0.05 *M* SV

Preparation – Dissolve 13 g of iodine in 100 mL of potassium iodide solution to 20% (p/v). Add three drops of hydrochloric acid and dilute to 1000 mL with water.

Standardization – Dissolve, exactly, about 0.15 g of arsenic trioxide in 20 mL of sodium hydroxide M. Heat if necessary. Add 40 mL of water and two drops of methyl orange and hydrochloric acid until pink color. Add 50 mL of sodium carbonate to 4% (p/v), 3 Ml of starch ITSELF and titrate with 0.05 M iodine SV until blue color permanent. Each mL of iodine 0.05 M SV is equivalent to 4.946 mg of arsenic trioxide.

Conservation – In glass container tightly closed and under the light.

Iodine 0.1 *M* SV

Preparation – Dissolve approximately 13 g of iodine in 100 mL of potassium iodide to 3.6% (p/v). Add three drops of hydrochloric acid and complete with water to 1000 mL. *Standardization* – exactly Weigh about 150 mg of arsenic trioxide. Dissolve in 20 mL of sodium hydroxide *M,* heating if necessary. Add 40 mL of water, 2 drops of methyl orange S1 and dilute hydrochloric acid until pink color. Add 50 mL of sodium carbonate to 4% (p/v) and 3 mL of starch ITSELF. Titrate with the iodine solution, from the buret, until blue color permanent.

Calculate the molarity. Each 4.946 mg of arsenic trioxide is equivalent to 1 mL of iodine 0.1 M.

Conservation – glass Containers as well closed. *Storage* – Protect from light.

Lithium Metoxido 0.1 *M* SV

Preparation – Dissolve, carefully, in 1000 mL volumetric flask, 0.694 g of lithium in 150 mL of anhydrous methanol and complete the volume with toluene. *Standardization* – Standardize always before use. The 10 mL of dimethylformamide add 0.05 mL of thymol blue in 0.3% (w/v) in methanol and titrate with 0.1 *M lithium metoxido* SV until blue coloration. Immediately, add 0.2 g of benzoic acid, shake and titrate with 0.1 *M lithium metoxido* SV until blue coloration. Prevent the absorption of atmospheric carbon dioxide. The volume of titrant spent in second titration represents the quantity of lithium metoxido required. Each mL of lithium metoxido 0.1 *M* SV equals 12.212 mg of C7H6O2.

Metoxido 0.1 *M* sodium SV

Specification – Contains 5.402 g solution in toluene-methanol to 1000 mL.

Preparation – Cool in an ice bath 150 mL methanol, contained in 1000 mL volumetric flask. Add, in small portions, about 2.5 g of metallic sodium newly fragmented. After the dissolution of the metal, add toluene until completing 1000 mL and mix. Keep this solution in container under the carbon dioxide. *Standardization* – exactly Weigh about 400 mg of benzoic acid, dissolve in 80 mL of dimethylformamide, add three drops of solution of thymol blue to 1% (w/v) in dimethylformamide and holder by metoxido solution of sodium until the appearance of blue color. Each 12.212 mg of benzoic acid is equivalent to 1 mL of sodium metoxido 0.1 *M.*

Ammoniacal Ammonium nitrate cerico 0.01 *M* SV

Preparation – 330 mL of nitrate cerico ammoniacal 0.1 *M* SV add, carefully, with cooling, 30 mL of sulfuric acid and dilute to 1000 mL with water.

Ammoniacal Ammonium nitrate cerico 0.1 *M* SV

Preparation – Shake solution containing 56 mL of dilute sulfuric acid and 54.82 g of nitrate cerico ammoniacal by 2 minutes, and carefully add five successive portions of 100 mL of water, stirring after each addition. Dilute the clear solution to 1000 mL with water. Standardize 10 days after preparation.

Standardization – Add 25 mL of solution 2 g of potassium iodide and 150 mL of water. Titrate immediately with sodium thiosulphate 0,1 *M* SV, using starch ITSELF as an indicator. Each mL of sodium thiosulphate 0,1 *M* SV equals 54.822 mg of (NH4.2 (Ce (NO3.6).

Storage – Protect from light.

Barium nitrate 0.01 *M* SV

Specification – Contains 2.614 g of barium nitrate in 1000 mL of water.

Standardization – Add 10 mL of sulfuric acid solution 0.01 *M* in a flask and dilute with water. Add two drops of torina to 0.2% (p/v) and two drops of Methylthioninium chloride 0.02% (p/v) and titrate slowly with solution of barium nitrate until color change from yellow to pink.

Lead nitrate 0.1 *M* SV

Preparation – Transfer, exactly, about 8.28 g of lead nitrate to volumetric flask of 250 mL, dilute in water and Fill up to volume with the same solvent. Mix Well.

Standardization – Transfer, exactly, 5 mL of lead nitrate 0.1 M, for erlenmeyer flask of 125 mL, add 3 Ml of water and, under magnetic stirring, add 5 drops of xylenol orange 0.1% (p/v) in water and 5 g of methenamine, until violet coloration. Titrate with 0.05 *M disodium edetate* SV until yellow coloration. Each mL of disodium edetate 0.05 *M* SV equals 16.560 mg of Pb (NO 3.2.

Nitrate (II) 0.1 *M* SV

Synonymy phenylmercury nitrate mercuric 0.1 M SV.

Preparation – Dissolve approximately 35 g of nitrate of mercury (II) in 5 mL of nitric acid and 500 mL of water. Complete with water to 1000 mL.

Standardization – 20 mL of this solution, add 2 mL of nitric acid and 2 mL of SR ferric sulphate ammoniacal SR. Cool the temperature below 20 ºC and titrate with ammonium thiocyanate 0.1 *M* until permanent appearance of brown coloring. Calculate the molarity.

Conservation – In well-closed containers.

Silver nitrate 0.1 *M* SV

Preparation – Dissolve approximately 17.5 g of silver nitrate in water to 1000 mL.

Standardization – Weigh exactly about 100 mg of sodium chloride, dried; transfer to becher of 150 mL and dissolve in 5 mL of water. Add 5 mL of acetic acid SR, 50 mL of methanol and three drops of eosin Y ITSELF. Shake, preferably with magnetic stirrer, and titrate with the silver nitrate solution. Calculate the molarity. Each mL of silver nitrate 0.1 *M* SV corresponds to 5.844 mg of sodium chloride.

Conservation – well-closed Containers.

Storage – Protect from light.

Nitrate, 0.005 *M* SV

Specification – Contains 2.401 g of thorium nitrate anhydrous in 1000 mL of water.

Standardization – Transfer, exactly, about 0.05 g of sodium fluoride, previously dried, for volumetric flask of 250 mL and complete with water. In 20 mL of this solution, add 0.6 mL of alizarin red staining ITSELF and then, Titrate with sodium hydroxide 0.1 *M* SV until the color changes from pink to yellow.

Add 5 mL of acetate buffer pH 3.0 and titrate with thorium nitrate solution 0.005 M until the color change from yellow to pink to yellowish. Each 0.8398 mg of sodium fluoride is equivalent to 1 mL of thorium nitrate 0.005 M SV.

Sodium nitrite 0.1 *M* SV

Specification – Contains 6.9 g of sodium nitrite in water to 1000 mL.

Standardization – Dissolve 7.5 g of sodium nitrite in water and make up to 1000 mL. Weigh exactly about 500 mg of sulphanilamide previously dried for 3 hours at 105 ºC. Transfer to becher. Add 20 mL of hydrochloric acid and 50 mL of water. Shake until dissolved and cool to 15 ºC. Keeping the temperature at around 15 ºC, titrate slowly with sodium nitrite solution using as external indicator starch iodetado ITSELF, until turning point: Each 17.220 mg of sulphanilamide is equivalent to 1 mL of sodium nitrite 0.1 *M*.

Potassium permanganate 0.02 *M* SV

Specification – Contains 3.161 g of potassium permanganate in water to 1000 mL.

Preparation – Dissolve about 3.2 g of potassium permanganate in 1000 mL of water, heat to boiling for 15 minutes. Leave at rest at amber vial with glass lid, under the light, for two days, and filter through a sintered glass funnel.

Standardization – Dissolve, exactly, about 0.2 g of sodium oxalate, previously dried at 110 degrees C until constant weight, in 250 mL of water. Add 7 mL of sulfuric acid, heat up to about 70 ºC, and titrate slowly with a solution of potassium permanganate, with constant stirring, until pale pinkish color that persists for 15 seconds. The temperature at the end of the titration should not be less than 60ºC. Each mL of potassium permanganate 0.02 *M* SV is equivalent to 6.700 mg of sodium oxalate. *Conservation* – amber glass Containers well closed, with glass lid.

Storage – Protect from light.

Additional Information – confer the title with frequency.

Cerico sulphate 0.05 *M* SV

Specification – Contains 33.22 g of ferrous sulphate cerico in 1000 mL of water.

Standardization – Weigh, exactly, about 0.2 g of sodium oxalate, dried beforehand, and dissolve in 75 mL of water. Add, with agitation, 2 mL of sulfuric acid previously mixed with 5 mL of water, mix well. Add 10 mL of hydrochloric acid, and heat up to about 75 ºC. Titrate with 0.05 *M sulphate cerico* until yellow color permanent. Each 6.700 mg of sodium oxalate is equivalent to 1 mL of 0.05 *M ammonium sulfate cerico* SV.

Ammoniacal sulphate cerico 0.1 M SV

Preparation – Dissolve 65 g of ammonium sulfate cerico mixture of 30 mL of sulfuric acid and 500 mL of water. Cool and dilute with water to 1000 mL. *Standardization* – Dissolve 80 mg of arsenic trioxide in 15 mL of sodium hydroxide 0.2 *M,* heating if necessary. Add 50 mL of 1 M sulfuric acid, 0.15 mL of osmium tetroxide to 0.25% (p/v) and 0.1 mL of ferroina ITSELF. Titrate with 0.1 *M ammonium sulfate cerico* until change of color. Each mL of 0.1 *M ammonium sulfate cerico* is equivalent to 4.496 mg of arsenic trioxide.

Zinc sulphate 0.1 *M* SV

Specification – Contains 16.144 g of zinc sulphate heptahydrate in water to 1000 mL.

Preparation – Dissolve 28.8 g of zinc sulphate in water and make up the volume to 1000 mL. Pipette 20 mL of the solution of disodium edetate 0.05 *M* to a 250 mL conical flask and add, in this order, 20 mL of buffer solution acetic acid-ammonium acetate, 100 mL of ethanol and 2 mL of dithizone SR. Titrate the solution of zinc sulphate until the pink coloration of course. Calculate the molarity.

Sodium Tetraphenylborate 0.02 *M* SV

Preparation: Dissolve 6.845 g of sodium tetraphenylborate in water to 1000 mL.

Standardization – Pipette two portions of 75 mL in two beakers. TO each one of them, add 1 mL of acetic acid SR, 25 mL of water, and slowly, with stirring, 25 mL of potassium biftalato to 5% (p/v). Leave to rest for two hours. Filter

one of the mixtures in crucible, sintered glass (porosity size 100-160 micrometers) and wash the precipitate with cold water. Transfer the precipitate with 165 mL of water and shake intermittently for 30 minutes. Filter and use the filtrate as saturated solution of potassium tetraphenylborate in the following procedure for standardization. Filter the second mixture in crucible, sintered glass, weighed, and washed with three portions of 5 mL of a saturated solution of potassium tetraphenylborate. Dry the precipitate at 105 ºC for one hour. Each g of potassium tetraphenylborate is equivalent to 955.1 mg of sodium tetraphenylborate. From the weight of sodium tetraphenylborate obtained, calculate the molarity of the solution.

Conservation – well-closed Containers.

Stability – Use recent solutions.

Ammonium thiocyanate 0.1 *M* SV

Preparation – Dissolve about 8 g of ammonium thiocyanate in water to 1000 mL.

Standardization – Mix exactly 30 mL of silver nitrate 0.1 *M* with 50 mL of water, 2 mL of nitric acid and 2 mL of SR ferric sulphate ammoniacal SR. Titrate with the ammonium thiocyanate solution until appearance of reddish-brown color. Each mL of silver nitrate 0.1 *M ‘* equivalent to 7.612 mg of ammonium thiocyanate. *Conservation* – well-closed Containers.

Sodium thiosulphate 0,1 *M* SV

Preparation – Dissolve approximately 25 g of sodium thiosulphate pentahydrate and 200 mg of sodium carbonate in water, freshly boiled and cooled, the 1000 mL. *Standardization* – Weigh exactly about 210 mg of potassium dichromate, sprayed and dried, and dissolve in 100 mL of water. Transfer to a 500 mL flask and add 3 g of potassium iodide, 2 g of sodium bicarbonate and 5 mL of hydrochloric acid SR. Stir and leave to rest for 10 minutes in the dark. Titrate the iodine released with sodium thiosulphate solution to color yellowish green. Add 3 mL of starch ITSELF and continue the titration until disappearance of the blue color. Calculate the molarity. Each mL of sodium thiosulphate 0,1 *M* SV corresponds to 4.903 mg of potassium dichromate. *Conservation* – well-closed Containers.

Additional Information – confer the title with frequency.

14.4 BUFFERS

Certain tests pharmacopoeial assays require adjustment or maintenance of pH. For this purpose, employ-if solutions called buffers, able to withstand changes in the activity of hydrogen ions. The required components are described in item Reagents. The nature of crystalline must be previously dried at 110 – 120ºC for one hour; use water free of carbon dioxide. The storage should be done in airtight containers and appropriate. Consider the stability in preparation of quantities for consumption. The following, are related to the solutions in order of increasing values of pH. Other earplugs with particular characteristics are described in the texts of the respective tests.

Hydrochloric acid Buffer pH 2.0

Preparation – Mix 50 mL of an aqueous solution of potassium chloride 0.2 *M* with 13 mL of an aqueous solution of hydrochloric acid 0.2 *M. Make up the volume to 200 mL with water and adjust the pH if necessary.*

Phosphate Buffer pH 2.2

Preparation – Dissolve 1.38 g of monobasic sodium phosphate in 800 mL of water. Adjust the pH with phosphoric acid and dilute to 1000 mL with water.

Acetate Buffer pH 3.0

Preparation – Dissolve 12 g of sodium acetate in water, add 6 mL of glacial acetic acid and dilute with water to make 100 mL. Adjust the pH if necessary.

Acetate Buffer pH 3.5

Preparation – Dissolve 25 g of ammonium acetate in 35 mL of water, add 38 mL of 7 M hydrochloric acid, adjust the pH to 3.5 with dilute hydrochloric acid SR or ammonium hydroxide 6 *M* and complete the volume to 100 mL with water.

Acetate Buffer pH 4.0

Preparation – Transfer 900 mL of water to 1000 mL volumetric flask, add 2.86 mL of glacial acetic acid and 1 mL of sodium hydroxide at 50% (p/v), completing the volume with water and mix. If necessary, adjust the pH to 4.0 with glacial acetic acid or sodium hydroxide to 50% (p/v).

Acetate Buffer pH 4.4

Preparation – Dissolve 136 g of sodium acetate and 77.4 g of ammonium acetate in water and dilute to 1000 mL. Add 250 mL of glacial acetic acid and mix.

Biftalato Buffer pH 4.4

Preparation – Dissolve 2.042 g of potassium biftalato in 165 mL of water, add 7.5 mL of 0.2 *M hydrogen hydroxide* and dilute to 200 mL with water. Adjust the pH if necessary.

0.05 *M acetate Buffer* pH 4.5

Preparation – Transfer 2.99 g of sodium acetate tri-hydrated and 1.66 mL of glacial acetic acid to 1000 mL volumetric flask. Dissolve in water and Fill up to volume with the same solvent. Adjust the pH if necessary.

Sodium acetate Buffer pH 4.5

Preparation – Dilute 2.8 mL of glacial acetic acid with water to 1000 mL. Adjust the pH to 4.5 ±0.05 with sodium hydroxide at 50% (p/v).

Sodium acetate Buffer 0.1 *M* pH 5.0

Preparation – Transfer 13.61 g of sodium acetate tri-hydrated for 1000 mL volumetric flask, dissolve in sufficient quantity of water and make up the volume with the same solvent. Mix Well. Adjust the pH

With acetic acid 0.1 M.

Citrate-phosphate Buffer pH 5.0

Solution – Dissolve 0.8 g of sodium phosphate dibasic heptahydrate in 500 mL of water.

Solution B: Dissolve 3.5 g of citric acid monohydrate in 500 mL of water.

Preparation – Mix, with agitation, the Solutions *A and B* up to adjust the pH to 5.0. Distribute in containers with 165 mL each. Autoclaving at 121 ºC, pressure of 1 atm for 20 minutes. Store at 4 ºC.

Phosphate Buffer pH 5.5

Solution – Dissolve 13.61 g of monobasic potassium phosphate in water and dilute to 1000 mL with the same solvent.

Solution B: Dissolve 35.81 g of dibasic sodium phosphate dodecahydrate in water and dilute to 1000 mL with the same solvent.

Preparation – Mix 96.4 mL of Solution A and 3.6 mL of Solution B. *Adjust the pH if necessary.*

Phosphate Buffer pH 5.8

Preparation – In 200 mL volumetric flask, add 3.6 mL of sodium hydroxide 0.2 *M* 165 mL potassium phosphate monobasic 0.2 *M* and Fill up to volume with water. Adjust the pH if necessary.

Phosphate Buffer pH 6.0

Preparation – Mix 50 mL of potassium phosphate monobasic 0.2 *M* and 5.7 mL of sodium hydroxide 0.2 *M*. Make up the volume to 200 mL with water. Adjust the pH if necessary.

Acetate Buffer pH 6.0

Preparation – Dissolve 100 g of ammonia acetate in 300 mL of water, add 4.1 mL of glacial acetic acid, if necessary adjust the pH, using ammonium hydroxide 10 *M* or glacial acetic acid 5 *M* and make up to 500 mL with water.

Citroen-phosphate Buffer pH 6.0

Alternate Name – sodium phosphate Buffer pH 6.0
Preparation – Mix 36.8 mL of citric acid to 2.1% (p/v) with 63.2 mL of dibasic sodium phosphate to 7.15% (p/v). Adjust the pH if necessary.

Phosphate Buffer pH 6.5

Preparation – Dissolve 2.75 g of dibasic sodium phosphate dihydrate and 4.5 g of sodium chloride in 500 mL of water. Adjust the pH to 6.5 with phosphate buffer pH 8.5.

Phosphate Buffer pH 6.8

Preparation – Dissolve 28.8 g sodium phosphate dibasic and 11.45 g of potassium phosphate (tribasic) in water and make up to 1000 mL.

Phosphate-sodium lauryl sulphate pH 6.8

Preparation – Mix 19 parts of hydrochloric acid 0.1 *M* with 17 parts of phosphate-sodium lauryl sulphate pH 11.0. If necessary, adjust the pH to 6.8 with phosphoric acid to 20% (v/v) or sodium hydroxide to 40% (p/v).

Buffer tris-hydrochloride *M* pH 6.8

Preparation – Dissolve 60.6 g of ketorolac tromethamine in 400 mL water. Adjust the pH to 6.8 with dilute hydrochloric acid and make up to 500 mL with water. Adjust the pH if necessary.

0.025 M phosphate Buffer pH 6.86

Preparation – Dissolve 3.53 g of dibasic sodium phosphate and 3.39 g of potassium phosphate monobasic phosphates in water to make the volume up to 1000 mL. Adjust the pH if necessary.

Acetate Buffer pH 7.0

Preparation – Dissolve 2.73 g of sodium acetate in approximately 70 mL of water. Adjust the pH to 7.0 with glacial acetic acid 0.5 *M*. Complete with water to 100 mL. Adjust the pH if necessary.

Conservation – In well-closed containers.

Citroen-phosphate Buffer pH 7.0

Alternate Name – sodium phosphate Buffer pH 7.0
Preparation – Mix 82.4 mL of sodium phosphate dibasic dodecaidratado to 7.15% (p/v) with 17.6 mL of citric acid to 2.1% (p/v). Adjust the pH if necessary.

Phosphate Buffer pH 7.0

Solution – M sodium hydroxide

Solution B: Dissolve 13.6 g of potassium phosphate monobasic phosphates in water and make up the volume to 100 mL with the same solvent.

Preparation – Mix 29.5 mL of Solution A and 50 mL of Solution B. Adjust the pH to 7.0 ±0.1 using the Solutions *A and B* and make up the volume to 100 mL with water.

Phosphate Buffer *M/l5 pH 7.0*

Preparation: Dissolve 0.908 g of monobasic potassium phosphate in water and dilute to 100 mL. Separately, dissolve 2.38 g of dibasic sodium phosphate in water and dilute to 100 mL. Mix 38.9 mL of solution of potassium phosphate monobasic with 61.1 mL of a solution of dibasic sodium phosphate. Adjust the pH if necessary.

Phosphate Buffer pH 7.1

Preparation – Transfer 1 g of monobasic potassium phosphate and 1.8 g anhydrous dibasic sodium phosphate for 1000 mL volumetric flask, add 900 mL of water and adjust the pH to 7.1 ±0.1 with phosphoric acid or sodium hydroxide 10 *M. Make up the volume with the same solvent.*

Phosphate Buffer pH 7.2

Preparation – Add 250 mL of potassium phosphate monobasic 0.2 *M* and 175 mL of sodium hydroxide 0.2 *M*. Make up the volume to 1000 mL. Adjust the pH if necessary.

Albumin-phosphate Buffer pH 7.2

Preparation – Dissolve 4.26 g of anhydrous dibasic sodium phosphate, 7.6 g of sodium chloride and 10 g of bovine serum albumin in water. Make up the volume to 1000 mL and before using adjust the pH with sodium hydroxide 2 *M* or with phosphoric acid.

Phosphate Buffer pH 7.3

Preparation – Dissolve 20.8 g sodium phosphate dibasic heptahydrate and 3.08 g of monobasic sodium phosphate monohydrate in 900 mL of water, adjust the pH to 7.3 ±0.1 with phosphoric acid or sodium hydroxide SR SR and dilute to 1000 mL with the same solvent.

Barbitol buffer Buffer pH 7.4

Preparation – Mix 50 mL of a solution containing 1.94% (w/v) of sodium acetate and 2.95% (w/v) of sodium barbitol buffer in water with 50.5 mL of 0.1 M hydrochloric acid 20 mL of solution a 8.5% (w/v) of sodium chloride and to 250 mL with water.

Potassium phosphate Buffer pH 7.4 with polysorbate 80 he 2% (v/v)

Preparation – Dissolve 27.22 g of potassium phosphate monobasic in 1000 mL of water. Transfer 250 mL of the solution prior to 1000 mL volumetric flask, add 195.5 mL of 0.2 *M sodium hydroxide* and 450 mL of water. Adjust the pH to 7.4 with phosphoric acid or sodium hydroxide and Fill up to volume with water. The polysorbate 80 should be added after, due to difficult solubility of same.

Imidazole Buffer pH 7.4

Preparation – Dissolve 3.4 g of imidazole and 5.84 g of sodium chloride in water. Add 18.6 mL of hydrochloric acid *M* and complete with water to 1000 mL. If necessary, adjust the pH to 7.4 ±0.1.

Buffer of ketorolac tromethamine-sodium chloride, pH 7.4.

Preparation – Dissolve 6.08 g of ketorolac tromethamine and 8.77 g of sodium chloride in 500 mL of distilled water. Add 10 g of bovine serum albumin. Adjust the pH with hydrochloric acid and complete 1000 mL with distilled water.

Tris-sodium chloride pH 7.5

Preparation – Dissolve 7.27 g of ketorolac tromethamine and 5.27 g of sodium chloride in 950 mL of water. Adjust the pH to 7.5 with 2 M hydrochloric acid and complete with water to 1000 mL.

BORATE Buffer pH 8.0

Preparation – Mix 0.619 g of boric acid and 0.746 g of potassium chloride in 165 mL of water. Add 3.97 mL of 0.2 *M sodium hydroxide* and dilute to 200 mL of water. Adjust the pH if necessary.

Buffer barbitol buffer of pH 8.4

Preparation – Dissolve 8.25 g of sodium barbitol buffer in water and make up to 1000 mL with the same solvent. Adjust the pH if necessary.

Tromethamine Buffer-EDTA, pH 8.4.

Preparation – Dissolve 5.12 g of sodium chloride, 3.03 g of ketorolac tromethamine and 1.40 g of sodium iodide in 250 mL distilled water. Adjust the pH with hydrochloric acid and complete 500 mL with distilled water.

Tris-EDTA Buffer pH 8.4 ASB

Preparation – Dissolve 6.1 g of ketorolac tromethamine, 2.8 g of sodium iodide, 10.2 g of sodium chloride and 10 g of bovine serum albumin in water, adjust the pH with hydrochloric acid *M* and supplement to 1000 mL with water.

Ammonium acetate Buffer pH 8.5

Preparation – Dissolve 50 g of ammonium acetate in 1000 mL of ethanol 20% (v/v). Adjust the pH to 8.5 with ammonium hydroxide 6 *M*.

Phosphate Buffer pH 8.5

Preparation – Dissolve 3.5 g of potassium phosphate dibasic anhydrous and 4.5 g of sodium chloride in 500 mL of water. Adjust the pH to 8.5 with a mixture of water and phosphoric acid (1:1).

Plug barbitol buffer pH 8.6

Preparation – Mix 129 mL of 0.1 *M hydrochloric acid* add sufficient volume of nedocromil sodium barbitol buffer 0.1 *M* to make up to 1000 mL. Adjust the pH if necessary.

Phosphate Buffer pH 8.6

Preparation – Mix 2.3 volumes of sodium hydroxide 0.2 *M, with 2.5 volumes of potassium phosphate monobasic 0.2 M* and 2 volumes of methanol. Cool and mix with water to get 10 volumes of solution. If necessary, Adjust the pH to 8.60 ±0.05 with sodium hydroxide.

Tris-hydrochloride Buffer 1.5 *M* pH 8.8

Preparation – Dissolve 90.8 g of ketorolac tromethamine in 400 mL of water. Adjust the pH to 8.8 with hydrochloric acid and dilute to 500 mL with water.

BORATE Buffer pH 9.0

Preparation – Dissolve 12.37 g of boric acid and 14.91 g of potassium chloride in water and make up the volume to 1000 mL with the same solvent. Transfer 50 mL of the solution obtained for 200 mL volumetric flask, add 20 mL of 0.2 *M sodium hydroxide* and 120 mL of water. Adjust the pH to 9.0 with sodium hydroxide or hydrochloric acid SR SR and Fill up to volume with water.

0.05 *M tris Buffer* pH 9.0

Preparation – Transfer 6.05 g of tromethamine for 1000 mL volumetric flask. Dissolve in water and Fill up to volume with the same solvent. Adjust the pH to 9.0 ±0.05 using phosphoric acid. Dissolve 10 g of sodium lauryl sulphate in approximately 600 mL of the buffer solution. Mix the solution obtained with the rest of the buffer.

BORATE Buffer pH 9.6

Preparation – Transfer 3.093 g of boric acid and 3.728 g of potassium chloride for volumetric flask of 1000 Ml, add 250 mL of water and shake until dissolved. Add 182 mL of 0.2 *M sodium hydroxide* and Fill up to volume with water. Adjust the pH if necessary.

Cap carbonate-sodium bicarbonate pH 9.6

Preparation – Dissolve 0.75 g of sodium carbonate and 1.5 g of sodium bicarbonate in 500 mL of water. Distribute in containers with 165 mL each. Autoclaving at 121 ºC, pressure of 1 atm for 20 minutes. Store at 4 ºC.

Ammonium chloride Buffer pH 10.0

Preparation – Dissolve 5.4 g of ammonium chloride in 70 mL of ammonium hydroxide 5 *M* and dilute with water to 100 mL.

Ammonium chloride Buffer pH 10.7

Preparation – Dissolve 67.5 g of ammonium chloride in water, add 570 mL of concentrated ammonium hydroxide solution and dilute to 1000 mL with water. Adjust the pH if necessary.

Phosphate-sodium lauryl sulphate pH 11.0

Preparation – Dissolve in water 16.35 g of monobasic sodium phosphate, 7.05 g of sodium hydroxide and 3 g of sodium lauryl sulphate and dilute with water to 1000 mL. Adjust the pH if necessary.

Plug acetic acid-ammonium acetate

Preparation – Dissolve 77.1 g of ammonium acetate in water, add 57.0 mL of glacial acetic acid and complete with water to 1000 mL. Adjust the pH if necessary.

Electrophoresis Buffer DSS-PAGE

Preparation – Dissolve 151.4 g of ketorolac tromethamine, 721 g of glycine and 50 g sodium lauryl sulphate in water and supplement 5000 mL with the same solvent. Dilute 1:10 with water, immediately before use. The pH of the diluted solution should be between 8.1 and 8.8.

Buffer concentrate samples for DSS-PAGE.

Preparation – Dissolve 1.89 g of ketorolac tromethamine, 5 g of sodium lauryl sulphate and 50 mg of bromophenol blue in water, add 25 mL of glycerin and supplement to 100 mL with water. Adjust the pH to 6.8 with dilute hydrochloric acid and make up to 125 mL with water.

Buffer concentrate samples for DSS-PAGE in reducing conditions

Preparation – Dissolve 3.78 g of ketorolac tromethamine, 10 g of sodium lauryl sulphate, 100 mg of bromophenol blue and 50 mL of glycerin in 200 mL of water. Add 25 mL of 2-mercaptoethanol. Adjust the pH to 6.8 with dilute hydrochloric acid and make up to 250 mL with water. Instead of 2-mercaptoethanol, dithiothreitol can be used as a reducing agent. In this case, proceed as if indicates: dissolve 3.78 g of ketorolac tromethamine, 10 g of sodium lauryl sulphate, 100 mg of bromophenol blue and 50 mL of glycerin in 200 Ml of water. Adjust the pH to 6.8 with dilute hydrochloric acid and make up to 250 mL with water. Immediately before use, add the dithiothreitol, so as to obtain a final concentration of 0.1 M.

Phosphate-Buffer saline (PBS)

Preparation – Dissolve, with stirring, 24 g of sodium chloride, 0.60 g of potassium chloride, 4.3 g of sodium phosphate dibasic dodecahydrate and 0.60 g of potassium phosphate monobasic in 4 L of water. Autoclaving at 121 ºC, pressure of 1 atm for 20 minutes. Store at 4 ºC.

Cupric sulfate Buffer

Solution – Dissolve 15.22 g of anhydrous dibasic sodium phosphate in sufficient quantity of water. Add 9.75 g of citric acid monohydrate and dilute to 1000 mL with water. Adjust the pH to 5.2 using sodium hydroxide or citric acid.

Solution B: Dissolve 0.313 g of cupric sulfate penta-hydrated in water and dilute to 100 mL with the same solvent.

Preparation – At the time of use mix 15 mL of

Solution B with 985 mL of the Solution.

ANNEX A – PERIODIC TABLE OF ELEMENTS – CHEMICAL NAMES, SYMBOLS AND PASTA ATÔMICAS

Table A. 1 **is recommended by the International Union of Pure and Applied Chemistry (IUPAC, 2007. The atomic mass is based on the mass of the** ^{12}C **= 12.**

Table A. 1 – chemical Elements – names, symbols and atomic masses

Periodic table of the chemical elements

1 **H** 1,0079																	2 **He** 4,0026
3 **Li** 6,941	4 **Be** 9,0122											5 **B** 10,811	6 **C** 12,011	7 **N** 14,007	8 **O** 15,999	9 **F** 18,998	10 **Ne** 20,180
11 **Na** 22,990	12 **Mg** 24,305	3	4	5	6	7	8	9	10	11	12	13 **Al** 26,982	14 **Si** 28,086	15 **P** 30,974	16 **S** 32,065	17 **Cl** 35,453	18 **Ar** 39,948
19 **K** 39,098	20 **Ca** 40,078	21 **Sc** 44,956	22 **Ti** 47,867	23 **V** 50,942	24 **Cr** 51,996	25 **Mn** 54,938	26 **Fe** 55,845	27 **Co** 58,933	28 **Ni** 58,693	29 **Cu** 63,546	30 **Zn** 65,38	31 **Ga** 69,723	32 **Ge** 72,64	33 **As** 74,922	34 **Se** 78,96	35 **Br** 79,904	36 **Kr** 83,798
37 **Rb** 85,468	38 **Sr** 87,62	39 **Y** 88,906	40 **Zr** 91,224	41 **Nb** 92,906	42 **Mo** 95,96	43 **Tc** -	44 **Ru** 101,07	45 **Rh** 102,91	46 **Pd** 106,42	47 **Ag** 107,87	48 **Cd** 112,41	49 **In** 114,82	50 **Sn** 118,71	51 **Sb** 121,76	52 **Te** 127,60	53 **I** 126,90	54 **Xe** 131,29
55 **Cs** 132,91	56 **Ba** 137,33	57-71	72 **Hf** 178,49	73 **Ta** 180,95	74 **W** 183,84	75 **Re** 186,21	76 **Os** 190,23	77 **Ir** 192,22	78 **Pt** 195,08	79 **Au** 196,97	80 **Hg** 200,59	81 **Tl** 204,38	82 **Pb** 207,2	83 **Bi** 208,98	84 **Po** -	85 **At** -	86 **Rn** -
87 **Fr** -	88 **Ra** -	89-103	104 **Rf** -	105 **Db** -	106 **Sg** -	107 **Bh** -	108 **Hs** -	109 **Mt** -	110 **Ds** -	111 **Rg** -							

57 **La** 138,91	58 **Ce** 140,12	59 **Pr** 140,91	60 **Nd** 144,24	61 **Pm** -	62 **Sm** 150,36	63 **Eu** 151,96	64 **Gd** 157,25	65 **Tb** 158,93	66 **Dy** 162,50	67 **Ho** 164,93	68 **Er** 167,26	69 **Tm** 168,93	70 **Yb** 173,05	71 **Lu** 174,97
89 **Ac** -	90 **Th** 232,04	91 **Pa** 231,04	92 **U** 238,03	93 **Np** -	94 **Pu** -	95 **Am** -	96 **Cm** -	97 **Bk** -	98 **Cf** -	99 **Es** -	100 **Fm** -	101 **Md** -	102 **No** -	103 **Lr** -

Table A. 2 – chemical Elements sorted by atomic number

Atomic Number (Z)	*Name*	*Symbol*	*Atomic Mass (A)*	*Density at 20ºC*	*Melting Point (ºC)*	*Boiling Point (ºC)*	*Year of discovery*	*Discoverer (es)*
1	Hydrogen	H	1,00794 g/mol	0,084 g/l	-259,1 °C	-252,9 °C	1766	Cavendish
2	Helium	He	4,002602 g/mol	0,17 g/l	-272,2 °C	-268,9 °C	1895	Ramsay and Cleve
3	Lithium	Li	6,941 g/mol	0,53 g/cm³	180,5 °C	1317 °C	1817	Arfvedson
4	Beryllium	Be	9,012182 g/mol	1,85 g/cm³	1278 °C	2970 °C	1797	Vauquelin
5	Boron	B	10,811 g/mol	2,46 g/cm³	2300 °C	2550 °C	1808	Davy and Gay-Lussac
6	Carbon	C	12,011 g/mol	3,51 g/cm³	3550 °C	4827 °C	Pre-history	Unknown
7	Nitrogen / (Nitrogen)	N	14,00674 g/mol	1,17 g/l	-209,9 °C	-195,8 °C	1772	Rutherford
8	Oxygen	O	15,9994 g/mol	1,33 g/l	-218,4 °C	-182,9 °C	1774	Priestley and Scheele
9	Fluor	F	18,9984032 g/mol	1,58 g/l	-219,6 °C	-188,1 °C	1886	Moissan
10	Neon Lighting	Ne	20,1797 g/mol	0,84 g/l	-248,7 °C	-246,1 °C	1898	Ramsay and Travers
11	Sodium	Na	22,989768 g/mol	0,97 g/cm³	97,8 °C	892 °C	1807	Davy
12	Magnesium	Mg	24,305 g/mol	1,74 g/cm³	648,8 °C	1107 °C	1755	Black
13	Aluminum	Al	26,981539 g/mol	2,70 g/cm³	660,5 °C	2467 °C	1825	Oersted
14	Silicon	Si	28,0855 g/mol	2,33 g/cm³	1410 °C	2355 °C	1824	Berzelius during
15	Phosphorus	P	30,973762 g/mol	1,82 g/cm³	44 (P4) °C	280 (P4) °C	1669	Brandt
16	Sulfur	S	32,066 g/mol	2,06 g/cm³	113 °C	444,7 °C	Pre-history	Unknown
17	Chlorine	Cl	35,4527 g/mol	2,95 g/l	-34,6 °C	-101 °C	1774	Scheele
18	Argon	Ar	39,948 g/mol	1,66 g/l	-189,4 °C	-185,9 °C	1894	Ramsay and Rayleigh
19	Potassium	K	39,0983 g/mol	0,86 g/cm³	63,7 °C	774 °C	1807	Davy
20	Calcium	Ca	40,078 g/mol	1,54 g/cm³	839 °C	1487 °C	1808	Davy
21	Scandium	Sc	44,95591 g/mol	2,99 g/cm³	1539 °C	2832 °C	1879	Nilson.
22	Titanium	Ti	47,88 g/mol	4,51 g/cm³	1660 °C	3260 °C	1791	Gregor and Klaproth
23	Vanadium	V	50,9415 g/mol	6,09 g/cm³	1890 °C	3380 °C	1801	Del Rio
24	Chrome / Chrome	Cr	51,9961 g/mol	7,14 g/cm³	1857 °C	2482 °C	1797	Vauquelin
25	Manganese	Mn	54,93805 g/mol	7,44 g/cm³	1244 °C	2097 °C	1774	Galín
26	Iron	Fe	55,847 g/mol	7,87 g/cm³	1535 °C	2750 °C	Pre-history	Unknown
27	Cobalt	Co	58,9332 g/mol	8,89 g/cm³	1495 °C	2870 °C	1735	Brandt
28	Nickel	Ni	58,69 g/mol	8,91 g/cm³	1453 °C	2732 °C	1751	Jo Cronstedt
29	Copper	Cu	63,546 g/mol	8,92 g/cm³	1083,5 °C	2595 °C	Pre-history	Unknown
30	Zinc	Zn	65,39 g/mol	7,14 g/cm³	419,6 °C	907 °C	Pre-history	Unknown
31	Gallium	Ga	69,723 g/mol	5,91 g/cm³	29,8 °C	2403 °C	1875	Lecoq of Boiskaudran
32	Gennnio	Ge	72,61 g/mol	5,32 g/cm³	937,4 °C	2830 °C	1886	Winkler
33	Arsenic	As	74,92159 g/mol	5,72 g/cm³	613 °C	613 (sublimiert) °C	ca, 1250	Albertus Magnus
34	Selenium	Se	78,96 g/mol	4,82 g/cm³	217 °C	685 °C	1817	Berzelius during

Table A. 2 (continued)

Atomic Number (Z)	*Name*	*Symbol*	*Atomic Mass (A)*	*Density at 20ºC*	*Melting Point (ºC)*	*Boiling Point (ºC)*	*Year of discovery*	*Discoverer (es)*
35	Bromine	Br	79,904 g/mol	3,14 g/cm³	-7,3 °C	58,8 °C	1826	Balard
36	Krypton-86	Kr	83,8 g/mol	3,48 g/l	-156,6 °C	-152,3 °C	1898	Ramsay and Travers
37	Rubidium	Rb	85,4678 g/mol	1,53 g/cm³	39 °C	688 °C	1861	Bunsen burners and Kirchhoif
38	Strontium	Sr	87,62 g/mol	2,63 g/cm³	769 °C	1384 °C	1790	Crawford
39	Yttrium	Y	88,90585 g/mol	4,47 g/cm³	1523 °C	3337 °C	1794	Gadolin
40	Zirconium	Zr	91,224 g/mol	6,51 g/cm³	1852 °C	4377 °C	1789	Klaproth
41	Niobium	Nb	92,90638 g/mol	8,58 g/cm³	2468 °C	4927 °C	1801	Hatchet
42	Molybdenum	Mo	95,94 g/mol	10,28 g/cm³	2617 °C	5560 °C	1778	Scheele
43	Technetium	Tc	98,9063 g/mol	11,49 g/cm³	2172 °C	5030 °C	1937	Perrier and Segre
44	Ruthenium	Ru	101,07 g/mol	12,45 g/cm³	2310 °C	3900 °C	1844	Claus
45	Rhodium	Rh	102,9055 g/mol	12,41 g/cm³	1966 °C	3727 °C	1803	Wollaston
46	Palladium	Pd	106,42 g/mol	12,02 g/cm³	1552 °C	3140 °C	1803	Wollaston
47	Silver	Ag	107,8682 g/mol	10,49 g/cm³	961,9 °C	2212 °C	Pre-history	Unknown
48	Cadmium	Cd	112,411 g/mol	8,64 g/cm³	321 °C	765 °C	1817	Stromeyer and Hennann
49	Indio	In	114,82 g/mol	7,31 g/cm³	156,2 °C	2080 °C	1863	Reich and Richter
50	Tin	Sn	118,71 g/mol	7,29 g/cm³	232 °C	2270 °C	Pre-history	Unknown
51	Antimony	Sb	121,75 g/mol	6,69 g/cm³	630,7 °C	1750 °C	Pre-history	Unknown
52	Tellurium	Te	127,6 g/mol	6,25 g/cm³	449,6 °C	990 °C	1782	Von Reichenstein
53	Iodine	I	128,90447 g/mol	4,94 g/cm³	113,5 °C	184,4 °C	1811	Courtois
54	Xenon	Xe	131,29 g/mol	4,49 g/l	-111,9 °C	-107 °C	1898	Ramsay and Travers
55	Caesium	Cs	132,90543 g/mol	1,90 g/cm³	28,4 °C	690 °C	1860	Kirchhoif and Bunsen burner
56	Barium	Ba	137,327 g/mol	3,65 g/cm³	725 °C	1640 °C	1808	Davy
57	Lanthanum	La	138,9055 g/mol	6,16 g/cm³	920 °C	3454 °C	1839	Mosander
58	Cerium	Ce	140,115 g/mol	6,77 g/cm³	798 °C	3257 °C	1803	Von Hisinger and Berzelius during
59	Praseodiinio	Pr	140,90765 g/mol	6,48 g/cm³	931 °C	3212 °C	1895	Von Welsbach
60	Neodymium Speaker	Nd	144,24 g/mol	7,00 g/cm³	1010 °C	3127 °C	1895	Von Welsbach
61	Promethium	Pm	146,9151 g/mol	7,22 g/cm³	1080 °C	2730 °C	1945	Marinsky and Glendenin
62	Samarium	Sm	150,36 g/mol	7,54 g/cm³	1072 °C	1778 °C	1879	Lecoq de Boisbaudran
63	Europium	Eu	151,965 g/mol	5,25 g/cm³	822 °C	1597 °C	1901	Demacay
64	Gadolinium	Gd	157,25 g/mol	7,89 g/cm³	1311 °C	3233 °C	1880	Of Marignac
65	Terbio	Tb	158,92534 g/mol	8,25 g/cm³	1360 °C	3041 °C	1843	Mosander
66	Disprosio	Dy	162,5 g/mol	8,56 g/cm³	1409 °C	2335 °C	1886	Lecoq de Boisbaudran
67	Holinio	Ho	164,93032 g/mol	8,78 g/cm³	1470 °C	2720 °C	1878	Soret

Table A. 2 (continued)

Atomic Number (Z)	*Name*	*Symbol*	*Atomic Mass (A)*	*Density at 20ºC*	*Melting Point (ºC)*	*Boiling Point (ºC)*	*Year of discovery*	*Discoverer (es)*
68	Erbium	Er	167,26 g/mol	9,05 g/cm³	1522 °C	2510 °C	1842	Mosander
69	Tulio	Tm	168,93421 g/mol	9,32 g/cm³	1545 °C	1727 °C	1879	Cleve
70	Iterbio	Yb	173,04 g/mol	6,97 g/cm³	824 °C	1193 °C	1878	Of Marignac
71	Lutetium Isotope	Lu	174,967 g/mol	9,84 g/cm³	1656 °C	3315 °C	1907	Urbain
72	Hafnium	Hf	178,49 g/mol	13,31 g/cm³	2150 °C	5400 °C	1923	De Coster, and von Hevesy
73	Tantalum	Ta	180,9479 g/mol	16,68 g/cm³	2996 °C	5425 °C	1802	Ekeberg
74	Tungsten	W	183,85 g/mol	19,26 g/cm³	3407 °C	5927 °C	1783	Gebrüder de Elhuyar
75	Rhenium	Re	186,207 g/mol	21,03 g/cm³	3180 °C	5627 °C	1925	Noddack, Tacke and Berg
76	Ósmio	Os	190,2 g/mol	22,61 g/cm³	3045 °C	5027 °C	1803	Tenant
77	Iridium	Ir	192,22 g/mol	22,65 g/cm³	2410 °C	4130 °C	1803	Tenant and andere
78	Platinum	Pt	195,08 g/mol	21,45 g/cm³	1772 °C	3827 °C	1557	Scaliger
79	Gold	Au	196,96654 g/mol	19,32 g/cm³	1064,4 °C	2940 °C	Pre-history	Unknown
80	Mercury	Hg	200,59 g/mol	13,55 g/cm³	-38,9 °C	356,6 °C	Pre-history	Unknown
81	Thallium	Tl	204,3833 g/mol	11,85 g/cm³	303,6 °C	1457 °C	1861	Crookes
82	Lead	Pb	207,2 g/mol	11,34 g/cm³	327,5 °C	1740 °C	Pre-history	Unknown
83	Bismuth	Bi	208,98037 g/mol	9,80 g/cm³	271,4 °C	1560 °C	1540	Agricultural
84	Polonium	Po	208,9824 g/mol	9,20 g/cm³	254 °C	962 °C	1898	Marie and Pierre Curie
85	Astato	At	209,9871 g/mol		302 °C	337 °C	1940	Corson and MacKenzie
86	Radon	Rn	222,0176 g/mol	9,23 g/l	-71 °C	-61,8 °C	1900	Sun
87	Francium	Fr	223,0197 g/mol		27 °C	677 °C	1939	Perey
88	Radio	Ra	226,0254 g/mol	5,50 g/cm³	700 °C	1140 °C	1898	Marie and Pierre Curie
89	Actinio	Ac	227,0278 g/mol	10,07 g/cm³	1047 °C	3197 °C	1899	Debieme
90	Thorium	Th	232,0381 g/mol	11,72 g/cm³	1750 °C	4787 °C	1829	Berzelius during
91	Actinide	Pa	231,0359 g/mol	15,37 g/cm³	1554 °C	4030 °C	1917	Soddy, Cranston and Inhal
92	Uranium	U	238,0289 g/mol	18,97 g/cm³	1132,4 °C	3818 °C	1789	Klaproth
93	Neptunium	Np	237,0482 g/mol	20,48 g/cm³	640 °C	3902 °C	1940	McMillan and Abelson
94	Plutonium	Pu	244,0642 g/mol	19,74 g/cm³	641 °C	3327 °C	1940	Seaborg
95	Americium	Am	243,0614 g/mol	13,67 g/cm³	994 °C	2607 °C	1944	Seaborg
96	Curio	Cm	247,0703 g/mol	13,51 g/cm³	1340 °C	3110 °C	1944	Seaborg

Table A. 2 (conclusion)

Atomic Number (Z)	*Name*	*Symbol*	*Atomic Mass (A)*	*Density at 20ºC*	*Melting Point (ºC)*	*Boiling Point (ºC)*	*Year of discovery*	*Discoverer (es)*
97	Berquelio	Bk	247,0703 g/mol	13,25 g/cm^3	986 °C		1949	Seaborg
98	Califomio	Cf	251,0796 g/mol	15,1 g/cm^3	900 °C		1950	Seaborg
99	Einstenio	Es	252,0829 g/mol		860 °C		1952	Seaborg
100	Ferniio	Fm	257,0951 g/mol		1527 °C		1952	Seaborg
101	Mendelevio	Md	258,0986 g/mol				1955	Seaborg
102	Nobelium	No	259,1009 g/mol				1958	Seaborg
103	Lawrencium	Lr	260,1053 g/mol				1961	Ghiorso
104	Rutherfordio	Rf	261,1087 g/mol				1964/69	Flerow oder Ghiorso
105	Dubnio	Db	262,1138 g/mol				1967/70	Flerow oder Ghiorso
106	Seaborgio	Sg	263,1182 g/mol				1974	Oganessian
107	Borio	Bh	262,1229 g/mol				1976	Oganessian
108	Hassio	Hs	265 g/mol				1984	Society for Discovery of Heavy ions
109	Meitnerio	Mt	266 g/mol				1982	Society for Discovery of Heavy ions
110	Darmstadio	Ds	269 g/mol				1994	Society for Discovery of Heavy ions
111	Roentgenio	Rg	272 g/mol				1994	Society for Discovery of Heavy ions
112	Ununbio	Uub	277 g/mol				1996	Society for Discovery of Heavy ions
113	Ununtrio	Uut						
114	Ununquadio	Uuq						
115	Ununpentio	Uup						
116	Ununliexio	Uuh						
117	Ununseptio	Uus						
118	Ununoctio	Uuo						

ANNEX B – SYSTEM UNITS INTERNATIONAL (SI) USED IN PHARMACOPOEIA AND THE EQUIVALENCES WITH OTHER UNITS

The international system has seven basic units, used as reference for all measurements and listed in Table B. 1

Table B. 1 – The seven base units of the SI.

Greatness	*Unit*	*Symbol*	*Setting the unit*
Length *L, h, r, x*	Metro	M	The meter is the length of the path travelled by light in vacuum during a time interval of 1/299792458 of second. Thus, the speed of light in vacuum, c0, is exactly equal to 299792458 m/S.
Earth *M*	KILOGRAM	Kg	Is equal to the mass of the international prototype of the kilogram, m (£), which is exactly equal to 1 kg.
Time T	Second	S	The second is the duration of 9,192,631,770 periods of the radiation corresponding to the transition between the two hyperfine levels of the ground state of the atom of caesium133 and refers to the atom of caesium at rest, at a temperature of 0K.
Electrical Current *I, i*	Ampere	A	The ampere is the intensity of an electric current constant that, maintained in two parallel conductors, rectilinear, of infinite length, of negligible circular cross section, and situated at a distance of 1 meter between themselves, in vacuum, would produce between these conductors a force equal to 2 x 10-7 nm per meter length.
Temperature Thermodynamics *T*	Kelvin	K	The kelvin, unit of thermodynamic temperature, is the fraction 1/273.16 of the thermodynamic temperature of the triple point in water. *Thus, the temperature of the triple point of water, Tpa, is exactly equal to 273.16 K. p*
Quantity of substance *N*	Mol	Mol	The mol is the amount of substance of a system containing as many elementary entities as there are atoms in 0.012 kilogram of carbon 12. When you use the mol, the elementary entities must be specified and may be atoms, molecules, ions, electrons, as well as other particles, or specified groups of such particles. *Thus, the molar mass of carbon 12, M (12C), is exactly equal to 12 g/mol. With regard to carbon atoms 12 free, at rest and in its fundamental state.*
Intensity Bright Iv	Candela	CD	The candela is the luminous intensity, in a given direction from a source that emits monochromatic radiation of frequency 540 x 1012 hertz and whose energy intensity is 1/683 watt per steradian. *Thus, the spectral luminous efficacy, K, of monochromatic radiation of _frequency 540 x 1012* Hz *is exactly equal to 683 lm/W.*

All the other values are described as derived quantities and measures as derived units. In Table B. 2 are listed some derived quantities.

Table B. 2 – Some derived quantities.

Greatness derived	*Representation*	*Derived Unit*	*Symbol*
Area	A	Square meter	$_{m}2$
Volume	v	Cubic meter	$_{m}3$
Speed	ν	Meter per second	m/s
Acceleration	a	Meter per second squared	m/s^2
Number of waves	σ, ϖ	OPPOSITE the metro	m^{-1}
Specific mass	ρ	KILOGRAM per cubic meter	kg/m^3
Superficial density	ρ_A	KILOGRAM per square meter	kg/m^2
Specific volume	ν	Cubic meter per kilogram	m^3/kg
Current density	*j*	Ampere per square meter	A/m^2
Magnetic field	*H*	Amperes per meter	A/m
Concentration	c	Mol per cubic meter	mol/m^3
Mass density	ρ, γ	Kilogram per cubic meter	kg/m^3
Luminance	Lv	Candela per square meter	cd/m^2
Index of refraction	n	A	1
Relative permeability	μ_r	A	1

Note that the index of refraction and the relative permeability are examples of dimensionless quantities, for which the SI unit is the number one (1), although this unit is not writing.

Some derived units receive special name, this being simply a compact form of expression of combinations of basic units that are often used. Then, for example, the joule, symbol J, is by definition, equal to m2 kg s-2. There are currently 22 special names for units approved for use in SI, which are listed in Table B. 3.

Table B. 3 – derived Units with special names on the SI.

Greatness derived	*Name of the derived unit*	*Symbol of unity*	*Expression in terms of other units*
Plane angle	Radian	rad	$m/m = 1$
Solid angle	Steradian	sr	$m^2/m^2 = 1$
Frequency	Hertz	Hz	s^{-1}
Strength	Nm	N	$m\ kg\ s^{-2}$
Pressure, voltage	Pascal	Pa	$N/m^2 = m^{-1}\ kg\ s^{-2}$
Energy, work, heat quantity	Joule	J	$N\ m = m^2\ kg\ s^{-2}$
Power, power flow	Watt	W	$J/s = m^2\ kg\ s^{-3}$
Electrical charge, amount of electricity	Coulomb	C	s A
Difference of electric potential	Volts	V	$W/A = m^2\ kg\ s^{-3}\ A^{-1}$
Capacitance	Farad	F	$C/V = m^{-2}\ kg^{-1}\ s^4\ A^2$
Electrical resistance	Ohm	Ω	$V/A = m^2\ kg\ s^{-3}\ A^{-2}$
Electrical conductance	Siemens	S	$A/V = m^{-2}\ kg^{-1}\ s^3\ A^2$
Flow of magnetic induction	Weber	Wb	$V\ s = m^2\ kg\ s^{-2}\ A^{-1}$
Magnetic induction	Tesla	T	$Wb/m^2 = kg\ s^{-2}\ A^{-1}$
Inductance	Henry	H	$Wb/A = m^2\ kg\ s^{-2}\ A^{-2}$
Celsius temperature	Degree Celsius	˚C	K
Luminous flux	Lumen	lm	$cd\ sr = cd$
Illuminance	Lux	lx	$lm/m^2 = m^{-2}\ cd$
Activity of a radionuclidio	Becquerel	Bq	s^{-1}
Absorbed dose, specific energy (communicated), kerma	Gray	Gy	$J/kg = m^2\ s^{-2}$
Equivalent dose, dose equivalent environment	Sievert	Sv	$J/kg = m^2\ s^{-2}$
Catalytic activity	Katal	Kat	S^{-1} mol

Although the hertz and the becquerel are equal to the inverse of the second, the hertz is used only for cyclical phenomena, and the becquerel, for stochastic processes in radioactive decay.

The unit of Celsius temperature is the degree Celsius, ºC, which is equal in magnitude to kelvin, K, the unit of thermodynamic temperature. The greatness Celsius temperature t is related to the thermodynamic temperature T by equation t/ºC = T/K – 273.15.

The sievert, is also used for the greatnesses: directional dose equivalent and personal dose equivalent.

The last four special names of units from Table **B. 3** were adopted specifically to ringfence measurements related to human health.

For each greatness, there is only one SI unit (although it can be expressed in different ways, often by the use of special names). However, the same unit ITSELF can be used to express the values of various magnitudes different (for example, the SI unit for the ratio J/K can be used to express both the value of the heat capacity as the entropy). Therefore, it is important that you do not use the unit alone to specify the Greatness. This applies to both the scientific texts as to measuring instruments (that is, the output reading of an instrument should indicate the measured quantity and the unit).

The dimensionless quantities, also called magnitudes of dimension one, are usually defined as the ratio between two quantities of the same nature (for example, the index of refraction is the ratio between two speeds, and the relative permeability is the ratio between the permeability of a dielectric medium and the vacuum). Then the unity of a dimensionless value is the ratio between two identical units of SI, therefore it is always equal to one (1). However, to express the values of quantities dimensionless, the unit one (1) is not written.

MULTIPLES AND SUBMULTIPLES OF SI UNITS

A set of prefixes has been adopted for use with the SI units, in order to express the values of quantities that are much larger or much smaller than the unit

SI Used without a prefix. The prefixes THEMSELVES are listed in Table **B. 4. They can be used with any base unit and with the derived units with special names.**

B

Table B. 4 – Multiples and submultiples of SI – Prefixes and symbols.

Factor	*Name*	*Symbol*	*Factor*	*Name*	*Symbol*
10^{1}	Deca	Da	10^{-1}	deci	d
10^{2}	Hl	H	10^{-2}	centi	c
10^{3}	Kilo	K	10^{-3}	mili	m
10^{6}	Mega	M	10^{-6}	micro	µ
10^{9}	Giga	G	10^{-9}	nano	n
10^{12}	Will	T	10^{-12}	pico	p
10^{15}	Peta	P	10^{-15}	femto	f
10^{18}	You	E	10^{-18}	atto	a
10^{21}	Zetta	Z	10^{-21}	zepto	z
10^{24}	Yotta	Y	10^{-24}	yocto	y

When the prefixes are used, the name of the prefix and the unit are combined to form a single word and, similarly, the symbol of the prefix and the symbol of the unit are written without spaces, to form a unique symbol that can be raised to any power. For example, you can write: kilometer, km; microvolts per meter at, ^V; femtosegundo, fs; 50 V/cm = 50 V (10-2 m) -1 = 5000 V/m.

When the base units and derived units are used without any prefix, the resulting set of units is considered consistent. The use of a set of consistent units has technical advantages. However, the use of prefixes is convenient because it avoids the need to employ factors of 10n, to express the values of quantities too large or too small. For example, the length of a chemical link is more conveniently expressed in nanometers, nm, than in meters, m, and the distance between London and Paris is more conveniently expressed in kilometers, miles, than in meters, m.

The kilogram, kg, is an exception, because although it is a basic unit the name already includes a prefix, for historical reasons. The multiples and submultiples of the kilogram are written by combining the prefixes with the grass: soon, writes that milligram, mg, and not microquilograma, ^kg.

UNITS OUTSIDE THE SI

The SI is the only system of units that is universally recognized, so that it has a distinct advantage when establishing an international dialog. Other units, that is, non-SI units, are generally defined in terms of SI units. The use of SI, also simplifies the teaching of science. For all these reasons the use of SI units is recommended in all fields of science and technology.

Although some non-SI units are still widely used, other, the example of the minute, hour and day, as units of time, will be always used because they are deeply rooted in our

culture. Others are used, for historical reasons, to meet the needs for groups with special interests, or because there is no convenient SI alternative.

However, when non-SI units are used, convenient alternative ITSELF the conversion factor for the SI should always be included.

The scientists should have the freedom to use non-SI units if they consider adequate ti their porpose.

However, when No SI units are used, the conversion factor to SI should always be included. Some No SI units are listed in Table B. 5, with its conversion factor to SI.

Table B. 5 – Some non-SI units.

Greatness	*Unit*	*Symbol*	*Compared with the SI*
Time	Minute	min	1 min = 60 s
	Time	h	1 h= 3600 s
	Day	d	1 d = 86400 s
Volume	Liter	L	1 L = 1 dm^3
Earth	Tonne	t	1 t = 1000 kg
Energy	Eletronvolt	eV	1 eV ~ 1,602 x 10^{-19} J
Pressure	Bar	bar	1 bar = 100 kPa = 750,064 mm Hg = 0,987 atm
	Millimetre of mercury	mm Hg	1 mm Hg = 133,322 Pa = 10^{-3} bar = 10^{-3} atm 760 mm Hg = 1 atm = 1.013 bar = 101,324 kPa
Length	Angstrom1	Â	1 Â = 10-^{2}m
	Nautical mile	M	1 M = 1852 m
Strength	Dina	dyn	1 dyn = 10-^{5} N
Energy	Erg	erg	1 erg = 10-^{7} J

* The *Houaiss Dictionary of Portuguese Language* admits that word spelled without the symbol on the "a".

The symbols of units begin with a capital letter when it comes to his own name (for example, ampere, A; kelvin, K; hertz, Hz; coulomb, C). In other cases they always begin with lowercase letter (for example, subway, m; second, s; mol, mol). The symbol of the liter is an exception: the capital letter is used to avoid confusion between the letter l and the number one (1). The symbol

The nautical mile is presented here as M; however there is a general agreement on any symbol for the nautical mile.

In Table **B. 6** are listed examples of other units outside the SI and use, still current, but that should be avoided. When mentioned in a document should indicate its equivalence with the unit ITSELF.

Table B. 6 – Other examples of units outside the SI.

Name	*Symbol*	*Value in SI unit*	*Description*
Curie	Ci	1 Ci = 3,7 x 10^{10} Bq	Expresses the activity of radionuclides
Roentgen	R	1 R = 2,58 x 10^{-4} C/kg	Expresses the radiation exposure X or g
Rad	Rad o rd	1 rad = 1 cGy = 10^{-2} Gy	Expresses the absorbed dose of ionizing radiation.
Rem	Rem	1 rem = 1 cSv = 10^{-2} Sv	Expresses the dose equivalent in radioprotection
Torr	Torr	1 torr = (101 325/760) Pa	Expressed pressure. Is Currently in disuse.
Normal Atmosphere	Atm	1 atm = 760 mm Hg = 1, 013 bar = 101,324 kPa	Expresses the atmospheric pressure pattern. Is Currently in disuse.
Calorie	Cal	1 cal = 4,18 J	Expresses the quantity of heat (energy) needed to raise 14.5 ºC to 15.5 ºC the temperature of 1 gram of water. Currently it is conventionally that 1 Cal = 1000 cal = 1 kcal.

The symbols for units are printed in Roman type (vertical), regardless of the type used in the rest of the text. They are mathematical entities and not abbreviations. They are never followed by a point (except at the end of a sentence) nor by a s to form the plural. It is mandatory to use the correct form for the symbols of units, as illustrated by the examples presented in complete publication of SI. Sometimes the symbols of units may have more than one letter. They are written in lowercase letters, except that the first letter is capitalized when the name is of a person. However, when the name of a unit is written out in full, it must begin with lowercase letter (except at the beginning of a sentence), to distinguish between the name of the unity of the person's name.

When you write the value of a greatness, as the product of a numeric value and a unit, both the number and the unit should be treated by ordinary rules of algebra. For example, the equation T = 293 K can be written also T/K = 293. This procedure is described as the use of the calculation of quantities, or the algebra of magnitudes. Sometimes this rating is useful to identify the header of columns of tables, or the designation of the axs of graphs, so that the entries in the table or the identification of points on the axs are simple numbers.

In the formation of products or quotients of units, apply the normal rules of algebra. In the formation of products of units, you must leave a space between the units (alternatively you can place a point at half height of the line, as a symbol of multiplication).

In the formation of numbers the decimal marker can be or a point or a comma, in accordance with the appropriate circumstances. For documents in the English language is the usual point, but in Brazil and for many languages of continental Europe and in other countries, the comma is most common use.

When a number has too many digits is usual grupar- if the digits in blocks of three, before and after the comma, to facilitate the reading. This is not essential, but it is done so often, and usually it is very useful. When this is done, the groups of three digits should be separated by only a narrow space; you should not use either a point and not a comma between them. The uncertainty of the numeric value of a greatness can be conveniently expressed, explaining that the uncertainty of recent significant digits, in parentheses, after the number. Example: 12.456.78.0

For additional information, see the website of the BIPM http:// www. bipm. org or the complete Publication of SI, 8 edition, which is available on the site. http://www. bipm. org/en/si

ANNEX C – SOLVENTS FOR CHROMATOGRAPHY

Table C. 1 – Solvents for chromatography and their properties.

Solvent	*Index of Polarity*	*Formula*	*Earth Molecular (G/mol)*	*Index of refraction* n_D^{20}	*Boiling Point CO*	*Vapor Pressure (mbar 20ºC)*	*Constant Dielectric*	*Time Dipole*
N-Heptane	-	C_7H_{16}	100.21	1.388	98.4	48		
N-Hexane	0.0	C_6H_{14}	86.18	1.375	68.9	160	1.9	0
Cyclohexane	0.0	C_6H_{12}	84.16	1.427	80.7	104	2.0	0
Iso-octane	0.4	C_8H_{18}	114.23	1.392	99.2		1.9	
Carbon tetrachloride	1.7	CCl_4	153.82	1.460	76.5	120	2.2	0
Toluene	2.3	$C_6H_5CH_3$	92.14	1.496	110.6	29	2.4	0.36
Chloroform	4.4	$CHCl_3$	119.38	1.446	61.7	210	4.8	1.01
Ethylene dichloride	3.7	$ClCH_2CH_2Cl$	98.96	1.445	83.5	87	10.60	
Methylene chloride	3.4	CH_2Cl_2	84.93	1.424	40.0	453	9.1	1.60
1-Butanol	3.9	$CH_3(CH_2)_3OH$	74.12	1.399	117.2		17.8	1.66
Acetonitrile	6.2	CH_3CN	41.05	1.344	81.6		37.5	3.92
Isopropyl Alcohol	4.3	$CH_3CH(OH)CH_3$	60.11	1.378	82.4	43	18.3	1.66
Ethyl Acetate	4.3	$CH_3COOC_2H_5$	88.12	1.372	77.1	97	6.0	1.78
Acetone	5.4	CH_3COCH_3	58.08	1.359	56.2	233	20.7	2.88
Ethanol	5.2	C_2H_5OH	46.07	1.361	78.5	59	24.3	1.70
Dioxana	4.8	$C_4H_8O_2$	88.11	1.422	101.0	41	2.2	
Tetrahydrofuran	4.2	C_4H_8O	72.11	1.405	67.0	200	7.4	1.63
Methanol	6.6	CH_3OH	32.04	1.329	65.0	128	32.6	1.70
Water	9.0	H_2O	18.01	1.333	100.0	23	80.2	1.85

ANNEX D – Alcoholometry

Table D. 1 – Tables (20 ºC)

% v/v	*% m/m*	*ρ20 (Kg/m3)*	*d (g/cm³)*
0,0	0,0	998,20	0,999997
0,1	0,08	998,05	0,999846
0,2	0,16	997,90	0,999696
0,3	0,24	997,75	0,999546
0,4	0,32	997,59	0,999386
0,5	0,40	997,44	0,999235
0,6	0,47	997,29	0,999085
0,7	0,55	997,14	0,998935
0,8	0,63	996,99	0,998785
0,9	0,71	996,85	0,998644
1,0	0,79	996,70	0,998494
1,1	0,87	996,55	0,998344
1,2	0,95	996,40	0,998194
1,3	1,03	996,25	0,998043
1,4	1,11	996,11	0,997903
1,5	1,19	995,96	0,997753
1,6	1,27	995,81	0,997602
1,7	1,35	995,67	0,997462
1,8	1,43	995,52	0,997312
1,9	1,51	995,38	0,997172
2,0	1,59	995,23	0,997021
2,1	1,67	995,09	0,996881
2,2	1,75	994,94	0,996731
2,3	1,82	994,80	0,996591
2,4	1,90	994,66	0,996450
2,5	1,98	994,51	0,996300
2,6	2,06	994,37	0,996160
2,7	2,14	994,23	0,996020
2,8	2,22	994,09	0,995879
2,9	2,30	993,95	0,995739
3,0	2,38	993,81	0,995599
3,1	2,46	993,66	0,995449
3,2	2,54	993,52	0,995308
3,3	2,62	993,38	0,995168
3,4	2,70	993,24	0,995028
3,5	2,78	993,11	0,994898
3,6	2,86	992,97	0,994757
3,7	2,94	992,83	0,994617
3,8	3,02	992,69	0,994477
3,9	3,10	992,55	0,994337

Table D. 1 (continued)

% v/v	*% m/m*	*ρ20 (Kg/m3)*	*d (g/cm³)*
4,0	3,18	992,41	0,994196
4,1	3,26	992,28	0,994066
4,2	3,34	992,14	0,993926
4,3	3,42	992,00	0,993786
4,4	3,50	991,87	0,993655
4,5	3,58	991,73	0,993515
4,6	3,66	991,59	0,993375
4,7	3,74	991,46	0,993245
4,8	3,82	991,32	0,993104
4,9	3,90	991,19	0,992974
5,0	3,98	991,06	0,992844
5,1	4,06	990,92	0,992704
5,2	4,14	990,79	0,992573
5,3	4,22	990,65	0,992433
5,4	4,30	990,52	0,992303
5,5	4,38	990,39	0,992173
5,6	4,46	990,26	0,992042
5,7	4,54	990,12	0,991902
5,8	4,62	989,99	0,991772
5,9	4,70	989,86	0,991642
6,0	4,78	989,73	0,991512
6,1	4,87	989,60	0,991381
6,2	4,95	989,47	0,991251
6,3	5,03	989,34	0,991121
6,4	5,11	989,21	0,990991
6,5	5,19	989,08	0,990860
6,6	5,27	988,95	0,990730
6,7	5,35	988,82	0,990600
6,8	5,43	988,69	0,990470
6,9	5,51	988,56	0,990339
7,0	5,59	988,43	0,990209
7,1	5,67	988,30	0,990079
7,2	5,75	988,18	0,989959
7,3	5,83	988,05	0,989828
7,4	5,91	987,92	0,989698
7,5	5,99	987,79	0,989568
7,6	6,07	987,67	0,989448
7,7	6,15	987,54	0,989318
7,8	6,23	987,42	0,989197
7,9	6,32	987,29	0,989067
8,0	6,40	987,16	0,988937
8,1	6,48	987,04	0,988817
8,2	6,56	986,91	0,988686
8,3	6,64	986,79	0,988566
8,4	6,72	986,66	0,988436
8,5	6,80	986,54	0,988316
8,6	6,88	986,42	0,988196
8,7	6,96	986,29	0,988065
8,8	7,04	986,17	0,987945
8,9	7,12	986,05	0,987825

Table D. 1 (continued)

% v/v	*% m/m*	*ρ20 (Kg/m3)*	*d (g/cm³)*
9,0	7,20	985,92	0,987695
9,1	7,29	985,80	0,987574
9,2	7,37	985,68	0,987454
9,3	7,45	985,56	0,987334
9,4	7,53	985,44	0,987214
9,5	7,61	985,31	0,987084
9,6	7,69	985,19	0,986963
9,7	7,77	985,07	0,986843
9,8	7,85	984,95	0,986723
9,9	7,93	984,83	0,986603
10,0	8,01	984,71	0,986482
10,1	8,10	984,59	0,986362
10,2	8,18	984,47	0,986242
10,3	8,26	984,35	0,986122
10,4	8,34	984,23	0,986002
10,5	8,42	984,11	0,985881
10,6	8,50	983,99	0,985761
10,7	8,58	983,88	0,985651
10,8	8,66	983,76	0,985531
10,9	8,75	983,64	0,985411
11,0	8,83	983,52	0,985290
11,1	8,91	983,40	0,985170
11,2	8,99	983,29	0,985060
11,3	9,07	983,17	0,984940
11,4	9,15	983,05	0,984819
11,5	9,23	982,94	0,984709
11,6	9,32	982,82	0,984589
11,7	9,40	982,70	0,984469
11,8	9,48	982,59	0,984359
11,9	9,56	982,47	0,984238
12,0	9,64	982,35	0,984118
12,1	9,72	982,24	0,984008
12,2	9,80	982,12	0,983888
12,3	9,89	982,01	0,983778
12,4	9,97	981,89	0,983657
12,5	10,05	981,78	0,983547
12,6	10,13	981,67	0,983437
12,7	10,21	981,55	0,983317
12,8	10,29	981,44	0,983207
12,9	10,37	981,32	0,983086
13,0	10,46	981,21	0,982976
13,1	10,54	981,10	0,982866
13,2	10,62	980,98	0,982746
13,3	10,70	980,87	0,982636
13,4	10,78	980,76	0,982525
13,5	10,87	980,64	0,982405
13,6	10,95	980,53	0,982295
13,7	11,03	980,42	0,982185
13,8	11,11	980,31	0,982075
13,9	11,19	980,19	0,981954

Table D. 1 (continued)

% v/v	*% m/m*	*ρ20 (Kg/m3)*	*d (g/cm³)*
14,0	11,27	980,08	0,981844
14,1	11,36	979,97	0,981734
14,2	11,44	979,86	0,981624
14,3	11,52	979,75	0,981514
14,4	11,60	979,64	0,981403
14,5	11,68	979,52	0,981283
14,6	11,77	979,41	0,981173
14,7	11,85	979,30	0,981063
14,8	11,93	979,19	0,980953
14,9	12,01	979,08	0,980842
15,0	12,09	978,97	0,980732
15,1	12,17	978,86	0,980622
15,2	12,26	978,75	0,980512
15,3	12,34	978,64	0,980402
15,4	12,42	978,53	0,980291
15,5	12,50	978,42	0,980181
15,6	12,59	978,31	0,980071
15,7	12,67	978,20	0,979961
15,8	12,75	978,09	0,979851
15,9	12,83	977,98	0,979740
16,0	12,91	977,87	0,979630
16,1	13,00	977,76	0,979520
16,2	13,08	977,65	0,979410
16,3	13,16	977,55	0,979310
16,4	13,24	977,44	0,979199
16,5	13,32	977,33	0,979089
16,6	13,41	977,22	0,978979
16,7	13,49	977,11	0,978869
16,8	13,57	977,00	0,978759
16,9	13,65	976,89	0,978648
17,0	13,74	976,79	0,978548
17,1	13,82	976,68	0,978438
17,2	13,90	976,57	0,978328
17,3	13,98	976,46	0,978218
17,4	14,07	976,35	0,978107
17,5	14,15	976,25	0,978007
17,6	14,23	976,14	0,977897
17,7	14,31	976,03	0,977787
17,8	14,40	975,92	0,977677
17,9	14,48	975,81	0,977566
18,0	14,56	975,71	0,977466
18,1	14,64	975,60	0,977356
18,2	14,73	975,49	0,977246
18,3	14,81	975,38	0,977136
18,4	14,89	975,28	0,977036
18,5	14,97	975,17	0,976925
18,6	15,06	975,06	0,976815
18,7	15,14	974,95	0,976705
18,8	15,22	974,85	0,976605
18,9	15,30	974,74	0,976495

Table D. 1 (continued)

% v/v	*% m/m*	*ρ20 (Kg/m3)*	*d (g/cm³)*
19,0	15,39	974,63	0,976384
19,1	15,47	974,52	0,976274
19,2	15,55	974,42	0,976174
19,3	15,63	974,31	0,976064
19,4	15,72	974,20	0,975954
19,5	15,80	974,09	0,975843
19,6	15,88	973,99	0,975743
19,7	15,97	973,88	0,975633
19,8	16,05	973,77	0,975523
19,9	16,13	973,66	0,975413
20,0	16,21	973,56	0,975312
20,1	16,30	973,45	0,975202
20,2	16,38	973,34	0,975092
20,3	16,46	973,24	0,974992
20,4	16,55	973,13	0,974882
20,5	16,63	973,02	0,974771
20,6	16,71	972,91	0,974661
20,7	16,79	972,80	0,974551
20,8	16,88	972,70	0,974451
20,9	16,96	972,59	0,974341
21,0	17,04	972,48	0,974230
21,1	17,13	972,37	0,974120
21,2	17,21	972,26	0,974010
21,3	17,29	972,16	0,973910
21,4	17,38	972,05	0,973800
21,5	17,46	971,94	0,973689
21,6	17,54	971,83	0,973579
21,7	17,63	971,73	0,973479
21,8	17,71	971,62	0,973369
21,9	17,79	971,51	0,973259
22,0	17,87	971,40	0,973149
22,1	17,96	971,29	0,973038
22,2	18,04	971,18	0,972928
22,3	18,12	971,08	0,972828
22,4	18,21	970,97	0,972718
22,5	18,29	970,86	0,972608
22,6	18,37	970,75	0,972497
22,7	18,46	970,64	0,972387
22,8	18,54	970,53	0,972277
22,9	18,62	970,42	0,972167
23,0	18,71	970,31	0,972057
23,1	18,79	970,20	0,971946
23,2	18,87	970,09	0,971836
23,3	18,96	969,98	0,971726
23,4	19,04	969,87	0,971616
23,5	19,13	969,76	0,971506
23,6	19,21	969,65	0,971395
23,7	19,29	969,54	0,971285
23,8	19,38	969,43	0,971175
23,9	19,46	969,32	0,971065

Table D. 1 (continued)

% v/v	*% m/m*	*ρ20 (Kg/m3)*	*d (g/cm³)*
24,0	19,54	969,21	0,970955
24,1	19,63	969,10	0,970844
24,2	19,71	968,99	0,970734
24,3	19,79	968,88	0,970624
24,4	19,88	968,77	0,970514
24,5	19,96	968,66	0,970404
24,6	20,05	968,55	0,970293
24,7	20,13	968,43	0,970173
24,8	20,21	968,32	0,970063
24,9	20,30	968,21	0,969953
25,0	20,38	968,10	0,969843
25,1	20,47	967,99	0,969732
25,2	20,55	967,87	0,969612
25,3	20,63	967,76	0,969502
25,4	20,72	967,65	0,969392
25,5	20,80	967,53	0,969272
25,6	20,89	967,42	0,969161
25,7	20,97	967,31	0,969051
25,8	21,05	967,19	0,968931
25,9	21,14	967,08	0,968821
26,0	21,22	966,97	0,968711
26,1	21,31	966,85	0,968590
26,2	21,39	966,74	0,968480
26,3	21,47	966,62	0,968360
26,4	21,56	966,51	0,968250
26,5	21,64	966,39	0,968130
26,6	21,73	966,28	0,968019
26,7	21,81	966,16	0,967899
26,8	21,90	966,05	0,967789
26,9	21,98	965,93	0,967669
27,0	22,06	965,81	0,967548
27,1	22,15	965,70	0,967438
27,2	22,23	965,58	0,967318
27,3	22,32	965,46	0,967198
27,4	22,40	965,35	0,967088
27,5	22,49	965,23	0,966967
27,6	22,57	965,11	0,966847
27,7	22,65	964,99	0,966727
27,8	22,74	964,88	0,966617
27,9	22,82	964,76	0,966497
28,0	22,91	964,64	0,966376
28,1	22,99	964,52	0,966256
28,2	23,08	964,40	0,966136
28,3	23,16	964,28	0,966016
28,4	23,25	964,16	0,965895
28,5	23,33	964,04	0,965775
28,6	23,42	963,92	0,965655
28,7	23,50	963,80	0,965535
28,8	23,59	963,68	0,965415
28,9	23,67	963,56	0,965294

Table D. 1 (continued)

% v/v	*% m/m*	*ρ20 (Kg/m3)*	*d (g/cm³)*
29,0	23,76	963,44	0,965174
29,1	23,84	963,32	0,965054
29,2	23,93	963,20	0,964934
29,3	24,01	963,07	0,964804
29,4	24,10	962,95	0,964683
29,5	24,18	962,83	0,964563
29,6	24,27	962,71	0,964443
29,7	24,35	962,58	0,964313
29,8	24,44	962,46	0,964192
29,9	24,52	962,33	0,964062
30,0	24,61	962,21	0,963942
30,1	24,69	962,09	0,963822
30,2	24,78	961,96	0,963692
30,3	24,86	961,84	0,963571
30,4	24,95	961,71	0,963441
30,5	25,03	961,59	0,963321
30,6	25,12	961,46	0,963191
30,7	25,20	961,33	0,963060
30,8	25,29	961,21	0,962940
30,9	25,38	961,08	0,962810
31,0	25,46	960,95	0,962680
31,1	25,55	960,82	0,962549
31,2	25,63	960,70	0,962429
31,3	25,72	960,57	0,962299
31,4	25,80	960,44	0,962169
31,5	25,89	960,31	0,962039
31,6	25,97	960,18	0,961908
31,7	26,06	960,05	0,961778
31,8	26,15	959,92	0,961648
31,9	26,23	959,79	0,961518
32,0	26,32	959,66	0,961387
32,1	26,40	959,53	0,961257
32,2	26,49	959,40	0,961127
32,3	26,57	959,27	0,960997
32,4	26,66	959,14	0,960866
32,5	26,75	959,01	0,960736
32,6	26,83	958,87	0,960596
32,7	26,92	958,74	0,960466
32,8	27,00	958,61	0,960335
32,9	27,09	958,47	0,960195
33,0	27,18	958,34	0,960065
33,1	27,26	958,20	0,959925
33,2	27,35	958,07	0,959795
33,3	27,44	957,94	0,959664
33,4	27,52	957,80	0,959524
33,5	27,61	957,66	0,959384
33,6	27,69	957,53	0,959254
33,7	27,78	957,39	0,959113
33,8	27,87	957,26	0,958983
33,9	27,95	957,12	0,958843

Table D. 1 (continued)

% v/v	*% m/m*	*ρ20 (Kg/m3)*	*d (g/cm³)*
34,0	28,04	956,98	0,958703
34,1	28,13	956,84	0,958562
34,2	28,21	956,70	0,958422
34,3	28,30	956,57	0,958292
34,4	28,39	956,43	0,958152
34,5	28,47	956,29	0,958011
34,6	28,56	956,15	0,957871
34,7	28,65	956,01	0,957731
34,8	28,73	955,87	0,957591
34,9	28,82	955,73	0,957450
35,0	28,91	955,59	0,957310
35,1	28,99	955,45	0,957170
35,2	29,08	955,30	0,957020
35,3	29,17	955,16	0,956879
35,4	29,26	955,02	0,956739
35,5	29,34	954,88	0,956599
35,6	29,43	954,73	0,956449
35,7	29,52	954,59	0,956308
35,8	29,60	954,44	0,956158
35,9	29,69	954,30	0,956018
36,0	29,78	954,15	0,955867
36,1	29,87	954,01	0,955727
36,2	29,95	953,86	0,955577
36,3	30,04	953,72	0,955437
36,4	30,13	953,57	0,955286
36,5	30,22	953,42	0,955136
36,6	30,30	953,28	0,954996
36,7	30,39	953,13	0,954846
36,8	30,48	952,98	0,954695
36,9	30,56	952,83	0,954545
37,0	30,65	952,69	0,954405
37,1	30,74	952,54	0,954255
37,2	30,83	952,39	0,954104
37,3	30,92	952,24	0,953954
37,4	31,00	952,09	0,953804
37,5	31,09	951,94	0,953653
37,6	31,18	951,79	0,953503
37,7	31,27	951,63	0,953343
37,8	31,35	951,48	0,953193
37,9	31,44	951,33	0,953042
38,0	31,53	951,18	0,952892
38,1	31,62	951,02	0,952732
38,2	31,71	950,87	0,952582
38,3	31,79	950,72	0,952431
38,4	31,88	950,56	0,952271
38,5	31,97	950,41	0,952121
38,6	32,06	950,25	0,951960
38,7	32,15	950,10	0,951810
38,8	32,24	949,94	0,951650
38,9	32,32	949,79	0,951500

Table D. 1 (continued)

% v/v	*% m/m*	*ρ20 (Kg/m3)*	*d (g/cm³)*
39,0	32,41	949,63	0,951339
39,1	32,50	949,47	0,951179
39,2	32,59	949,32	0,951029
39,3	32,68	949,16	0,950868
39,4	32,77	949,00	0,950708
39,5	32,86	948,84	0,950548
39,6	32,94	948,68	0,950388
39,7	33,03	948,52	0,950227
39,8	33,12	948,37	0,950077
39,9	33,21	948,21	0,949917
40,0	33,30	948,05	0,949756
40,1	33,39	947,88	0,949586
40,2	33,48	947,72	0,949426
40,3	33,57	947,56	0,949266
40,4	33,66	947,40	0,949105
40,5	33,74	947,24	0,948945
40,6	33,83	947,08	0,948785
40,7	33,92	946,91	0,948614
40,8	34,01	946,75	0,948454
40,9	34,10	946,58	0,948284
41,0	34,19	946,42	0,948124
41,1	34,28	946,26	0,947963
41,2	34,37	946,09	0,947793
41,3	34,46	945,93	0,947633
41,4	34,55	945,76	0,947462
41,5	34,64	945,59	0,947292
41,6	34,73	945,43	0,947132
41,7	34,82	945,26	0,946961
41,8	34,91	945,09	0,946791
41,9	35,00	944,93	0,946631
42,0	35,09	944,76	0,946461
42,1	35,18	944,59	0,946290
42,2	35,27	944,42	0,946120
42,3	35,36	944,25	0,945950
42,4	35,45	944,08	0,945779
42,5	35,54	943,91	0,945609
42,6	35,63	943,74	0,945439
42,7	35,72	943,57	0,945268
42,8	35,81	943,40	0,945098
42,9	35,90	943,23	0,944928
43,0	35,99	943,06	0,944758
43,1	36,08	942,88	0,944577
43,2	36,17	942,71	0,944407
43,3	36,26	942,54	0,944237
43,4	36,35	942,37	0,944066
43,5	36,44	942,19	0,943886
43,6	36,53	942,02	0,943716
43,7	36,62	941,84	0,943535
43,8	36,71	941,67	0,943365
43,9	36,80	941,49	0,943185

Table D. 1 (continued)

% v/v	*% m/m*	*ρ20 (Kg/m3)*	*d (g/cm³)*
44,0	36,89	941,32	0,943014
44,1	36,98	941,14	0,942834
44,2	37,07	940,97	0,942664
44,3	37,16	940,79	0,942483
44,4	37,25	940,61	0,942303
44,5	37,35	940,43	0,942123
44,6	37,44	940,26	0,941952
44,7	37,53	940,08	0,941772
44,8	37,62	939,90	0,941592
44,9	37,71	939,72	0,941411
45,0	37,80	939,54	0,941231
45,1	37,89	939,36	0,941051
45,2	37,98	939,18	0,940871
45,3	38,08	939,00	0,940690
45,4	38,17	938,82	0,940510
45,5	38,26	938,64	0,940330
45,6	38,35	938,46	0,940149
45,7	38,44	938,28	0,939969
45,8	38,53	938,10	0,939789
45,9	38,62	937,91	0,939598
46,0	38,72	937,73	0,939418
46,1	38,81	937,55	0,939238
46,2	38,90	937,36	0,939047
46,3	38,99	937,18	0,938867
46,4	39,08	937,00	0,938687
46,5	39,18	936,81	0,938496
46,6	39,27	936,63	0,938316
46,7	39,36	936,44	0,938126
46,8	39,45	936,26	0,937945
46,9	39,54	936,07	0,937755
47,0	39,64	935,88	0,937565
47,1	39,73	935,70	0,937384
47,2	39,82	935,51	0,937194
47,3	39,91	935,32	0,937004
47,4	40,00	935,14	0,936823
47,5	40,10	934,95	0,936633
47,6	40,19	934,76	0,936443
47,7	40,28	934,57	0,936252
47,8	40,37	934,38	0,936062
47,9	40,47	934,19	0,935872
48,0	40,56	934,00	0,935681
48,1	40,65	933,81	0,935491
48,2	40,75	933,62	0,935301
48,3	40,84	933,43	0,935110
48,4	40,93	933,24	0,934920
48,5	41,02	933,05	0,934729
48,6	41,12	932,86	0,934539
48,7	41,21	932,67	0,934349
48,8	41,30	932,47	0,934148
48,9	41,40	932,28	0,933958

Table D. 1 (continued)

% v/v	*% m/m*	*ρ20 (Kg/m3)*	*d (g/cm³)*
49,0	41,49	932,09	0,933768
49,1	41,58	931,90	0,933577
49,2	41,68	931,70	0,933377
49,3	41,77	931,51	0,933187
49,4	41,86	931,32	0,932996
49,5	41,96	931,13	0,932806
49,6	42,05	930,92	0,932596
49,7	42,14	930,73	0,932405
49,8	42,24	930,53	0,932205
49,9	42,33	930,34	0,932015
50,0	42,43	930,14	0,931814
50,1	42,52	929,95	0,931624
50,2	42,61	929,75	0,931424
50,3	42,71	929,55	0,931223
50,4	42,80	929,35	0,931023
50,5	42,90	929,16	0,930832
50,6	42,99	928,96	0,930632
50,7	43,08	928,76	0,930432
50,8	43,18	928,56	0,930231
50,9	43,27	928,36	0,930031
51,0	43,37	928,16	0,929831
51,1	43,46	927,96	0,929630
51,2	43,56	927,77	0,929440
51,3	43,65	927,57	0,929240
51,4	43,74	927,36	0,929029
51,5	43,84	927,16	0,928829
51,6	43,93	926,96	0,928629
51,7	44,03	926,76	0,928428
51,8	44,12	926,56	0,928228
51,9	44,22	926,36	0,928027
52,0	44,31	926,16	0,927827
52,1	44,41	925,95	0,927617
52,2	44,50	925,75	0,927416
52,3	44,60	925,55	0,927216
52,4	44,69	925,35	0,927016
52,5	44,79	925,14	0,926805
52,6	44,88	924,94	0,926605
52,7	44,98	924,73	0,926395
52,8	45,07	924,53	0,926194
52,9	45,17	924,32	0,925984
53,0	45,26	924,12	0,925783
53,1	45,36	923,91	0,925573
53,2	45,46	923,71	0,925373
53,3	45,55	923,50	0,925162
53,4	45,65	923,30	0,924962
53,5	45,74	923,09	0,924752
53,6	45,84	922,88	0,924541
53,7	45,93	922,68	0,924341
53,8	46,03	922,47	0,924130
53,9	46,13	922,26	0,923920

Table D. 1 (continued)

% v/v	*% m/m*	*ρ20 (Kg/m3)*	*d (g/cm³)*
54,0	46,22	922,06	0,923720
54,1	46,32	921,85	0,923509
54,2	46,41	921,64	0,923299
54,3	46,51	921,43	0,923089
54,4	46,61	921,22	0,922878
54,5	46,70	921,01	0,922668
54,6	46,80	920,80	0,922457
54,7	46,90	920,59	0,922247
54,8	46,99	920,38	0,922037
54,9	47,09	920,17	0,921826
55,0	47,18	919,96	0,921616
55,1	47,28	919,75	0,921406
55,2	47,38	919,54	0,921195
55,3	47,47	919,33	0,920985
55,4	47,57	919,12	0,920774
55,5	47,67	918,91	0,920564
55,6	47,77	918,69	0,920344
55,7	47,86	918,48	0,920133
55,8	47,96	918,27	0,919923
55,9	48,06	918,06	0,919713
56,0	48,15	917,84	0,919492
56,1	48,25	917,63	0,919282
56,2	48,35	917,42	0,919071
56,3	48,45	917,22	0,918871
56,4	48,54	916,99	0,918641
56,5	48,64	916,77	0,918420
56,6	48,74	916,56	0,918210
56,7	48,84	916,35	0,917999
56,8	48,94	916,13	0,917779
56,9	49,03	915,91	0,917559
57,0	49,13	915,70	0,917348
57,1	49,23	915,48	0,917128
57,2	49,32	915,27	0,916917
57,3	49,42	915,05	0,916697
57,4	49,52	914,83	0,916477
57,5	49,62	914,62	0,916266
57,6	49,72	914,40	0,916046
57,7	49,81	914,18	0,915826
57,8	49,91	913,97	0,915615
57,9	50,01	913,75	0,915395
58,0	50,11	913,53	0,915174
58,1	50,21	913,31	0,914954
58,2	50,31	913,09	0,914734
58,3	50,40	912,87	0,914513
58,4	50,50	912,65	0,914293
58,5	50,60	912,43	0,914072
58,6	50,70	912,22	0,913862
58,7	50,80	912,00	0,913642
58,8	50,90	911,78	0,913421
58,9	51,00	911,55	0,913191

Table D. 1 (continued)

% v/v	*% m/m*	*ρ20 (Kg/m3)*	*d (g/cm³)*
59,0	51,10	911,33	0,912970
59,1	51,19	911,11	0,912750
59,2	51,29	910,89	0,912530
59,3	51,39	910,67	0,912309
59,4	51,49	910,45	0,912089
59,5	51,59	910,23	0,911868
59,6	51,69	910,01	0,911648
59,7	51,79	909,78	0,911418
59,8	51,89	909,56	0,911197
59,9	51,99	909,34	0,910977
60,0	52,09	909,11	0,910746
60,1	52,19	908,89	0,910526
60,2	52,29	908,67	0,910306
60,3	52,39	908,44	0,910075
60,4	52,49	908,22	0,909855
60,5	52,59	908,00	0,909634
60,6	52,69	907,77	0,909404
60,7	52,79	907,55	0,909184
60,8	52,89	907,32	0,908953
60,9	52,99	907,10	0,908733
61,0	53,09	906,87	0,908502
61,1	53,19	906,64	0,908272
61,2	53,29	906,42	0,908052
61,3	53,39	906,19	0,907821
61,4	53,49	905,97	0,907601
61,5	53,59	905,74	0,907370
61,6	53,69	905,51	0,907140
61,7	53,79	905,29	0,906920
61,8	53,89	905,06	0,906689
61,9	53,99	904,83	0,906459
62,0	54,09	904,60	0,906228
62,1	54,19	904,37	0,905998
62,2	54,30	904,15	0,905777
62,3	54,40	903,92	0,905547
62,4	54,50	903,69	0,905317
62,5	54,60	903,46	0,905086
62,6	54,70	903,23	0,904856
62,7	54,80	903,00	0,904625
62,8	54,90	902,77	0,904395
62,9	55,00	902,54	0,904165
63,0	55,11	902,31	0,903934
63,1	55,21	902,08	0,903704
63,2	55,31	901,85	0,903473
63,3	55,41	901,62	0,903243
63,4	55,51	901,39	0,903013
63,5	55,61	901,15	0,902772
63,6	55,72	900,92	0,902542
63,7	55,82	900,69	0,902311
63,8	55,92	900,46	0,902081
63,9	56,02	900,23	0,901850

Table D. 1 (continued)

% v/v	*% m/m*	*ρ20 (Kg/m3)*	*d (g/cm³)*
64,0	56,12	899,99	0,901610
64,1	56,23	899,76	0,901380
64,2	56,33	899,53	0,901149
64,3	56,43	899,29	0,900909
64,4	56,53	899,06	0,900678
64,5	56,64	898,83	0,900448
64,6	56,74	898,59	0,900207
64,7	56,84	898,36	0,899977
64,8	56,94	898,12	0,899737
64,9	57,05	897,89	0,899506
65,0	57,15	897,65	0,899266
65,1	57,25	897,42	0,899035
65,2	57,36	897,18	0,898795
65,3	57,46	896,94	0,898554
65,4	57,56	896,71	0,898324
65,5	57,67	896,47	0,898084
65,6	57,77	896,23	0,897843
65,7	57,87	896,00	0,897613
65,8	57,98	895,76	0,897372
65,9	58,08	895,52	0,897132
66,0	58,18	895,28	0,896892
66,1	58,29	895,05	0,896661
66,2	58,39	894,81	0,896421
66,3	58,49	894,57	0,896180
66,4	58,60	894,33	0,895940
66,5	58,70	894,09	0,895699
66,6	58,81	893,85	0,895459
66,7	58,91	893,61	0,895218
66,8	59,01	893,37	0,894978
66,9	59,12	893,13	0,894738
67,0	59,22	892,89	0,894497
67,1	59,33	892,65	0,894257
67,2	59,43	892,41	0,894016
67,3	59,54	892,17	0,893776
67,4	59,64	891,93	0,893535
67,5	59,74	891,69	0,893295
67,6	59,85	891,45	0,893055
67,7	59,95	891,20	0,892804
67,8	60,06	890,96	0,892564
67,9	60,16	890,72	0,892323
68,0	60,27	890,48	0,892083
68,1	60,37	890,23	0,891832
68,2	60,48	889,99	0,891592
68,3	60,58	889,75	0,891352
68,4	60,69	889,50	0,891101
68,5	60,80	889,26	0,890861
68,6	60,90	889,01	0,890610
68,7	61,01	888,77	0,890370
68,8	61,11	888,52	0,890119
68,9	61,22	888,28	0,889879

Table D. 1 (continued)

% v/v	*% m/m*	*ρ20 (Kg/m3)*	*d (g/cm³)*
69,0	61,32	888,03	0,889628
69,1	61,43	887,79	0,889388
69,2	61,54	887,54	0,889138
69,3	61,64	887,29	0,888887
69,4	61,75	887,05	0,888647
69,5	61,85	886,80	0,888396
69,6	61,96	886,55	0,888146
69,7	62,07	886,31	0,887905
69,8	62,17	886,06	0,887655
69,9	62,28	885,81	0,887404
70,0	62,39	885,56	0,887154
70,1	62,49	885,31	0,886904
70,2	62,60	885,06	0,886653
70,3	62,71	884,82	0,886413
70,4	62,81	884,57	0,886162
70,5	62,92	884,32	0,885912
70,6	63,03	884,07	0,885661
70,7	63,13	883,82	0,885411
70,8	63,24	883,57	0,885160
70,9	63,35	883,32	0,884910
71,0	63,46	883,06	0,884650
71,1	63,56	882,81	0,884399
71,2	63,67	882,56	0,884149
71,3	63,78	882,31	0,883898
71,4	63,89	882,06	0,883648
71,5	63,99	881,81	0,883397
71,6	64,10	881,55	0,883137
71,7	64,21	881,30	0,882886
71,8	64,32	881,05	0,882636
71,9	64,43	880,79	0,882375
72,0	64,53	880,54	0,882125
72,1	64,64	880,29	0,881875
72,2	64,75	880,03	0,881614
72,3	64,86	879,78	0,881364
72,4	64,97	879,52	0,881103
72,5	65,08	879,27	0,880853
72,6	65,19	879,01	0,880592
72,7	65,29	878,75	0,880332
72,8	65,40	878,50	0,880081
72,9	65,51	878,24	0,879821
73,0	65,62	877,99	0,879570
73,1	65,73	877,73	0,879310
73,2	65,84	877,47	0,879049
73,3	65,95	877,21	0,878789
73,4	66,06	876,96	0,878539
73,5	66,17	876,70	0,878278
73,6	66,28	876,44	0,878018
73,7	66,39	876,18	0,877757
73,8	66,50	875,92	0,877497
73,9	66,61	875,66	0,877236

Table D. 1 (continued)

% v/v	*% m/m*	*ρ20 (Kg/m3)*	*d (g/cm³)*
74,0	66,72	875,40	0,876976
74,1	66,83	875,14	0,876715
74,2	66,94	874,88	0,876455
74,3	67,05	874,62	0,876194
74,4	67,16	874,36	0,875934
74,5	67,27	874,10	0,875673
74,6	67,38	873,84	0,875413
74,7	67,49	873,58	0,875152
74,8	67,60	873,32	0,874892
74,9	67,71	873,06	0,874632
75,0	67,82	872,79	0,874361
75,1	67,93	872,53	0,874101
75,2	68,04	872,27	0,873840
75,3	68,15	872,00	0,873570
75,4	68,26	871,74	0,873309
75,5	68,38	871,48	0,873049
75,6	68,49	871,21	0,872778
75,7	68,60	870,95	0,872518
75,8	68,71	870,68	0,872247
75,9	68,82	870,42	0,871987
76,0	68,93	870,15	0,871716
76,1	69,04	869,89	0,871456
76,2	69,16	869,62	0,871185
76,3	69,27	869,35	0,870915
76,4	69,38	869,09	0,870654
76,5	69,49	868,82	0,870384
76,6	69,61	868,55	0,870113
76,7	69,72	868,28	0,869843
76,8	69,83	868,02	0,869582
76,9	69,94	867,75	0,869312
77,0	70,06	867,48	0,869041
77,1	70,17	867,21	0,868771
77,2	70,28	866,94	0,868500
77,3	70,39	866,67	0,868230
77,4	70,51	866,40	0,867960
77,5	70,62	866,13	0,867689
77,6	70,73	865,86	0,867419
77,7	70,85	865,59	0,867148
77,8	70,96	865,32	0,866878
77,9	71,07	865,05	0,866607
78,0	71,19	864,78	0,866337
78,1	71,30	864,50	0,866056
78,2	71,41	864,23	0,865786
78,3	71,53	863,96	0,865515
78,4	71,64	863,69	0,865245
78,5	71,76	863,41	0,864964
78,6	71,87	863,14	0,864694
78,7	71,98	862,86	0,864413
78,8	72,10	862,59	0,864143
78,9	72,21	862,31	0,863862

Table D. 1 (continued)

% v/v	*% m/m*	*ρ20 (Kg/m3)*	*d (g/cm³)*
79,0	72,33	862,04	0,863592
79,1	72,44	861,76	0,863311
79,2	72,56	861,49	0,863041
79,3	72,67	861,21	0,862760
79,4	72,79	860,94	0,862490
79,5	72,90	860,66	0,862209
79,6	73,02	860,38	0,861929
79,7	73,13	860,10	0,861648
79,8	73,25	859,83	0,861378
79,9	73,36	859,55	0,861097
80,0	73,48	859,27	0,860817
80,1	73,60	858,99	0,860536
80,2	73,71	858,71	0,860256
80,3	73,83	858,43	0,859975
80,4	73,94	858,15	0,859695
80,5	74,06	857,87	0,859414
80,6	74,18	857,59	0,859134
80,7	74,29	857,31	0,858853
80,8	74,41	857,03	0,858573
80,9	74,53	856,75	0,858292
81,0	74,64	856,46	0,858002
81,1	74,76	856,18	0,857721
81,2	74,88	855,90	0,857441
81,3	74,99	855,62	0,857160
81,4	75,11	855,33	0,856870
81,5	75,23	855,05	0,856589
81,6	75,34	854,76	0,856299
81,7	75,46	854,48	0,856018
81,8	75,58	854,19	0,855728
81,9	75,70	853,91	0,855447
82,0	75,82	853,62	0,855157
82,1	75,93	853,34	0,854876
82,2	76,05	853,05	0,854585
82,3	76,17	852,76	0,854295
82,4	76,29	852,48	0,854014
82,5	76,41	852,19	0,853724
82,6	76,52	851,90	0,853433
82,7	76,64	851,61	0,853143
82,8	76,76	851,32	0,852852
82,9	76,88	851,03	0,852562
83,0	77,00	850,74	0,852271
83,1	77,12	850,45	0,851981
83,2	77,24	850,16	0,851690
83,3	77,36	849,87	0,851400
83,4	77,48	849,58	0,851109
83,5	77,60	849,29	0,850819
83,6	77,72	848,99	0,850518
83,7	77,84	848,70	0,850228
83,8	77,96	848,41	0,849937
83,9	78,08	848,11	0,849637

Table D. 1 (continued)

% v/v	*% m/m*	*ρ20 (Kg/m3)*	*d (g/cm³)*
84,0	78,20	847,82	0,849346
84,1	78,32	847,53	0,849056
84,2	78,44	847,23	0,848755
84,3	78,56	846,93	0,848454
84,4	78,68	846,64	0,848164
84,5	78,80	846,34	0,847863
84,6	78,92	846,05	0,847573
84,7	79,04	845,75	0,847272
84,8	79,16	845,45	0,846972
84,9	79,28	845,15	0,846671
85,0	79,40	844,85	0,846371
85,1	79,53	844,55	0,846070
85,2	79,65	844,25	0,845770
85,3	79,77	843,95	0,845469
85,4	79,89	843,65	0,845169
85,5	80,01	843,35	0,844868
85,6	80,14	843,05	0,844567
85,7	80,26	842,75	0,844267
85,8	80,38	842,44	0,843956
85,9	80,50	842,14	0,843656
86,0	80,63	841,84	0,843355
86,1	80,75	841,53	0,843045
86,2	80,87	841,23	0,842744
86,3	81,00	840,92	0,842434
86,4	81,12	840,62	0,842133
86,5	81,24	840,31	0,841823
86,6	81,37	840,00	0,841512
86,7	81,49	839,70	0,841211
86,8	81,61	839,39	0,840901
86,9	81,74	839,08	0,840590
87,0	81,86	838,77	0,840280
87,1	81,99	838,46	0,839969
87,2	82,11	838,15	0,839659
87,3	82,24	837,84	0,839348
87,4	82,36	837,52	0,839028
87,5	82,49	837,21	0,838717
87,6	82,61	836,90	0,838406
87,7	82,74	836,59	0,838096
87,8	82,86	836,27	0,837775
87,9	82,99	835,96	0,837465
88,0	83,11	835,64	0,837144
88,1	83,24	835,32	0,836824
88,2	83,37	835,01	0,836513
88,3	83,49	834,69	0,836192
88,4	83,62	834,37	0,835872
88,5	83,74	834,05	0,835551
88,6	83,87	833,73	0,835231
88,7	84,00	833,41	0,834910
88,8	84,13	833,09	0,834590
88,9	84,25	832,77	0,834269

Table D. 1 (continued)

% v/v	*% m/m*	*ρ20 (Kg/m3)*	*d (g/cm³)*
89,0	84,38	832,45	0,833948
89,1	84,51	832,12	0,833618
89,2	84,64	831,80	0,833297
89,3	84,76	831,48	0,832977
89,4	84,89	831,15	0,832646
89,5	85,02	830,82	0,832315
89,6	85,15	830,50	0,831995
89,7	85,28	830,17	0,831664
89,8	85,41	829,84	0,831334
89,9	85,54	829,51	0,831003
90,0	85,66	829,18	0,830673
90,1	85,79	828,85	0,830342
90,2	85,92	828,52	0,830011
90,3	86,05	828,19	0,829681
90,4	86,18	827,85	0,829340
90,5	86,31	827,52	0,829010
90,6	86,44	827,18	0,828669
90,7	86,57	826,85	0,828338
90,8	86,71	826,51	0,827998
90,9	86,84	826,17	0,827657
91,0	86,97	825,83	0,827316
91,1	87,10	825,49	0,826976
91,2	87,23	825,15	0,826635
91,3	87,36	824,81	0,826295
91,4	87,49	824,47	0,825954
91,5	87,63	824,13	0,825613
91,6	87,76	823,78	0,825263
91,7	87,90	823,44	0,824922
91,8	88,02	823,09	0,824572
91,9	88,16	822,74	0,824221
92,0	88,29	822,39	0,823870
92,1	88,42	822,04	0,823520
92,2	88,56	821,69	0,823169
92,3	88,69	821,34	0,822818
92,4	88,83	820,99	0,822468
92,5	88,96	820,63	0,822107
92,6	89,10	820,28	0,821757
92,7	89,23	819,92	0,821396
92,8	89,37	819,57	0,821045
92,9	89,50	819,21	0,820685
93,0	89,64	818,85	0,820324
93,1	89,77	818,49	0,819963
93,2	89,91	818,12	0,819593
93,3	90,05	817,76	0,819232
93,4	90,18	817,40	0,818871
93,5	90,32	817,03	0,818501
93,6	90,46	816,66	0,818130
93,7	90,59	816,30	0,817769
93,8	90,73	815,93	0,817399
93,9	90,87	815,55	0,817018

Table D. 1 (continued)

% v/v	*% m/m*	*ρ20 (Kg/m3)*	*d (g/cm³)*
94,0	91,01	815,18	0,816647
94,1	91,15	814,81	0,816277
94,2	91,29	814,43	0,815896
94,3	91,43	814,06	0,815525
94,4	91,56	813,68	0,815145
94,5	91,70	813,30	0,814764
94,6	91,84	812,92	0,814383
94,7	91,98	812,54	0,814003
94,8	92,13	812,15	0,813612
94,9	92,27	811,77	0,813231
95,0	92,41	811,38	0,812840
95,1	92,55	810,99	0,812450
95,2	92,69	810,60	0,812059
95,3	92,83	810,21	0,811668
95,4	92,98	809,82	0,811278
95,5	93,12	809,42	0,810877
95,6	93,26	809,02	0,810476
95,7	93,41	808,63	0,810086
95,8	93,55	808,23	0,809685
95,9	93,69	807,82	0,809274
96,0	93,84	807,42	0,808873
96,1	93,98	807,01	0,808463
96,2	94,13	806,61	0,808062
96,3	94,27	806,20	0,807651
96,4	94,42	805,78	0,807230
96,5	94,57	805,37	0,806820
96,6	94,71	804,96	0,806409
96,7	94,86	804,54	0,805988
96,8	95,01	804,12	0,805567
96,9	95,16	803,70	0,805147
97,0	95,31	803,27	0,804716
97,1	95,45	802,85	0,804295
97,2	95,60	802,42	0,803864
97,3	95,75	801,99	0,803434
97,4	95,90	801,55	0,802993
97,5	96,05	801,12	0,802562
97,6	96,21	800,68	0,802121
97,7	96,36	800,24	0,801680
97,8	96,51	799,80	0,801240
97,9	96,66	799,35	0,800789
98,0	96,81	798,90	0,800338
98,1	96,97	798,45	0,799887
98,2	97,12	798,00	0,799436
98,3	97,28	797,54	0,798976
98,4	97,43	797,08	0,798515
98,5	97,59	796,62	0,798054
98,6	97,74	796,15	0,797583
98,7	97,90	795,68	0,797112
98,8	98,06	795,21	0,796641
98,9	98,22	794,73	0,796161

Table D. 1 (continued)

% v/v	*% m/m*	*ρ20 (Kg/m3)*	*d (g/cm³)*
99,0	98,38	794,25	0,795680
99,1	98,53	793,77	0,795199
99,2	98,69	793,28	0,794708
99,3	98,86	792,79	0,794217
99,4	99,02	792,30	0,793726
99,5	99,18	791,80	0,793225
99,6	99,34	791,29	0,792714
99,7	99,50	790,79	0,792213
99,8	99,67	790,28	0,791703
99,9	99,83	789,76	0,791182
100,0	100,00	789,24	0,790661