COLLEGIATE BOARD RESOLUTION – RDC NO. 166 OF 24 JULY 2017

Provides for the validation of analytical methods and gives other provisions.

The Collegiate Board of Directors of the Brazilian Health Regulatory Agency, in the use of the attributions vested in it under Article 15, items III and IV, and Article 7, items III and IV of Law no. 9,782 of 26 January 1999, and item V, paragraphs 1 and 3 of Article 53 of the Internal Regulation approved by Collegiate Board Resolution – RDC no. 61 of 3 February 2016, adopts the following Collegiate Board Resolution, as decided upon in a meeting held on 11 July 2017, and I, Director-President, determine its publication.

CHAPTER I

INITIAL PROVISIONS

Section I

Objective

Article 1. This resolution establishes criteria for the validation of analytical methods.

Sole paragraph. Failure to meet any criterion provided for in this Resolution must be technically justified and shall be subject to analysis by Anvisa.

Section II

Scope

Article 2. This resolution is applicable to analytical methods used for pharmaceutical ingredients, medicinal products, and biological products in all production stages.

Paragraph 1. The validation parameters and their respective acceptance criteria must be defined according to the characteristics of the analyte and the method's nature.

Paragraph 2. The analytical methods applied to investigational products used in clinical trials must have their adequacy shown in accordance with this Resolution, as applicable for each phase of clinical development.

I – The use of an alternative approach must be technically justified, based on recognized scientific references.

Paragraph 3. The use of alternative approaches for the validation of analytical methods applied to biological products, such as biological trials and immunoassays, shall be allowed.

Paragraph 4. Microbiological methods, for which technical justification must be presented for the approach chosen, based on the Brazilian Pharmacopeia or other official compendia recognized by Anvisa, are excluded from this Resolution.

Section III

Definitions

Article 3 For the purposes of this Resolution, the following definitions are adopted:

I – sample: representative amount of pharmaceutical ingredient, intermediate or finished product, duly identified, within the shelf life determined;

II – analyte: substance or set of substances of interest whose identification or quantification is intended;

III – chemical substance characterization: set of tests that undoubtedly assures the authenticity and quality of the substance regarding its identity, purity, content, and potency, which must include data obtained from techniques applicable to each substance characterization, such as, for example, thermogravimetric analysis, melting point, differential scanning calorimetry, infrared spectroscopy, mass spectrometry, nuclear magnetic resonance, elemental analysis (carbon/ hydrogen/ nitrogen), X-ray diffraction, optical rotation, chromatographic test, among others;

IV – analytical run: set of measurements performed in a pool of samples in a predetermined time interval under the same conditions of repeatability, such as method, analyst, instruments, place, and use conditions;

V – matrix effect: effect of matrix components on the analytical response;

VI – test: technical operation consisting of the determination of one or more characteristics of a certain ingredient or product, according to a specified method;

VII – limit test: test that allows checking if the amount of analyte is above or below a pre-determined level without quantifying such analyte precisely;

VIII – response factor: ratio between the analytical sign and the analyte concentration;

IX – relative response factor: ratio between two response factors, which is used as correction in the calculation of the concentration of a substance when such substance is measured through the analytical response of another substance;

X – quality management: it determines the implementation of “Quality Policy”, that is, global intentions and guidelines related to quality, which is formally expressed and authorized by the company’s high administration;

XI – impurities: any component present in the pharmaceutical ingredient of finished product that is not the active pharmaceutical ingredient nor the excipient(s);

XII – pharmaceutical ingredient: any substance that is part of the formulation of a dosage form;

XIII – active pharmaceutical ingredient (API): pharmaceutical ingredient that, when given to a patient, acts as active ingredient, and may have pharmacological activity or direct effect on the

diagnosis, cure, treatment, or prevention of a disease or also affect structure and function of the human body;

XIV – complex matrix: the one containing an indefinite number of unmonitored substances that cannot be obtained without the presence of the analyte;

XV – matrix: composition that mimics the sample without the presence of the analyte;

XVI – investigational products: experimental drug, placebo, active comparator, or any other product to be used in the clinical trial;

XVII – chromatographic purity: absence of interference with the analyte chromatographic signal;

XVIII – peak purity: spectral homogeneity of a chromatographic peak indicating its chromatographic purity, and the criteria to define if there is spectral homogeneity and the parameters adopted for purity calculation are defined as pre-determined in the software used or through a scientifically-based technical assessment;

XIX – validation report: document that consolidates and summarizes procedures, records, results, and validation assessment;

XX – analytical method revalidation: partial or total repetition of the validation of an analytical method to assure that it still meeting the requirements established;

XXI – chemical reference substance (CRS): substance or mixture of chemical or biological substances with high purity level that is carefully characterized to assure its identity, quality, content, and potency, including the characterized chemical reference substance and the pharmacopeial chemical reference substance;

XXII – characterized chemical reference substance (CCRS): substance or mixture of chemical or biological substances whose identity, quality, purity, content, and potency have been carefully assured through a characterization process;

XXIII – pharmacopeial chemical reference substance (PCRS): substance or mixture of chemical or biological substances established and distributed by official compendia recognized by Anvisa;

XXIV – working chemical substance (WCS): substance or mixture of chemical or biological substances used in lab routine, standardized based on a pharmacopeial chemical reference substance or, in its absence, based on a characterized chemical reference substance, being traceable to the CRS used in its standardization;

XXV – method transfer: documented process that qualifies a laboratory (receiving unit) for the use of an analytical method provided by another laboratory (transferring unit), thus assuring that the receiving unit has the required knowledge and is able to execute the analytical method according to the intended purpose;

XXVI – analytical validation: systematic assessment of a method by means of experimental tests in order to confirm and provide objective evidence that the specific requirements for its intended use are complied with;

XXVII – partial validation: demonstration, by means of some validation parameters, that the analytical method previously validated has the characteristics needed for the obtention of results with the required quality under the conditions of its use; and

XXVIII – system suitability: procedure to be performed prior to an analytical run to show that the system is proper for the intended use, and the parameters for such procedure must be defined during method development and validation.

CHAPTER II

GENERAL PROVISIONS

Article 4. The validation must show, in a documented way and according to objective criteria, that the analytical method produces reliable results and is appropriate for the intended purpose.

Article 5. The use of an analytical method not described in an official compendium recognized by Anvisa requires an analytical validation, in accordance with the parameters established in this Resolution, considering technical and operational conditions.

Article 6. Typical parameters to be considered for validation depend on the test to be performed and are shown in Table 1 of Annex I.

Article 7. Compendial analytical methods must have their suitability shown for the intended use, under the laboratory operating conditions, through a partial validation study.

Sole paragraph. The provisions in the caption of this article exclude basic general compendial methods such as pH measurement, loss on drying, sulphated ash, moisture, disintegration, among others, as well as analytical methods described in compendial individual monographs of non-active pharmaceutical ingredients.

Article 8. Partial validation must assess at least the parameters of precision, accuracy, and selectivity.

Paragraph 1. In the case of analytical methods destined for impurity quantification, partial validation must include the limit of quantification.

Paragraph 2. In the case of limit test, the parameters of selectivity and detection limit must be assessed instead of the parameters referred to in the caption of this article.

Article 9. In the case of method transfer between laboratories, the method shall be deemed as validated, provided that a partial validation study is conducted in the receiving laboratory's facilities.

Paragraph 1. Method transfer between laboratories that have the same quality management system may be done by means of a partial validation study in accordance with Article 8 or through reproducibility assessment.

Paragraph 2. A different approach may be accepted upon justification and presentation of protocol and report of transfer, based on risk assessment and considering prior experience, knowledge of the receiving unit, the product's and method's complexity, and specifications, as well as other relevant aspects that may be applicable.

Paragraph 3. If the transfer also uses comparative tests, the similarities of the results must be confirmed through a statistical tool.

Paragraph 4. Documents of method transfer must be provided including the copy of the validation report of the transferred method, as evidence that such method has been originally validated in compliance with specific rules and regulations approved/ attested by Anvisa.

Paragraph 5. In the case of transfer of methods already approved by Anvisa, a copy of the approved validation report, or the petition number under which the final version of such report was filed, must be provided.

Article 10. Revalidation of analytical method may consider the following circumstances:

I – alterations in the synthesis or obtainment of API;

II – alterations in product composition;

III – alterations in analytical method; and

IV – other alterations that may significantly impact the validated method.

Sole paragraph. Validation parameters to be assessed depend on the nature of the alterations carried out.

Article 11. The system must be verified at each analytical run.

Article 12. Documents of validation and partial validation must describe procedures, analytical parameters, acceptance criteria, and results with details enough to allow their reproduction and, as applicable, their statistical assessment.

Article 13. The validation report to be submitted, in accordance with resolutions providing for marketing authorization and post- marketing authorization, must include data and calculations obtained during the analytical validation conduction, as well as the statistical approach used for data assessment.

Paragraph 1. Raw data related to selectivity parameter must be included in the report referred to in the caption of this article.

Paragraph 2. Raw data related to other parameters must be available at the company for assessment upon request by Anvisa.

CHAPTER III

CHEMICAL REFERENCE SUBSTANCES

Article 14 For the validation of analytical methods, the Pharmacopeial Chemical Reference Substance (PCRS) officialized by the Brazilian Pharmacopeia should be used preferably, or other compendia officially recognized by Anvisa.

Paragraph 1. The use of Characterized Chemical Reference Substance (CCRS) is permitted upon the presentation of conclusive characterization report for the batch under study, including the technical reasons for the choice of tests used and relevant raw data.

Paragraph 2. Anvisa and members of the Brazilian Health Surveillance System shall be able to request samples of the CCRS in order to assess the characterization process in the hypotheses of the previous paragraph and, when a fiscal analysis is required, a sample of the CCRS must be provided for the purposes of carrying out the necessary tests.

Article 15. Depending on the analyte, the characterization report must include data obtained from techniques applicable to the characterization of each chemical substance, such as, for instance, thermogravimetric analysis, melting point, differential scanning calorimetry, infra-red spectroscopy, mass spectrometry, nuclear magnetic resonance, elemental analysis (carbon/ hydrogen/ nitrogen), X- ray diffraction, optical rotation, chromatographic methods, among others.

Paragraph 1. In addition to characterization data, the following information must be included in the report:

I – number and shelf life of the substance batch used in the characterization;

II – Brazilian non-exclusive name or international non-proprietary name;

III – CAS number;

IV – chemical name;

V – synonyms;

VI – molecular and structural formula;

VII – molecular weight;

VIII – physical form;

IX – physicochemical properties;

X – impurity profile;

XI – handling and maintenance information, and

XII – analytical report attesting identity, content, and shelf-life of the CCRS.

Paragraph 2. For biological products, characterization of reference standard/ material must be carried out using appropriate state-of-the-art methods.

Article 16. For medicinal gases, analytical verification of instruments and analytical determinations must be conducted using traceable reference materials distributed by metrology institutes or organisms recognized as certified producers of reference materials.

Sole paragraph. In the absence of reference materials, internal standards produced according to bibliographic references and guidelines may be used.

Article 17. For biological products, the words material or standard replace the term chemical substance in the definitions of CRS, PCRS, CCRS, and WCS.

Article 18. The use of WCS is not allowed for the purposes of analytical method validation.

CHAPTER IV

ANALYTICAL VALIDATION PARAMETERS

Section I

Selectivity

Article 19. Analytical method selectivity must be confirmed by means of its ability to identify or quantify the analyte of interest, undoubtedly, in the presence of components that may be present in the sample, such as impurities, diluents, and matrix components.

Sole paragraph. In the case of chromatographic methods, the chromatographic purity of the analyte signal must be proven, except for biological products.

Article 20. For identification methods, the ability to obtain a positive result for the sample containing the analyte and a negative result for other substances present in the sample must be confirmed.

Paragraph 1. The CRS must be used in the comparison with the response obtained for the analyte under the terms of Chapter III.

Paragraph 2. In order to demonstrate the selectivity of identification methods, the tests must be carried out in substances that are structurally similar to the analyte, and the acceptance criterion is a negative test result.

Paragraph 3. For active pharmaceutical ingredients of vegetal origin and medicinal products containing such APIs, the method's ability to distinguish the material of interest from other similar vegetal species, especially those that may be present as adulterants or substituents, must be demonstrated.

Paragraph 4. In order to reach the required level of selectivity, a combination of two or more analytical methods of identification may be needed.

Article 21. For quantitative methods and limit tests, selectivity must be demonstrated by evidencing that the analytical response is due to the analyte only, without interference of diluent, matrix, impurities, or degradation products.

Paragraph 1. In order to show the lack of interference of degradation products, the sample needs to be exposed to degradation conditions in a wide range of pH, oxidation, heat, and light.

Paragraph 2. The following are exempted from the demonstration described in Paragraph 1 above:

I – products whose adequacy to the resolution establishing parameters for notification, identification, and qualification of degradation products in medicinal products has already been shown;

II – performance methods;

III – non-chromatographic methods.

Paragraph 3. The use of a method with selectivity technical limitation, under the terms of the caption of this article, is accepted only upon technical justification and concomitant application of an additional method.

Article 22. For medicinal gases, selectivity must be evidenced by comparing the result of sample reading with the CRS reading response under the terms of Chapter III.

Sole paragraph. The maximum value of a potential interference must be justified.

Section II

Linearity

Article 23. The linearity of a method must be demonstrated by its ability to obtain analytical responses directly proportional to the concentration of one analyte in a sample.

Article 24. A linear relationship must be assessed within the entire range established for the method.

Article 25. In order to establish linearity, at least five (5) different concentrations of CRS must be used for solutions prepared in at least triplicate.

Sole paragraph. The solutions used to assess linearity must be prepared independently, and diluted solutions of the same SQR stock solution may be used.

Article 26. All calculations for linearity assessment must be made based on the actual concentration data and individual analytical responses.

Article 27. For linearity assessment, the following data must be provided:

I – graphic representation of responses in relation to analyte concentration;

II – residue dispersion graphic accompanied by its statistical assessment;

III – equation of regression line of y on x, estimated by the least squares method;

IV – assessment of linear association between variables through correlation coefficient (r) and determination coefficient (r^2);

V – assessment of the significance of angular coefficient.

Paragraph 1. Data homoscedasticity must be investigated for the use of an appropriate model.

Paragraph 2. In statistical tests, a significance level of 5% (five per cent) must be used.

Paragraph 3. The correlation coefficient must be above 0.990.

Paragraph 4. The angular coefficient must be significantly different from zero.

Section III

Matrix Effect

Article 28. The provisions in this section apply to complex matrices.

Article 29. Matrix effect must be determined by comparing the angular coefficients of calibration curves constructed with the analyte CRS in solvent and with the sample spiked with the analyte CRS.

Sole paragraph. The curves must be established the same way as in linearity for the same levels of concentration, using at least five (5) different concentrations in at least triplicate.

Article 30. Parallelism of lines indicates absence of interference of matrix components and its demonstration must be carried out through proper statistical assessment.

Sole paragraph. A significance level of 5% (five per cent) must be adopted in the hypotheses test.

Section IV

Working range

Article 31. The working range must be established based on linearity studies together with precision and accuracy results, depending on the intended application.

Article 32. The following working range must be considered:

I – for content: from 80% (eighty per cent) to 120% (one hundred and twenty per cent);

II – for content uniformity: from 70% (seventy per cent) to 130% (one hundred and thirty per cent);

III – for dissolution test: from -20% (minus twenty per cent) of the lowest concentration expected to +20% (plus twenty per cent) of the highest concentration expected from the dissolution profile; and

IV – for impurity determination: from the limit of quantification up to 120% (one hundred and twenty per cent) of the concentration at the specification limit of each individual impurity;

V – for simultaneous determination of content and impurities through the area normalization procedure: from the limit of quantification (LQ) up to 120% (one hundred and twenty per cent) of the expected concentration of the active ingredient.

Paragraph 1. Working ranges wider than those defined in the caption of this article may be used if technical justification is provided.

Paragraph 2. For medicinal gases, alternative working ranges shall be accepted, provided that the approach for interval choice is justified.

Section V

Precision

Article 33. Precision must assess the proximity between results obtained from tests with samples prepared according to the description of the analytical method to be validated.

Article 34. Precision must be expressed by means of repeatability, intermediate precision, or reproducibility.

Article 35. Precision must be demonstrated through dispersion of results, by calculating the relative standard deviation (RSD) of the series of measurements according to the formula "RSD=(SD/DMC)X100", where SD is the standard deviation and DMC is the determined mean concentration.

Article 36. Samples for precision assessment must be prepared independently, since the beginning of the procedure described in the method.

Sole paragraph. For solid and semi-solid samples, the use of diluted solutions derived from the same stock solution is not allowed.

Article 37. When precision assessment involves matrix contamination with a very low amount of substance making a direct weighing impossible, a concentrated solution of such substance may be used, following the procedure described in the analytical method for sample extraction and dilution.

Paragraph 1. In the case of known impurities not found in the sample or present in concentrations lower than the limit of specification, the sample must be spiked with known concentrations of impurity standard.

Paragraph 2. In the case of unknown impurities, the sample must be assessed by using the response of the active ingredient added to the matrix at a concentration corresponding to the limit of specification established for such impurities, provided that the same response factor is considered for both the impurity and the active ingredient.

Article 38. Repeatability determination must meet the following criteria:

I – assess samples under the same operational conditions, with the same analyst and same instruments, in a single analytical run;

II – use at least 9 (nine) determinations covering the linear interval of the analytical method, that is, 3 (three) concentrations: low, medium, and high, with 3 (three) replicates in each level or 6 (six) replicates at 100% (one hundred per cent) of the test concentration prepared individually.

Article 39. The acceptance criteria must be defined and justified according to the following aspects:

I – method's objective;

II – method's intrinsic variability;

III – working concentration; and

IV – analyte concentration in the sample.

Article 40. The determination of intermediate precision must meet the following criteria:

I – express the proximity of results obtained from the analysis of the same sample, in the same laboratory, in at least two different days, performed by different operators; and

II – include the same concentrations and the same number of determinations described in the repeatability assessment.

Article 41. Reproducibility must be obtained by the proximity of results obtained in different laboratories.

Paragraph 1. Reproducibility is applicable to collaborative studies or in the standardization of analytical methods for their inclusion in official compendia, upon appropriate statistical tests.

Paragraph 2. The acceptance criterion for relative standard deviation must be justified as provided for in Article 39.

Section VI

Accuracy

Article 42. The accuracy of an analytical method must be reached through the level of conformity between individual results of the method being tested in relation to a value accepted as true.

Article 43. The accuracy must be checked from at least 9 (nine) determinations, including the linear interval of the analytical method, that is, 3 (three) concentrations: low, medium, high, with 3 (three) replicates at each level.

Article 44. Samples for accuracy assessment must be prepared independently, and diluted solutions derived from the same CRS stock solution may be used.

Article 45. For accuracy determination, the most appropriate approach must be used, according to the analytical method under study:

I – for API:

a) apply the proposed method by using a substance with known purity (CRS);

b) compare the results obtained with those from a second validated method whose accuracy has been established; or

c) in the case of analyte in complex matrix, carry out an analysis using the CRS addition method in which known amounts of CRS are added to the sample.

II – for finished product:

a) apply the proposed method in the analysis of one sample which had a known amount of CRS added to the matrix;

b) in the event there are not samples from all components of the medicinal product available, the analysis may be carried out through the CRS addition method, in which known amounts of CRS are added to the finished product solution; or

c) compare the results obtained with those from a second validated method.

III – for impurities:

a) apply the method of standard addition in which known amounts of impurities or degradation products are added to the sample;

b) in the event there are not samples of certain impurities or degradation products, the analysis may be carried out by comparing the results obtained with those from a second validated method and using the response factor related to the API;

c) for unknown impurities, the accuracy must be assessed by comparing the CRS response of API or of a known impurity, according to the proposed method, within a concentration range that includes the working range of the method, provided that the same response factor is considered.

Sole paragraph. In all cases, the form of calculation of analyte concentrations must be the same described in the analytical method at issue.

Article 46. The accuracy must be expressed by the percentage relation of recovery of an analyte of known concentration added to the sample or by the relation between the mean concentration, determined experimentally, and the corresponding theoretical concentration, given by Formula 1 of Annex II.

Sole paragraph. When the accuracy is determined based on a previously validated method, the concentration of analyte determined by such method must be considered instead of the term “theoretical concentration”.

Article 47. The relative standard deviation (RSD) must be calculated for each concentration.

Article 48. The acceptance criteria for recovery percentages and relative standard deviation obtained must be justified in accordance with the criteria provided for in Article 39.

Section VII

Limit of Detection

Article 49. The limit of detection must be shown by obtaining the lowest amount of analyte present in a sample that may be detected but not necessarily quantified under the experimental conditions established.

Article 50. The determination of the limit of detection may be carried out through visual method, signal-to-noise ratio, based on the determination of blank or on calibration curve parameters, considering the particularities of the analytical method used.

Article 51. For visual methods, the limit of detection is determined by the lowest concentration at which it is possible to notice the expected visual effect.

Article 52. For instrumental methods, the limit of detection may be determined by the signal-to-noise ratio.

Paragraph 1. The method used to determine the signal-to-noise ratio must be described and justified.

Paragraph 2. The signal-to-noise ratio must be higher than or equal to 2:1.

Article 53. For the determination based on analytical curve parameters, the limit of detection may be calculated by Formula 2 of Annex II.

Article 54. In the cases where an estimated value for the limit of detection is obtained through calculation or extrapolation, such estimate must be confirmed in accordance with Article 52.

Section VIII

Limit of Quantification

Article 55. The limit of quantification is the lowest amount of analyte in a sample that may be determined with acceptable precision and accuracy under the experimental conditions established.

Article 56. The limit of quantification must be coherent with the limit of specification of impurity.

Sole paragraph. For products adequate to the Resolution that establishes parameters for notification, identification, and qualification of degradation products in medicinal products, the limit of quantification must be lower than or equal to the limit of notification.

Article 57. For the determination of such parameter, the procedure described in Article 53 must be followed, and the signal-to-noise ratio must be at least 10:1.

Article 58. For the determination based on analytical curve parameters, the limit of quantification may be calculated through Formula 3 of Annex II.

Article 59. In the cases where an estimated value for the limit of quantification is obtained through calculation or extrapolation, such estimate must be confirmed in accordance with Article 57.

Article 60. Precision and accuracy must be tested in the concentrations corresponding to the limit of quantification.

Section IX

Robustness

Article 61. Robustness is a parameter usually carried out in the development of the analytical method that indicates its ability to resist minor and deliberated variations of analytical conditions.

Sole paragraph. If the method is susceptible to variations in analytical conditions, such conditions must be controlled through the precautions described in the method.

Article 62. For quantitative methods, the impact of the proposed variations on the results obtained must be assessed using the same criteria used for accuracy.

Article 63. In the case of qualitative methods, there must be a verification whether the variations proposed interfere with the analytical response.

Article 64. Compliance with the system verification characteristics must be demonstrated.

Article 65. The assessment of the parameters described in Table 1 of Annex III must be included in the validation report.

Paragraph 1. Parameters deemed as relevant for the result, according to the method's characteristics, must be also assessed.

Paragraph 2. The lack of assessment of any variation must be justified.

CHAPTER V

TRANSITIONAL PROVISIONS

Article 66. Validations of an analytical method will be accepted if in accordance with Resolution RE No. 899/2003, provided that they have been completed before the enforcement of this Resolution and the petitions that include them are submitted within up to 550 (five hundred and fifty) calendar days from the date this Resolution enters into force.

Paragraph 1. If it is necessary to execute and resubmit one or more validation parameters, provided that submitting a new validation is not necessary, the company may follow Resolution RE No. 899 of 29 May 2003.

Paragraph 2. If it is necessary to execute and submit a new validation, the company must follow this Resolution.

Paragraph 3. After the deadline established in the caption of this article, for products under investigation whose validation of the analytical method used in clinical development has been initiated before the enforcement of this Resolution, the analytical validations carried out in accordance with Resolution RE no. 899 of 29 May 2003 shall be accepted at the moment of submission.

CHAPTER VI

FINAL PROVISIONS

Article 67. Documents and additional tests may be requested at any time by Anvisa.

Article 68. All relevant data obtained during the analytical validation procedure, as well as the formulas used for calculations, must be submitted together with the petition of interest to be assessed by Anvisa.

Article 69. Failure to comply with the provisions contained in this Resolution constitutes a health infraction, pursuant to Law No. 6,437, of 20 August 1977, without prejudice to the applicable civil, administrative, and criminal liabilities.

Article 70. Resolution RE no. 899 of 29 May 2003, item XXXI of Article 1, the Sole Paragraph of Article 11 and Annex I of Resolution RDC no. 31 of 11 August 2010 are hereby revoked.

Article 71. This Resolution enters in force one hundred and eighty (180) calendar days from the date of its publication.

JARBAS BARBOSA DA SILVA JR.

ANNEX I

Table 1. Parameters to be considered in analytical validation.

Parameter Assessed	**Identification**	**Impurity Test Assay**		**Dosing**
		Quantitative	Limit test	- dissolution (quantification) - content uniformity - potency
Accuracy	no	yes	no	yes
Repeatability Precision	no	yes	no	yes
Intermediate Precision	no	yes(1)	no	yes(1)

Selectivity[2]	yes	yes	yes	yes
Limit of Detection	no	no[3]	yes	no
Limit of Quantification	no	yes	no	no[3]
Linearity	no	yes	no	yes
Interval	no	yes	no	yes

(1) If reproducibility was conducted, intermediate precision does not need to be carried out.

(2) In the cases of identification tests, combining one or more analytical procedures may be needed to reach the required level of discrimination.

(3) It may be required in some cases.

ANNEX II

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Formula 1. Accuracy calculation.

$$\text{Recovery} = \frac{\text{Experimental mean concentration}}{\text{Theoretical concentration}} \times 100$$

Or

$$\text{Recovery} = \frac{\text{CA (added sample)} - \text{CA (sample)}}{\text{TCA}} \times 100$$

Where: CA is the experimental concentration of analyte and TCA is the theoretical concentration of analyte added.

Formula 2. Limit of detection calculation.

$$LD = \frac{3.3.\sigma}{CI}$$

Where: CI is the calibration curve inclination, σ is the standard deviation and may be obtained in 3 ways:

I – from the standard deviation of the intercept with Y-axis of at least 3 calibration curves constructed containing analyte concentrations near the assumed limit of detection;

II – from the residual standard deviation of the regression line;

III – from the estimated noise resulting from the analysis of an appropriate number of blank samples.

Formula 3. Limit of quantification calculation.

$$LQ = \frac{10.\sigma}{CI}$$

Where: CI is the calibration curve inclination, σ is the standard deviation and may be obtained in 3 ways:

I – from the standard deviation of the intercept with Y-axis of at least 3 calibration curves constructed containing analyte concentrations near the assumed limit of detection;

II – from the residual standard deviation of the regression line;

III – from the estimated noise resulting from the analysis of an appropriate number of blank samples.

ANNEX III

Table 1. Conditions for the assessment of method robustness

Sample Preparation	Stability of analytical solutions
	Extraction time
	Filter compatibility
Spectrophotometry	Variation of solution pH
	Different solvent batches or manufacturers
Liquid Chromatography	Variation of mobile phase pH
	Variation of mobile phase composition
	Different column batches or manufacturers
	Temperature
	Mobile phase flow
Gas Chromatography	Different column batches or manufacturers
	Temperature
	Carrier gas velocity
Other analytical techniques	The variations to be tested must be assessed critically, and their results must be presented