RESOLUTION OF THE COLLEGIATE BOARD - RDC Nº. 166, DATED JULY 24th, 2017

Providing for the validation of analytical methods and other provisions.

The **Collegiate Board of the National Agency for Sanitary Surveillance**, as per its duties conferred by items III and IV of Article 15, allied to items III and IV of Article 7, Law no. 9.782, dated January 26, 1999, and to Article 53, V, §§ 1 and 3 of the Internal Regulations approved in accordance with Annex I of the Resolution of the Collegiate Board of Directors - RDC No. 61, of February 3, 2016, resolves to adopt the following Resolution of the Collegiate Board of Directors, as resolved at a meeting held On July 11, 2017, and I, Director President, determine its publication.

CHAPTER I

INITIAL PROVISIONS

Section I

Objective

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

Sole paragraph. The non-fulfillment of any criterion provided for herein shall be technically justified and will be subject to analysis by Anvisa.

Section II

Scope

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

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

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

I – The use of 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 biology and immunology test will be admitted.

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

Section III

Definitions

Art. 3 For purposes of this resolution, the following definitions are adopted:

I - sample: representative amount of pharmaceutical ingredient, intermediate or finished product, duly identified, before the determined expiration date;

II - analyte: substance or group of substances of interest whose identification or quantification is intended;

III - chemical substance characterization: the set of tests that undoubtedly assures the authenticity and quality of substance regarding its identity, purity, content and potency, which must include data obtained through techniques applicable to each substance characterization, such as, for example, 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 test, among others;

IV - analytical run: set of measurements performed in a pool of samples in a pre-determined 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 response factors that is used as correction in the calculation of the concentration of a substance when such substance is measured by the analytical response of another substance;

X - quality management: 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 the structure and function of 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 the purity calculation are defined as pre-determined in the software used or by scientifically-based technical evaluation;

XIX - validation report: document that consolidates and summarizes the procedures, records, results and validation evaluation;

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

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 pharmacopeia 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 by a characterization process;

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

XXIV - working standard (WS): substance or mixture of chemical or biological substances used in lab routine standardized based on a pharmacopeia 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 can execute the analytical method according to the intended purpose;

XXVI - analytical validation: the systematic evaluation of a method by means of experimental tests in order to confirm and provide objective evidence that the specific requirements for its use have been met;

XXVII -partial validation: demonstration, by means of some validation parameters, that the analytical method previously validated has the characteristics needed for providing 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 shall be defined during method development and validation.

CHAPTER II

GENERAL PROVISIONS

Art. 4 Validation shall show, in a documented way and according to objective criteria, that the analytical method produces reliable results and is proper for the intended purpose.

Art. 5 The use of an analytical method not described in the official compendium recognized by Anvisa requires an analytical validation, as per the parameters established in this resolution, considering the technical and operational conditions.

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

Art. 7 Compendial analytical methods shall have their suitability for the intended use shown, under the laboratory operating conditions, by a partial validation study.

Sole paragraph. The provision in the caput excludes 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.

Art. 8 Partial validation must evaluate at least the parameters of precision, accuracy and selectivity.

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

Paragraph 2. For limit test, the parameters of selectivity and limit of detection shall be assessed instead of the parameters mentioned in the caput.

Art. 9 For method transfer between laboratories, the method will be deemed as validated if a partial validation study is conducted in the receiving unit area.

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 reproducibility evaluation.

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

Paragraph 3. If the transfer also used comparative tests, results similarities shall be evidenced by statistical tool.

Paragraph 4. Documents of method transfer shall 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. For the 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.

Art. 10. Revalidation of analytical method can consider the following cases:

I - changes in the synthesis or obtainment of API;

II - changes in product composition;

III - changes in analytical method; and

IV - other changes that may significantly impact the validated method.

Sole paragraph. Validation parameters to be evaluated depend on the nature of the changes performed.

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

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

Art. 13. The validation report to be filed, as per registration and post-registration resolutions, must include data and calculations obtained during the analytical validation process, as well as the statistical approach used for data evaluation.

Paragraph 1. Raw data related to selectivity parameter must be included in the report mentioned in the caput.

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

CHAPTER III

CHEMICAL REFERENCE SUBSTANCES

Art. 14 For the validation of analytical methods, the Pharmacopeia 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 test and relevant raw data.

Paragraph 2. Anvisa and members of the Sanitary National System will be able to request samples of the CCRS in order to evaluate the characterization process in the hypotheses of the previous paragraph and when a fiscal analysis is required, a sample of CCRS should be provided for the purposes of carrying out the necessary tests.

Art. 15. Depending on the analyte, the characterization report shall 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 test, among others.

Paragraph 1. Additionally to characterization data, the following information shall be included in the report:

I - number and expiration date 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 - physical-chemical properties;
X - impurity profile;
XI - handling and maintenance information, and analytical report attesting identity, content and shelf-life of CCRS.

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

Art. 16. For medicinal gases, analytical verification of instruments and analytical determinations should be performed using traceable reference materials distributed by metrology institutes or organs 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.

Art. 17. For biological products, the words material or standard are used instead of the word chemical substance in the definitions of CRS, PCRS, CCRS and WS.

Art. 18. The use of WS is not allowed for purposes of analytical method validation.

CHAPTER IV

ANALYTICAL VALIDATION PARAMETERS

Section I

Selectivity

Art. 19. Analytical method selectivity shall be shown by means of its ability of identifying or quantifying the analyte of interest undoubtedly in the presence of components that may be found in the sample, such as impurities, diluents and matrix components.

Sole paragraph. For chromatographic methods, the chromatographic purity of the analyte signal shall be proven, except for biologic products.

Art. 20 For identification methods, its ability of obtaining a positive result for the sample containing the analyte and a negative result for other substances present in the sample shall be demonstrated.

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

Paragraph 2. To demonstrate the selectivity of identification methods, the test shall be carried out with 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 drug products containing such APIs, the method's ability to distinguish the material of interest from other similar vegetal materials, especially those found as adulterants and substituents, shall be demonstrated.

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

Art. 21 For quantitative methods and limits test, selectivity shall be demonstrated by evidencing that the analytical response is due to the analyte only, without interference of the diluent, matrix, impurities or other degradation products.

Paragraph 1. To show the lack of interference of degradation products, the sample has to be exposed to degradation conditions with a wide range of pH, oxidation, heat and light.

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

I - products whose adequacy to the resolution establishing parameters for notification, identification and qualification of degradation products in drug 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 caput, is accepted only upon technical justification and concomitant application of an additional method.

Art. 22. For medicinal gases, selectivity shall 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

Art. 23 Linearity of a method shall be demonstrated by its ability of obtaining analytical responses directly proportional to the concentration of one analyte in the sample.

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

Art. 25 To establish linearity, at least five (5) different concentrations of CRS shall be used for solutions in at least triplicate.

Sole paragraph: The solutions used to evaluate the linearity must be prepared independently, and diluted solutions of the same SQR mother solution can be used.

Art. 26 All calculations for linearity assessment shall be made based on actual concentration data and individual analytical responses.

Art. 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 evaluation;

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

IV - evaluation of linear association between variables correlation coefficient (r) and determination coefficient (r^2);

V - evaluation of significance of angular coefficient.

Paragraph 1. Data homocedasticity shall be investigated for the use of a proper model.

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

Paragraph 3. Correlation coefficient shall be above 0.990.

Paragraph 4. Angular coefficient shall be significantly different from zero.

Section III

Matrix Effect

Art. 28 The provisions of this section apply to complex matrixes.

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

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

Art. 30 Parallelism of lines indicates absence of interference of matrix components and its demonstration shall be carried out by proper statistical evaluation.

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

Section IV

Working range

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

Art. 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 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 by area normalization method: from the limit of quantification (LQ) to 120% (one hundred and twenty per cent) of the expected concentration of active ingredient.

Paragraph 1. Working ranges wider than those defined in the caput may be used if technical justification is provided.

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

Section V

Precision

Art. 33 Precision shall evaluate the proximity of results obtained by test of samples prepared according to the description of the analytical method to be validated.

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

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

Art. 36 Samples for precision evaluation 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.

Art. 37 When precision evaluation 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. For known impurities not found in the sample or present in concentrations lower than the limit of the specification, the sample should be spiked with known concentrations of impurities standard.

Paragraph 2. For unknown impurities, the sample must be evaluated 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.

Art. 38 Repeatability determination must meet the following criteria:

I - evaluate samples under the same operational conditions, with the same analyst and same instruments, in a single analytical run.

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

Art. 39 Acceptance criteria must be defined and justified according to the following aspects:

I – method purpose;

II – method intrinsic variability;

III – working concentration; and

IV – analyte concentration in the sample.

Art. 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 evaluation.

Art. 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 on official compendia upon proper statistical tests.

Paragraph 2. Acceptance criterion for relative standard deviation must be justified as provided for in Art. 39.

Section VI

Accuracy

Art. 42 Accuracy of an analytical method must be reached by the level of conformity between individual results of the method being tested in relation to a value accepted as true.

Art. 43 Accuracy shall be checked based on at least nine (9) determinations, including the linear interval of analytical method, that is, three (3) concentrations: low, medium, high, with three (3) replicates in each level.

Art. 44 Samples for accuracy evaluation shall be prepared independently, and diluted solutions derived from the same CRS stock solution may be used.

Art. 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) for 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 ingredients of the drug product available, the analysis may be carried out by 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 API relative response factor;

c) for unknown impurities, accuracy must be evaluated by comparing the CRS response of API or of a known impurity, according to the proposed method, within a concentration range including 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 shall be the same described in the analytical method in question.

Art. 46 Accuracy shall 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. If accuracy is determined based on a previously validated method, the concentration of analyte determined by such method shall be considered instead of the “theoretical concentration”.

Art. 47 The relative standard deviation (RSD) shall be calculated for every concentration.

Art. 48 Acceptance criteria for recovery percentages and relative standard deviation obtained must be justified as per the criteria set forth in Art. 39.

Section VII

Limit of Detection

Art. 49 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 established experimental conditions.

Art. 50 The determination of the limit of detection may be carried out by 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.

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

Art. 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.

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

Art. 54 When an estimated value for the limit of detection is obtained by calculation or extrapolation, such estimate must be confirmed as per Art. 52.

Section VIII

Limit of Quantification

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

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

Sole paragraph. For products applicable to the resolution that establishes parameters for notification, identification and qualification of degradation products in drug products, the limit of quantification shall be lower than or equal to the limit of notification.

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

Art. 58 For the determination based on analytical curve parameters, the limit of quantification may be calculated by formula 3 of Annex II.

Art. 59 When an estimated value for the limit of quantification is obtained by calculation or extrapolation, such estimate must be confirmed as per Art. 57.

Art. 60 Precision and accuracy shall be tested in the concentrations corresponding to the limit of quantification.

Section IX

Robustness

Art. 61 Robustness is a parameter usually performed in the development of the analytical method and indicates its ability to resist minor and deliberated variations of analytical conditions.

Sole paragraph. If the method is susceptible to variations of analytical conditions, such conditions shall be controlled by the precautions described in the method.

Art. 62 For quantitative methods, the impact of the proposed variations on the results achieved shall be assessed using the same criteria used for accuracy.

Art. 63 In the case of qualitative methods, it must be verified whether the variations proposed interfere with the analytical response.

Art. 64 Compliance with system verification characteristics must be demonstrated.

Art. 65 Evaluation of parameters described in Table 1 of Annex III shall be included in the validation report.

Paragraph 1. Parameters deemed as relevant for the result, according to method characteristics, shall be assessed additionally.

Paragraph 2. The lack of evaluation of any variation shall be justified.

CHAPTER V

TEMPORARY PROVISIONS

Art. 66 Validations of an analytical method will be accepted if in consonance with Resolution RE No. 899/2003, provided that they have been completed before the enforcement of this resolution and the petitions that filed within up to 550 (five hundred and fifty) calendar days from the enforcement of this resolution.

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, May 29, 2003.

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

Paragraph 3. After the deadline established in the caput for products under investigation whose validation of the analytical method used in clinical development has been initiated before the validity of this resolution, the analytical validations performed in accordance with Resolution RE 899/2003 will be accepted.

CHAPTER VI

FINAL PROVISIONS

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

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

Art. 69 The non-compliance with the provisions set forth in this resolution constitutes a health violation under the terms of Law no. 6.437, dated August 20, 1977, without prejudice to any civil and criminal liability that may be applicable.

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

Art. 71 This resolution enter in force within 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 -dissolution (quantification) - content uniformity - potency**
		Quantitative	**Limit test**	
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 carried out, intermediate precision does not need to be carried out.

[2] In the identification test, 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

Formula 1. Accuracy calculation.

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

Or

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

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

Formula 2. Limit of detection calculation.

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

Where: IC 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 concentration 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 a proper number of blank samples.

Formula 3. Limit of quantification calculation.

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

Where: IC 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 concentration 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 a proper number of blank samples.

ANNEX III

Table 1. Conditions for method robustness evaluation

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
	Inert gas velocity
Other analytical techniques	Variations to be tested shall be assessed critically, and their results shall be provided