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THE EURASIAN ECONOMIC COMMISSION
BOARD

DECISION

July 17, 2018 **No. 113** Moscow

On approval of the Guidelines for validation of the analytical procedures for medicinal products testing

In accordance with Article 30 of the Treaty on the Eurasian Economic Union dated May 29, 2014, and paragraph 2 of Article 3 of the Agreement on common principles and rules for the circulation of medicinal products within the framework of the Eurasian Economic Union dated December 23, 2014, the Board of the Eurasian Economic Commission decided to:

1. Approve the attached Guidelines for validation of the analytical procedures for medicinal products testing.

2. This Decision shall come into effect 6 months after its official publication.

Chairman of the Board
of the Eurasian Economic Commission T. Sarkisian

Seal: *EURASIAN ECONOMIC COMMISSION * FOR DOCUMENTS*

APPROVED

by Decision of the Board
of the Eurasian Economic Commission No.
113 dated July 17, 2018

GUIDELINES
on validation of the analytical procedure for medicinal products testing

I. General provisions

1. These Guidelines define the rules for validation of the analytical procedure for medicinal products testing, as well as a list of characteristics to be evaluated during validation of these methods and included in the marketing authorisation application submitted to the authorised bodies of the Member States of the Eurasian Economic Union (hereinafter, respectively, the Member States, the Union).

2. The purpose of validation of the analytical procedure for medicinal products testing is to provide documented proof of its suitability for the intended purpose.

II. Definitions

3. For the purposes of these Guidelines, the terms below shall have the following meaning:

"analytical procedure" means a procedure of medicinal products testing, which includes a detailed description of the sequence of actions required to perform an analytical test (including a description of the preparation of test samples, reference standards, reagents, the use of

equipment, calibration curve construction, calculation of formulas used, etc.);

"reproducibility" means a property characterizing the precision in interlaboratory tests;

"range" means an interval between the highest and lowest concentrations (quantity) of the substance being identified in the sample (including these concentrations), for which it is shown that the analytical procedure has an acceptable level of precision, correctness, and linearity;

"linearity" means a directly proportional dependence of the analytical signal on the concentration (quantity) of the substance being identified in the sample within the range of the procedure;

"recovery" means the ratio between the recovered average and true (reference) values, taking into account the corresponding confidence intervals;

"repeatability (intra-assay precision))" means the procedure precision when repeated tests are performed under the same operating conditions (for example, by the same analyst or a group of analysts, using the same equipment, with the same reagents, etc.) within a short period of time;

"accuracy, trueness" means the proximity between the accepted true (reference) value and the recovered value, which is expressed by the value of recovery;

"quantitation limit" means the smallest amount of substance in a sample that can be

determined quantitatively with appropriate precision and accuracy;

"detection limit" means the smallest amount of detectable substance in a sample that can be detected, but not necessarily accurately quantified;

"precision" means the expression of the proximity (degree of dispersion) of the results (values) between a series of measurements made on a set of samples taken from the same homogeneous sample, under the conditions prescribed by the procedure;

"intermediate precision" means the effect of variations within the laboratory (different days, different analysts, different equipment, different batches of reagents, etc.) on the test results of identical samples taken from the

same batch;

"specificity" means the ability of the analytical procedure to unambiguously evaluate a defined substance independently of the other substances (impurities, degradation products, excipients, matrix (medium), etc.) present in the test sample;

"robustness" means the ability of the analytical procedure to be resistant to the influence of small specified changes in the test conditions, which indicates its reliability under normal (standard) use.

III. Types of the analytical procedures to be validated

4. These Guidelines discuss the approaches to validation of the 4 most common types of analytical procedures:

a) identification tests;

b) quantitative tests for impurities content;

c) limit tests for the control impurities;

d) quantitative tests of the active moiety to determine an active part of the active substance molecule in the test sample.

5. All analytical procedures used for the quality control of medicinal products must be validated. These Guidelines do not address the validation of analytical procedures for the types of tests not included in paragraph 4 hereof (for example, tests for dissolution or determination of the particle size (dispersion) of an active substance, etc.).

6. Identification tests usually provide for comparison of the properties (for example, spectral characteristics, chromatographic behavior, chemical activity, etc.) of the test sample and reference standard.

7. Quantitative tests for impurities content and limit tests for the control impurities in the sample shall be aimed at correct description of the sample purity. The requirements for validation of the procedures for quantitation of

impurities differ from the requirements for validation of the procedures to determine the limit of impurities content in a sample.

8. Quantitative testing procedures are aimed at measuring the content of the substance being identified in the test sample. For the purposes hereof, the quantitation refers to the quantitative measurement of the main components of an active substance. Similar validation parameters are applicable to the quantitation of the active substance or other components of the medicinal product. The validation parameters of the quantitation may be used in the other analytical procedures (for example, in the dissolution test).

The purpose of the analytical procedure should be clearly defined, since this determines the choice of validation characteristics to be evaluated during validation.

9. The following typical validation characteristics of the analytical procedure are subject to evaluation:

a) accuracy (trueness);

b) precision: repeatability;

intermediate precision

;

c) specificity;

d) detection limit;

e) quantitation limit;

f) linearity;

g) range.

10. The most important validation characteristics to validate various types of analytical procedures are shown in the table.

Table

Validation characteristics to validate various types of analytical procedures

Validation	Analytical procedure type

characteristic	identification tests	impurity tests		quantitative tests
		quantitative content	limit	dissolution (measurement only), content (activity)
Accuracy	-	+	-	+
Precision				
repeatability	-	+	-	+
intermediate precision	—	+*	—	+*
Specificity	+	+	+	+
Detection limit	-	–***	+	-
Quantitation limit		+		—
Linearity	-	+	-	+
Range	-	+	-	+

* If the reproducibility is defined, the determination of intermediate precision is not required.

** Insufficient specificity of a single analytical procedure can be compensated by using one or more additional analytical procedures.

*** May be required in some cases (for example, when the detection limit and the normalized limit of the impurity to be determined are close).

Note. "-" means that the characteristics is not evaluated, and "+" means that the characteristics is evaluated.

This list should be considered as a standard list for validation of analytical procedures. There may be exceptions that require separate justification by the manufacturer of medicinal products.

Such characteristics of the analytical procedure as robustness is not given in the table, but it should be considered at the appropriate stage of the analytical procedure development.

Re-validation may be required in the following cases (including, but noted limited to):

change of the active substance synthesis pattern;

change in the medicinal product composition;

change in the analytical procedure.

Re-validation is not performed if the manufacturer has provided the appropriate rationale. The scope of re-validation depends on the nature of the

changes made.

IV. Analytical procedure validation methodology

1. General requirements for the analytical procedure validation methodology

11. This section describes the characteristics that are taken into account when validating analytical procedures, as well as some approaches and recommendations for establishing different validation characteristics of each analytical procedure.

12. In some cases (for example, when proving specificity), a combination of several analytical procedures may be used to ensure the quality of an active substance or medicinal product.

13. All relevant data collected during validation and the formulas used to calculate the validation characteristics should be presented and analyzed.

14. The approaches other than the approaches outlined herein may be used. The applicant is responsible for selecting the validation procedure and protocol. At the same time, the main purpose of the analytical procedure validation is to confirm the method suitability for the intended purpose. Due to their complexity, the approaches to analytical procedures for biological and biotechnological preparations may differ from those described herein.

15. Reference standards with known characteristics confirmed by documents should be used throughout the study of validation characteristics. The required degree of the standard sample purity depends on the intended purpose.

16. Individual subsections of this section consider various validation characteristics. The structure of this section reflects the progress of the analytical procedure development and evaluation process.

17. Experimental work should be planned in such a way that the relevant

validation characteristics are studied simultaneously, obtaining reliable data on the capabilities of the analytical procedure (for example, specificity, linearity, range, accuracy, and precision).

2. Specificity

18. Specificity should be studied during the validation of tests for identification, impurity, and quantitation. The procedures of confirming specificity depend on the purpose of the analytical method.

19. The method of confirming specificity depends on the tasks for which this analytical procedure is intended. It is not always possible to confirm that the analytical procedure is specific to the substance being identified (complete selectivity). In this case, we recommend using a combination of 2 or more analytical procedures.

Insufficient specificity of a single analytical procedure can be compensated by using one or more additional analytical procedures.

20. Specificity for different types of tests means the following:

a) for the identification test – a proof that the procedure allows the identification of the substance being identified;

b) for the impurity test – a proof that the procedure allows correct recognition of impurities in the sample (for example, testing for related compounds, heavy metals, residual solvent content, etc.);

c) for quantitative tests – a proof that the procedure allows determining the content or activity of the substance being identified in the sample.

Identification

21. Satisfactory identification tests must be able to distinguish between structurally closely related compounds that may be present in a sample. The selectivity of the analytical procedure can be confirmed by obtaining positive

results (possibly by comparing with a known reference standard) for samples containing the component being identified, and negative results for samples not containing the same.

22. To confirm the absence of false positive results, an identification test may be performed for substances with a similar structure or substances accompanying the substance being identified.

23. The choice of the potentially interfering substances must be justified.

Quantitation and impurity tests

24. When confirming specificity for an analytical procedure using chromatographic separation, representative chromatograms with appropriate indication of individual components should be provided. It is required to apply similar approaches to the other procedures based on separation.

25. Critical separations in chromatography shall be studied at the appropriate level. In the case of critical separations, the resolution value of the 2 most closely eluted components must be set.

26. When using a non-specific quantitation method, additional analytical procedures should be used and the specificity of the entire set of procedures should be confirmed. For example, if the active substance quantitation is performed titrimetrically, it can be supplemented with an appropriate impurity test.

27. The approach is similar for both the quantitation and the impurity tests.

The presence of impurity samples

28. In the presence of impurity samples, the determination of the analytical procedure specificity is as follows:

a) for quantitation, it is required to confirm the selectivity of the

substance identification in the presence of impurities and (or) other sample components. In practice, this is done by adding impurities and (or) excipients to the sample (active substance or medicinal product) in the appropriate amount, and if there is evidence that they do not affect the result of the active ingredient quantitation;

b) for impurity tests, specificity can be established by adding certain amounts of impurities to the active substance or medicinal product, and if there is evidence of separation of these impurities from each other and (or) from the other sample components.

Lack of impurity samples

29. If reference standards of impurity samples or degradation products are absent, specificity can be confirmed by comparing the results of testing the samples containing impurities or degradation products with the results of another validated procedure (for example, a pharmacopoeia or another validated analytical (independent) procedure). Where appropriate, reference impurity samples should include samples that have been stored under certain stressful conditions (light, heat, humidity, acid (base) hydrolysis, and oxidation).

30. In the case of quantitation, 2 results must be compared.

31. In the case of the impurity tests, the profiles of the impurities must be compared.

32. To prove that the peak of the identified substance corresponds to only one component, it is advisable to investigate the purity of peaks (for example, the use of diode-matrix detection, mass spectrometry).

3. Linearity

33. A linear relationship should be evaluated within the entire analytical

procedure application range. It can be confirmed directly on the active substance (by diluting the main standard solution) and (or) on separate aliquots of artificial (model) mixtures of medicinal product components using the proposed procedure. The latter aspect can be studied in the course of determining the range (analytical area) of the procedure.

34. The linearity is evaluated visually according to the graph of dependence of the analytical signal as a function of the concentration or quantity of the substance being identified. If there is a clear linear relationship, the results must be processed using suitable statistical methods (for example, by calculating the regression line using the least squares method). A mathematical transformation of the test results may be required to obtain linearity between the quantitation results and the sample concentrations prior to regression analysis.

The results of the regression line analysis can be used for mathematical assessment of the linearity degree.

35. If there is no linearity, the test data should be subjected to mathematical transformation before performing a regression analysis.

36. To confirm linearity, the correlation coefficient or the determination coefficient, the free term of the linear regression, the tangent of the slope of the regression line and the residual sum of the squares of deviations must be determined and presented, and a graph with all experimental data must be attached.

37. If linearity is not observed in any types of mathematical transformations (for example, in the validation of immune analytical procedures), the analytical signal must be described using the appropriate function of the concentration (quantity) of the component being identified in the sample.

38. It is recommended to use at least 5 concentrations to establish

linearity. The use of the other approaches requires justification.

V. Range

39. The analytical procedure range depends on its intended purpose and is determined when studying the linearity. Within the range, the procedure must provide the required linearity, accuracy, and precision.

40. The following ranges of analytical procedures should be considered as the minimum acceptable ones:

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a) for the quantitation of the active ingredient in the active substance or medicinal product - from the 80 percent concentration (content) to the 120 percent concentration (content) of the nominal concentration (content);

b) for the dosage uniformity - from the 70 percent concentration (content) to the 130 percent concentration (content), if a wider range depending on the dosage form is not justified for the medicinal product (for example, dosed inhalers);

c) for the dissolution test - ±20 percent (absolute) of the nominal range. For example, if the specifications of a modified release product cover an area from 20 percent in the first hour to 90 percent of the declared content in 24 hours, the validated range of use should be from 0 to 110 percent of the declared content;

d) for the impurity identification - from the impurity detection limit to the 120 percent of the value indicated in the specification;

e) for impurities that have an extremely strong or toxic or unforeseen pharmacological effect, the detection limit and the quantitation limit should be proportional to the level at which these impurities should be controlled. In order to validate the impurity tests procedures used during development, it may be required to set an analytical area near the intended (possible) limit;

e) if the quantitation and purity are studied simultaneously using a single test and only a 100 % standard is used, the linear relationship should be maintained throughout the range of the analytical procedure application, starting from the information threshold for the impurity (in accordance with the rules for the study of impurities in medicinal products and setting requirements thereto in specifications approved by the Eurasian Economic Commission) to the 120 % content indicated in the specification for quantitation.

VI. Accuracy

41. Accuracy must be established for the entire range of the analytical procedure application.

1. Active substance quantitation

Active substance

42. Accuracy may be assessed in several ways by:

applying analytical procedures to a test substance with a known degree of purity (for example, to a standard material);

comparison of analysis results obtained using a validated analytical procedure with results obtained using a procedure with known accuracy and/or an independent procedure.

A conclusion about accuracy can be made after establishing the precision, linearity, and specificity.

Medicinal product

43. Accuracy may be assessed in several ways by:

application of analytical procedure to artificial (model) mixtures of medicinal product components, in which a pre-known amount of the substance

to be determined was added;

in the absence of samples of all drug components, a pre-determined amount of active substance may be added to the medicinal product or the results obtained using another procedure, the accuracy of which is known, and/or an independent procedure may be compared.

A conclusion about accuracy can be made after establishing the precision, linearity, and specificity.

2. Impurity quantitation

44. The accuracy is determined using the samples (of an active substance and medicinal product), to which a known amount of impurities is added.

45. In the absence of samples of the impurities and/or degradation products being identified, it is acceptable to compare the results with those obtained using an independent procedure. It is allowed to use an analytical signal of the active ingredient.

46. It is required to indicate a specific way of expressing the content of individual impurities or their sum (for example, in mass percentages or as a percentage of the peak area, but in all cases in relation to the main ingredient being identified).

3. Recommended scope of studies and accuracy indicators

47. Accuracy is evaluated for at least 9 determinations of 3 different concentrations covering the entire range (i.e. 3 concentrations and 3 repetitions for each concentration). Determinations should include all procedure stages.

48. Accuracy is expressed by the recovery value as a percentage based on the results of quantitation of the ingredient added in a known amount to the sample being analyzed, or the difference between the recovered average and true (reference) values, taking into account the corresponding confidence

intervals.

VII. Precision

49. Validation of quantitation and impurity tests provides for the determination of precision.

50. Precision is set at 3 levels: repeatability, intermediate precision, and reproducibility. Precision should be established using homogeneous authentic samples. When it is impossible to obtain a homogeneous sample, precision can be determined using artificially prepared (model) samples or sample solution. The precision of an analytical procedure is usually expressed by the amount of variance, standard deviation, or coefficient of variation of a series of measurements.

VIII. Repeatability

51. Repeatability is determined by performing at least 9 determinations of concentrations within the analytical procedure application range (3 concentrations and 3 repetitions for each concentration), or at least 6 determinations of concentration for samples with 100 % content of the ingredient being identified.

IX. Intermediate precision

52. The degree to which intermediate precision is established depends on the conditions of using the analytical procedure. The applicant must establish the influence of random factors on the precision of the analytical procedure. Typical (variable) factors studied are different days, analysts, equipment, etc. These effects do not need to be studied separately. When studying the effect of various factors, the experiment planning is preferred.

X. Reproducibility

53. Reproducibility characterizes precision in an interlaboratory experiment. Reproducibility should be determined in the case of standardization of the analytical procedure (for example, when it is included in the Pharmacopoeia of the Union or in the Pharmacopoeia of the Member States). The reproducibility data do not need to be included into the marketing authorisation application dossier.

XI. Data presentation

54. For each type of precision, the standard deviation, relative standard deviation (coefficient of variation), and confidence interval must be specified.

XII. Detection limit

55. Different approaches to determining the detection limit are possible, depending on whether the procedure is instrumental or non-instrumental. The other approaches are allowed, too.

XIII. Visual assessment

56. Visual assessment can be used for both non-instrumental and instrumental procedures. The detection limit is established by analyzing samples with known concentrations of the ingredient being identified and determining its minimum content at which it is reliably detected.

XIV. Estimation of the detection limit by signal/noise ratio

57. This approach is only applicable to the analytical procedures for which the baseline noise is observed.

58. The signal/noise ratio shall be determined by comparing signals received from samples with known low concentrations and signals received

from blank samples, and establishing the minimum concentration at which the ingredient can be reliably detected. The signal/noise ratio of 3:1 to 2:1 is considered acceptable for evaluating the detection limit.

XV. Estimation of the detection limit based on the standard deviation of the analytical signal and calibration curve slope

59. The detection limit (LoD) may be expressed as follows:

$$LoD = 3.3 \cdot \frac{\mathbf{S}}{k},$$

where:

s means a standard deviation of the analytical signal;

k means the tangent of the calibration curve slope.

60. The k value is calculated from the calibration curve for the ingredient being identified. There are several ways to evaluate s:

a) by the standard deviation of the blank sample. The value of the analytical signal for a sufficient number of blank samples is measured, and the standard deviation of their values is calculated;

b) by the calibration curve. It is required to analyze the resulting calibration curve mapped for samples with the content of the ingredient being identified close to the detection limit. The residual standard deviation of the regression line or the standard deviation of the intersection point with the ordinate axis (the standard deviation of a free term of the linear regression) can serve as the standard deviation.

XVI. Data presentation

61. The detection limit and its determination method must be specified. If the determination of the detection limit is based on a visual or signal/noise ratio assessment, the presentation of the relevant chromatograms is considered sufficient to justify the same.

62. If the value of the detection limit is obtained by calculation or extrapolation, the assessment outcome must be confirmed by independent testing of a sufficient number of samples with the content of the ingredient being identified corresponding or close to the detection limit.

XVII. Quantitation limit

63. The quantitation limit is a required validation characteristic of the methods used to determine the low content of ingredients in the sample, in particular, to identify the impurities and (or) degradation products.

64. There may be several approaches to determining the quantitation limit, depending on whether the procedure is instrumental or non-instrumental. The other approaches are allowed.

XVIII. Visual assessment

65. A visual assessment can be used for both non-instrumental and instrumental procedures.

66. The quantitation limit is usually established by analyzing samples with known concentrations of the ingredient being identified and evaluating the minimum content at which the ingredient being identified can be quantified with acceptable accuracy and precision.

XIX. Estimation of the quantitation limit by signal/noise ratio

67. This approach is only applicable to the measurement methods where the baseline noise is observed.

68. The signal/noise ratio shall be determined by comparing the measured signals received from samples with known low concentrations of the ingredient being identified and signals received from blank samples, and establishing the minimum concentration at which the ingredient can be reliably

quantified. The normal signal/noise ratio is 10:1.

XX. Estimation of the quantitation limit based on the standard signal deviation and the calibration curve slope

69. The quantitation limit (LoQ) can be expressed as follows:

$$LoQ = 10 \cdot \frac{s}{k},$$

where:

s means a standard deviation of the analytical signal;

k means the tangent of the calibration curve slope.

70. The k value is calculated from the calibration curve for the ingredient being identified. There are several ways to evaluate s:

a) by the standard deviation of the blank sample. The value of the analytical signal for a sufficient number of blank samples is measured, and the standard deviation of their values is calculated;

b) by the calibration curve. It is required to analyze the resulting calibration curve mapped for samples with the content of the ingredient being identified close to the quantitation limit. The residual standard deviation of the regression line or the standard deviation of the intersection point with the ordinate axis (the standard deviation of a free term of the linear regression) can serve as the standard deviation.

XXI. Data presentation

71. The quantitation limit and its determination method must be specified.

72. The quantitation limit must be subsequently confirmed by analyzing a sufficient number of samples with a content of the ingredient being identified equal or close to the quantitation limit.

73. The approaches other than those listed above can be acceptable.

XXII. Robustness

74. The robustness must be studied at the development stage; the scope of research depends on the analytical procedure under consideration. It is required to show the analysis robustness in the case of deliberate variations of the procedure parameters (conditions).

75. If the measurement results depend on changes in the analytical procedure application conditions, it is required to strictly monitor the compliance with such conditions or to specify precautionary measures during the test.

76. In order to maintain the validity of the analytical procedure when using it, one of the consequences of studying the robustness should be the establishment of a series of parameters for the system suitability (for example, the resolution test).

77. The common variations of parameters are:

stability of solutions used in analytical procedures;

the time of extraction.

The variation parameters for liquid chromatography are: change in the mobile phase pH;

change of the mobile phase composition;

different columns (different batches and suppliers);

temperature;

mobile phase flow rate.

The variation parameters for gas chromatography are: different columns (different batches and suppliers);

temperature;

carrier gas flow rate.

XXIII. System suitability evaluation

78. System suitability evaluation is an integral part of many analytical procedures. These tests are based on the concept that equipment, electronics, analytical operations, and samples being analyzed constitute an integral system and need to be evaluated as such. The system suitability criteria must be set for a specific procedure and depend on the type of analytical procedure being validated. Additional information can be obtained from the Pharmacopoeia of the Union and in the Pharmacopoeia of the Member States.