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Validation of analytical methods for pharmacopoeial analysis of pharmaceutical substances of plant origin and herbal drugs in the Russian Federation

D.O. Bokov[1,2], O.A. Smyslova[1], O.G. Potanina[3], T.M. Litvinova[1], I.A. Samylina[1]

[1] *Sechenov First Moscow State Medical University, 8 Trubetskaya St., bldg. 2, Moscow, 119991, Russian Federation*
[2] *Federal Research Center for Nutrition, Biotechnology and Food Safety, 2/14 Ustyinsky pr., Moscow, 109240, Russian Federation*
[3] *Peoples' Friendship University of Russia (RUDN University), 6 Miklukho-Maklaya Street, Moscow, 117198, Russian Federation*

Abstract

Analytical control of pharmaceutical substances and drugs of plant origin plays an important role in the quality assurance system for pharmaceutical products. The use of validated analytical methods for pharmacopoeial analysis is necessary in accordance with modern requirements for the medicines production. This article provides data on the validation procedure, the main parameters (specificity, detection limit, quantitation limit, range, linearity, trueness, precision, robustness). We discuss the parameters that must be assessed in order to validate the method for determining biologically active compounds in pharmaceutical substances of plant origin and herbal medicinal products taking into account specific requirements in the Russian Federation.

Keywords: validation, pharmacopoeial analysis, pharmaceutical substances of plant origin, herbal drugs

INTRODUCTION

Validation of the analytical method is an experimental proof that the procedure is suitable for solving the proposed problems. Validation of the analytical method is carried out in case of introducing a new method (development of new drugs), and when changing the conditions for analyzing drugs. The practical value of validation is to identify shortcomings in the process of developing new analytical methods at the early stages; it helps to improve significantly the method. Validation experiments provide an understanding of the critical method points and the need for strict adherence to its parameters. As a result, error probability is significantly reduced during the subsequent operation of a validated method. There are many domestic and international regulatory documents describing the validation procedure [1-9].

«ICH Q2 (R1) Guide Validation of Analytical Procedures: Text and Method» is one of the most authoritative regulatory document [10]. Another one is «Analytical Procedures and Methods Validation: Chemistry, Manufacturing, and Controls» adopted by FDA [11]; GMP in the USP [12].

On July 17th 2018 the College of the Eurasian Economic Commission (EEC) approved «The Guidance on Analytical Method Validation» [13]. The Guidance determines the approaches for validation of the 4 most common tests types, including the identification, quantitative tests for content of impurities, limit tests for the impurities controlling, and determination of concentration or activity of the active substance in the drugs. Document reports the conditions when method revalidation should be performed.

Such re-validation is necessary because of a change in the pharmaceutical substance scheme synthesis or the composition of the drug.

Validation in the Russian Federation is regulated by GOST R ISO 5725-1-2002 «Accuracy (accuracy and precision) of measurement methods and results» [14], which is the full authentic text of the international standard ISO 5725-1-1994 [15].

In the Russian Federation the State Pharmacopoeia of the Russian Federation XIII edition (SPRF XIII ed.) General Pharmacopoeial Monograph (GMP) 1.1.0012.15 «Validation of analytical methods» is used to validate the analytical methods for the quantitative determination of biologically active compounds in medicinal plant materials [16]. Our article analyzes the validation processes in accordance with this regulatory document.

METHODS

In the present work the information-analytical method and the system analysis method were used. In the course of our study, standard technical documentation and validation guidelines for analytical methods were analyzed.

RESULTS AND DICUSSION

The SPRF XIII ed. GPM 1.1.0012.15 «Validation of analytical methods» regulates the characteristics of analytical methods, criteria of methods validity, which are designed to control the quality of pharmaceutical substances and herbal medicines.

Quantification methods, including methods for determining impurities and methods for determining the limit of content, are subject to validation. Identification methods are validated if necessary to confirm their specificity.

During validation the analytical method is assessed according to the parameters listed below. Their selection is based on model recommendations given in charts 1 and 2.

It should be noted that the lack of specificity of one analytical method can be compensated by using another analytical method.

Revalidation (repeat validation) of the methods is carried out when changing:

- technology for obtaining the object of analysis;
- composition of the medicinal product (object of analysis);
- previously approved method of analysis.

Specificity – the ability of an analytical method to definitely evaluate a substance to be determined in the presence of accompanying components.

The specificity proof of a method is usually based on a consideration of data obtained from its analysis of model mixtures of known composition.

The method specificity can also be proved by appropriate statistical processing of theanalyzes results, performed with its use and, in parallel, using a different, obviously specific, method (a method whose specificity has been proven).

For identity tests, the validated method should provide reliable information about the presence of the active substance in a pharmaceutical substance or dosage form (if it contains any components prescribed in the formulation). The identity of the

active ingredient in a pharmaceutical substance or drug is established in comparison with a standard sample or by evaluating physicochemical or chemical properties that are not characteristic for other components.

The same approaches are used for the quantitative determination and testing of impurities. Specificity must be evaluated with respect to the analyte, i.e. it must be experimentally confirmed that the presence of concomitant components does not inadvertently affect the result of the analysis.

It is allowed to assess the specificity of the validated method by:

1) analysis of model mixtures of known composition containing the substance to be determined;

2) comparison of analyzes results obtained simultaneously using a validated and another, obviously specific method.

The results of relevant experiments should be statistically processed. The lack of specificity of the test can be compensated by other additional test.

If appropriate, drugs samples subjected for the impurities accumulation can be used during validation. Impurities accumulation can be induced by extreme conditions (light, temperature, humidity) or chemical modification by any suitable method.

The resolution should be observed between the two most eluting substances at appropriate concentrations for chromatographic methods.

The detection limit – the smallest amount (concentration) of the analyte in a sample that can be detected (or approximately estimated) using a validated method.

The detection limit in the cases indicated in the chart 2 is usually expressed as the concentration of the analyte (in % relative or parts per million – ppm).

Different methods of determining the detection limit are used depending on the method type (visual or instrumental).

1) For methods with a visual assessment of the analysis result. Samples are tested with various known analyte quantities (concentrations) and the minimum value is determined when the result of the analysis can be evaluated visually. This value is an estimation of the detection limit.

2) For methods with instrumental evaluation of the analysis result.

By the signal-to-noise ratio (SNR). This approach is applicable to methods for which baseline noise is observed. The values of the signals are compared: a) obtained for the control experiment, b) for samples with low analyte concentrations. Then the minimum amount (concentration) of the analyte in the sample is established when the ratio of the analytical signal to the noise level is 3. The found value is an estimation of the detection limit.

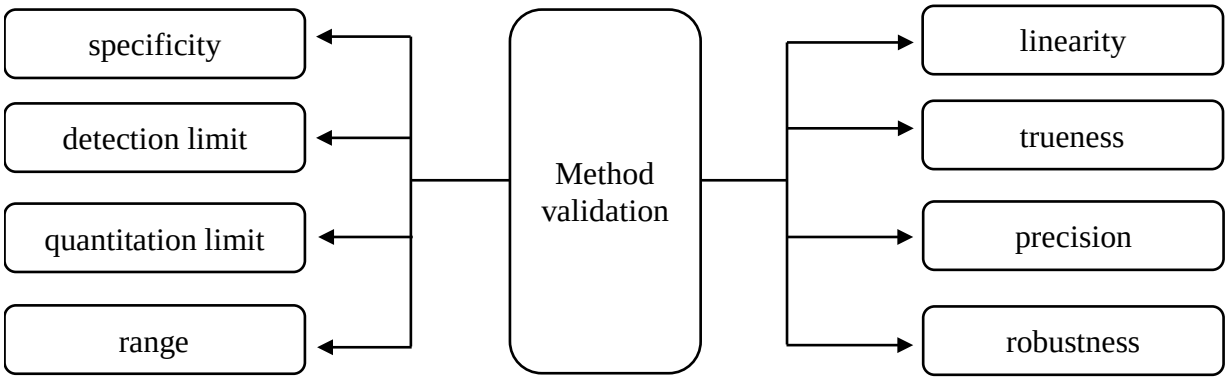

Chart 1. Parameters assessed during analytical method validation

Chart 2. Methods parameters determined during validation:

Parameter	**The main methods types**				
	Identity test	Impurities		Quantitation	
		Quantitative methods	Content limit	Main active ingredient, components	Active substance in the test «Dissolution»
Specificity	+	+	+	+	+
Detection limit quantitation limit;- analytical area (range)	–	–	+	–	–
Quantitation limit	–	+	–	–	–
Range	–	+	–	+	+
Linearity	–	+	–	+	+
Trueness	–	+	*	+	+
Precision: - repeatability - intermediate (interlaboratory) precision	– –	+ +	– –	+ +	+ –
Robustness	–	*	*	*	*

«+» - is required; «–» - not required; «*» - can be determined if necessary

By the value of the standard deviation of the signal and the angular coefficient of the calibration graph. The detection limit (DL) is found by the equation:

$$DL = 3.3 \cdot S/b,$$

where

S – the standard deviation of the analytical signal;

b – the sensitivity coefficient, the ratio of the analytical signal to the determined value (tangent of the angle of inclination of the calibration curve).

S and *b* can be estimated by the method of least squares in the presence of experimental data in a wide range of measured values.

For a linear calibration graph, the value of *S* is taken equal to the standard deviation S_a of the free term of the equation of this graph. The obtained value of the detection limit, if necessary, can be confirmed by a direct experiment with quantities (concentrations) of the analyte close to the found value of the detection limit.

As a rule, it is not necessary to determine the real DL for method if there are data on the suitability for reliable substance determination in concentrations that are both above and below its normative value (established by specification).

The quantitation limit – the smallest amount (concentration) of a substance in a sample that can be quantified using a validated method with the required accuracy and internal laboratory (intermediate) precision.

The quantitation limit is a necessary validation characteristic of the methods used to estimate small quantities (concentrations) of substances in a sample and, in particular, to estimate the content of impurities.

There are the following methods for finding the quantitation limit (depending on the method type):

1) For methods with a visual assessment of the analysis result. Samples are tested with various known quantities (concentrations) of the analyte and the minimum value is established at which the result of the analysis can be obtained visually with the required accuracy and intra-laboratory (intermediate) precision.

2) For methods with instrumental assessment of the analysis result

By the signal-to-noise ratio (SNR). The minimum concentration of the analyte in the sample is established, at which the ratio of the analytical signal to the noise level is about 10:1.

By the value of the standard deviation of the signal and the angular coefficient of the calibration graph. The quantitation limit (QL) is calculated by the equation:

$$QL = 10 \cdot S/b,$$

where

S – the standard deviation of the analytical signal;

b – the coefficient of sensitivity, which is the ratio of the analytical signal to the determined value.

S and *b* can be estimated by the method of least squares in the presence of experimental data in a wide range of measured values.

For a linear calibration graph, the value of *S* is taken equal to the standard deviation S_a of the free term of the equation of this graph. The obtained QL value, if necessary, can be confirmed by a direct experiment with quantities (concentrations) of the analyte close to the found QL value.

It is not necessary to determine the real QL for method if there are data on the suitability for reliable substance determination in concentrations that are both above and below its normative value (established by specification).

The range – the interval between the upper and lower values of the analytical characteristics of the component being determined in the object of analysis (its quantity, concentration, activity, etc.). In this interval, the results obtained using a validated method should have an acceptable level of accuracy and interlaboratory (intermediate) precision.

The following requirements are imposed for range:

– quantitative determination methods should be applicable in the range from 80 to 120% of the nominal value of the analytic characteristic;

– methods for assessing the uniformity of dosing should be applicable in the range from 70 to 130% of the nominal dose;

– quantitative determination methods used during the «Dissolution» test should usually be applicable in the range from 50 to 120% of the expected concentration of the active substance in the dissolution medium;

– test procedures for purity should be applicable in the range from the «Quantitation limit» or «Detection Limit» to 120% of the permissible impurity content.

The range can be established by diapason of experimental data satisfying a linear model.

The linearity – the linear dependence of the analytical signal on the concentration or amount of the analyte in the analyzed sample within the method range.

When validating a method, its linearity in the range is checked experimentally by measuring analytical signals for at least 5 samples with different analyte amounts or concentrations. The experimental data are processed by the method of least squares using a linear model:

$$y = b \cdot x + a,$$

where

x – the analyte amount or concentration;

y – the value of the response;

b – the slope;

a – the free term.

The values of *b*, *a* and the correlation coefficient *r* must be calculated and presented. In most cases, linear dependencies that meet the condition $|r| \geq 0.99$ are used, and only, when analyzing trace amounts, linear dependencies with $|r| \geq 0.9$ are considered.

In some cases, the possibility of linear approximation of experimental data is provided only after their mathematical transformation (for example, logarithmization).

A linear relationship between experimental data cannot be used as a basis in principle for some analysis methods. In this case, the concentration or amount of a substance is determined using non-linear calibration graphs. The graph of the analytical signal versus the amount or concentration of the analyte can be approximated by a suitable nonlinear function using the least squares method. This is possible if the appropriate validated software is available.

The trueness of the method is characterized by the deviation of the average result of the determinations from the true value.

Validated method is recognized as true if the values taken as true are within the confidence intervals of the corresponding average test results obtained experimentally with this method.

The following approaches are applicable to assessing the trueness of quantitative methods:

a) analysis of standard samples or model mixtures with a known content (concentration) of the analyte;

b) comparing the results obtained using the validated method and the model method with previously established trueness;

c) consideration of the linearity studying results of the validated method. If the free term in the linearity equation does not statistically reliably differ from zero, then the use of such a method gives results free from a systematic error.

For approaches «a» and «b» it is possible to present the obtained data as an equation of linear dependence (regression) between the experimentally found and true values. For this equation, we test 2 hypotheses: 1) the equality of the unit of the slope *b* tangent; 2) the equality to zero of the free term *a*. As a rule, if these hypotheses are recognized as valid (with a degree of reliability equal to 0.05) then the validated method gives the correct (free from systematic error) results.

The precision of the method is characterized by the scattering of the results obtained with its use, relative to the value of the average result. The measure of such scattering is a standard deviation value of an individual determination result; it is obtained for large volume sampling.

Precision is evaluated for any quantitative method based on the results of at least 3 definitions for each of the 3 levels of determined values (lower, middle and upper) within the method range. Repeatability can also be assessed for any quantitative method based on the results of at least 6 determinations for samples with a content of the analyte close to the nominal one. In many cases, a precision can be estimated by the results of experimental data processing using the least squares method.

Precision should be investigated for homogeneous samples and can be evaluated in three variants:

– as repeatability;

– as interlaboratory (intermediate) precision;

– as interlaboratory precision (reproducibility).

The results of the analysis method evaluation for each precision variant are usually characterized by the value of the standard deviation of individual determination result.

Usually, when developing an original method, the repeatability of the results obtained with its use is determined. Internal laboratory (intermediate) precision is additionally determined if it is necessary to include the developed method in the regulatory documentation. Interlaboratory precision (reproducibility) of the method is evaluated when it is supposed to be included in the GPM draft, pharmacopoeial monograph or in the normative documentation for pharmacopoeial standard samples.

Repeatability

The repeatability of the analytical method is assessed by independent results obtained in the same regulated conditions in the same laboratory (the same researcher, the same equipment, the same set of reagents) within a short period of time.

Intermediate precision. Intermediate precision of the validated method is evaluated in the working conditions of one laboratory (different days, different researchers, different equipment, etc.).

Interlaboratory precision (reproducibility). Interlaboratory precision (reproducibility) of the validated method is evaluated during testing in different laboratories.

The robustness of a validated method is the ability to maintain the characteristics in optimal (nominal) conditions with probable small deviations from these conditions of analysis.

The method robustness should not be determined in relation to the easily controlled analysis conditions. It reduces the need for a special study of robustness.

Robustness should be studied only in cases when the validated method is particularly sensitive to external conditions, such as various types of chromatography and functional analysis. If necessary, the assessment of the method robustness is carried out at the stage of its development. If the method is characterized with low robustness, the suitability test is carried out directly in practical use.

The verification of the analytical system suitability – the verification of the fulfillment of the basic requirements. The system is a set of specific instruments, reagents, standards and analyzed samples. Requirements for system are usually specified in the GPM for the corresponding analytical method. Thus, the verification of the analytical system suitability becomes a procedure that is included in the validated method.

Presentation of validation results

The protocol of analytical method validation should contain:

- full description that is sufficient to reproduce and reflect all the conditions necessary for the analysis;
- estimated characteristics;
- all primary results that are included in the statistical data processing;
- statistical processing results of data obtained experimentally in the development or verification of a validated method;
- illustrative materials such as copies of chromatograms obtained by high performance liquid chromatography or gas chromatography, electrophoregrams, electronic and infrared spectra, photographs or drawings of chromatograms obtained by thin layer or paper chromatography, titration curves, calibration graphs;
- conclusion for the suitability of a validated method for inclusion in a regulatory document.

Validation materials of individual analytical methods should be arranged in the form of a combined validation report.

CONCLUSION

The validation process is mandatory in the practice of quality production of herbal medicines and it is an important part of the quality assurance and pharmacopoeial control system. The introduction of validated methods helps to obtain reliable analysis results.

Using validated quality control methods will ensure that unsafe drugs are removed from circulation before they reach the pharmacy chains and can be purchased by patients.

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