MANUFACTURING & QUALITY AUDIT DIVISION
HEALTH PRODUCTS REGULATION GROUP

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GUIDANCE NOTES ON ANALYTICAL METHOD VALIDATION

GUIDANCE NOTES

Subject: ANALYTICAL METHOD VALIDATION

1. Introduction

Analytical methods include identification test, quantitative test for impurities, limit test for impurities, assay and dissolution test. This guide on analytical method validation presents the characteristics for consideration during the validation of an analytical procedure. Please also refer to the Guidance Notes on Analytical Method Validation: Methodology. (GUIDE-MQA-012B).

Note: All analytical equipment must have completed Installation Qualification (IQ) and Operational Qualification (OQ) before the commencement of Analytical Method Validation.

2. Purpose

The principal purpose of analytical method validation is to ensure that test methods, which are used for assessing compliance of pharmaceutical products with established specifications, will give accurate, reliable and reproducible results.

3. Scope

3.1 Exclusion

The laboratory of a manufacturer or a contract laboratory is expected to use compendial methods. Full validation of compendial methods is not required. However, the laboratory should verify that it could achieve the performance characteristics of the method and its suitability for the intended analytical applications.

3.2 Inclusion

In cases where the laboratory has to develop in-house methods, modify compendial methods or use them beyond their intended usage, the laboratory should carry out full validation of the methods. Full validation shall include the establishment of specifications and performance characteristic of the method and confirmation of its suitability for the intended analytical applications.

3.2.1 For identification test (ID), the following performance characteristics should be covered:

- Selectivity (Specificity)

3.2.2 For quantitative tests for impurities' content, the following performance characteristics should be covered:

- accuracy
- precision
- selectivity
- quantitation limit
- linearity and range

3.2.3 For limit tests for impurities' content, the following performance characteristics should be covered:

- selectivity
- detection limit

3.2.4 For assay (quantitation of active ingredients in finished products) and dissolution test, the following performance characteristics should be covered:

- accuracy
- precision
- robustness
- linearity and range
- selectivity

3.2.5 For dissolution test, the following performance characteristics should be covered:

- precision

3.2.6 For quantitative test involving microbiological analysis, the following performance characteristics should be covered:

- accuracy
- precision
- robustness
- linearity and range
- selectivity

- detection limit
- quantitation limit

3.2.7 For qualitative or limit test involving microbiological analysis (a test with two outcomes, either positive or negative), the following performance characteristics should be covered:
- robustness
- selectivity
- detection limit

3.3 System Suitability

System suitability tests must be performed on all liquid and gas chromatographic system. Sample solution stability should be determined. Other tests include capacity factor, precision/injection repeatability, relative retention, resolution, tailing factor and theoretical plate number.

3.4 Bacterial Endotoxins Test and Methods

The bacterial endotoxins test should be validated and carried out in accordance with the requirements of the compendial methods. The validation of gelation method should include initial qualification of the laboratory such as equipment qualification and technician qualification, test for confirmation of labelled sensitivity of the LAL reagent, inhibition and enhancement testing and determination of noninhibitory concentration and maximum valid dilution.

At present, the gelation test for bacterial endotoxins is the official method of analysis. Four types of quantitative assays may be recognised, namely turbidimetric end point method, kinetic turbidimetric method, chromogenic peptide end-point method and kinetic chromogenic peptide method. The method used should be shown to meet the requirement for linear regression, i.e, a significant slope and non-significant deviations from linear regression. For end point methods, an additional requirement of having an intercept not significantly different from zero should be fulfilled

Test for confirmation of labelled sensitivity of the LAL reagent must be repeated for every new lot of reagent used. Qualification of the

laboratory technician is required for new technician before carrying out the test.
Revalidation of bacterial endotoxins test is required when conditions that are likely to influence the test result change (e.g., change in manufacturer of the LAL reagent or the formula of the product).

Routine bacterial endotoxins test should include verification of labelled LAL reagent sensitivity and positive and negative controls in duplicates as per requirement of the compendial method.

4. Documentation

The following validation documents relating to analytical method validation would be necessary.

4.1 Validation Protocol

This document shall have the following elements:

(a) Purpose of validation
(b) A description of the main principle of the test procedure/method
(c) A description of the test procedures and the test conditions (including precautions, reagents, reference and preparations substances).
(d) Details of the equipment/facilities to be used (including measuring/recording equipment) together with its calibration status
(e) The variable(s) to be monitored
(f) The samples to be taken - where, when, how and how many
(g) The product performance characteristics/attributes to be mentioned, together with the best methods
(h) The acceptance limits
(i) Time schedules
(j) Personnel responsibilities
(k) Details of methods for recording and evaluating results, including statistical analysis.

4.2 Validation Report

This document shall have the following elements:

(a) Purpose of the validation
(b) A description of the test methods
(c) Test data from validation batches
(d) Evaluation, including comparison with the reference substances and preparations (in-house standards), acceptance criteria and recommendations
(e) Formal acceptance/rejection of the work by the team/persons designated as being responsible for the validation.

4.3 Validation Summary

This document shall be an abbreviated version of the validation report.

5. Definitions:

5.1 Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

The accuracy of a microbiological method is the closeness of the actual test results obtained by the test method to the results predicted from the dilution of the microbial suspension or the results obtained by the current compendial method.

This is sometimes termed trueness.

For biological analysis, a suspension of microorganisms with an appropriate count in cfu per ml or g is assigned a value of 100% and is serially diluted to five levels. Accuracy is expressed as the percentage recovery of microorganisms by the assay method.

5.2 Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous samples or suspensions of laboratory organisms, across the range of test, under

the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample it may be investigated using artificially prepared samples or a sample solution.

The precision of analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

A coefficient of variation (C.V.) of $\leq$ 5% is desirable for chemical test methods. For low level impurities, higher variations may be acceptable.

5.2.1 Repeatability

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

5.2.2 Intermediate precision

Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, etc.

5.2.3 Reproducibility

Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardization of methodology).

5.3 Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

5.4 Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte or microorganisms in the sample. Under most circumstances, regression coefficient (r) is $\geq$ 0.995 for chemical test methods.

5.5 Range

The range of an analytical procedure is the interval between the upper and lower concentrations (amounts) of analyte or microorganisms in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

5.6 Selectivity

Selectivity is the ability to assess unequivocally the analyte in the presence of components, which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.

The selectivity of a microbiological method is its ability to detect a range of microorganisms, which demonstrates that the method is fit for purpose.

Lack of selectivity of an individual analytical procedure may be compensated by other supporting analytical procedure(s).

This definition has the following implications:

Identification: to ensure the identity of an analyte.

Assay (content or potency): to provide an exact result, which allows an accurate statement on the content or potency of the analyte in a sample.

5.7 Detection Limit

The detection limit of an individual analytical procedure is the lowest amount of analyte or microorganisms in a sample, which can be detected but not necessarily quantitated as an exact value.

Due to the nature of microbiology, the detection limit refers to the number of organisms in the original sample, before any incubation step, not the number of organisms present at the point of assay. Also, the amount of sample tested and the dilution of that sample may determine the detection limit. For example, when 10 g of test material is diluted in 90 ml of diluent and 1 ml is plated, the absence of colonies on the plate would be reported as <10 cfu per g.

5.8 Quantitation Limit

The quantitation limit of an individual analytical procedure is the lowest amount of analyte or microorganisms in a sample which can be quantitatively determined with suitable precision and accuracy under the stated experimental conditions.

5.9 Sample Solution Stability

This study determines the time period after sample preparation during which the compound of interest remains stable in the HPLC apparatus under the described analytical conditions. Data to support the sample solution stability under normal laboratory conditions for the duration of the test procedures, e.g., 24 hours should be generated.

5.10 Capacity Factor (k')

The capacity factor is a measure of where the peak of interest is located with respect to the void volume, i.e., elution time of the non-retained components. The peak should be well resolved from other peaks and the void volume. Generally, the value of k' is >2.

5.11 Precision/Injection repeatability (RSD)

Injection precision expressed as RSD (relative standard deviation) or coefficient of variation (C.V.) indicates the performance of the HPL chromatograph which includes the plumbing, column, and environmental conditions, at the time the samples are analysed. It should be noted that sample preparation and manufacturing variations are not considered. A C.V. of $\leq 1\%$ for $n \geq 5$ is desirable.

5.12 Relative retention (α)

Relative retention is a measure of the relative location of two peaks. This is not an essential parameter as long as the resolution (R_S) is stated.

5.13 Resolution (R_S)

R_S is a measure of how well two peaks are separated. For reliable quantitation, well separated peaks are essential for quantitation. This is a very useful parameter if potential interference peak(s) may be of concern. The closest potential eluting peak to the analyte should be selected.

R_S of >2 between the peak of interest and the closest potential interfering peak (impurity, excipient, degradation product, internal standard, etc.) is desirable.

5.14 Tailing factor (T)

The tailing factor, a measure of peak symmetry, is unity for perfectly symmetrical peaks and its value increases as tailing becomes more pronounced. As peaks asymmetry increases, integration and hence precision becomes less reliable. T of ≤ 2 is desirable.

5.15 Theoretical plate number (N)

Theoretical plate number is a measure of column efficiency, i.e., how many peaks can be located per unit run-time of the chromatogram.

N is fairly constant for each peak on a chromatogram with a fixed set of operating conditions. H or HETP, the height equivalent of a theoretical plate, measures the column efficiency per unit length (L) of the column.

Parameters which can affect N or H include peak position, particle size in column, flow rate of mobile phase, column temperature, viscosity of mobile phase and molecular weight of the analyte.

The theoretical plate number depends on elution time but in general should be >2000.

REFERENCES

1. ICH Harmonised Tripartite Guideline. Q2A: Text on Validation of Analytical Procedures

2. USP 24

3. PDA Technical Report 33. Evaluation, Validation and Implementation of New Microbiological Testing Methods

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