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Stability Indicating Analytical Method Development and Validation

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Abstract: This write-up provides a review on the stability indicating analytical method development and validation. This review provides a basic detail about analytical method development, stability indicating analytical methods and validation as per regulatory guidelines and current scenario. What kind of content required for analytical procedure, steps involve in development of stability indicating analytical procedure also described. Details of Validation and its parameter like Specificity, Linearity, Range, Accuracy, Precision, Detection limit, Quantitation limit, Robustness, System suitability testing given.

Keywords: Analytical method development, Stability indicating analytical methods, Validation, Regulatory guidelines.

Introduction:

Analytical method development

An analytical procedure is developed to test a defined characteristic of the drug substance or drug product against established acceptance criteria for that characteristic (like identification test, quantitative tests, qualitative tests and limit tests etc.).

In the development of a new analytical procedure, the choice of analytical instrumentation and methodology should be chosen based on the intended requirement and scope of the analytical method.

Content of analytical procedures

Analytical procedures should be described in sufficient detail to permit a competent analyst to redeveloped the require conditions and obtain results within the proposed acceptance criteria as well as described aspects of the analytical procedures that require special attention.

An analytical method procedure may be referenced from FDA-recognized sources (e.g., USP/NF, Association of Analytical Communities (AOAC) International). The following is a list of essential information that researcher should be include for an analytical procedure:

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A. Principle/Scope

A description of the basic principles and theories of the analytical test/technology (i.e., separation, detection); target analyte(s) and sample(s) type (e.g., drug substance, drug product, impurities or compounds in biological fluids).

B. Apparatus/Equipment

All required qualified instruments, equipment and components (e.g., instrument type, detector, column type, dimensions, and alternative column, filter type).

C. Operating parameters

Qualified optimal settings and ranges (include allowed adjustments supported by compendial sources or development and/or validation studies) critical to the analysis (e.g., flow rate, column type, components temperatures, run time, detector settings, gradient, head space sampler). A drawing with experimental outline and integration parameters may be used, as applicable.

D. Reagents/Standards

The following should be listed where applicable:

• Description of chemical and reagent or standard

• Grade of chemical (e.g., USP/NF, American Chemical Society, High Performance or Pressure Liquid Chromatography, or Gas Chromatography and preservative-free)

• Source and make (e.g., USP reference standard, qualified in-house reference material (working standards), WHO International Standard/Reference Material, CBER standard)

• Purity (for pure chemicals only like active pharmaceutical ingredients, excipients), State (e.g., dried, undried), and concentration

• Potencies (where required by CFR, USP)

• Storage conditions

• Directions for safe use (as per current Safety Data Sheet)

• Validated or documented shelf life

E. Sample preparation

Procedures (e.g., extraction method, digestion method, dilution or concentration, desalting procedures and mixing by sonication, shaking or sonication time) for the preparations for individual sample tests. A single preparation for qualitative and replicate preparations for quantitative tests with appropriate units of

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concentrations for working solutions (e.g., µg/ml or mg/ml or gm/ml) and information on stability of solutions and storage conditions.

F. Standards control solution preparation

Procedures for the preparation and use of all standard and control solutions with appropriate units of concentration like µg/ml, mg/ml, gm/ml etc and information on stability of standards and storage conditions, including calibration standard chemicals, internal standard chemicals, system suitability standard chemicals etc.

G. Procedure

A stepwise description of the method (e.g., equilibration times, and scan/injection sequence with blanks, placeboes, samples, controls, sensitivity solution (for impurity method) and standards to maintain validity of the system suitability during the duration of analysis) and allowable operating ranges, limits and adjustments if applicable.

H. System suitability

Confirmatory test(s) procedures and parameters to ensure that the system (instrument, equipment, electronics, and analytical operations and controls to be analyzed) will function correctly as an integrated system at the time of use. The system suitability acceptance criteria applied to standards controls and samples, such as peak tailing, percent RSD, precision and resolution acceptance criteria, may be required as applicable.

I. Calculations

The integration method and representative calculation formulas for data analysis (standards, controls, samples) for tests based on label claim and specification (e.g., assay, specified and unspecified impurities, total impurities and relative response factors). This includes a explanation of any mathematical transformations or formulas used in data analysis, along with a scientific justification for any correction factors used.

J. Data reporting

A presentation of numeric data that is consistent with instrumental capabilities, instrumental method and acceptance criteria. The method should indicate what format to use to report results (e.g., percentage label claim, weight/weight, and weight/volume) with the specific number of significant figures required. For chromatographic methods, retention times (RTs) for identification with reference standard comparison basis, relative retention times (RRTs) (known and unknown impurities) acceptable ranges and sample results reporting criteria should include.[1]

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Stability indicating analytical method

Validated analytical test procedures that can detect the changes with time in the chemical, physical or microbiological properties of the active pharmaceutical ingredient (API) or finished pharmaceutical product, and that are specific so that the content of the API, degradation products and other components of interest can be accurately measured without interference.[2]

The main objective of a stability indicating method is to monitor all observations and results during stability studies in order to guarantee safety, efficacy and quality. It represents also a powerful tool when investigating lab incidence, out-of-trend (OOT) or out-of-specification (OOS) results in quality control processes.

There are basically 3 steps necessary for developing a SIM:

Step 1: Generation of degraded samples for testing selectivity of the method

Stress tests should produce representative samples to assess drug substance and drug product stability, deliver information about possible degradation pathway and demonstrate the stability indicating power of the analytical procedures applied.

Determination of Limit of Quantification (LoQ)

In close relation to the determination of the amount of degradation is the evaluation of Limit of Detection (LoD) and Limit of Quantification (LoQ) of the method. These limits should be closely related to the Reporting, Identification and Qualification of degradation products, as stated in ICH Q3B (R2). These thresholds are determined either as percentage of drug substance or total daily intake (TDI) of degradation product.

Overstressing/Understressing

Precaution should be taken in order to avoid overstressing or understressing samples, with may lead to non-representative or non-purposeful degradation. So, the use of a properly designed and executed forced degradation study will produce representative samples that will help to ensure that resulting method reflects adequately long-term stability.

About the forced degradation (or stress test, both terms will be used in the text) design, it is recommended to include alkaline and acidic hydrolysis, photolysis, oxidation, humidity and temperature stress. Table 1 compiles the more often used conditions to perform forced degradation studies. These conditions can be used as a starting point in the development of a SIM. Changing conditions to harsher or softer levels, can be applied, when too little or too much degradation are obtained.

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Solid State			
Stress		**Condition**	**Period of time**
Heat		60°C	Up to 1 month
Humidity		75 % RH	Up to 1 month
Photostability		33 mm (Powder) Exposed and non-exposed sample ("Control")	Follow ICH requirements (Q1B)
Solution State			
Stress		**Condition**	**Period of time**
Hydrolysis	Acid	0.1 to 1 M HCl	Up to weeks and 60 °C
	Alkaline	0.1 to 1 M NaOH	Up to weeks and 60 °C
Oxidation		H2O2 3% (v/v)	Up to 24 hours
Photostability		Exposed and non-exposed sample	Follow ICH requirements (Q1B)
Heat		60 °C	Up to 1 month

Table 1. "More often" used conditions for forced degradation studies

Photostability studies

Photostability tests should follow ICH requirements (EMEA, 1998), i.e., should be complete in a sequential way, initiating with the fully exposed product and proceeding, if necessary to the immediate pack and then to the market pack, until studies prove the drug product is adequately protected from exposure to light.

Apart from this, design of forced degradation test should consider the previous knowledge of the substance or product being tested, since photoreactivity is wavelength dependent and degradation pathways can be different for UV and visible ranges (case by case basis).

Step 2: Method development (manipulating and evaluating selectivity/specificity)

Liquid chromatography is the most appropriate technique for developing/validating a stability indicating method. The use of diode-array-detector and additionally mass spectrometers, gives best performances for people working with stability indicating method development.

The aim is to manipulate selectivity by changing mobile phase composition, wavelength of detection and pH. Related to mobile phase pH, it can be said, that the advances in LC column technology have made possible the use of pH as a true selectivity tool for the separation of ionizable compounds. Columns mechanically strong, with high efficiency and that are operate over an extended pH range, should be preferred. Acidic compounds are more retained at low pH; while basic compounds are more retained at higher pH (neutral compounds are unaffected). At traditionally used pH values (pH 4 - 8), a slight change in pH would result in a significant shift

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in retention. Type of chromatography used (e. g. HPLC or GC) and arrangements/detectors (GC/FID, GC/MS, LC/DAD or LC/MS) are certainly useful tools.

For HPLC, different modes of chromatography can be used (normal or reversed phase, ion par or HILC). Other powerful tool is the use of Light Scattering Detector (LSD) coupled to HPLC to monitor compounds without light absorption in uv/Vis region.

Gas chromatography may only be used when no additional thermal degradation of the test sample is produced (sample inlet works on high temperatures). The use of HPLC coupled to diode-array detectors (DAD) in the attainment of peak purity usually give reasonable results, mainly related to reliable determination of the main active ingredient. It is possible to guarantee no co-elution with degradation peaks and other impurities. Indeed, the main feature of DAD detectors is that it is possible to collect spectra across a range of wavelengths at each data point collected across a peak, and through software manipulations involving multidimensional vector algebra, to compare each of the spectra to determine peak purity. In this way, DAD detectors can differentiate spectral and chromatographic differences not readily observable by simple overlay comparisons. DAD detectors can be limited on occasion the more similar the spectra, and the lower the relative absorbance, the more difficult it can be to differentiate co-eluted compounds. MS detection overcomes many of these limitations.

MS can deliver unequivocal peak purity information, exact mass, structural and quantitative information depending upon the type of instrument, equipment used. MS is also a very useful tool to track peaks to selectivity manipulations in method development. As disadvantage, MS detectors cannot handle non-volatile buffers, which are frequently used as mobile phase in drug analysis. The combination of both DAD and MS on a single instrument and software platform provides the type of valuable orthogonal information required when evaluating specificity on stability indicating method development. After determination of peak purity, in fact, the identification of degradation products and also mass balance determination usually are more complexes steps of analytical development, as in most of cases, commercial reference standards of degradation products are not available. Calculations using area-percent-normalization (area %) are not precise, since it is necessary to take into consideration the response factors (area relative to amount). Degradation products may have not the same ultra-violet spectra of that of the parent drug and even if the UV spectra are similar, the absorptivity coefficient may have different values.

Step 3: Method validation

Validation is not a efficient way to do method development so efforts should be dedicated in Specificity step of the method, working with the stressed samples. The validation routine may start with a protocol based on pharmacopeia and/or ICH guidelines (Q2B). For assay procedures, that are intended do measure the analyte present in a given sample, typical validation items should be considered: Accuracy, Precision (repeatability and intermediate precision), Specificity, Detection and Quantitation Limits, Robustness, Linearity and Range. For

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related substances procedure, that are intended do measure known impurities and unknown impurities of given drug substance and drug product, typical validation items should be considered: Specificity, Detection and Quantitation Limits, Accuracy, Precision (repeatability and intermediate precision), Robustness, Linearity and Range. [3]

Validation of analytical procedure

A validation study is designed to provide sufficient evidence that the analytical procedure meets its predetermine objectives. These objectives are described with a suitable set of performance characteristics and related performance criteria, which can vary depending on the intended use of the analytical procedure and the specific technology selected (like sophisticated instruments, software's etc).

The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose.

Types of analytical procedures to be validated

The validation of analytical procedures is directed to the four most common types of analytical procedures:

A. Identification tests;

B. Quantitative tests for impurities' content;

C. Limit tests for the control of impurities;

D. Quantitative tests of the active moiety in samples of drug substance or drug product or other selected component(s) in the drug product.

- Identification tests are intended to ensure the identity of an analyte in a sample (drug substance or drug product). This is normally attained by comparison of a property of the sample (e.g., spectrum, chromatographic behavior, chemical reactivity, etc) to that of a reference standard;

- Testing for impurities can be either a quantitative test or a limit test for the impurity in a sample. Either test is intended to accurately reflect the purity characteristics of the sample. Different validation characteristics are required for a quantitative test than for a limit test;

- Assay procedures are used to measure the analyte present in a given sample. The assay represents a quantitative measurement of the major component(s) in the drug substance. For the drug product, similar validation characteristics also apply when assaying for the active or other selected component(s). The same validation characteristics may also apply to assays associated with other analytical procedures (e.g., dissolution).

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The objective of the analytical procedure should be clearly understood since this will govern the validation characteristics which need to be evaluated. Typical validation characteristics which should be considered are listed below:

Accuracy

Precision

Repeatability

Intermediate Precision

Specificity

Detection Limit

Quantitation Limit

Linearity

Range

The table lists those validation characteristics regarded as the most important for the validation of different types of analytical procedures. This list should be considered typical for the analytical procedures cited but occasional exceptions should be dealt with on a case-by-case basis. It should be noted that robustness is not listed in the table but should be considered at an appropriate stage in the development of the analytical procedure.

Furthermore, revalidation may be necessary in the following circumstances:

- changes in the synthesis of the drug substance;
- changes in the composition of the finished product;
- changes in the analytical procedure.

The degree of revalidation required depends on the nature of the changes. Certain other changes may require validation as well.

Type of analytical Procedure **Characteristics**	**Identification**	**Testing for impurities**		**Assay** **-Dissolution (Measurement only)** **-Content / Potency**
		Quantitative tests	**Limit tests**	
Accuracy	-	+	-	+
Precision				
Repeatability	-	+	-	+

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Intermediate Precision	-	+ (1)	-	+ (1)
Specificity (2)	+	+	+	+
Detection Limit	-	- (3)	+	-
Quantitation limit	-	+	-	-
Linearity	-	+	-	+
Range	-	+	-	+

- signifies that this characteristic is not normally evaluated

+ signifies that this characteristic is normally evaluated

(1) in cases where reproducibility has been performed, intermediate precision is not needed

(2) lack of specificity of one analytical procedure could be compensated by other supporting analytical procedure(s)

(3) may be needed in some cases

Validation of analytical procedures: methodology

Analytical procedure

The analytical procedure refers to the way of performing the analysis. It should describe in detail the steps necessary to perform each analytical test. This may include but is not limited to: the sample, the reference standard and the reagents preparations, use of the apparatus, generation of the calibration curve, use of the formulae for the calculation, etc.

1. Specificity

Specificity 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. Lack of specificity 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.

Purity Tests: to ensure that all the analytical procedures performed allow an accurate statement of the content of impurities of an analyte, i.e. related substances test, heavy metals, residual solvents content, etc.

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.

An investigation of specificity should be conducted during the validation of identification tests, the determination of impurities and the assay. The procedures used to demonstrate specificity will depend on the

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intended objective of the analytical procedure. It is not always possible to demonstrate that an analytical procedure is specific for a particular analyte (complete discrimination). In this case a combination of two or more analytical procedures is recommended to achieve the necessary level of discrimination.

1.1. Identification

Suitable identification tests should be able to discriminate between compounds of closely related structures which are likely to be present. The discrimination of a procedure may be confirmed by obtaining positive results (perhaps by comparison with a known reference material) from samples containing the analyte, coupled with negative results from samples which do not contain the analyte. In addition, the identification test may be applied to materials structurally similar to or closely related to the analyte to confirm that a positive response is not obtained. The choice of such potentially interfering materials should be based on sound scientific judgement with a consideration of the interferences that could occur.

1.2. Assay and Impurity Test(s)

For chromatographic procedures, representative chromatograms should be used to demonstrate specificity and individual components should be appropriately labelled. Similar considerations should be given to other separation techniques. Critical separations in chromatography should be investigated at an appropriate level. For critical separations, specificity can be demonstrated by the resolution of the two components which elute closest to each other. In cases where a non-specific assay is used, other supporting analytical procedures should be used to demonstrate overall specificity. For example, where a titration is adopted to assay the drug substance for release, the combination of the assay and a suitable test for impurities can be used. The approach is similar for both assay and impurity tests:

1.2.1 Impurities are available

For the assay, this should involve demonstration of the discrimination of the analyte in the presence of impurities and/or excipients; practically, this can be done by spiking pure substances (drug substance or drug product) with appropriate levels of impurities and/or excipients and demonstrating that the assay result is unaffected by the presence of these materials (by comparison with the assay result obtained on unspiked samples). For the impurity test, the discrimination may be established by spiking drug substance or drug product with appropriate levels of impurities and demonstrating the separation of these impurities individually and/or from other components in the sample matrix.

1.2.2 Impurities are not available

If impurity or degradation product standards are unavailable, specificity may be demonstrated by comparing the test results of samples containing impurities or degradation products to a second well-characterized procedure e.g.: pharmacopoeial method or other validated analytical procedure (independent procedure). As appropriate,

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this should include samples stored under relevant stress conditions: light, heat, humidity, acid/base hydrolysis and oxidation.

- for the assay, the two results should be compared;

- for the impurity tests, the impurity profiles should be compared. Peak purity tests may be useful to show that the analyte chromatographic peak is not attributable to more than one component (e.g., diode array, mass spectrometry).

2. 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 in the sample.

A linear relationship should be evaluated across the range of the analytical procedure. It may be demonstrated directly on the drug substance (by dilution of a standard stock solution) and/or separate weighings of synthetic mixtures of the drug product components, using the proposed procedure. The latter aspect can be studied during investigation of the range. Linearity should be evaluated by visual inspection of a plot of signals as a function of analyte concentration or content. If there is a linear relationship, test results should be evaluated by appropriate statistical methods, for example, by calculation of a regression line by the method of least squares. In some cases, to obtain linearity between assays and sample concentrations, the test data may need to be subjected to a mathematical transformation prior to the regression analysis. Data from the regression line itself may be helpful to provide mathematical estimates of the degree of linearity. The correlation coefficient, y-intercept, slope of the regression line and residual sum of squares should be submitted. A plot of the data should be included. In addition, an analysis of the deviation of the actual data points from the regression line may also be helpful for evaluating linearity. Some analytical procedures, such as immunoassays, do not demonstrate linearity after any transformation. In this case, the analytical response should be described by an appropriate function of the concentration (amount) of an analyte in a sample. For the establishment of linearity, a minimum of 5 concentrations is recommended. Other approaches should be justified.

3. Range

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte 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.

The specified range is normally derived from linearity studies and depends on the intended application of the procedure. It is established by confirming that the analytical procedure provides an acceptable degree of linearity, accuracy and precision when applied to samples containing amounts of analyte within or at the extremes of the specified range of the analytical procedure.

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The following minimum specified ranges should be considered:

- for the assay of a drug substance or a finished (drug) product: normally from 80 to 120 percent of the test concentration;

- for content uniformity, covering a minimum of 70 to 130 percent of the test concentration, unless a wider more appropriate range, based on the nature of the dosage form (e.g., metered dose inhalers), is justified;

- for dissolution testing: +/-20 % over the specified range;

e.g., if the specifications for a controlled released product cover a region from 20%, after 1 hour, up to 90%, after 24 hours, the validated range would be 0-110% of the label claim.

- for the determination of an impurity: from the reporting level of an impurity1 to 120% of the specification;

- for impurities known to be unusually potent or to produce toxic or unexpected pharmacological effects, the detection/quantitation limit should be commensurate with the level at which the impurities must be controlled;

Note: for validation of impurity test procedures carried out during development, it may be necessary to consider the range around a suggested (probable) limit.

- if assay and purity are performed together as one test and only a 100% standard is used, linearity should cover the range from the reporting level of the impurities1 to 120% of the assay specification.

4. 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. This is sometimes termed trueness.

Accuracy should be established across the specified range of the analytical procedure.

4.1. Assay

4.1.1 Drug substance

Several methods of determining accuracy are available:

a) application of an analytical procedure to an analyte of known purity (e.g. reference material);

b) comparison of the results of the proposed analytical procedure with those of a second well-characterized procedure, the accuracy of which is stated and/or defined;

c) accuracy may be inferred once precision, linearity and specificity have been established.

4.1.2 Drug product

Several methods for determining accuracy are available:

a) application of the analytical procedure to synthetic mixtures of the drug product components to which known quantities of the drug substance to be analysed have been added;

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b) in cases where it is impossible to obtain samples of all drug product components , it may be acceptable either to add known quantities of the analyte to the drug product or to compare the results obtained from a second, well characterized procedure, the accuracy of which is stated and/or defined;

c) accuracy may be inferred once precision, linearity and specificity have been established.

4.2. Impurities (Quantitation)

Accuracy should be assessed on samples (drug substance/drug product) spiked with known amounts of impurities.

In cases where it is impossible to obtain samples of certain impurities and/or degradation products, it is considered acceptable to compare results obtained by an independent procedure. The response factor of the drug substance can be used. It should be clear how the individual or total impurities are to be determined e.g., weight/weight or area percent, in all cases with respect to the major analyte.

4.3. Recommended data

Accuracy should be assessed using a minimum of 9 determinations over a minimum of 3 concentration levels covering the specified range (e.g., 3 concentrations/3 replicates each of the total analytical procedure). Accuracy should be reported as percent recovery by the assay of known added amount of analyte in the sample or as the difference between the mean and the accepted true value together with the confidence intervals.

5. 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 sample 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 an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements

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

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

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

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Validation of tests for assay and for quantitative determination of impurities includes an investigation of precision.

5.1 Repeatability

Repeatability should be assessed using:

a) a minimum of 9 determinations covering the specified range for the procedure (e.g., 3 concentrations/3 replicates each); or

b) a minimum of 6 determinations at 100% of the test concentration.

5.2. Intermediate precision

The extent to which intermediate precision should be established depends on the circumstances under which the procedure is intended to be used. The applicant should establish the effects of random events on the precision of the analytical procedure. Typical variations to be studied include days, analysts, equipment, etc. It is not considered necessary to study these effects individually. The use of an experimental design (matrix) is encouraged.

5.3. Reproducibility

Reproducibility is assessed by means of an inter-laboratory trial. Reproducibility should be considered in case of the standardization of an analytical procedure, for instance, for inclusion of procedures in pharmacopoeias. These data are not part of the marketing authorization dossier.

5.4. Recommended data

The standard deviation, relative standard deviation (coefficient of variation) and confidence interval should be reported for each type of precision investigated.

6. Detection limit

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

Several approaches for determining the detection limit are possible, depending on whether the procedure is a non-instrumental or instrumental. Approaches other than those listed below may be acceptable.

6.1. Based on visual evaluation

Visual evaluation may be used for non-instrumental methods but may also be used with instrumental methods. The detection limit is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.

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6.2. Based on signal-to-noise

This approach can only be applied to analytical procedures which exhibit baseline noise. Determination of the signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be reliably detected. A signal-to-noise ratio between 3 or 2:1 is generally considered acceptable for estimating the detection limit.

6.3 Based on the standard deviation of the response and the slope

The detection limit (DL) may be expressed as:

$DL = 3.3 \sigma / S$

where

σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte. The estimate of σ may be carried out in a variety of ways, for example:

6.3.1 Based on the standard deviation of the blank

Measurement of the magnitude of analytical background response is performed by analyzing an appropriate number of blank samples and calculating the standard deviation of these responses.

6.3.2 Based on the calibration curve

A specific calibration curve should be studied using samples containing an analyte in the range of DL. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines may be used as the standard deviation.

6.4 Recommended data

The detection limit and the method used for determining the detection limit should be presented. If DL is determined based on visual evaluation or based on signal to noise ratio, the presentation of the relevant chromatograms is considered acceptable for justification.

In cases where an estimated value for the detection limit is obtained by calculation or extrapolation, this estimate may subsequently be validated by the independent analysis of a suitable number of samples known to be near or prepared at the detection limit.

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7. Quantitation limit

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.

Several approaches for determining the quantitation limit are possible, depending on whether the procedure is a non-instrumental or instrumental. Approaches other than those listed below may be acceptable.

7.1. Based on visual evaluation

Visual evaluation may be used for non-instrumental methods but may also be used with instrumental methods. The quantitation limit is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision.

7.2. Based on signal-to-noise approach

This approach can only be applied to analytical procedures that exhibit baseline noise. Determination of the signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and by establishing the minimum concentration at which the analyte can be reliably quantified. A typical signal-to-noise ratio is 10:1.

7.3. Based on the standard deviation of the response and the slope

The quantitation limit (QL) may be expressed as:

$QL = 10\ \sigma / S$

where σ = the standard deviation of the response S = the slope of the calibration curve The slope S may be estimated from the calibration curve of the analyte. The estimate of σ may be carried out in a variety of ways for example:

7.3.1 Based on standard deviation of the blank

Measurement of the magnitude of analytical background response is performed by analyzing an appropriate number of blank samples and calculating the standard deviation of these responses.

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7.3.2 Based on the calibration curve

A specific calibration curve should be studied using samples, containing an analyte in the range of QL. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines may be used as the standard deviation.

7.4 Recommended data

The quantitation limit and the method used for determining the quantitation limit should be presented. The limit should be subsequently validated by the analysis of a suitable number of samples known to be near or prepared at the quantitation limit.

8. 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.

The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters.

If measurements are susceptible to variations in analytical conditions, the analytical conditions should be suitably controlled or a precautionary statement should be included in the procedure. One consequence of the evaluation of robustness should be that a series of system suitability parameters (e.g., resolution test) is established to ensure that the validity of the analytical procedure is maintained whenever used.

Examples of typical variations are:

- stability of analytical solutions;
- extraction time.

In the case of liquid chromatography, examples of typical variations are:

- influence of variations of pH in a mobile phase;
- influence of variations in mobile phase composition;
- different columns (different lots and/or suppliers);
- temperature;
- flow rate.

In the case of gas-chromatography, examples of typical variations are:

- different columns (different lots and/or suppliers);
- temperature;
- flow rate.

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9. System suitability testing

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated.[4]

Conclusion

In the field of pharmaceutical sciences, analytical method development plays a huge role. Every analytical method used for testing of pharmaceutical product or drug should be stability indicating and validated too. This literature is provided basic details about analytical method development, stability indicating analytical method and validation.

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