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**Review Aritcle**

# Method Development and validation- An Overview

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Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics. Validation of an analytical method is the process by which it is established by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical application Validation is required for any new or amended method to ensure that it is capable of giving reproducible and reliable results, when used by different operators employing the same equipment in the same or different laboratories. The type of validation programme required depends entirely on the particular method and its proposed applications.

**Key Words**: Method Development, Validation, Analytical Validation, reproducible, reliable results.

## INTRODUCTION

Method development involves a series of sample steps; based on what is known about the sample, a column and detector are chosen; the sample is dissolved, extracted, purified and filtered as required; an eluent survey (isocratic or gradient) is run; the type of final separation (isocratic or gradient) is determined from the survey; preliminary conditions are determined for the final separation; retention efficiency and selectivity are optimized as required for the purpose of the separation (quantitative, qualitative or preparation); the method is validated using ICH guidelines. The validated method and data can then be documented.[1]

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Validation is the confirmation by examination and the provision of objective evidence that the particular requirements for a specific intended use are fulfilled. A process of evaluating method performance and demonstrating that it meets a particular requirement. In essence, it is knowing what your method is capable of delivering, particularly at low concentrations.[2]

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice.

**Analytical methods need to be validated or revalidated**

– before their introduction into routine use;

– whenever the conditions change for which the method has been validated (e.g., an instrument with different characteristics or samples with a different matrix)

– whenever the method is changed and the change is outside the original scope of the method.[3]

Method validation has received considerable attention in the literature and from industrial committees and regulatory agencies.

**1. The U.S. FDA CGMP** request in section 211.165 (e) methods to be validated: the accuracy, sensitivity, specificity, and reproducibility of test methods employed by the firm shall be established and documented. Such validation and documentation may be accomplished in accordance with Sec. 211.194(a). These requirements include a statement of each method used in testing the sample to meet proper standards of accuracy and reliability, as applied to the tested product. The U.S. FDA has also proposed an industry guidance for Analytical Procedures and Methods Validation.

**2. ISO/IEC 17025** includes a chapter on the validation of methods with a list of nine validation parameters. The ICH has developed a consensus text on the validation of analytical procedures. The document includes definitions for eight validation characteristics. ICH also developed a guidance with detailed methodology.

**3. The U.S. EPA** prepared a guidance for method's development and validation for the Resource Conservation and Recovery Act (RCRA). The AOAC, the EPA and other scientific organizations provide methods that are validated through multi-laboratory studies.[4]

**Type of analytical procedures to be validated.**

Validation of analytical procedures is directed to the four most common types of analytical procedures.

- Identification test.
- Quantitative test for impurities content.
- Limit test for the control of impurities.
- Quantitative test of the active moiety in samples of drug substance on drug product on other selected components in the drug product.[5]

**Objective of validation**

The objective of validation of analytical procedure is to demonstrate that it is suitable for its intended purpose. Validation is documented evidence, which

provide a high degree of assurance for specific method. Any developed method may be influenced by variables like different elapsed assay times, different days, reagents lots, instruments, equipments, environmental conditions like temperature, etc so it is expected that after the method has been developed and before it is communicated or transferred from one lab to the other, it is properly validated and the result of validity tests reported.

The primary objective of validation is to form a basis for written procedure for production and process control which are designed to assure that the drug products have the identity, strength, quality and purity they purport or are represented to possess quality, safety and efficacy must be designed to build into the product. Each step of the manufacturing process must be controlled to maximize the probability that the finished products meet all quality and design specification.[6]

**Benefits of Validation:**

a) Produces quality products
b) Helps in process improvement technology transfer, related product validation, failure investigation, and increased employee awareness.
c) Cost reduction by increasing efficacy, few reject, longer equipment life, production of cost effective products
d) Helps in optimization of process or method.
e) Regulatory affairs-produces approved products and increased ability to export.[7]

**Performance characteristics examined when carrying out method validation are.**

1. Accuracy / Precision
2. Repeatability / Reproducibility
3. Linearity / Range
4. Limit of detection (LOD)/ Limit of quantification (LOQ)
5. Selectivity / Specificity
6. Robustness / Ruggedness

**Significance of Method Validation:**

The quality of analytical data is a key factor in the success of a drug development programme. The process of method development and validation has a direct impact on the quality of these data.

- To trust the method.
- Regulatory requirement.

Analytical validation is a very important feature of any package of information submitted to international regulatory agencies in support of new product marketing or clinical trials applications. A thorough method development can almost rule out all potential problems, at the same time, a thorough validation programme can address the most common ones and

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**Table 1: - Characteristics That Should Be Considered For Different Types Of Analytical Procedure: (As per WHO guidelines)**

| Sr. No | Parameter | Class A | Class B | | Class C | Class D |
|---|---|---|---|---|---|---|
| | | | Quantitative tests | Limit Tests | | |
| 1. | Accuracy | -- | Yes | -- | Yes | Yes |
| 2. | Precision | -- | Yes | -- | Yes | Yes |
| 3. | Robustness | Yes | Yes | Yes | Yes | Yes |
| 4. | Linearity and Range | -- | Yes | -- | Yes | Yes |
| 5. | Selectivity | Yes | Yes | Yes | Yes | Yes |
| 6. | Limit of Detection | Yes | -- | Yes | -- | -- |
| 7. | Limit of Quantification | -- | Yes | -- | -- | |

provide assurance to the intended purpose (can be used with 100% confidence). In other words, a thorough validation can fulfill all the technical and regulatory objectives. A direct consequence and most significant out come from any method validation exercise is 'the development of meaningful specifications can be predicted upon the use of validated analytical procedures that can assess changes in a drug substance or drug product during its life time.

Analytical characteristics listed below may not be applicable to every test procedure or every particular material. It will mostly depend on the purpose for which the procedure is required, however, these following aspects of validation should be given due importance.[8]

The different parameters which are to be considered in analytical method validation of an as per USP and ICH guidelines can be summarized as follows.

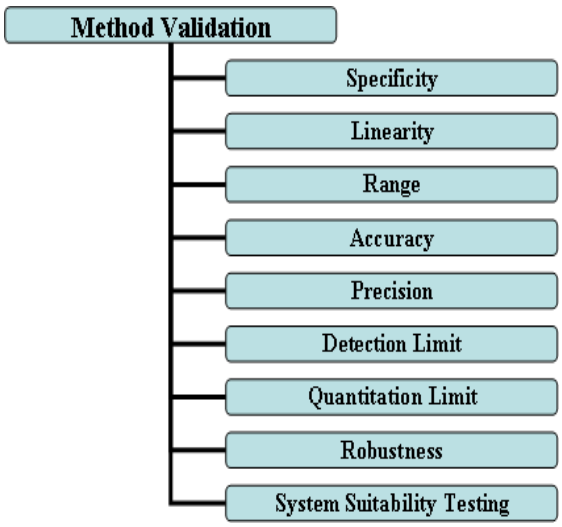


**Fig. 1: The USP and ICH Method Validation Parameters**

The different parameters of analytical method development are discussed below as per ICH guideline:-

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**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**.**

**Method:** When the impurities are available: Spiking of pure substance (drug substance or drug product) with appropriate levels of impurities/excipients and demonstrate the result is unaffected.

- When the impurities are not available: Comparing the test results of sample containing impurities or degradation product to second well-characterized procedure. These comparisons should include sample under relevant stress condition.
- In chromatographic method: Peak purity test to be done by diode array and mass spectrometry.

**Expression/calculation:** Proof of discrimination of analyte in the presence of impurities. e.g. for chromatography chromatogram should be submitted.

- Peak purity test helps in demonstrating that the peak is not attributable to more than one component.
- For assay two results should be compared and for impurity tests two profiles should be compared.

**Acceptance criteria:** Not specified

**2) Linearity:** The linearity of an analytical procedure is its ability (within given range) to obtain test results, which are directly proportional to the concentration (amount) of analyte in the sample.

**Method:** Drug (different dilution) and/or separately weighed synthetic mixture. Measurement of response and plot response vs. concentration of analyte and demonstration of linearity by

- Visual inspection of plot
- Appropriate statistical methods

**Recommendation:** Minimum of 5 concentrations are recommended

**Expression/calculation:** Correlation coefficient, y-intercept, slope of regression line, residual sum of squares.

**Acceptance criteria:** Not specified

**3) Range:** The range of 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.

**Method:** Drug (different dilution) and/or separately weighed synthetic mixture.

Measurement of response and plot response vs. concentration of analyte and demonstration of linearity by

- Visual inspection of plot
- Appropriate statistical methods

**Recommendation:** Assay of drug/finished product: 80 – 120% of test concentration.

- For content uniformity: 70 – 130% of test concentration.
- For dissolution testing: ± 20% over specified range.
- For impurity: from reporting level to 120% of specification.

**Expression/calculation:** Correlation coefficient, y-intercept, slope of regression line, residual sum of squares.

**Acceptance criteria:** Not specified

**4) Accuracy:**The accuracy of 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.

**Method:** Application of procedure to analyze synthetic mixture of known purity.

- Comparison of result with already established procedure.
- Accuracy may be inferred once precision, linearity and specificity have been established.

**Recommendation:** Minimum of nine determinations

- Low concentration of range × 3 replicates
- Medium concentration of range × 3 replicates
- High concentration of range × 3 replicates

**Expression/calculation:** Percent recovery by the assay of known added amount of analyte

- Mean – Accepted true value with confidence interval

**Acceptance criteria:** Not specified

**5) Precision:** The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between the series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

**Method:** Determination of % relative standard deviation (RSD) of response of multiple aliquots

**Recommendation:**

**a) Repeatability** (Same operating condition over short interval of time):

Minimum of nine determinations

- Low concentration of range × 3 replicates
- Medium concentration of range × 3 replicates
- High concentration of range × 3 replicates (Or)
- At target concentration × 6 determinations

**b) Intermediate precision** (within laboratory variation):

- Different Days
- Different Analysts
- Different Equipment etc.

**Expression/calculation:** Standard deviation, % RSD and confidence interval

**Acceptance criteria:** Not specified

**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 under stated experimental conditions.

**Method:**

1. By visual evaluation
2. Based on S/N ratio

- Applicable to procedure, which exhibit baseline noise.
- Actual lowest concentration of analyte detected in compared with blank response

3. Based on S.D. of response and slope

LOD = 3.3 σ/s

s = Slope of calibration curve

σ = S.D. of response

**Expression/calculation:** If based on visual examination or S/N ratio – relevant chromatogram is to be presented.

- If by calculation/extrapolation – estimate is validated by analysis of suitable no. of samples known to be near or prepared at detection limit.

**Acceptance criteria:** S/N ratio > 3 or 2:1; not specified in other cases

**7) Quantitation Limit:** The quantitation limit of an individual analytical procedure is defined as the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy.

**Method:**

1. By visual evaluation
2. Based on S/N ratio

- Applicable to procedure, which exhibit baseline noise.
- Actual lowest concentration of analyte detected in compared with blank response

3. Based on S.D. of response and slope

LOQ = 10 σ/s

s = Slope of calibration curve

σ = S.D. of response

**Recommendation:** Limit should be validated by analysis of suitable no. of samples known to be near or prepared at quantitation limit.

**Expression/calculation:**Limits of quantitation and method used for determining should be presented.

- Expresses as analyte concentration.

**Acceptance criteria:** S/N ratio > 10:1; not specified in other cases

**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.

**Method:** It should show the reliability of an analysis with respect to deliberate variations in method parameters.

In 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.

**Recommendation:** Robustness should be considered early in the development of a method.

If the results of a method or other measurements are susceptible to variations in method parameters, these parameters should be adequately controlled and a precautionary statement included in the method documentation.

**Expression/calculation:** Effect of these changed parameters on system suitability parameters.

**Acceptance criteria:** The method must be robust enough to withstand slight changes and allow routine analysis of sample.

**9) Ruggedness:** The ruggedness of an analytical method is the degree of reproducibility of test results obtained by analysis of the same samples under a variety of conditions.

**Method:** Analysis of aliquots of homogenous lots in different laboratories by different analysts under different operational and environmental conditions.

**Expression/calculation:** % RSD

**Note:** In the guideline on definitions and terminology, the ICH did not address ruggedness specifically. This apparent omission is really a matter of semantics, however, as ICH chose instead to cover the topic of ruggedness as part of precision, as discussed previously.

**10) Stability:** During the earlier validation studies, the method developer gained some information on the stability of reagents, mobile phases, standards, and sample solutions. For routine testing in which many samples are prepared and analyzed each day, it is often essential that solutions are stable enough to allow for delays such as instrument breakdowns or overnight analyses using auto-samplers.

Stability has not been given due importance in ICH guidelines but the USFDA has discussed stability parameters for bio samples. It is important to determine the stability of an analyte in a particular matrix by comparison with freshly prepared standards.

Samples and standards should be tested over at least a 48 h period, and the

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quantitation of components should be determined. If the solutions are not stable over 48 h, storage conditions or additives should be identified that can improve stability.

**11) System Suitability Testing:** The system has to be tested for its suitability for the intended purpose. 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.

Numerous approaches may be used to set the limits for system suitability tests. This depends on experience with the method, material available and personal preference. Parameters such as plate count, tailing factors, resolution and reproducibility (% RSD retention time and area for six repetitions) are determined and compared against the specifications set for the method.

**A. Retention Time ($R_t$):** Retention time is the time of elution of peak maximum after injection of compound.

**B. Theoretical Plates (N):** It is also called as column efficiency. A column can be considered as being made up of large number of theoretical plates where distribution of sample between liquid-liquid or solid-solid phase occurs. The number of theoretical plates in column is given by the relationship

$$N = 16 (t / w)^2$$

Where, t is retention time and w is width at the base of the peak.

$$HETP = L / N$$

Where L=length of column.

The theoretical plates should be more than 2000.

**C. Resolution(R):** It is a function of column efficiency and is specified to ensure that closely eluting components are resolved from each other to establish the general resolving power of the system. Resolution of two components in mixture is determined by equation.

$$Rs = \frac{2 (t_2 - t_1)}{W_1 + W_2}$$

Where, $t_2$ and $t_1$ is the retention time of second and first compound respectively, where as $W_2$ and $W_1$ are the corresponding widths at the bases of peak obtained by extrapolating straight sides of the peaks to baselines.

R should be more than 2 between peaks of interest and the closest eluted potential interferences (impurities, excipients, degradation products or internal standard).

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**Table 2: System Suitability Parameters and their recommended limits**

| Parameter | Recommendation |
|---|---|
| Capacity Factor (K') | The peak should be well-resolved from other peaks and the void volume, generally K' > 2 |
| Repeatability | RSD ≤ 1%<br>N ≥ 5 is desirable |
| Relative Retention | Not essential as the resolution is stated. |
| Resolution($R_s$) | $R_s$ of > 2 between the peak of interest and the closest eluting potential interferent (impurity, excipients, degradation product, internal standard, etc.) |
| Tailing Factor(T) | T ≤ 2 |
| Theoretical Plates(N) | In general should be > 2000. |

**D. Tailing Factor (T):** It is the measure of peak symmetry, is unity for perfectly symmetrical peaks and its value increases as tailing become more pronounced.

$$T = \frac{W_{0.05}}{2F}$$

Where, $W_{0.05}$ is the width of peak at 5% height and F is the distance from the peak maximum to the leading edge of the peak height from baseline.

Tailing factor should be less than 2.

**E. Capacity Factor (K'):** It is calculated by using the formula

$$K' = t/t_a - 1$$

Where, t is the retention time of Drug Xnd $t_a$ is the retention time of non-retarded component, air with thermal conductivity detection.[9]

The following table lists the terms generally used and their recommended limits obtained from the analysis of the system suitability sample.

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