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ANALYTICAL METHOD VALIDATION: AN UPDATED REVIEW

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ABSTRACT: The development of sound Analytical method(s) is of supreme importance during the process of drug discovery, release to market and development, culminating in a marketing approval. The objective of this paper is to review the method development, optimize and validation of the method for the drug product from the developmental stage of the formulation to commercial batch of the product. Method development for the interested component in finished product or in process tests and the sample preparation of drug product and to provide practical approaches for determining selectivity, specificity, limit of detection, limit of quantitation, linearity, range accuracy, precision, recovery solution stability, ruggedness, and robustness of liquid chromatographic methods to support the Routine, in process and stability analysis.

INTRODUCTION: The prime objective of any pharmaceutical plant is to manufacture products of requisite attribute and quality consistently, at the lowest possible cost.

Although validation studies have been conducted in the pharmaceutical industry for a long time, there is an ever increasing interest in validation owing to their industry's greater emphasis in recent years on quality assurance program and is fundamental to an efficient production operation.

Validation is a concept that has evolved in united states in 1978.

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The concept of validation has expanded through the years to embrace a wide range of activities from analytical methods used for the quality control of drug substances and drug products to computerized systems for clinical trials, labeling or process control, Validation is founded on, but not prescribed by regulatory requirements and is best viewed as an important and integral part of cGMP.

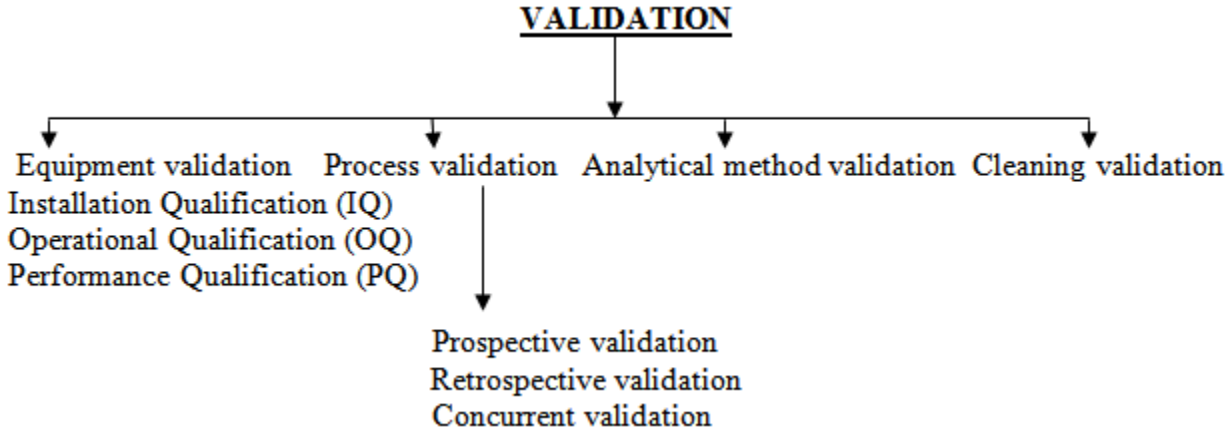
The word validation simply means assessment of validity or action of proving effectiveness. Validation is a team effort where it involves people from various disciplines of the plant.

Method validation is the process of "establishing documented evidence" which provides high degree of assurance that product (equipment) will meet the requirements for the intended analytical applications.[1]

IMPORTANCE OF VALIDATION

1. Assurance of quality
2. Time bound

3. Process optimization
4. Reduction of quality cost.
5. Nominal mix-ups, and bottle necks
6. Minimal batch failures, improved efficiently and productivity.
7. Reduction in rejections.
8. Increased output.
9. Avoidance of capital expenditures
10. Fewer complaints about process related failures.
11. Reduced testing in process and in finished goods.
12. More rapid and reliable start-up of new equipments
13. Easier scale-up form development work.
14. Easier maintenance of equipment.
15. Improved employee awareness of processes.
16. More rapid automation.
17. Government regulation (Compliance with validation requirements is necessary for obtaining approval to manufacture and to introduce new products) [2, 3]



Process Validation: "Process validation" is establishing documented evidence which provides a high degree of assurance that specific processes consistently produce a product meeting its predetermined specifications and quality attributes" [4, 5].

Analytical Method Validation: There are many reasons for the need to validate analytical procedures. Among them are regulatory requirements, good science, and quality control requirements. The *Code of Federal Regulations* (CFR) 311.165c explicitly states that "the accuracy, sensitivity, specificity, and reproducibility of test methods employed by the firm shall be established and documented. "Of course, as scientists, we would want to apply good science to demonstrate that the analytical method used had demonstrated accuracy, sensitivity, specificity, and reproducibility.

Finally management of the quality control unit would definitely want to ensure that the analytical methods that the department uses to release its products are properly validated for its intended use so the product will be safe for human use [6-9].

Analytical methods need to be validated, verified, or revalidated in the following instances:

- Before initial use in routine testing
- When transferred to another laboratory

Whenever the conditions or method parameters for which the method has been validated change (for example, an instrument with different characteristics or samples with a different matrix) and the change is outside the original scope of the method [10].

Types of Analytical procedures to be Validated: Discussion of the validation of analytical procedures is directed to the four most common types of analytical procedures:

1. Identification tests
2. Quantitative tests for impurities content
3. Limit tests for the control of impurities
4. Quantitative tests of the active moiety in samples of drug

Identification tests are intended to ensure the identity of an analyte in the sample. This normally achieved 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 intended to measure the analyte present in a given sample. In the perspective of this document the assay presents a quantitative measurement of the major components in the drug substances.

For the drug products similar characteristics also apply when assaying for the active or other selected components. The same validation characteristics also apply to assay associated with other analytical procedures [11].

Steps in Method Validation:

1. Develop a validation protocol or operating procedure for the Validation
2. Define the application, purpose and scope of the method
3. Define the performance parameters and acceptance criteria
4. Define validation experiments
5. Verify relevant performance characteristics of equipment
6. Qualify materials, e.g. standards and reagents
7. Perform pre-validation experiments
8. Adjust method parameters or/and acceptance criteria if necessary
9. Perform full internal (and external) validation experiments
10. Develop SOPs for executing the method in the routine
11. Define criteria for revalidation
12. Define type and frequency of system suitability tests and/or analytical quality control (AQC) checks for the routine
13. Document validation experiments and results in the validation.[12]

Advantages of Analytical Method Validation: The biggest advantage of analytical method validation is that it builds a degree of confidence, not only for the developer and also to the user. Although the validation exercise may appear costly and time consuming, it results expensive, eliminate frustrating repetitions and leads to better time management in the end.

Minor changes in the conditions such as reagent suppliers or grade, analytical setup are unavoidable due to obvious regions but in the method validation absorb the shock of such conditions and pays for more than invested on the process [10].

Parameters for Method Validation: The various validation parameters are;

1. Accuracy
2. Precision (repeatability and reproducibility)
3. Linearity
4. Range
5. Limit of detection (LOD)
6. Limit of Quantitation (LOQ)
7. Selectivity/ specificity
8. Robustness

9. Ruggedness

10. System Suitability Studies

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. This is sometimes termed trueness. Accuracy should be established across the specified range of the analytical procedure.

   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.

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 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.

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

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.

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

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

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

3. **Specificity (Selectivity):** 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).

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 in the sample. 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. 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.

For the establishment of linearity, a minimum of 5 concentrations is recommended.

5. **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 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;

6. **Detection Limit (LOD):** 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.

   - Based on Visual Evaluation
   - Based on Signal-to-Noise
   - 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.

7. **Quantitation Limit (LOQ):** 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.

   - Based on Visual Evaluation
   - Based on Signal-to-Noise Approach
   - Based on the Standard Deviation of the Response and the Slope

The quantitation limit (QL) may be expressed as:

$$QL=10/S$$

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.

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.

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.

9. **Ruggedness:** Ruggedness is measure of reproducibility test results under the variation in conditions normally expected from laboratory to laboratory and from analyst to analyst. The Ruggedness of an analytical method is degree of reproducibility of test results obtained by the analysis of the same samples under a variety of conditions, such as; different laboratories, analysts, instruments, reagents, temperature, time etc.

10. **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. See Pharmacopoeias for additional information. [13-15]

CONCLUSION: Analytical method validation and method transfer data playing a fundamental role in pharmaceutical industry for releasing the commercial batch and long term stability data therefore, the data must be produced to acceptable scientific standards. For this reason and the need to satisfy regulatory authority requirements, all analytical methods should be properly validated and documented. The aim of this article is to provide simple to use approaches with a correct scientific background to improve the quality of the analytical method development and validation process. This article gives an idea about number of sample preparation, procedure and acceptance criteria for all analytical method validation parameters in wider range. Applications of analytical method and method transfer are also taken into consideration in this article. These various essential development and validation characteristics for analytical methodology have been discussed with a view to improving the standard and acceptance in this area of research.

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