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Review Article

# ANALYTICAL METHOD VALIDATION: PLANNING AND ITS SIGNIFICANCE

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**Abstract:** Analytical method validation is demonstration for analytical procedure that it is suitable for intended purpose, method validation is must for the all analytical methods for which we requires precise and accurate results. Every analytical method should either verified or revalidated to check its appropriate use for its intended purpose.

Analytical method validation initiated with development of Analytical method and through process of drug commercialization and finally culminated in market. This study provides the thorough review of method development, optimization and validation of the analytical method for the drug substance as well as drug product from initial development to commercialization of the drug product. This process includes the intermediate stages including in-process analysis and stability study for better quality control of the drug product.

Analytical method is documentation process proving that equipment, system, materials, procedure, activities and process are performing as per expectation and provides the specific, linear, precise, accurate, robust, etc. results. Analytical method should provide reproducible results even when analysis performed in same or different laboratories by suing different make chemicals, equipments and by different analyst in different environmental conditions.

In this review article, we discussed about analytical method validation, importance of planning and its consequences.

**Keywords:** Planning, Validation, Analysis, Significance, Methodology.

**Introduction:** Any method newly introduced into a laboratory should also be documented and all analysts who will use it must receive adequate training. Demonstration is necessary to prove the capability of the analytical method at site before commercialization. For existing commercial analytical methods either verification or revalidation is required. Analytical methods should be followed as per manufacturer recommendation, because if there are significant changes in the manufacturing

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process, complete validation is required. For any modification in analytical method, verification or revalidation is required based on the change in the analytical method. Revalidation is required when there is change in analyte even all chromatographic conditions are same. Verification is required when analyte is same but there is small change in chromatographic column with different nature and or dimension is used. For minor modification in analytical method, no action is required, e.g. for change is analytical column with different make but column type is same.

For analytical method validation or verification, needs to follow the standard experimental test for data generation related to specificity, linearity, accuracy, precision, robustness etc. The analytical procedure to follow this validation or verification activity should be written in standard format like SOP (Standard operating procedure). After complete activity of validation or verification, methodology should be approved by authorized person and then only it can be release for routine use.

Analytical procedure consists of the detailing for performing the activity. For any analytical procedure like herbal products, new process, new reaction, new chemical entity, active pharmaceutical ingredients, impurity analysis, for content analysis in sample. Analytical method consist of analytical requirements, procedure, and precautions o be taken during analysis.

Different organization like International Conference on Harmonization (ICH), Regulatory bodies of different countries like united state pharmacopoeia and European pharmacopoeia, World Health Organization (WHO) etc made guidelines by individual, which includes procedure for performing experimental sets with acceptance criteria.

This article gives the detail information about review for planning and its significance of analytical method validation for standard method as well as in-house developed methods. Also includes the documentation and recommendation for performing analytical method validation activity

**Validation:** Analytical method validation is process for establishment by the laboratory studies, so that it fulfills the requirements of analytical procedure for intended purpose. Verification of compendial methods includes recommendation to perform the analytical method successfully in laboratory with demonstration for suitability at laboratory so that analytical procedure can run successfully.

Analytical procedure validation is required mostly for below three types,

1. Identification
2. Testing for impurities
3. Assay

Analytical procedure for Identification test ensures identity of an analyte in sample. This test includes comparison of the sample with standard, e.g., chromatographic spectrum, retention time, specific chemical activity of sample, etc. Generally in impurity testing quantification is done or either limit tests are performed for better control of the impurity in the sample. During analytical method validation for testing of impurities, there are different parameters for quantification of impurity and limit test. Assay is performed to know the exact content or potency of the analyte in the sample. Assay test represents the activity of the sample. For drug substance and drug product, same validation parameters can be applied for assay or component determination.

Analytical methods are available in various types based on application; we can distinguish the analytical methods in two major types like qualitative and qualitative. Detail is mentioned in Tabel-1

Additionally some more parameters for analytical method valuation can be performed like robustness, filter interference, extensive solution stability etc. For both type like qualitative and quantitative methods, it should be verified for accuracy, precision and at different laboratories.

**Planning and Execution of Method Validation:** Validation plan includes

preparation of protocol, which includes the step by step instruction and guidance for performing the analytical method validation activity and execution for the same.

Validation protocol consist of below steps,

- Objective, scope of the method
- Parameters and its acceptance criteria
- Experimental observations and raw data
- Elaboration in method parameters
- Specify the system suitability for routine use
- Specify the revalidation criteria
- Report all validation experiments and results

**Objective:** It includes applicability of the method in specific area. Method validation parameters to be performed are specificity / selectivity, Detection limit (LOD)/ Quantitation limit (LOQ), Precision, Linearity, Range, Accuracy, Robustness, Solution stability, System suitability study, etc. Parameters are to be modified based on the method parameters as well as variables. There is no any specific sequence for parameters or guideline to perform complete analytical method validation. Experimental sequence is totally depending on the analytical method, its parameters and variables, and as per intended purpose.

**Scope:** Before routine use of analytical method, we should have the analytical method validation. Responsibility is with the user to ensure that method is validated and is intended for in-house use. For standard methods, it needs to verify by user laboratory for its intended purpose and ensure its validity.

**Revalidation:** Revalidation is to be performed when the parameters are out of working range. Working range provides important information and guidance for revalidation. For example, the working range for column temperature is mentioned between 25°C and 30°C, due to some reason, new working range is 32°C, and then the analytical method has t be revalidated. This revalidation includes instrument change, instrument parameters, sample matrix etc.

**Method validation parameters:** For analytical method validation, understanding of validation parameters is important for initiating of this activity. Various parameters elaborated in validation process are mentioned a s below,

- Accuracy
- Precision
- Determination of repeatability
- Determination of reproducibility
- Linearity
- Range
- Limit of detection and limit of quantitation:
- Selectivity and specificity
- Robustness
- Stability and system suitability tests

**Accuracy:** Accuracy is closeness of test results and the true results. Exactness of the analytical method is measured by this parameter, accuracy. By different following ways, accuracy can be determined,

- Sample to be analyze and by comparing this results with the true value (reference standard should be well characterized).
- For drug product, active drug substance is added with known quantity in formulation placebo (all ingredients except drug substances) and is analyzed for test assay, obtained assay value compared with the expected assay value.
- Standard with known amount is added in sample and results compared with as such analyzed sample. Assay results with addition method should be comparable within the range.

Except first method, recovery is calculated in percentage by ratio of observed value and true value (expected value).

Accuracy of the analytical method varies between the ranges; hence accuracy is determined at different levels like at LOQ, 50%, 75%, 80%, 100%, 110%, 120%, 125%, 150% etc. Minimum three concentration levels are required and more can be captured as per requirement for fortification. By comparing the test requires with another validated method, accuracy can be determined.

As per ICH guidelines, accuracy should cover minimum nine determinations with at least three

concentration levels i.e. for each three concentration, three replicate determinations are mandatory.

**Precision:** Precision is measure for degree of agreement between individual test analyses by using the analytical method repeatedly for multiple sampling of homogeneous sample.

Precision is measured in terms of deviation or variation, i.e. standard deviation and relative standard deviation. Analytical method shows reproducible results under normal operational conditions, only when method is precise, i.e. standard deviation value is on lower side with respect to the acceptance criteria for standard deviation.

Method repeatability can be done by performing repeat analysis by same analysts by using same instrument and method within in short period whereas reproducibility can be performed by different analyst on different day and using different instrument, different chemicals and reagents, different laboratory, etc.

**Determination of Repeatability:** To prove repeatability, needs to carry out six replicates determinations, and by calculating mean, standard deviation and correlation of variation.

Relative standard deviation shows the degree of variation, when repetitions applied to analytical procedure. Generally for drug substances RSD to be kept for replicate measurement is less than 1.0% and for drug product RSD to be kept for replicate measurement is less than 2.0%

As per ICH recommendation, minimum nine determinations to be access for repeatability, which should cover the specified range. Either three replicate for each three concentration level or six determination over the concentration at specification level at 100% level).

**Determination of Reproducibility:** It is one type of precision, when analytical procedure applied to same homogeneous sample in different laboratories under different conditions.

Results are compared and may give valuable information to prevent the further incidence, which are obtained from different laboratories, under different conditions like difference temperature, different analyst, different equipments, different chemicals etc.

**Linearity:** Linearity is ability of analytical procedure, where analytical results are directly proportional to the test concentration.

It measures the variations calculated as over test results obtained at different test concentrations.

Detectability of compound and detector used in liner range follows Beers law. This proves that detector response and sample concentration are linearly increasing or decreasing and is calculated by measuring squared correlation coefficient or regression coefficient. For the analytical method which follows beers law, correlation coefficient should be nearby ±1.0

**Range:** Range of analytical procedure can be defined as the interval between the lower concentration and higher concentration of analyte shows the linear, accurate and precise response.

Range can be specified for specific tests in drug product or drug substances are as below,

For test assay, range can be specified between 80% and 120% of analyte concentration. For test content uniformity in drug product, range can be specified between 70% and 130% of analyte concentration.

For the test dissolution for drug product, range can be specified ± 20% of label claim.

For impurity profile, range can be specified between quantification level and 120% of target concentration.

**Limit of Detection and Limit of Quantitation:**

Limit of detection (LOD) can be defined as lowest concentration of analyte in homogeneous sample that can be detected by using the defined analytical procedure. LOD can be determined based on visual evaluation, signal to noise ratio, standard deviation of response and slope, and recommended data.

Visual evaluation is generally considered for non instrumental methods. Signal to noise ratio technique is used when analytical procedure having the baseline noise. LOD evaluated based on signal to noise ratio and 3:1 or 2:1 is considered in most of the analytical procedures.

Limit of quantitation (LOQ) can be defined as lowest concentration of analyte in homogeneous sample that can be quantified with acceptable accuracy and precision by using the defined analytical procedure. LOQ can be determined based on visual evaluation, signal to noise ratio, standard deviation of response and slope, and recommended data.

LOQ evaluated based on signal to noise ratio and 10:1 is considered in most of the analytical procedures. For most of the analytical procedures, LOQ is approximately double of the limit of detection (LOD).

**Selectivity and Specificity:** Selectivity can be referred for analytical procedure where number of impurities or chemical entities shows response; whereas it may or may not be separated from each other. The term specificity is used when analytical procedure is for single analyte. Generally there are very few methods which show response for single analyte; hence use of selectivity is more accurate as compare to specificity.

**Robustness:** Robustness is capacity measurement of analytical method when small deliberate changes in method parameters have been done and results produced are unaffected. Robustness is evaluated by determination of results by small change in analytical method parameters like, change in mobile phase composition, change in pH, change is solvent ratio, change in column brand, ionic strength, column oven temperature, sample cooler temperature, etc. For any significant variation in results due to change in specific parameters, it should be suitably controlled and precautionary note should be added in the analytical procedure. It is recommended to measure the system suitability like theoretical plates, resolution, etc for each parameter specified in analytical procedure.

**Stability and System Suitability Tests:** For any analytical method, evaluation of solution stability as well as system suitability tests is integral part. For example, 24 h stability is desired for solutions and reagents that need to be prepared for each analysis.

Test for system suitability assures that analytical method can provide reproducible, precise accurate results.

**Documentation:** Report summary should contain detail of validation protocol, report, details of standard and sample used, and development history of the method.

Validation report summary should include, forced degradation studies, incident occurred during activity with legible chromatograms.

**Validation Characteristics and Requirements**

During pharmaceutical material testing, various analytical methods or techniques are used Not all the characteristics referred above will need to be considered in all cases. Analytical method generally distributed in four major classes,

Class A: Identification test for drug substance and or drug product.

Class B: Detection and quantification of related impurities in drug substance and or drug product

Class C: Quantification of major drug content in drug substance and or drug product Class D: Measurement of dissolution, content uniformity of drug in drug product

Table 1: Characteristic for different types of analytical procedure

| Parameters | Class-A | Class-B | | Class-C | Class-D |
|---|---|---|---|---|---|
| | | Quantitative tests | Limit tests | | |
| Accuracy | - | Yes | - | Yes | Yes |
| Precision | Yes | Yes | - | Yes | Yes |
| Robustness | Yes | Yes | Yes | Yes | Yes |
| Linearity | Yes | Yes | - | Yes | Yes |
| Range | - | - | - | - | - |
| Selectivity | Yes | Yes | Yes | Yes | Yes |
| Limit of detection | - | Yes | Yes | - | - |
| Limit of quantization | - | Yes | - | - | - |

**Significance of Validation:** Capability of analytical procedures are identified during analytical method validation, hence this activity increases analyst confidence for further analytical activities.

Though it is expensive study, it avoids the further failure related to analytical methods. Minor changes in the conditions such as make of reagent / chemicals and its grade, can be taken care by analytical method validation.

The importance of analytical method validation is to produce reliable and reproducible results for routine as well as stability study. Based on analytical method validation, elaboration in analytical methodology can be incorporate such as solutions preparation, use of chemical and reagents, use of instrument and apparatus, use of calculation formula, etc. The use of analytical method during drug development and manufacturing provide information on, Potency, impurities, degradation products, effect of manufacturing parameters, drug characteristic like polymorphism and its particle shape and size which can compromise the bioavailability.

**Conclusion:** Validation is continuous evolving process includes purchasing of instrument, method development, validation, technology transfer. From the discussion in this review article we can conclude method development; validation is very important step in pharmaceutical developments. Success in these areas can be attributed to several important factors, which in turn will contribute to regulatory compliance. Analytical method validation data playing an important role in quality department for release of drug product and its stability study. Hence all the test methods should be validated as per ICH guidelines. The aim of this article is to provide documented support for method, includes sample preparation, procedure and its acceptance criteria

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