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HPLC METHOD VALIDATION FOR PHARMACEUTICALS: A REVIEW

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ABSTRACT

Validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. Chromatographic methods play significant role in the pharmaceutical industry from the drug discovery, development, formulations and quality control. A validated analytical method ensures that it provides consistent, reliable and accurate data. So these methods help pharmaceutical analyst to ensure quality products are released for market. This review describes general approach towards validation process and validation parameters to be considered during validation of a HPLC method. It also refers to various regulatory requirements. The parameters described here are according to ICH guidelines and include accuracy, precision, specificity and limit of detection, limit of quantitation, linearity, range and robustness.

INTRODUCTION:

In pharmaceutical industry, Validation is an important part of quality control and quality assurance. Various regulatory authorities give special emphasis on the validation of all the processes used in the industry. Validation is a formal and systematic way to demonstrate suitability of the method to provide useful data to ensure that the process or the method gives satisfactory and consistent results within the scope of the process. The analytical methods refer to the way of performing the analysis. “Validation is the process by which it is established, by laboratory studies, that the performance characteristics of the method meet the requirements for the intended application”[1-3]. All the analytical methods that are intended for analyzing any sample will need to be validated. The current good manufacturing practices suggest that quality should be built into the product, and testing alone cannot be relied on to ensure product quality. Pharmaceutical products need to maintain high quality in order to provide safe and effective usage. From the analytical point of view, analytical methods used to test these products should have quality attributes built into them. Validation ensures these quality attributes are built into the method. Validation of analytical methods is an essential but time consuming activity for most analytical laboratories. But it results inexpensive, eliminates frustrating repetitions and leads to better time management in the end. The analytical methods need to be validated or revalidated before initial use of the method in routine analysis, when transferred from one laboratory to another, whenever the conditions or method parameters for which the method has been validated change and change is outside the original scope of the method [4].

Chromatography is defined as a procedure by which solutes are separated by dynamic differential migration process in a system consisting of two or more mobile phases, one of which moves continuously in a given direction and in which the individual substances exhibit different mobilities by reason of differences in absorption, partition, solubility, vapor pressure, molecular size or ionic charge density [5]. When mobile phase used is liquid the type of chromatography is called liquid chromatography. High performance liquid chromatography (HPLC) is a modern form of liquid chromatography that uses small particle columns through which the mobile phase is pumped at high pressure. The separation of components depends on the extent of interaction between the solute component and the stationary phase. The component that has lowest affinity for the stationary phase will elute first. HPLC is becoming a preferred method of analysis among various analytical methods for pharmaceuticals. HPLC methods provide rapid analysis, greater sensitivity, high resolution, easy sample recovery, precise and reproducible results [6].

Method validation has received considerable attention from industrial committees and regulatory agencies. The USFDA have developed guidelines for the analytical method validation.

The United State Pharmacopoeia had developed methodology for specific applications and general chapters on different analytical aspects of FDA-regulated industry. In USP, chapter <1225> on “validation of compendial methods” describes parameters to be used for the validation of analytical methods [7]. Chapter <621> on “chromatography” has useful recommendation on how GC and HPLC methods can be studied and modified [5]. ICH published two guidelines for method validation. Q2A describes terminologies and definitions for the various parameters to be considered for validation [8]. Q2B gives description of the methods to be used for estimating the validation characteristics. It also provides flexibility in the procedures [9]. The EURACHEM has published detailed guide as Fitness for Purpose of Analytical Methods. It describes how important it is for the analytical performance and the analytical problem to be suited [10]. It describes the importance of method validation and indicates when, how and who should perform validation[11].

1. Validation Process:

Successful validation requires cooperative efforts of several departments of organization including regulatory affairs, quality control, quality assurance and analytical development. Therefore a well planned process should be followed during validation. Possible steps for a complete method validation are listed below:

Steps in method validation [12-13]:

1. Develop a validation protocol or operating procedure for the validation
2. Define the application, purpose and scope of the method
3. Define 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 and acceptance criteria if necessary
9. Perform full validation experiments
10. Develop SOPs for executing method in routine
11. Define criteria for revalidation
12. Define type and frequency of system suitability tests for routine
13. Document validation experiments and results of validation.

1.1. Validation protocol

Validation protocol prepared is a document that indicates the company’s approach for validation [12]. It ensures consistent and efficient execution of validation projects and also answers auditor during audits. The validation protocol is an ideal tool for training all the employees working for

validation. The validation protocol should include:

1. Introduction: Firms validation policy, general description
2. Organizational structure: Description of all personal responsibilities for all validation activities
3. Process and product description: Makes a brief description of the process and product or reference to adequate documents.
4. Specific process considerations: describes critical characteristics of the process.
5. Key acceptance criteria: General statement on acceptance criteria for the process.
6. Documentation format: The format used for protocol and report is described.
7. Required SOPs: a list of relevant SOPs should be mentioned.
8. Planning and Scheduling: describes the resources, equipments and chemicals to be used, including time plan of the project.
9. Change control: includes description or reference to the critical parameters variations in the process or product.

For each individual validation project a project plan should be developed. It outlines what is to be done in order to get a specific method or procedure validated. The plan should be include a time table with specific tasks, deliverables and owners.

1.2. Revalidation

Revalidation is necessary whenever a method is changed and the new parameter is outside the operating range. The operating parameters need to be specified with ranges clearly defined. In case of methods for quantitation of impurities, if a new impurity is found that makes the method deficient in its specificity, it needs modification and revalidation. Changes in equipment or chemical quality may also have critical effects on method. So any such change needs revalidation.

2. Validation Parameters

The analytical methods which need to be validated are classified as per ICH are classified as following [8]:

Identification tests: To ensure identity of an analyte.

Quantitative test for impurities: to accurately and quantitatively reflect the purity of a sample

Limit test for impurities: to reflect purity characteristics of the sample

Assay of drug substance and drug products: to measure accurately and quantitatively the analyte present in the sample. These methods also include analysis for content uniformity and measurement of analyte from dissolution samples.

The characteristics which need to be validated for the different types of method are also mentioned in ICH guidelines [8].

These are tabulated below in table 1.

Table 1: ICH characteristics

Characteristics	Identification	Test for impurities		Assay
		Quantitative	Limit test	
Accuracy	X	√	X	√
Precision				
Repeatability	X	√	X	√
Intermediate precision	X	√	X	√
Specificity	√	√	√	√
Detection limit	X	X	√	X
Quantitation limit	X	√	X	X
Linearity	X	√	X	√
Range	X	√	X	√

√ indicate this need to be evaluated

x indicate this need not to be evaluated

The united state pharmacopoeia (USP) has classified these methods into four categories and also specifies which parameters to be considered for validation of different types of methods [7].

Category I: Analytical methods for quantitation of measurement of bulk drug substances or active ingredients including preservatives in finished pharmaceutical products.

Category II: Analytical methods for determination of impurities in bulk drugs or for the determination of degradation compounds in finished pharmaceutical products.

Category III: Analytical methods for the determination of performance characteristics (e.g. dissolution, drug release).

Category IV: identification tests.

Table 2: characteristics required for the validation as per USP

Analytical performance characteristics	Category I	Category II		Category III	Category IV
		Quantitative	Limit test		
Accuracy	√	√	*	*	
Precision	√	√		√	
Specificity	√	√	√	*	√
Limit of detection			√	*	
Limit of quantitation		√		*	
Linearity	√	√		*	
Range	√	√	*	*	
Ruggedness	√	√		√	

√ indicates the parameter need to be considered

* indicates parameter may be considered depending on the nature of the test.

The parameters for validation need to be selected as per the regulatory requirements. The parameters considered in chromatographic method validation are discussed below.

2.1. Selectivity and Specificity

Selectivity of the analytical method is defined as the degree to which a method can quantify the analyte in the presence of interferences [14]. The other components which may be present include impurities, degradants, matrix, etc. The term specificity and selectivity is often used interchangeably. The term specific generally refers to a method that produces a response for a single analyte only, while the term selective refers to a method that provides responses for a number of chemical entities that may or may not be distinguished from each other. The International Union of Pure and Applied Chemistry (IUPAC) have expressed the view that “Specificity is the ultimate of Selectivity”. The IUPAC discourages use of the term specificity and instead encourages the use of the term selectivity [15].

Specificity study of the chromatographic method is performed by the separation of the analyte from the other potential components such as impurities, degradants or excipients etc. In addition forced degradation studies are carried out to challenge the method. The forced degradation studies are of particular importance when the impurities are not available. During forced degradation studies, the sample is subjected to the stressed conditions of light, heat, humidity, acid/base hydrolysis and oxidation. The scheme which is generally used for forced degradation studies for drug substances and drug products are summarized in table 3 below [15]. The selectivity of chromatographic methods may be assessed by examination of peak homogeneity or peak purity test. Peak purity test shows that there is no co-elution of any sample component. For this, peak purity assessment is done by using PDA or MS detectors. Representative chromatograms with peaks labeled should be included with resolution, plate count and tailing factor reported in the validation report.

Table 3: Table showing different forced degradation conditions to be used for drug substances and drug products

Sample	Forced degradation study
Drug substances	
Solid	Photolytic, thermal, humidity
Solution/suspension	Acid/Base hydrolysis, oxidative
Drug products	
Solid	Photolytic, oxidative, thermal, humidity
Semisolid	Photolytic, oxidative, thermal, humidity
Solution/suspension	Photolytic, thermal, oxidative, hydrolysis

2.2. Linearity

Linearity of a method is its ability to obtain test results that are directly proportional to the sample concentration over a given range. For HPLC methods, the linear relationship between detector

response (peak area and height) and sample concentration is determined. The relationship can be demonstrated directly on drug substance by dilution of standard stock or by separate weighing of the sample components, using the proposed procedures.

Linearity should be evaluated by visual inspection of a plot of signals as a function of analyte concentration or content. If there is linear relationship, test results should be evaluated by appropriate statistical methods, for example, by regression analysis. Data from the regression line is helpful to provide mathematical estimates of the degree of linearity. It is generally expressed in terms of variance around the slope of regression line. In some cases, the analytical responses should be described by the appropriate function of the analyte concentration. The widely used linearity ranges and acceptance criteria for various pharmaceutical methods are listed in the table 4 [16].

Table 4: Linearity ranges and Acceptance criteria for various pharmaceutical methods

Test	Linearity levels and ranges	Acceptance criteria
Assay	Five levels, 50-150% of label claim	Correlation coefficient, $R \geq 0.999$
Dissolution	Five to eight levels, 10-150% of label claim	% y intercept NMT 2.0% $R \geq 0.99$
Related substances	Five levels, LOQ to acceptance criteria	% y intercept NMT 5.0% $R \geq 0.99$

2.3. Precision

Precision of an analytical method expresses the closeness of agreement 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.

Repeatability is the precision under the same operating conditions over a short interval of time. It is also termed as intra-assay precision. It is assessed by making six sample determinations at 100% concentration or by preparing three samples at three concentrations in triplicates covering the specified range for the procedure. It involves repeated determination of same sample.

Intermediate precision expresses within laboratories variation: different days, different analyst, different equipments, etc. It is the term synonymous with the term 'ruggedness', defined by USP. The extent to which intermediate precision should be established depends on the circumstances under which the procedure is intended to be used. To study intermediate precision, use of an experimental design is encouraged. The intermediate precision is generally studied by multiple preparations of sample and standard solution.

Reproducibility is the precision obtained by analysis between laboratories. It is generally assessed during collaborative studies at the time of technology or method transfer. It is assessed by means of an inter-laboratory trial.

The precision data is generally expressed in the form of standard deviation, relative standard deviation and confidence intervals. To ensure precision of method for major analytes, RSD should be $\leq 2\%$. For low level impurities, RSD of 5-10 % is usually acceptable [17].

2.4. Range

Range of an analytical method is the interval between the upper and lower concentration 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 range is normally derived from the linearity studies and depends on the intended application of the procedure. The following minimum specified ranges should be considered [10]:

- For the assay method, normally covering from 80 to 120 percent of the test concentration.
- For content uniformity, covering minimum of 70 to 130 percent of the test concentration, based on the nature of the dosage form.
- For dissolution testing, $\pm 20\%$ over the specified range.
- For impurity determination, from reporting level of impurity to 120 % of the specification.

The range of a method is confirmed when linearity, accuracy and precision criteria are fulfilled [2].

2.5. Accuracy

The accuracy of an analytical method expresses the closeness of agreement between the value accepted either as a conventional true value or an accepted reference value and the value found. Practically no measurement process is ideal, therefore, the true or actual value cannot be exactly known in any particular measurement. The accepted true value for accuracy assessment can be assessed by analyzing a sample with known concentration. The accuracy studies are usually carried out by determining the recovery of the spiked sample of analyte into the matrix of the sample (a placebo) or by comparing the result to the results of a certified reference material of known purity. If the placebo of the sample is not available, the technique of standard addition is used. In case of methods for quantitation of impurities, the sample with known amount of impurities is assessed. Accuracy should be assessed using minimum of nine determinations over a minimum of three concentration levels covering the specified range (for e.g., three concentrations/ three 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 means and the accepted true value together with the confidence intervals. The concentration should cover the range of concern. The expected recovery depends on the sample matrix, the sample processing procedure, and the analyte concentration. The reported limits for accuracy for drug substances and products are 98.0 – 102.0 % and 97.0 – 103.0 % respectively. For the impurity determination, range from 50 - 150 % of average recovery may be accepted [2].

2.6. Limit of Detection

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

The simplest approach is based on the signal to noise ratio. The signal to noise ratio is determined by comparing measured signals from samples with known low concentration of analyte with those of blank samples. The concentration showing signal to noise ratio between 3:1 or 2:1 is generally considered as acceptable detection limit.

The other approach is based on the standard deviation of the response and the slope. The detection limit may be expressed as:

$$\text{LOD} = \frac{3.3 \sigma}{S}$$

Where, σ = the standard deviation of the response

S = the slope of the calibration curve

The slope may be estimated from the calibration curve of the analyte. The σ can be estimated as the standard deviation of the blank. The value of σ can also be estimated based on the calibration curve. For this the specific calibration curve should be studied using sample containing analyte in the range of detection limit. The residual standard deviation of a regression line or the standard deviation of the y-intercept of regression lines may be used as standard deviation.

Another approach for the estimation of the detection limit is base on visual evaluation. This method is applicable to non-instrumental methods but may be applied to the instrumental methods. The LOD 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. The relevant chromatograms are sufficient for the justification of the detection limit.

2.7. Limit of Quantitation

The Quantitation limit of an individual analytical procedure is the lowest amount of analyte in the sample which can be quantitatively determined with suitable precision and accuracy.

It is mainly affected by the detector sensitivity and accuracy of sample preparation. The Quantitation limit can be determined in the similar way as that of the detection limit. It is the concentration showing signal to noise ratio of 10:1. Based on the standard deviation of the response and the slope it is calculated by the formula:

$$\text{LOQ} = \frac{10 \sigma}{S}$$

Where, σ = the standard deviation of the response

S = the slope of the calibration curve

The value of S and σ are estimated as for the detection limit.

The LOQ can also be established from the visual evaluation as the LOD. The analyte concentration should be quantifiable with acceptable accuracy and precision at LOQ level. Typical acceptance criteria for LOQ are mean recovery at this level between 50 – 150 % with % RSD of ≤ 25 %.

2.8. 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. It is partially evaluated during method development stages. The aim of the robustness study is to identify the critical operating parameters for the successful implementation of the method. These parameters should be adequately controlled and a precautionary statement included in the method documentation. In case of an HPLC method, robustness study involves method parameters like pH, flow rate, column temperature and mobile phase composition which are varied within a reasonable range. The system suitability parameters obtained for each condition are studied to check the parameter which significantly affects the method.

Stability of the analytical solution and extraction time are other parameters which are also evaluated as additional parameters during robustness study. Stability of analytical solution is determined by assessing the results obtained by subjecting the analytical solution to the method parameters for longer period of time e.g. 4 hrs, 12 hrs, 24 hrs, 48 hrs, etc. The acceptance criteria are based on relative difference between initial value and the value at specified solution stability time. For drug substances and products difference should be ≤ 2.0 % and for impurity determination, it should be ≤ 10 %.²

When filtration is done during sample preparation filter paper study can be carried out. It involve analysis by filtering sample solution through different types of filter paper.

2.9. System suitability

System suitability testing (SST) is an integral part of many analytical procedures. The tests are based on the concept that the equipment, analytical operations and samples are the integral part of the system that can be evaluated as such. System suitability test provide the added assurance that on a specific occasion the method is giving, accurate and precise results. System suitability test are run every time a method is used either before or during analysis. The results of each system suitability test are compared with defined acceptance criteria and if they pass, the method is deemed satisfactory on that occasion. In case of HPLC methods, system suitability tests ensure the adequacy for performing the intended application on daily basis. The primary SST parameters considered are resolution (R_s), repeatability (% RSD of peak response and retention time), column efficiency (N), and tailing factor (T_f). The other SST parameters include retention factor (k) and separation factor (α). The limits which are considered for the SST parameters are listed table 5 [17].

Table 5: Limits for system suitability tests

SST	Limits
Resolution (R_s)	>2.0
Repeatability (RSD)	<1.0% for five replicates
Plate count (N)	>2000
Tailing factor (T_f)	≤ 2.0
Separation factor (α)	>1.0

3. Conclusion

The growing pharmaceutical industry demands various analytical methods for various pharmaceutical products. To ensure quality of the product, it is necessary that the analytical method used for assuring quality should give accurate and predictable results. For this the method need to be validated. The HPLC methods are the preferred methods of analysis due to their responsiveness. This review guides analysts to validate chromatographic methods in order to comply with regulatory requirements.

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