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Review Article

ANALYTICAL METHOD VALIDATION OF COMPENDIAL HPLC METHOD FOR PHARMACEUTICALS AS PER RECENT USP AND ICH GUIDELINES

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Analytical methods validation is a main regulatory requirement in pharmaceutical analysis in quality control laboratory. High-Performance Liquid chromatography (HPLC) is usually used as an analytical technique to evaluate the assay and organic impurities of drug product and drug substances. Method validation provides documented proof, and a high degree of assurance that an analytical method for a particular test is appropriate for its intended use. As per USP 43 NF 38 and ICH Q2 (R1) guideline of Analytical method Validation provides the elaborate guidance of method validation of any compendial or non-compendial method. This review focuses on approach to the validation of HPLC method with the compliance of restrictive needs and accepted pharmaceutical practices. The information during this review provides the explanations for performing analytical method validation. The validation parameters needed to be performed in validation for assay and organic impurities strategies. Individual validation parameters are mentioned in reference to the kind of method such assay and organic impurities method to be valid. This review was written to assist chemists/analysts to perform for method validation. This review study might facilitate to academics and pharmaceutical industry personnel to know the analytical method validation of HPLC as per USP and ICH guidelines.

Key Words: Validation, HPLC, USP, ICH, Compendial, Regulatory, QC Lab

INTRODUCTION

High-Performance Liquid chromatography (HPLC) is usually used as an analytical technique to evaluate the assay and organic impurities of drug product and drug substances. High performance liquid chromatography system (HPLC) is a modern form of liquid chromatography that uses small particle cylinders through that the mobile phase is elevated at high pressure. HPLC is changing into a most well-liked method of study among varied analytical methods for pharmaceuticals.[1]

In the pharmaceutical industry, validation is an important a part of quality control and quality assurance. Various regulatory authorities offer particular emphasis on the validation of all the processes utilized in the industry. The analytical techniques discuss with the approach of performing the analysis. All the investigative procedures that are planned for examining any specimen ought to be approved. Successful validation needs cooperative efforts of many

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departments of the organization as well as regulatory affairs, quality control, quality assurance and analytical development. Therefore, a well-planned method ought to be followed throughout validation.[2]

Validation protocol could be a document that indicates the company's approach to validation of analytical procedures. It ensures consistent and efficient execution of validation projects and conjointly answers auditor throughout audits.[2]

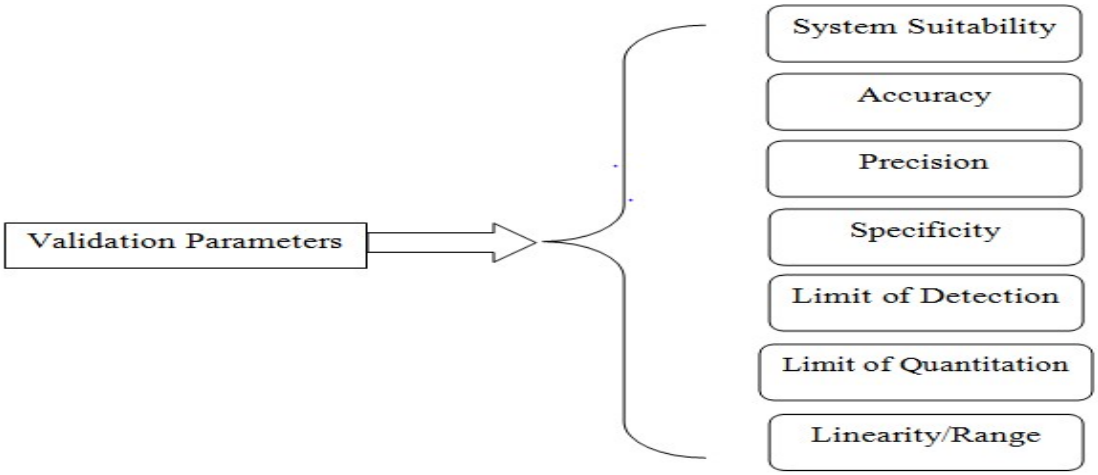

Fig. 1: Validation Parameters for Compendial Method

Validation Parameters:

1. System Suitability (as per ICH and USP)

For Assay Method

System suitability tests will be performed on both HPLC systems to determine the accuracy and precision of the system by injecting 5 replicate of a standard preparation. The subsequent parameters are determined: plate count, tailing factors reproducibility (percent RSD of retention time, peak area for 5 injections). The acceptance criteria are same as mentioned within the USP monograph of drug compound.[2]

For Organic Impurities Method

System suitability tests for organic impurities are same as assay method. The parameters are determined are area Response, Retention Time, and resolution between impurities (percent RSD of retention time, peak area for 5 injections). The acceptance criteria are same as mentioned within the USP monograph of drug compound. [3]

2. Accuracy

For Assay Method

ICH - The Accuracy of an analytical procedure expresses the closeness of agreement between value that is accepted either as a standard true value or an accepted reference value and therefore the value found. This can be typically termed trueness. [2]

USP - The Accuracy of an analytical procedure is that the closeness of test results obtained by that procedure to actuality value. The accuracy

$$\%\text{Recovery} = \frac{\text{Analytical Result} \times 100}{\text{True Value}}$$

$$\text{Sample Concentration Recovered} = \frac{\text{Spl. Peak Area} \times \text{Standard conc.}}{\text{Std Peak Area}}$$

of an analytical procedure ought to be established across its range. [2]

The procedure of accuracy is by preparing individual sample of 3 concentrations over the range of 80 %, 100% and 120 and prepare standard of 100 percent concentrations. Inject standard five injections and inject samples of every concentration. The recovery is determined by the equation: [4]

Acceptance Criteria: The mean recovery is within 90 to 110th of the theoretical value for non-regulated product. Recovery at every level, mean recovery and overall mean recovery ought to be 97.0% to 103.0%. Mean recovery and overall mean recovery should be between 98.0% and 102.0%.

For Organic Impurities Method

ICH – The Accuracy of an analytical procedure expresses the closeness of agreement between the value that is accepted either as a standard true value or an accepted reference value and the value found. This is often typically termed trueness. [2]

USP - The Accuracy of an analytical procedure is the closeness of test results obtained by that procedure to truth value. The accuracy of an analytical procedure ought to be established across it's vary. [4]

Prepare individually sample of 3 concentrations over the range of 50, 100% and 150 %. Inject five System suitability solution injections, working standard (WS) five injections and inject a pair of replicates of samples with 3 different preparations unspiked and spiked at each impurity concentration. The recovery will be determined by the equation: [6]

Acceptance Criteria: The mean recovery is within 80 to 120 of the theoretical value for non-regulated products. Recovery at each level, mean recovery and overall mean recovery ought to be 80.0% to 120.0%. Mean recovery and overall mean recovery ought to be between 80.0% and 120.0%.

3. Precision (As per ICH and USP)

For Assay and Organic Impurities Method

A. Method Precision (Repeatability)

It is the precision beneath the same operating conditions for a short period of time. ICH recommends a minimum of nine measurements inside the given vary of the procedure (3 concentrations/3 replications) or a minimum of six replications at 100%. One sample method containing the 100% target level of analyte is

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prepared. Six replicates are made from this sample solution according to the final method procedure. [2,6]

B. Intermediate Precision (Ruggedness)

It indicates intra-laboratory variations; different days, different analysts, totally different equipment. Intermediate precision (within-laboratory variation) are demonstrated by 2 analysts, using 2 HPLC systems on different. Inject the standard preparation for 5 replicates and Sample preparation for 3 replicates. [2,6]

Acceptance Criteria:

The acceptance criteria of method precision for Assay method is the RSD for the area and retention time of the principle peak in Sample preparation for 6 replicate injections should not be more than 1.0%. The acceptance criteria of Intermediate precision for Assay method are the assay results obtained by 2 operators using 2 instruments on totally different days ought to have a statistical RSD NMT 2.0%.

The acceptance criteria of method precision for Organic Impurities method is that the RSD for the recovery % of the impurity should not be more than 5.0 you care for the replicates of six preparations. The acceptance criteria of Intermediate precision are the organic impurities results obtained by 2 operators using 2 instruments on different days ought to have a statistical RSD NMT 5.0%.

4. Specificity

For Assay and Organic Impurities Method

ICH – Specificity is the ability to assess unequivocally the analyte within the presence of components which can be expected to be present. Generally these may embody impurities, degradants, matrix etc. Specificity is to produce a certain result that permits an accurate statement on the content or efficiency of the analyte in a sample. [2]

$$\text{Impurity (\%)} = \frac{\text{Impurity area in SPL} \times \text{Concentration of Impurity STD} \times 100}{\text{Impurity STD area} \times \text{Concentration of Test SPL}}$$

$$\text{\% Obtained Impurity} = \frac{\text{Impurity area in Spiked SPL} \times \text{Conc. of Impurity STD} \times 100}{\text{Impurity standard area} \times \text{Concentration of Spiked Sample}}$$

$$\text{\% Impurity added} = \frac{\text{Concentration of impurity} \times 100}{\text{Concentration of sample}}$$

$$\text{Recovery (\%)} = \frac{(\text{\% impurity obtained} - \text{Impurity \% in Test sample}) \times 100}{(\text{\% Impurity added})}$$

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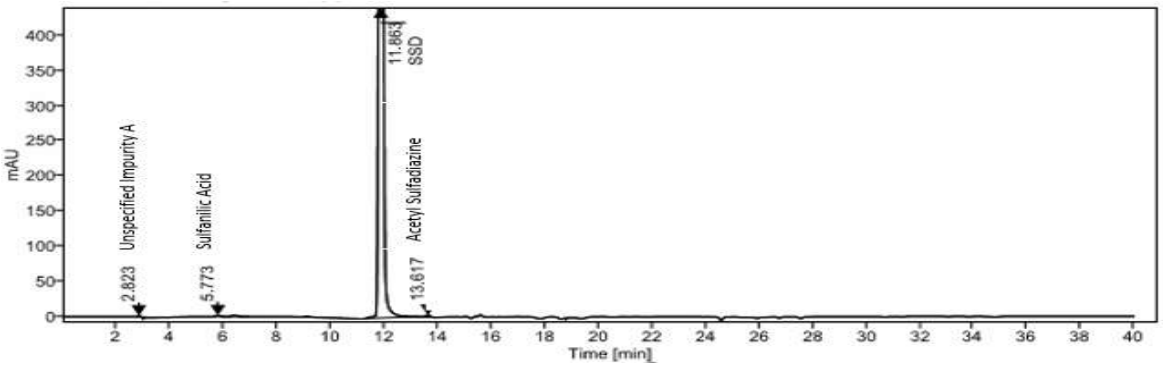

Fig. 2: Chromatogram of Spiked Impurity Solution

USP - It references the ICH definition.

The specificity of the organic impurity method are investigated by injecting of the sample with spiked impurities to demonstrate the absence of interference with the elution of analyte. Inject 5 replicates of standard solution, 3 replicates of sample solution with spiked impurities and 3 replicates of un-spiked sample solution.

Acceptance Criteria:

There should not be any interference from Blank and known impurities at the Retention Time (RT) of Sulfadiazine peak.

5. Limit of Detection

For Organic Impurities Method

ICH –"The detection limit of an individual analytical procedure is the lowest quantity of analyte in a sample which may be detected however not essentially quantitated as an exact value" [2]

USP - "The detection limit could be a characteristic of limit tests. It's the lowest quantity of analyte in a very sample that stated experimental conditions. Thus, limit tests simply substantiate that the number of analyte is above or below a certain level. The detection limit is typically expressed because the concentration of analyte (e.g., percentage, components per billion) within the sample." [4]

Limit of Detection:

The Limit of Detection is established by signal-to-noise (S/N) ratio obtained from baseline noise using following formula (Instrumental Output will be acceptable).

$$S/N = 2H / h$$

Where,

S/N = Signal-to-noise ratio,

H = Height of the Peak of Interest in mm.

h = Height of the noise in mm.

Acceptance Criteria:

Limit of Detection (S/N ratio), should be about 2 to 3.

6. Limit of Quantitation

ICH: The quantitation limit of an individual analytical procedure is the lowest quantity of analyte in a sample which may be quantitative determined with appropriate precision and accuracy. The quantitation limit could be a

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parameter of quantitative assays for low levels of compounds in sample matrices, and is employed particularly for the determination of impurities and/or degradation products." [2]

USP:

The quantitation limit could be a characteristic of quantitative assay for low levels of compounds in sample matrices, like impurities in bulk drugs substances and degradation products in finished pharmaceuticals. It's the lowest quantity of analyte in a very sample that may be determined with acceptable precision and accuracy under the expressed experimental conditions. The quantitation limit is expressed as the concentration of analyte (e.g., percentage, components per billion) within the sample.[4]

Limit of Quantitation:

The Limit of Quantification is established by Signal-to-noise (S/N) ratio obtained from baseline noise using following formula (Instrumental Output can be acceptable).

$$S/N = 2H / h$$

Where,

S/N = Signal-to-noise ratio,

H = Height of the Peak of Interest in mm.

h = Height of the noise in mm.

Acceptance Criteria:

The Limit of Quantitation (S/N ratio), should be about 10 and the RSD of Area should NMT 10.0%

7. Linearity and Range

Linearity:

ICH –The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration of analyte in the sample. [2]

USP – The linearity of an analytical procedure is its ability to elicit test results that are directly,

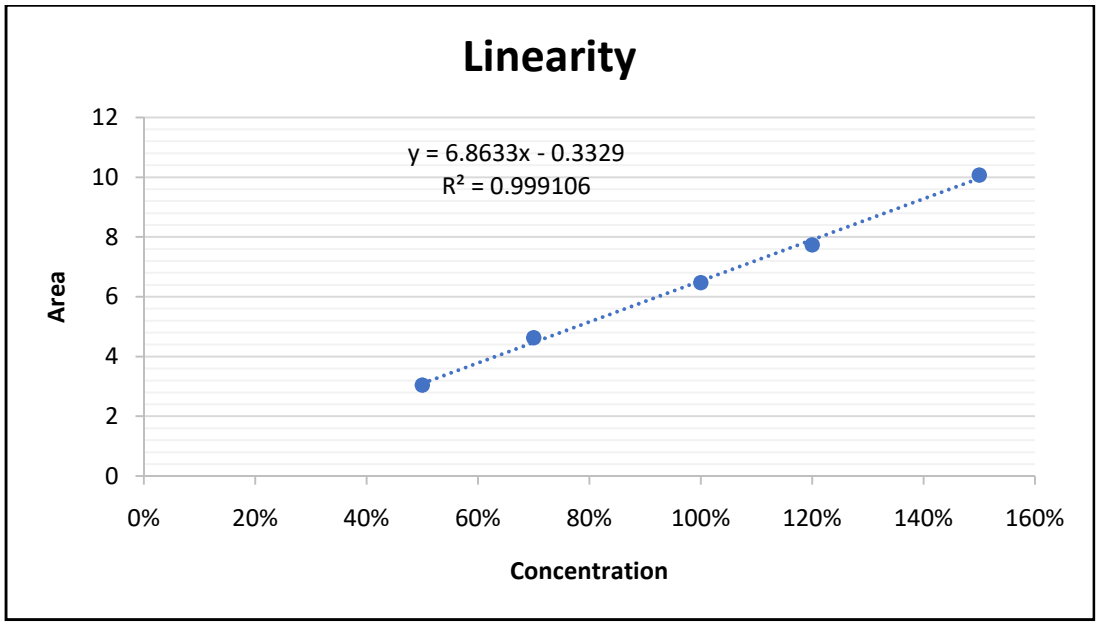

Fig. 3: Linearity Curve of Organic Impurity for reference

or by a well-defined mathematical transformation, proportional to the concentration of analyte in sample within a given range. [4]

Inject first and last level in six replicates and remaining all other levels in triplicates, adequately bracketed by the standard. Calculate the % RSD at each concentration. Plot the analyte concentration for each set of dilutions separately versus the signal response (average of each set of injections). Perform linear regression analysis,

Acceptance Criteria:

The Correlation Coefficient of linearity (r2) of assay should be greater than 0.9999 and organic impurities should be greater than 0.999. The Correlation Coefficient of Range (r2) of assay should be greater than 0.9998 and organic impurities should be greater than 0.998. The y intercept should not significantly depart from zero (e.g., area response of y intercept should be less than 5% of the response of the midrange concentration value).

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Conflict of Interest

The authors declare that they have no conflict of interest.