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Review Article

VALIDATION OF HPLC METHOD AND UV-VISIBLE METHOD FOR PHARMACEUTICALS AS PER OFFICIAL GUIDELINES

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Methods Validation is a critical quality attribute for the evaluation of any drug substance through an established method in the quality control laboratory. Method Validation is also the main regulatory requirement in pharmaceutical analysis with compliance as per the guidelines or chapter any pharmacopeia of the same scope. Method on UV spectrophotometer can be developed. Validation is establishing documented evidences, which provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality characteristics. Validation is considered a good manufacturing practice (GMP) activity; validation experiments must be properly documented and performed on qualified and calibrated instrumentation and equipment. At this stage, there should be documented evidence that the method is robust. USP defines eight steps for validation which are Accuracy, Precision, Specificity, Limit of detection, Limit of Quantitation, Linearity and range, Ruggedness, Robustness. 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 validated. This review was written to assist chemists/analysts to perform for method validation on UV spectrophotometer and HPLC. This review study may facilitate to academics and pharmaceutical industry personnel to know the analytical method validation as per Official guidelines.

Keywords: Validation, UV Spectroscopy, HLC, ICH, Regulatory, QC Lab

INTRODUCTION

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. All the investigative procedures that are planned for examining any specimen ought to be approved.

Successful validation needs cooperative efforts of many 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. Validation protocol could be a document that indicates the

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company's approach to validation of analytical procedures. It ensures consistent and efficient execution of validation projects and conjointly answers auditor throughout audits.[1]

Method development and Validation by HPLC Methods:

1. System Suitability

For Assay Method

System suitability tests will be performed on both HPLC systems to determine the accuracy and precision of the system by determining: plate count, tailing factors reproducibility (percent RSD of retention time, peak area for 5 injections).[1]

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). [2]

2. Accuracy

For Assay Method

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.The accuracy of an analytical procedure ought to be established across its range. [1]

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: [3,4]

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

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

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

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and overall mean recovery should be between 98.0% and 102.0%.

For Organic Impurities Method

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

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

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

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

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

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: [5]

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

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 prepared. Six replicates are made from this sample solution according to the final method procedure. [1,6]

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 different.HPLC systems. Inject the standard preparation for 5 replicates and Sample preparation for 3 replicates. [1,6]

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

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]

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

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

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expressed because the concentration of analyte (e.g., percentage, components per billion) within the sample. 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 [1,4]

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

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 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." [1,4]

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:

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the

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concentration (amount) of analyte in the sample. [1,3]

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).

Method development by UV-Spectrophotometric methods:

Spectroscopy may be a branch of science addressing the study of interactions of electromagnetic radiation with matter.[6] UV spectroscopy is sort of absorption chemical analysis in which light of ultra-violet region (200-400 nm.) is absorbed by the molecule. Absorption of the ultra-violet radiations ends up in the excitation of the electrons from the ground state to higher energy. The energy of the ultra-violet radiation that's absorbed is up to the energy distinction between the bottom state and higher energy states.[7]

Validation is a vital a part of quality control and quality assurance. Various regulatory authorities offer particular emphasis on the validation of all the processes utilised within the industry. The analytical techniques talk to the approach of performing arts the analysis. All the investigatory procedures that are planned for examining any specimen need to be approved. [8]

Analytical method Validation may be outlined as (ICH) "Establishing documented proof that provides a high degree of assurance that a particular activity can systematically produce a desired result or product meeting its preset specifications and quality characteristics". [5]

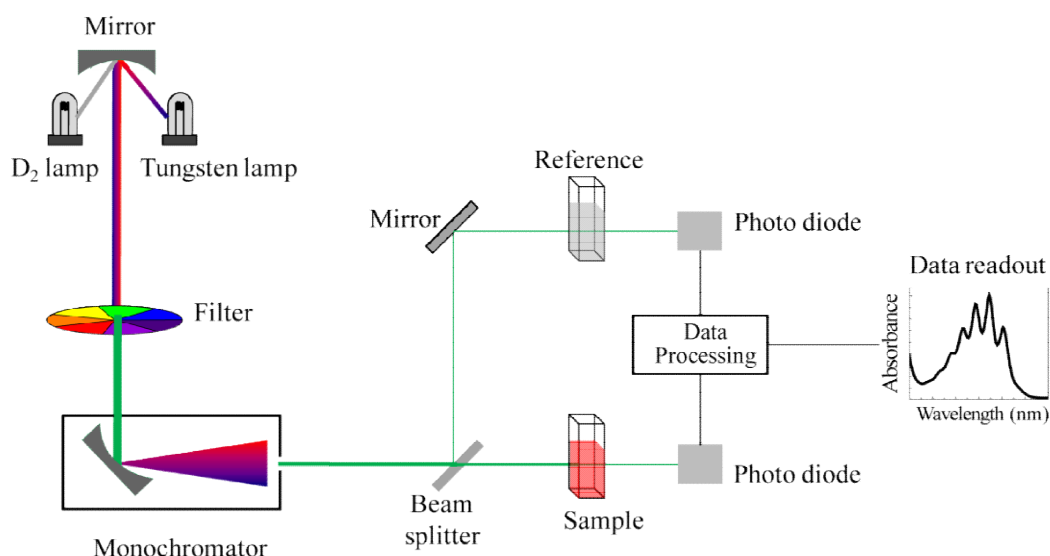

Fig. 1: Schematic Diagram of UV Spectroscopy[6]

Validation Parameters:

1. Accuracy

This is outlined as the closeness of agreement between a test result and also the accepted reference value (combination of random and systematic errors). The measure of the trueness is expressed by the bias, that is that the distinction between the expectation of the test results and an accepted reference value. The accuracy of a technique may be determined by performing recovery experiments, implementing standard addition calibration procedures, testing reference materials, etc. It's conjointly potential to compare the test results of a new method with those of an existing totally valid reference method through "cross validation" experiments. Accuracy is usually determined by recovery studies during which the analytes are spiked into a solution containing the matrix. [9]

2. Precision

A. Method Precision (Repeatability)

The repeatability of the analytical procedure is assessed by measuring the concentrations of six independently prepared sample solutions at

100% of the assay test concentration. Alternatively, it may be assessed by measuring the concentrations of 3 replicates of 3 separate sample solutions at totally different concentrations. The 3 concentrations ought to be close enough in order that the repeatability is constant across the concentration range. If this is often done, the repeatability at the 3 concentrations is pooled for comparison to the acceptance criteria. Six sample solutions containing the 100% target level of analyte will be prepared. Three replicates will be made from these sample solutions according to the final method procedure. [9]

B. Intermediate Precision (Ruggedness)

The effect of random events on the analytical precision of the method should be established. Typical variables embrace performing the analysis on completely different days, and/or having the method performed by 2 or more analysts. At a minimum, any combination of a minimum of 2 of those factors totalling six experiments can give an estimation of intermediate precision. Intermediate precision (within-laboratory variation) are demonstrated by 2 analysts, using UV-Visible spectrophotometer systems on completely different days. standard preparation, Sample Preparation are present with three replicates. [9]

3. Specificity

Specificity is the ability to assess unequivocally analyse within the presence of parts which can be expected to be present. In UV-Vis measurements, specificity is ensured by the utilization of reference standard where possible and is demonstrated by the lack of interference from alternative components present within the matrix. The specificity is investigated by analyzing the sample to demonstrate the absence of interference with the elution of analyte. [9]

4. Limit of Detection

The detection limit (DL) will be estimated by calculating the standard deviation of NLT six

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replicate measurements of a blank solution and multiplying by 3.3. as an alternative, the standard deviation will be determined from the error of the intercept from a calibration curve or by determining that the signal-to-noise is >3.3. The estimated DL should be confirmed by analyzing samples at the calculated concentration. Six Replicates of Blank solution which can show the absorbance. Calculate the standard Deviation of six Replicates and multiply by 3.3 for Limit of Detection. [10]

LOD = Standard Deviation × 3.3

5. Limit of Quantitation

The Quantitation Limit (QL) will be estimated by calculating the standard deviation of NLT six replicate measurements of a blank solution and multiplying by ten. Alternatively, the standard deviation will be determined from the error of the intercept from a calibration curve or by decisive that the signal-to-noise is >10. Measure of a test solution prepared from a sample matrix spiked at the specified QL concentration should be performed to substantiate sufficient sensitivity and adequate precision. The ascertained signal-to-noise at the specified QL ought to be >10. Six Replicates of Blank solution which can show the absorbance. Calculate the standard Deviation of six Replicates and multiply by ten for Quantification Limit. The Limit of Quantification is established by variance obtained from six replicates of blank using following formula.[10]

LOQ = Standard Deviation × 10

6. Linearity and Range

Linearity:

A linear relationship between the analyte concentration and UV-Vis response should be demonstrated by preparation of NLT 5 standard solutions at concentrations encompassing the anticipated concentration of the check solution. The standard curve is then evaluated using acceptable statistical methods like a least-squares regression. Deviation from linearity results from either instrumental or sample factors, or both, and might be reduced to acceptable levels by reducing the analyte concentration and thereby the associated

absorbance values.[11]

Range:

The operational range of an analytical instrument (and the analytical procedure as a whole) is that the interval between the higher and lower concentrations (amounts) of analyte within the sample (including these concentrations) that it's been demonstrated that the instrumental response perform contains a appropriate level of precision, accuracy, and linearity.[11]

7. Robustness

The reliability of an analytical measure is demonstrated by deliberate changes to experimental parameters. For UV-Vis this may include measure the stability of the analyte underneath given storage conditions, varying pH, and adding possible interfering species, to list some examples. Robustness is set at the same time employing a appropriate design for the procedure.[12]

Summary and Conclusion:

Analytical methods validation is a main regulatory requirement in pharmaceutical analysis in quality control laboratory. UV Spectroscopic and 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. This review was written to assist chemists/analysts to perform for method validation on UV spectrophotometer and HPLC. This review study may facilitate to academics and pharmaceutical industry personnel to know the analytical method validation of UV Spectrophotometer and HPLC as per official guidelines.

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