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Analytical Method Developement and Validation by RP-HPLC in Combination Dosage Form a Review

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ABSTRACT: Obtaining the desired quality is always a difficult task for any pharmaceutical industry. The development of an RP HPLC method for combined dosage forms begins with understanding the physicochemical properties of each API, including solubility, polarity, pKa, and pH. This knowledge guides the selection of appropriate chromatographic conditions, including the choice of mobile phase composition, buffer systems, and gradient elution parameters. Method optimization is crucial to achieving optimal separation and sensitivity, often involving adjustments to chromatographic conditions and detector settings. The pursuit of desired quality is a continual challenge for pharmaceutical industries, necessitating a meticulous approach known as validation. Validation encompasses the art of implementing planned actions and thorough documentation to ensure overall product quality alongside quality assurance. Process validation emphasizes designing processes that yield high-quality products, while maintaining control throughout commercialization. The ultimate goal is to ensure consistent product quality in combined dosage form throughout the product's lifecycle, underscoring the critical role of validation in pharmaceutical manufacturing

KEYWORDS: Validation, Method, UV Spectroscopy, Half Life, % RSD, RP-HPLC.

I. INTRODUCTION

Analytical Chemistry is defined as "The science and the art of determining the composition of materials in terms of the elements or compounds contained." This branch of chemistry, which deals with both theoretical, practical science and practiced in a large number of laboratories in many diverse ways. Methods of analysis are routinely developed, improved, validated, collaboratively studied and applied. In analytical chemistry it is of prime importance to gain information about the qualitative and quantitative composition of substances and chemical species that is to find out what substance is composed and exactly how much. In quantitative analysis the question is how much is present.[1]Medicines are key part of the health care system. Numerous medicines are introduced into the world-market and also, that is increasing every year. These medicines are being either new entities or partial structural modification of the existing one. So, evaluation of quality and efficacy of these medicines are important Right from the beginning of discovery of any medicine, quality and efficacy of the same are checked by quantification means. Quality and efficacy are checked by either observing effect of drug on various animal models or by analytical means the option of animal models is not practically suitable for every batch of medicine as it requires long time, high cost and more man-power. Later option of analytical way is more suitable, highly precise, and safe and selective.[1, 2] The HPLC (High-Performance Liquid Chromatography) systems consists of several key components this combination of components allows for precise and efficient separation and analysis of different components in a sample[3]

The HPLC (High-Performance Liquid Chromatography) system consists of several key components this combination of components allows for precise and efficient separation and analysis of different components in a sample.[3]

1. Mobile Phase Reservoir: Stores the liquid (mobile phase) used for the analysis.

2. Degasser: Removes dissolved gases from the mobile phase to prevent interference with the analysis.

3. Pump: Propels the mobile phase through the system, maintaining a constant flow rate.

4. Injector: Introduces the sample into the mobile phase flow, initiating the separation process.

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5. Column Temperature Control: Regulates the temperature of the chromatographic column to optimize separation.
6. Detector: Identifies and quantifies the individual components in the sample as they elute from the column.
7. Data Processor: Analyses and converts the raw data from the detector into meaningful results.
8. Waste Collection: Gathers the liquid waste generated during the analysis.

METHOD DEVELOPMENT

The method development process involves several key steps:
1. Understanding the physicochemical properties of the drug molecule: This entails studying properties like solubility, polarity, pKa, and pH, which influence method development decisions such as solvent selection and mobile phase composition.
2. Setting up HPLC conditions: This involves choosing buffers based on buffering capacity and pH considerations, considering the effect of pH on analyte retention, and selecting appropriate detectors and mobile phase components.
3. Preparation of sample solutions for method development: Ensuring the stability of the drug substance in solution, filtering the sample solution to remove particulates, and investigating the effectiveness of syringe filters in removing contaminants.
4. Method optimization: Experimentally optimizing conditions to achieve desired separations and sensitivity, which may involve systematic examination of parameters such as pH, mobile phase components and ratio, gradient, flow rate, sample amounts, injection volume, and diluents solvent type to develop stability indicating assays.

METHOD VALIDATION

Validation is a key process for effective quality assurance. "Validation" is established documented evidence, which provides a high degree of assurance that a specific process or equipment will consistently produce a product or result meeting its predetermined specification and quality attributes. Method validation is the process to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Analytical testing of a pharmaceutical product is necessary to ensure its purity, stability, safety and efficacy. Analytical method validation is an integral part of the quality control system. Although a thorough validation cannot rule out all potential problems, the process of method development and validation should address the most common ones.

Parameters Used For Assay Validation

The validation of the assay procedure was carried out using the following parameters.

A. SPECIFICITY

Definition:

Specificity is the ability to asses unequivocally the analyte in the presence of impurities, degradants, matrix etc (components) which may be expected to be present. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedures.

Determination:

The demonstration of specificity requires that the procedure is unaffected by the presence of impurities or excipients. In practice this can be done by spiking the drug substance or product with appropriate levels of impurities or excipients and demonstrating that the assay result is unaffected by the presence of these extraneous materials.

ICH Requirement:

The ICH documents state that when chromatographic procedures are used, representative chromatograms should be presented to demonstrate the degree of selectivity, and peaks should be appropriately labelled. Peak purity tests (e.g., using diode array or mass spectrometry) may be useful to show that the analyte chromatographic peak is not attributable to more than one component.

B. ACCURACY

Definition:

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.

Determination:

In case of assay of a drug in a formulated product, accuracy may be determined by application of the analytical method to synthetic mixtures of the drug product components to which the known amount of analyte have been added within the range of the method. If it is not possible to obtain all drug product components, it may be acceptable cither to add known quantities of the analyte to the drug product or to compare results with those of a second, well characterized method, the accuracy of which has been stated or defined. Accuracy is the measure of how close the experimental value is to the true value. Accuracy

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studies for drug substance and drug product are recommended to be performed at the 80, 100 and 120% levels of label claim as stated in the Guideline for Submitting Samples and Analytical Data for Methods Validation. For the drug product, this is performed frequently by the addition of known amounts of drug by weight or volume (dissolved in diluents) to the placebo formulation working in the linear range of detection of the analyte. This would be a true recovery for liquid formulations. For formulations such as tablet, suppository, transdermal patch, this could mean evaluating potential interaction of the active drug with the excipients in the diluents. From a practical standpoint, it is difficult to manufacture a single unit with known amount of active drug to evaluate recovery .This test evaluates the specificity of the method in the presence of the excipients under the chromatographic conditions used for the analysis of the drug product. It will pick up recovery problems that could be encountered during the sample preparation and the chromatographic procedures. However, it does not count the effect of the manufacturing process. At each recommended level studied, replicate samples are evaluated. The RSD of the replicates will provide the analysis variation or how precise the test method is. The mean of the replicates, expressed as % label claim, indicates how accurate the test method is.

ICH Requirement:

The ICH documents recommended that accuracy should be assessed using a minimum of nine determinations over a minimum of three concentration levels, covering the specified range (i.e., three concentrations and three replicates of each concentration.

C. PRECISION

Definition:

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

Precision can be categorized into two types as follows,

System precision: A system precision is evaluated by measuring the peak response for six replicate injection of the same standard solution prepared as per the proposed method. The RSD is calculating it should not be more than 2%.

Method precision: A method precision is evaluated by measuring the peak response for six replicate injection of six different weight of sample solution prepared as per the proposed method. The RSD is calculating it should not be more than 2%.

Determination:

The precision of an analytical method is determined by assaying a sufficient number of aliquots of a homogenous sample to be able to calculate statistically valid estimates of standard deviation or relative standard deviation.

ICH Requirement:

The ICH documents recommended that repeatability should be assessed using a minimum of nine determinations covering the specified range for the procedure (i.e. three concentrations and three replicates of each concentration or using a minimum of six determinations at 100% of the test concentration).

D. LINEARITY

Definition:

The linearity of an analytical procedure is its ability (within a given range) to obtain the test results which are directly proportional to the concentration (amount) of analyte in the sample. Linearity of an analytical procedure is established, using a minimum of five concentrations. It is established initially by visual examination of a plot of signals as a function of analyte concentration of content. If there appears to be a linear relationship, test results are established by appropriate statistical methods, (i.e. by calculation of a regression line by the method of least squares).

ICH Requirement:

ICH recommends that, for the establishment of linearity, a minimum of five concentrations normally be used. It is also recommended that the following minirnum specified ranges should be considered Assay of drug substance (or a finished product) from 80% to 120% of the test concentration.

E. LIMIT OF DETECTION (LOD)

Definition:

LOD is the lowest concentration of the substance that the method can detect but not necessarily quantify. LOD simply indicates that the sample is below or above a certain level.

Determination:

For non-instrumental methods, the detection limit is generally determined by the analysis of samples with known concentrations of

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analyte and by establishing the minimum level at which the analyte can be reliably detected.

ICH Requirement:

The ICH describes a common approach, which is to compare measured signal from samples with known low concentrations of analyte with those of blank samples. The minimum concentration at which the analyte can reliably be detected is established. Typically acceptable signal-to-noise ratios are 2:1 or 3:1.

Measurement is based on:

Signal to noise ratio

Visual evaluation (relevant chromatogram acceptable)

The standard deviation of the response and the slope.

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

Where,

σ - The standard deviation of the response

S. - The slope of the calibration curve (of the analyte)

F. LIMIT OF QUANTITATION (LOQ)

Definition:

LOQ is the lowest concentration of the substance that can be estimated quantitatively with acceptable precision, accuracy and reliability by the proposed method: LOQ is determined by analysis of samples containing decreasing known quantity of the substance and determining the lowest level at which acceptable level of accuracy and precision is attained.

Determination:

For non-instrumental methods, the quantization limit is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be determined with acceptable accuracy and precision.

ICH Requirement:

The ICH describes a common approach, which is to compare measured signal from samples with known low concentrations of analyte with those of blank samples. The minimum concentration at which the analyte can reliably be quantified is established. Typically acceptable signal-to-noise ratios are 10:1.

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

Where,

σ - The standard deviation of the response

S-The slope of the calibration curve (of the analyte)

G. RANGE

Definition:

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of anlayte 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.

Determination:

The range of the method is validated by verifying that the analytical method provides acceptable precision, accuracy and linearity when applied to samples containing analyte at the extremes of the range as well as within the range.

H. ROBUSTNESS:

Definition:

The robustness of an analytical procedure is a measure of its capacity to remain unchanged by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Determination:

The robustness of method is determined by performing the assay by deliberately altering parameters (change in flow rate ±10%, change in mobile phase ratio of 2, change in pH of mobile phase ±0.2, change in wave length detection +5nm, change in temperature 1 to 5°) that the results are not influenced by the changes in the above parameters.

I. RUGGEDNESS:

Definition:

The ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of conditions, such as different laboratories, different analysts, different instruments, different lots of reagents, different elapsed assay times, different assay temperatures, different days, etc.

Determination:

The Ruggedness of an analytical method is determined by the analysis of aliquots from homogeneous lots in different laboratories, by different analysts, using operational and environmental condition that may differ but are still within the specified parameters of the assay. The degree of reproducibility of the result is then determined as a function of assay variables. This reproducibility may be compared to the precision of assay under normal condition to obtain a

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measure of the ruggedness of the analytical method.

J. SAMPLE SOLUTION STABILITY:

Solution stability of the drug substance or drug product after preparation according to the test method should be evaluated according to the test method. Most laboratories utilize auto samplers with overnight runs and the sample will be in solution for hours in the laboratory environment before the test procedure is completed. This is of concern especially for drugs that can undergo degradation by hydrolysis, photolysis or adhesion to glassware.

K. SYSTEM SUITABILITY SPECIFICATIONS AND TESTS

The accuracy and precision of HPLC data collected begin with a well behaved chromatographic system. The system suitability specifications and tests are parameters that provide assistance in achieving this purpose. It consists of following factors.

1. Capacity factor.
2. Precision/injection repeatability
3. Relative retention
4. Resolution
5. Tailing factor
6. Theoretical plate number.

1. Capacity Factor (K')

$K' = (tR-t0) / tf$

The capacity factor is a measure of where the peak of interest is located with respect to the void volume, i.e., elution time of the non-retained components.

2. Precision/Injection repeatability (RSD)

Injection precision expressed as RSD (relative standard deviation) indicates the performance of the HPL chromatograph which includes the plumbing, column, and environmental conditions, at the time the samples are analyzed. It should be noted that sample preparation and manufacturing variations are not considered.

3. Relative retention (a)

$\alpha = k'1 / k'2$

Relative retention is a measure of the relative location of two peaks. This is not an essential parameter as long as the resolution (Rs) is stated.

4. Resolution (Rs)

$Rs = (t_{R2} - t_{R1}) / (1/2) (t_{W1} + t_{W2})$

Rs is a measure of how well two peaks are separated. For reliable quantitation well-separated peaks are essential for quantitation. This is a very useful parameter if potential interference peak(s) may be of concern.

5. Tailing factor (1)

$T = Wx / 2r$

The accuracy of quantitation decreases with increase in peak tailing because of the difficulties encountered by the integrator in determining where/when the peak ends and hence the calculation of the area under the peak. Integrator variables are preset by the analyst for optimum calculation of the area for the peak of interest. If the integrator is unable to determine exactly when an upslope or down slope occurs, accuracy drops.

6. Theoretical plate number (N)

$N = 16(tR / tW) - L/H$

Theoretical plate number is a measure of column efficiency, that is, how many peaks can be located per unit run-time of the chromatogram.

Where,

N= Constant for each peak on a chromatogram with a fixed set of operating conditions

H= Height equivalent of a theoretical plate.

L= Length of column.

Stability Indication and Degradation Products:

The RP HPLC method must also serve as a stability-indicating assay to detect and quantify degradation products or impurities that may arise during formulation, storage, or use of combined dosage forms. Stress testing under various conditions, such as temperature, humidity, and light exposure, helps identify potential degradation pathways and validate the method's stability-indicating capability.

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Table 1.1 ICH VALIDATION GUIDELINE.

Type Of Analytical Procedure	Identification	Testing For Impurities		Assay
Characteristics		Quantitation	limit	
Accuracy	-	+	-	+
Precision Repeatability Intermediate precision	- -	+ +(1)	- -	 + +(1)
Specificity	+	+		+
Detection limit	-	-(3)	+	-
Quantitation limit	-	+		-
Linearity	-	+		+
Range	-	+		+

Comparative Analysis and Regulatory Compliance:

Comparative analysis with existing methods or compendial standards demonstrates the superiority of the developed RP HPLC method for combined dosage forms in terms of sensitivity, selectivity, and efficiency. Regulatory compliance with international guidelines, such as those outlined by the International Council for Harmonisation (ICH), ensures that the method meets stringent quality standards and regulatory requirements for pharmaceutical analysis.

FUTURE PERSPECTIVES:

Future advancements in RP HPLC methodology for combined dosage forms may involve exploring novel stationary phases, detector technologies, or sample preparation techniques to further enhance method performance and efficiency. Integration with complementary analytical techniques, such as mass spectrometry or spectroscopic methods, could offer additional insights into complex pharmaceutical formulations.

II. CONCLUSION:

In conclusion, RP HPLC is a versatile and reliable technique for the development and validation of analytical methods for combined dosage forms. Through careful method development, validation, and compliance with regulatory standards, RP HPLC enables accurate and reliable quantification of multiple APIs in complex pharmaceutical formulations, contributing to the quality, safety, and efficacy of pharmaceutical products.

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