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A Review On Development And Validation Of Hplc Method For Analysis Of Pharmaceutical Drug

Manoj Phadtare[*1], Prof. Smita Aher[2], Dr. Dipti G.Phadtare[3], Dr. R.S.Bacchav[4], Dr. Anita Patil[5]

[*1]KCT's RG Sapkal College of Pharmacy,Anjaneri,NashiK

[2]KCT's RG Sapkal College of Pharmacy,Anjaneri,Nashik

[3]KCT'S RG Sapkal Institute of Pharmacy,Anjaneri,Nashik

[4]KCT'S RG Sapkal College of Pharmacy,Anjaneri,Nashik

[5]Head of Department,Distance Education BVDU ,YMIM, Karad

***Corresponding Author:**

Manoj Phadtare

Email ID: mnppharma1@gmail.com

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ABSTRACT

High-Performance Liquid Chromatography (HPLC) is the predominant method employed for the detection, separation, and quantification of pharmaceuticals. To enhance the methodology, many chromatographic parameters were examined, including sample pre-treatment, mobile phase selection, column selection, and detector selection. This article aims to discuss the processes of method development, optimisation, and validation. The HPLC method is advantageous due to its speed, specificity, accuracy, precision, and ease of automation, making it suitable for analysing most medicines in multicomponent dosage forms. The development and validation of HPLC methods are essential in the discovery, development, and production of novel drugs, as well as in many human and animal investigations. Validation of analytical methods is essential during medication development and production to confirm that these procedures are suitable for their intended purpose. To comply with GMP standards, pharmaceutical industries must establish a comprehensive validation policy outlining the validation process. This article focusses on the optimisation of HPLC conditions.

Keywords: *Development, Validation, HPLC Method, Analysis and Pharmaceutical Drug.*

1. INTRODUCTION

HPLC is an analytical method that is capable of separating, detecting, and quantifying different pharmaceuticals and their associated degradation products. Separating produced pharmaceuticals from drug-related impurities, detecting and quantifying synthesised drugs, and reducing other contaminants during separation are all uses of this technique that are commonplace. Analyses were carried out to determine the best chromatographic parameters for the procedure. In order to ensure the compatibility and stability of the medicine as well as the degradants and contaminants, an adequate mobile phase, column, column temperature, wavelength, and gradient must be identified (1).

The goal of pharmaceutical development is to create a high-quality product and manufacturing process that deliver on the product's promise of performance time and time again. To complement the design space, specifications and production controls, pharmaceutical development research and manufacturing experience give scientific insight. Quality risk management might be based on data from pharmaceutical development research. Quality cannot be tested into things; instead, it should be included into the design from the outset. In the course of product development and lifecycle management, it is important to see changes in formulation and manufacturing processes as chances to learn more and contribute to the creation of the design space. Experiments with unexpected findings that provide important information might also be included in the study. The applicant proposes the design space, which is then evaluated and approved by regulatory authorities. Working in the design industry is not seen as a shift in one's career path. Post-approval modification processes often begin when a product moves out of the design area (2).

Product development strategies differ from business to company and from product to product, but they should always be geared toward meeting the demands of patients and achieving the desired performance of the product. The method may also

differ, and this should be explained in the submission as a whole in the body of the document. A product developer might use an empirical technique, a more systematic approach, or a mix of the two to design their product. Prior knowledge, the outcomes of trials employing design of experiments, quality risk management, and the usage of knowledge management (ICH Q10) may all be incorporated into a more systematic approach to development. To achieve the intended product quality, a methodical approach might assist regulators better comprehend a company's plan. It is possible to keep up-to-date on product and process knowledge over the product's lifespan (3).

HPLC principle: The distribution of the analyte (sample) between a mobile phase (eluent) and a stationary phase is the basis for the separation principle of HPLC (packing material of the column). The molecules are retarded while passing through the stationary phase depending on the chemical structure of the analyte (4).

CLASSIFICATION OF HPLC:

- ✓ Preparative HPLC and analytical HPLC (based on scale of operation)
- ✓ Affinity chromatography, adsorption chromatography, size exclusion chromatography, ion exchange chromatography, chiral phase chromatography (based on principle of separation)
- ✓ Gradient separation and isocratic separation, (based on elution technique)
- ✓ Normal phase chromatography and reverse phase chromatography (based on modes of operation) (5)

Normal phase chromatography:

In normal phase chromatography, mobile phase is non-polar and stationary phase is polar. Hence, the station phase retains the polar analyte. An increase in polarity of solute molecules increases the adsorption capacity leading to an increased elution time. Chemically modified silica (cyanopropyl, aminopropyl and diol) is used as a stationary phase in this chromatography. For example. A typical column has an internal diameter of around 4.6 mm, and a length in the range of 150 to 250 mm. Polar compounds in the mixture that are passed through the column will stick longer to the polar silica than the non-polar compounds. Therefore, the non-polar ones will pass more quickly through the column (6).

RP-HPLC

RP-HPLC has a non-polar stationary phase and polar or moderately polar mobile phase. RP-HPLC is based on the principle of hydrophobic interaction [9].In a mixture of components those analytes which are relatively less polar will be retained by the non-polar stationary phase longer than those which are relatively more polar. Therefore the most polar component will elute first (7).

Size exclusion chromatography:

SEC, also known as gel permeation or gel filtration chromatography, is a technique for separating particles based on their size. It is also capable of determining the tertiary and quaternary structures of proteins and amino acids. This method is commonly used to calculate polysaccharide molecular weight (8).

Ion exchange chromatography:

Retention in ion-exchange chromatography is based on the attraction of solute ions to charged sites bound to the stationary phase. Ions with the same charge are not allowed. This chromatography technique is widely used in water purification, ligand-exchange chromatography, protein ion-exchange chromatography, high-pH anion-exchange chromatography of carbohydrates and oligosaccharides, and other applications (9).

Bio-affinity chromatography:

Separation based on reversible protein-ligand interactions. Ligands are covalently attached to solid support on an abio-affinity matrix, which keeps proteins that interact with the column bound ligands in place (10).

INSTRUMENTATION OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

In the field of biological separation and purification, reversed phase chromatography has been used for both analytical and preparative purposes. Reversed phase chromatography is an effective method for separating hydrophobic molecules with high recovery and resolution. Solute molecules in the mobile phase interact with the stationary phase's hydrophobic ligand (the stationary phase) to form hydrophobic binding interactions (11).

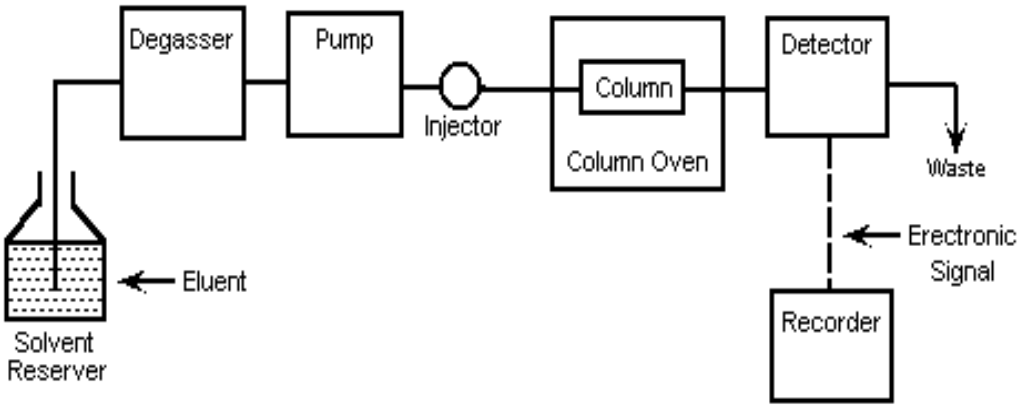

Figure 1 Components of HPLC System

Conventional wisdom is that the hydrophobic binding contact is the consequence of a favourable entropy effect, however the true nature of the binding interaction is up to question. A considerable degree of water structure is seen around both the solute molecule and the immobilised ligand in reversed-phase chromatography's early mobile phase binding conditions. An immobilised hydrophobic ligand reduces exposure of the hydrophobic ligand to the solvent. As a result, the level of organised water structure decreases, which results in an increase in system entropy that is beneficial. For quantitative analysis, HPLC is one of the most helpful technologies available. RPC refers to the use of reverse phase chromatography, which is a kind of chromatography that employs polar mobile phases and non-polar stationary phases. For quantitative and qualitative analysis, HPLC is always injected with another analytical equipment. The system's mode of operation is isocratic, which means that a single solvent or mixture is pumped throughout the analysis in order to make a specific determination. In order to achieve gradient elution, it is possible to progressively alter the solvent composition. Diffusion regulates the rate of dispersion between the stationary and mobile phases. Decreases in diffusion allow for quicker and more efficient purification (12).

The HPLC technique has the characteristics listed below:

- ✓ High resolution, small diameter, stainless steel, and glass column
- ✓ Quick analysis
- ✓ Significantly higher mobile phase pressure
- ✓ Mobile phase flow rate control. (13)

HPLC has many advantages, including:

- ✓ Simultaneous Analysis
- ✓ High Resolution
- ✓ Extreme Sensitivity
- ✓ Excellent repeatability
- ✓ Limited sample size
- ✓ The analysis condition is moderate.
- ✓ It is simple to fractionate and purify the sample. (14)

2. HPLC METHOD DEVELOPMENT

Methods are developed for new products when no official methods are available. Alternate method for existing (Non-Pharmacopoeial) products are to reduce the cost and time for better precision and ruggedness. When alternate method proposed is intended to replace the existing procedure comparative laboratory data including merit/demerits are made available. The goal of the HPLC-method is to try & separate, quantify the main active drug, any reaction impurities, all available synthetic inter-mediates and any degradants (15).

Understanding the physicochemical properties of drug molecules:

Physicochemical properties of a drug molecule play an important role in method development. For Method development one has to study the physical properties like solubility, polarity, pKa and pH of the drug molecule. Polarity is a physical property of a compound. It helps an analyst, to decide the solvent and composition of the mobile phase. The solubility of molecules

can be explained on the basis of the polarity of molecules. Polar, e.g. water, and nonpolar, e.g. benzene, solvents do not mix. In general, like dissolves like i.e., materials with similar polarity are soluble in each other. Selection of diluents is based on the solubility of analyte. The acidity or basicity of a substance is defined most typically by the pH value. Selecting a proper pH for ionizable analytes often leads to symmetrical and sharp peaks in HPLC (16).

Selection of chromatographic conditions

During initial method development, a set of initial conditions (detector, column, mobile phase) is selected to obtain the first "scouting" chromatograms of the sample. In most cases, these are based on reversed-phase separations on a C18 column with UV detection. A decision on developing either an isocratic or a gradient method should be made at this point (17).

Developing the approach for analysis:

While developing the analytical method on RP-HPLC the first step which is followed, the selections of various chromatographic parameters like selection of mobile phase, selection of column, selection of flow rate of mobile phase, selection of pH of mobile phase. All of these parameters are selected on the basis of trials and followed by considering the system suitability parameters. Typical parameters of system suitability are e.g. retention time should be more than 5 min, the theoretical plates should be more than 2000, the tailing factor should be less than 2, resolution between 2 peaks should be more than 5, % R.S.D. of the area of analyte peaks in standard chromatograms should not be more than 2.0 %.like other. Detection wavelength is usually isobestic point in the case of simultaneous estimation of 2 components (18).

Sample preparation:

Sample preparation is a critical step of method development that the analyst must investigate. For example, the analyst should investigate if centrifugation (determining the optimal rpm and time) shaking and/or filtration of the sample is needed, especially if there are insoluble components in the sample. The objective is to demonstrate that the sample filtration does not affect the analytical result due to adsorption and/or extraction of leachable. The effectiveness of the syringe filters is largely determined by their ability to remove contaminants/insoluble components without leaching undesirable artifacts (i.e., extractable) into the filtrate. The sample preparation procedure should be adequately described in the respective analytical method that is applied to a real in-process sample or a dosage form for subsequent HPLC analysis. The analytical procedure must specify the manufacturer, type of filter, and pore size of the filter media. The purpose of sample preparation is to create a processed sample that leads to better analytical results compared with the initial sample. The prepared sample should be an aliquot relatively free of interferences that is compatible with the HPLC method and that will not damage the column (19, 20).

Method optimization

Most of the optimization of HPLC method development has been focused on the optimization of HPLC conditions. The mobile phase and stationary phase compositions need to be taken into account. Optimization of mobile phase parameters is always considered first as this is much easier and convenient than stationary phase optimization. To minimize the number of trial chromatograms involved, only the parameters that are likely to have a significant effect on selectivity in the optimization must be examined. Primary control variables in the optimization of liquid chromatography (LC) methods are the different components of the mobile phase determining acidity, solvent, gradient, flow rate temperature, sample amounts, injection volume, and diluents solvent type. This is used to find the desired balance between resolution and analysis time after satisfactory selectivity has been achieved. The parameters involved include column dimensions, column-packing particle size and flow rate. These parameters may be changed without affecting capacity factor or selectivity (21).

3. VALIDATION OF HPLC

An evaluation of validity or an activity to demonstrate efficacy are both examples of validation. Assuring that an analytical technique may be used for its intended purpose is known as method validation. Pharmacopeia (USP), ICH (International Conference on Harmonization) and FDA (Food and Drug Administration) standards offer a framework for completing such validations for pharmaceutical techniques.The validation of the assay procedure was carried out as per ICH guidelines using the following parameters. The process of validating an analytical method is the process of establishing, through laboratory studies, that the method's performance characteristics meet the requirements for the intended analytical application. Any new or modified method must be validated to ensure that it is capable of producing reproducible and reliable results when used by different operators using the same equipment in the same or different laboratories. The type of validation program required is entirely dependent on the specific method and its proposed applications. (22) Method validation results can be used to assess the quality, reliability, and consistency of analytical results; it is an essential component of any good analytical practice. The use of equipment that is within specification, working properly, and properly calibrated is critical to the method validation process. Validation or revalidation of analytical methods is required. (23)

Specificity

Specificity is the capacity to access the analyte in the presence of the components that may be anticipated to be present. It is

important for chromatographic procedures to demonstrate specificity, which is the capacity to precisely detect the analyte response in the presence of all possible sample components. All possible sample components (placebo formulation, process contaminants, etc.) are compared to the analyte's reaction in a test mixture including the analyte and the analyte alone. The analyte peak must have a baseline chromatographic resolution of at least 1.5 from all other sample components to be considered specific. If this isn't possible, the final test result will be affected by no more than 5% by the unresolved components at their highest predicted level. (24)

Linearity

Analysis procedures are linear when they can provide findings that are directly correlated with analyte concentration (amount) within a predetermined range. Typically, standard solutions are prepared at five concentration levels for test procedures. Curvature in the displayed data can only be detected at a level of five. Testing for linearity by plotting the response vs. concentration means looking at the linear regression line's y-intercept and correlation. Evidence of a relationship between the data and a regression line is usually deemed to have a correlation value of >0.999. More than one or two percent of analyte responses obtained at target level should not be used to calculate the y-intercept. (25)

Accuracy

The accuracy of an analytical procedure expresses the closeness of the agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. The accuracy of the method is the closeness of the measured value to the true value for the sample. Accuracy is usually determined in one of four ways.

- ✓ Analysing a sample with a known concentration and comparing the measured result to the real amount may be used to determine accuracy.
- ✓ To compare test findings from the new approach with data from an alternative method proven to be accurate.
- ✓ Spiking analyte into blank matrices is the most common method of conducting a recovery investigation. At the 50-150 percent target concentration, a spiking sample is generated in triplicate at three levels.
- ✓ Standard additions, which may also be used to assess spiking analyte recovery. If the analyte cannot be removed from the blank sample matrix, this method is utilised.

At concentrations between 80% and 120% of the target concentration, the mean recovery must be more than or equal to 100+2% to be considered accurate. (26, 27)

Range

For example, the higher and lower concentrations of analyte in the sample, as well as those values for which the analytical technique has been shown to be accurate, precise, and linear are included in the range of analytical procedure. Data from linearity and accuracy studies are used to define the range. If linearity and accuracy can be achieved with a 3 percent RSD and a precision of an assay technique above the acceptable range, then the assay method's range requirements will be considered to be acceptable. (28)

Precision

A procedure's accuracy is expressed as the degree to which successive measurements of the same homogeneous sample under the same circumstances provide similar results. It is common to describe the accuracy of an analytical technique in terms of standard deviation, standard error, or coefficient of variation. An injection repeatability study is the initial precision investigation. A minimum of 10 injections of one sample solution are conducted to test the chromatographic instrument's performance. Reproducibility or intra-assay precision is the second form of consistency. One day is all that is needed to collect intra-assay precision data. Samples of homogeneity, each created according to the technique process, are aliquots. The number of duplicate samples to be created and the number of injections needed for each sample in the final technique process will be determined by these precision studies. For an assay procedure, the instrument precision (RSD) will be 1% and the intra-assay precision (RSD) will be 2%, as an example. (29)

Detection limit

Each analyte technique has a detection limit, which is the smallest quantity of analyte in a sample that can be detected but is not necessarily regarded as a precise measurement. Limit of detection based on the response and slope standard derivation. (30) Detection limit (or) limit of detection may be expressed as,

DL= (3.3σ/ S)

Were,

σ =standard deviation of the response

S =slope of the calibration curve (of the analyte)

Quantitation limit

The quantitation of an analytical procedure is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy. (31) Quantitation limit based on the standard deviation of the response and the slope. It can be expressed as,

QL= (10 σ /S)

Were,

σ =standard deviation of the response

S =slope of the calibration curve (of the analyte)

Ruggedness

There are several factors that contribute to an analytical method's ruggedness, such as the degree of repeatability of test results and the ability to analyse data from various sources. Different reagent sources, assay timeframes, and assay temperature conditions are all factors that might affect the results of an experiment. Ruggedness is the ability of a test result to be reliably reproduced in a variety of testing environments. The RSD should not exceed 2% as a roughness criterion. (32)

Robustness

The capacity of a technique to withstand slight variations in parameters such as the percentage of organic content, pH of the mobile phase, buffer concentration, temperature, and injection volume is known as robustness. The RSD should not exceed 2% as a measure of robustness (33).

System suitability testing

Analytical processes often include system suitability assessment as a component of the process. All aspects of testing are examined as a single integrated system that can be evaluated as a whole. This is how the tests are conducted. Typically, five injections of a standard solution are made and chromatographic characteristics such as resolution, area percent repeatability, the number of theoretical plates, and the tailing factor are evaluated (34).

4. APPLICATIONS OF HPLC METHOD

- ✓ HPLC is the most widely used chromatographic technology for purification of all sorts of biological molecules because of its versatility, speed, and sensitivity.
- ✓ Because biological fluids like serum and urine may be applied directly to the column in the system, it is frequently employed in clinical and pharmaceutical operations.
- ✓ In terms of oligopeptide and protein separation, RP-HPLC has the greatest influence (35).
- ✓ Wide variety of organic-chemistry-related uses.
- ✓ Ion exclusion chromatography and ion exchange ion pair chromatography may be used to separate anions in chromatography (36).
- ✓ For the cation separation of inert polymer resins, chromatography has been utilised.
- ✓ Most widely used in Agri-chemicals i.e., analysis of pesticides in cleaning water.
- ✓ In the field of agriculture, pesticide analysis in cleansing water is the most common use.
- ✓ For the most part, it's used in food testing Morphine and metabolites isolated from blood plasma are widely used in forensic research.
- ✓ Modern uses are mostly in the pharmaceutical industry (37, 38).

5. SUMMARY AND CONCLUSION

The development of analytical methods for drug identification, purity evaluation, and quantification has received a lot of attention in the field of pharmaceutical analysis in recent years. This review provides a general overview of HPLC method development and validation. A general and very simple approach to developing HPLC methods for compound separation was discussed. Before developing an HPLC method, it is critical to understand the physicochemical properties of the primary compound. The composition of the buffer and mobile phase (organic and pH) has a significant impact on separation selectivity. Finally, the gradient slope, temperature, and flow rate, as well as the type and concentration of mobile phase modifiers, can be optimized. The optimized method is validated using various parameters (e.g., specificity, precision, accuracy, detection limit, linearity, and so on) following ICH guidelines (39, 40).

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