



A REVIEW ON HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) METHOD DEVELOPMENT AND VALIDATION

Mariyamath Rafeeqa K A

Department of pharmaceutical analysis, Malik Deenar college of pharmacy, seethangoli, bela, kasaragod, kerala, 671321

ABSTRACT :

The most widely used separation method for drug detection, separation, and quantification is high-performance liquid chromatography. A number of chromatographic parameters, including sample pretreatment, mobile phase selection, column selection, and detector selection, were examined in order to optimize the procedure. This essay's goal is to examine the procedures that go into developing, refining, and validating methods. The majority of medications in multicomponent dosage forms can be tested using the HPLC method due to its benefits, which include speed, specificity, accuracy, precision, and ease of automation. In addition to several other human and animal research, the development and validation of HPLC procedures is essential for the discovery, development, and production of novel drugs.

To make sure that analytical techniques are appropriate for the purposes for which they are intended, validation is necessary during the phases of drug development and manufacture. Numerous aspects, such as the chemical structure of the molecules, the synthesis process, solubility, polarity, pH and pKa values, and the activity of functional groups, influence the creation of an HPLC technology. According to the ICH Guidelines, the validation process for an HPLC process includes testing for accuracy, specificity, linearity, range, limit of detection, limit of quantification, robustness, and system adaptability.

INTRODUCTION :

High-performance liquid chromatography has become one of the most useful tools in analytical chemistry. This technique can be used to separate, identify, and quantify the constituent portions of any chemical that dissolves in a liquid. High-performance liquid chromatography, or HPLC, is one of the most accurate analytical methods for both quantitative and qualitative pharmaceutical product examination. The basic idea is that a liquid (mobile phase) is pushed through a porous material column at high pressure after a sample solution (stationary phase) has been placed inside. Various sample partitioning between the stationary and mobile phases results in various migration rates through the column, which are used to segregate the sample. Depending on how various components divide, elution happens at different times. The HPLC procedure compresses the solvent at high pressures of up to 400 atmospheres in order to separate the sample into distinct constituents based on variations in relative affinities. The solvent typically flows through the column due to gravity. Because it may use a greater variety of mobile and stationary phases and is not restricted to volatile and thermally stable materials, high performance liquid chromatography is more adaptable than gas chromatography.

TYPES OF HPLC

HPLC can be classified as follows:

- i. Based on a scale of operation :Preparative HPLC and analytical HPLC
- ii. Based on the principle of separation
Affinity chromatography, adsorption chromatography, size exclusion chromatography, ion-exchange chromatography, chiral phase chromatography.
- iii. Based on the elution technique Gradient separation and isocratic separation
- iv. Based on modes of operation Normal phase chromatography and reverse-phase chromatography.

1. Size exclusion chromatography:

SEC is a method for sorting particles according to size. It is often referred to as gel permeation or gel filtration chromatography. Additionally, it can identify the quaternary and tertiary structures of amino acids and proteins. The molecular weight of polysaccharides is frequently determined using this method.

2. Ion exchange chromatography:

The attraction of solute ions to charged sites attached to the stationary phase is the basis for retention in ion-exchange chromatography. The same-charged ions are prohibited. Water purification, ligand-exchange chromatography, protein ion-exchange chromatography, high-pH anion-exchange chromatography of carbohydrates and oligosaccharides, and other applications all make extensive use of this chromatography technique.

3. Normal phase chromatography:

The stationary phase in normal phase chromatography is polar, while the mobile phase is non-polar. The polar analyte is thus retained in the station phase. The adsorption capacity of solute molecules rises with increasing polarity, which lengthens the elution time. This chromatography uses chemically modified silica (diol, aminopropyl, and cyanopropyl) as a stationary phase. For instance. A normal column is between 150 and 250 mm long, with an interior diameter of about 4.6 mm. When the combination passes through the column, the polar compounds will adhere to the polar silica for a longer period of time than the non-polar ones. As a result, the non-polar ones will move through the column faster.

4. RP-HPLC (Reversed phase HPLC):

The mobile phase of RP-HPLC is polar or somewhat polar, while the stationary phase is non-polar. The foundation of RP-HPLC is the hydrophobic interaction principle. The non-polar stationary phase will hold onto analytes that are comparatively less polar in a combination of components for a longer period of time than analytes that are comparatively more polar. Consequently, the component that is more polar will elute first.

Among the many benefits of HPLC are:

- ❖ Instantaneous Analysis
- ❖ High Resolution
- ❖ Extreme Sensitivity
- ❖ Excellent repeatability
- ❖ Small sample size
- ❖ Moderate analysis condition
- ❖ Easy sample fractionation and purification.

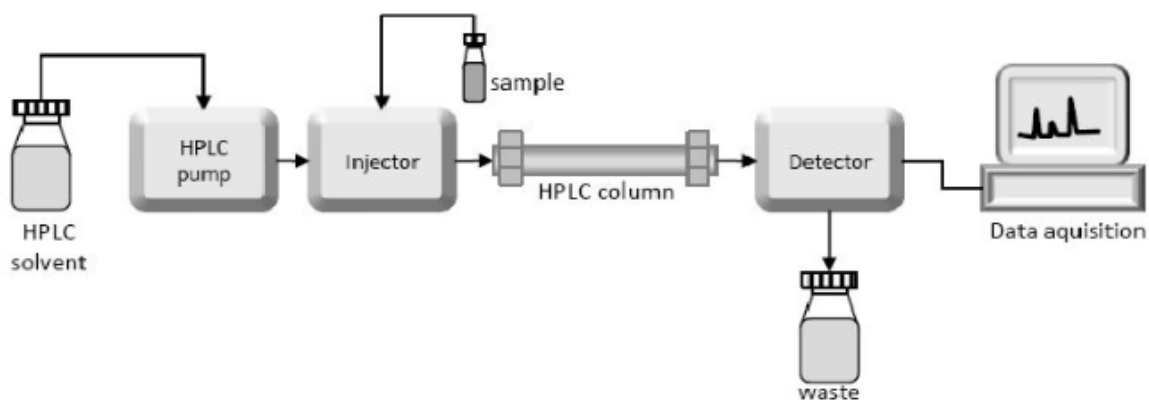
Instrumentation of High-Performance Liquid Chromatography (HPLC):

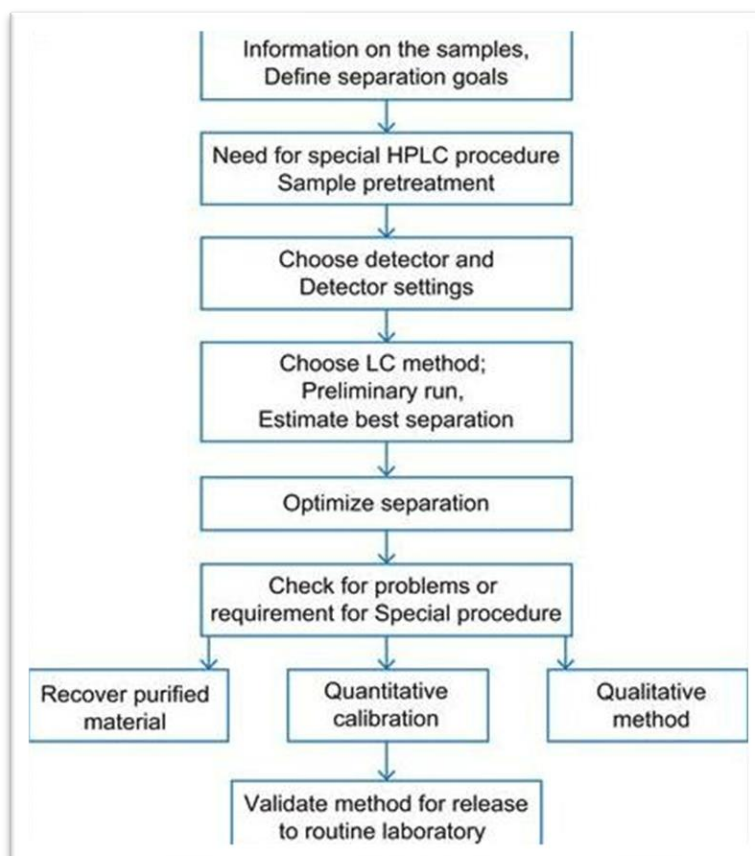
Analytical separation uses a high-pressure liquid flow through a column that contains a stationary phase. The stationary phase may be a solid (LSC) or a liquid (L) (LLC). A mixture of compounds placed at one end of the column separates as the chemicals pass through it. The separated compounds are electronically detected as they elute at the opposite end of the column. High-performance liquid chromatography is a more adaptable technique than gas chromatography. The selection of permanent and moveable phases has increased.

METHOD DEVELOPMENT ON HPLC :

A step involved in method development of HPLC is as follows:

1. Understanding the Physicochemical properties of drug molecule.
2. Selection of chromatographic conditions.
3. Developing the approach of analysis.
4. Sample preparations
5. Method optimization
6. Method validation





1. Understanding the Physicochemical Characteristics of Drug Molecules:

When developing a procedure, the physicochemical characteristics of a medicinal molecule must be considered. The physical characteristics of the drug molecule, such as its polarity, pH, pKa, and solubility, must be examined prior to developing a technique. A compound's polarity is one of its physical characteristics. It helps an analyst ascertain the composition of the solvent and mobile phase. Molecular solubility is explained by the polarity of molecules. Nonpolar solvents like benzene and polar solvents like water do not mix. Materials with similar polarity dissolve in one another because, generally speaking, like dissolves like. The choice of diluents or the mobile phase is influenced by the analyte's solubility. The analyte must not react with any of its constituents and be soluble in diluents. The pH and pKa levels determine how HPLC procedures develop. The pH value is the inverse of the logarithm of the hydrogen ion concentration to base 10.

$$\text{pH} = -\log_{10}[\text{H}_3\text{O}^+].$$

2. Selection of chromatographic conditions

To generate the sample's first "scouting" chromatograms, a set of beginning conditions (detector, column, and mobile phase) is chosen early in the technique development process. These typically use reversed phase separations on a C18 column that is detected by UV light. At this point, a choice between the gradient and isocratic methods must be made.

i. Selection of column

The first and most crucial step in creating a technique is selecting the stationary phase or column. A dependable and repeatable process cannot be established without a stable, high-performance column. Columns must be stable and repeatable to avoid issues caused by irreproducible sample retention during technique development. A C8 or C18 column, made of carefully purified, less acidic silica and intended for the separation of basic chemicals, is generally suitable for all samples.

ii. Selection of Chromatographic mode

The analyte's polarity and molecular weight dictate the chromatographic modes. Reversed-phase chromatography (RPC), the most widely used method for small organic molecules, will be the focus of all case studies. Using ion-pairing reagents or buffered mobile phases (to prevent the analytes from becoming ionized), RPC is commonly used to separate ionizable compounds (bases and acids).

iii. Buffer Selection

Acetate, potassium phosphate, sodium phosphate, and other buffers were examined for overall chromatographic performance and system compatibility aspects.

iv. Selection of Mobile Phase

Efficiency, selectivity, and resolution are all impacted by the mobile phase. In RP-HPLC separation, the solvent's strength or the mobile phase's composition are crucial. With UV cut-offs of 190, 205, and 212 nm, respectively, acetonitrile (ACN), methanol (MeOH), and tetrahydrofuran (THF) are frequently employed solvents in RPHPLC. Water is miscible with these solvents. An acetonitrile-water mixture is the best first option for the mobile phase when developing a method.

v. Selection of detectors

In HPLC, the detector is an essential part. The chemical makeup of the research, possible interference, the necessary detection limit, the availability of the detector, and/or its cost all influence the choice of detector. Commercial detectors used in LC include mass spectrometry (MS) detectors, UV detectors, fluorescence detectors, electrochemical detectors, and refractive index (RI) detectors. The sample and the analysis's objective dictate the detector to be employed.

3. Developing the approach of analysis.

Choosing different chromatographic parameters, including the mobile phase, column, mobile phase flow rate, and mobile phase pH, is the initial stage in creating an analytical method for RP-HPLC. Trials are used to determine each of these parameters, which are subsequently contrasted with the system suitability parameters. A retention time of more than five minutes, a theoretical plate count of more than two thousand, a tailing factor of less than two, a resolution of more than five, and an R.S.D. of no more than two percent of the area of analyte peaks in standard chromatograms are examples of typical system suitability parameters. The detection wavelength is typically an isobestic point when two components are being estimated simultaneously. The range of concentrations at which the drug exhibits the linear pattern is then ascertained by examining the drug's linearity. To ascertain whether the established approach for simultaneous estimation is feasible, the laboratory mixture is also examined. The commercial product is then diluted to the linearity concentration range for analysis.

4. Sample preparation:

Because it guarantees that the solution is repeatable and sufficiently homogeneous to be injected onto the column, sample preparation is a crucial step in HPLC analysis. In order to ensure that the sample solvent dissolves in the mobile phase without compromising sample retention or resolution, the sample preparation process aims to produce an aliquot that is compatible with the planned HPLC method, reasonably devoid of interferences, and won't harm the column. Sample collection is the first step in sample preparation, which then moves on to sample injection onto the HPLC column.

5. Method optimization:

Determine the "weaknesses" of the approach and use experimental design to improve it. Recognize how the technique works with various samples, equipment configurations, and environmental factors.

6. Method validation :

The process by which laboratory tests demonstrate that an analytical procedure's performance characteristics satisfy the specifications for its intended usage is known as validation. The applicant's deliberate and methodical gathering of the validation data to support analytical procedures is the first step in the techniques validation process for analytical procedures. Validation is required for all analytical techniques meant to be applied to the analysis of clinical samples. Analytical methods are validated in accordance with ICH criteria.

Components of method validation

The following are typical analytical performance characteristics which may be tested during methods validation:

1. Accuracy
2. Precision
3. Repeatability
4. Intermediate precision
5. Linearity
6. Detection limit
7. Quantitation limit
8. Specificity
9. Range
10. Robustness
11. Solution stability studies

1. ACCURACY :

The degree of agreement between the value found and the value that is recognized as either a conventional true value or an acceptable reference value is a measure of an analytical procedure's accuracy. This is referred to as trueness at times.

2. PRECISION :

An analytical procedure's precision is typically expressed as the variance, standard deviation, or coefficient of variation of a series of measurements. It describes the degree of agreement (or scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the specified conditions. Precision can be categorized into three levels: repeatability, intermediate precision, and reproducibility. If a homogeneous, authentic sample cannot be obtained, it can be investigated using artificially prepared samples or a sample solution.

3. REPEATABILITY :

Repeatability is the ability to convey precision over a brief period of time under the same operational conditions. Another name for repeatability is intra-assay precision.

4. INTERMEDIATE PRECISION :

Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, etc.

5. REPRODUCIBILITY :

Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardization of methodology).

6. DETECTION LIMIT :

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

7. QUANTITATION LIMIT

The lowest quantity of analyte in a sample that can be quantitatively identified with appropriate precision and accuracy is known as the quantitation limit of a particular analytical process. For low concentrations of chemicals in sample matrices, the quantitation limit is a parameter of quantitative assays that is specifically used to identify contaminants and/or degradation products.

8. LINEARITY

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

9. RANGE

The range of an analytical technique is the range between the sample's higher and lower analyte concentrations (amounts), including these values, for which it has been shown that the analytical method has an adequate degree of linearity, precision, and accuracy. The range of an analytical technique is the range between the sample's higher and lower analyte concentrations (amounts), including these values, for which it has been shown that the analytical method has an adequate degree of linearity, precision, and accuracy.

10. 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.

CONCLUSION:

In recent years, the discipline of pharmaceutical analysis has focused a lot of attention on developing analytical techniques for drug identification, purity assessment, and quantification. An overview of the development and validation of HPLC methods is given in this paper. There was discussion of a broad and extremely basic strategy for creating HPLC techniques for compound separation. Understanding the primary compound's physicochemical characteristics is essential before creating an HPLC technique. Separation selectivity is significantly impacted by the organic and pH composition of the buffer and mobile phase. Lastly, it is possible to optimize the gradient slope, temperature, and flow rate in addition to the kind and concentration of mobile phase modifiers. In accordance with ICH requirements, the optimized method is verified using a variety of criteria (such as specificity, precision, accuracy, detection limit, linearity, and so forth).

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