WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

HPLC METHODDEVELOPMENT AND VALIDATION: A REVIEW

[1]DEEPAK KUMAR, [2]Dr.KAUSHAL KIASHORE CHANDRUL, [3]DR. GAURAV KUMAR SHARMA

[1]STUDENT OF B. PHARMA (4TH YEAR), [2]PROFESSOR, [3]H.O.D,
DEPAETMENT OF PHARMACY MEWAR UNIVERSITY CHITTORGRH(RJ),INDIA

ABSTRACT:

The most common method for locating, separating, and measuring the medication is HPLC.To improve the approach, a number of chromatographic factors including sample pretreatment, mobile phase selection, column selection, and detector selection were examined. Goals of this piece includereview the creation, improvement, and validation of the approach. Development of HPLC techniques entailson the molecular composition, synthesis process, solubility, polarity, pH, and pKa values, as well asActivities of functional groups, etc. Information is provided through HPLC technique validation using ICH Guidelines.Understanding qualities like Accuracy, Specificity, and Linearity Limit with respect to various stages and detection and quantification's upper limit.

KEYWORDS: High Pressure Liquid Chromatography (HPLC), Method validation, Method development

Introduction

The qualitative and quantitative composition of the sample under investigation is ascertained using analytical chemistry. To comprehend the sample content, one must be aware of both of these factors. Quantitative and qualitative analytic chemistry are the two fields. By determining the existence or absence of specific components, a qualitative analysis enables us to learn more about the characteristics of the sample. An analysis using numbers can quantify the relative amounts of one or more of these components. Different analytical techniques are frequently employed to analyse drug samples in bulk, pharmaceutical formulations, and biological fluids.In non-instrumental analysis, the sample is analysed using conventional and physicochemical factors. The measurements of a substance's physical properties using an instrument to ascertain its chemical composition form the basis of instrumental methods of analysis. When compared to traditional approaches, instrumental methods are more straightforward, accurate, and repeatable. In order to ensure the quality and quantity of raw materials and finished goods, analytical procedures created employing advanced instruments such as spectrophotometers, HPLC, GC, and HPTLC have a wide range of applications.

Chromatography

Chromatography is a method for separating mixture components by evenly distributing each component between two phases over time. Continuously, one phase (mobile phase) advances over the other phase (stationary phase).
The USP defines chromatography as a technique whereby solute are separated by a differential migration process in a system made up of two or more phases, one of which moves continuously in a specific direction.

Principle of Chromatography

Absorption Principal: -Adsorption chromatography is used when the stationary phase is a solid and the mobile phase is a liquid or gaseous phase.
Examples:Thinlayerchromatography,ColumnChromatography,Gas-solidchromatography.
Partition Chromatography:-Partition chromatography is used when both the stationary phase and the mobile phase are liquids.
Example:Paperpartitionchromatography,Gas-liquidchromatography.

Classification of HPLC can be done as:

- Analytical HPLC and preparative HPLC (based on scale of operation)
- Affinity, adsorption, size exclusion, ion exchange, and chiral phase chromatography are a few examples of chromatographic techniques (based on principle of separation)
- Isocratic separation and gradient separation (based on elution technique)
- Both reverse phase chromatography and normal phase chromatography (based on modes of operation).

A. **Normal phase chromatography:**Mobile phase in normal phase chromatography is non-polar, while stationary phase is polar. Thus, the polar analyte is retained during the station phase. The adsorption capacity and elution time both increase as the polarity of the solute molecules increases. In this chromatography, cyanopropyl, aminopropyl, and diol-modified silica are utilised as the stationary phase. An illustration. A typical column is between 150 and 250 mm long, with an internal diameter of about 4.6 mm. When a combination is run through a column, polar silica will retain polar chemicals longer than non-polar ones. Therefore, the non-polar ones will move through the column more quickly.

B. **RP-HPLC (Reversed phase HPLC**:The stationary phase of RP-HPLC is non-polar, and the mobile phase is either polar or somewhat polar. The foundation of RP-HPLC is the hydrophobic interaction theory. The non-polar stationary phase will hold analytes that are comparatively less polar in a combination of components for a longer period of time than analytes that are considerably more polar. The most polar component will so elute first.

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

Understanding the Physicochemical properties of drug molecule

The physicochemical characteristics of a drug's molecule are crucial for developing procedures. One must research the physical characteristics of the drug molecule, such as its solubility, polarity, pKa, and pH, in order to build a method. A compound's physical characteristic of polarity. An analyst can use it to choose the mobile phase's solvent and chemical makeup. The polarity of the molecules can be used to explain their solubility. Solvents that are nonpolar, like benzene, and polar, like water, do not combine. Like generally dissolves like, which means that substances with comparable polarities can be dissolved in one another. The choice of mobile phase or diluents depends on how soluble the analyte is.The analyte must be soluble in diluents and must not react with any of its component. pH and pKa plays an important role in HPLC method development. The pH value is defined as the negative of the logarithm to base 10 of the concentration of the hydrogen ion.
pH = - log10[H3O+].
In HPLC, choosing the right pH for ionizable analytes frequently produces symmetrical and acute peaks. For quantitative analysis to achieve low detection limits, low relative standard deviations between injections, and consistent retention times, sharp, symmetrical peaks are required.

Selection of chromatographic conditions:

Choosing the stationary phase or column is the first and most crucial step in developing a method. Without the availability of a stable, high-performance column, it is impossible to build a robust and reproducible procedure. Column stability and reproducibility are crucial for avoiding issues caused by inconsistent sample retention while developing methods. It is generally acceptable for all samples and highly advised to use a C8 or C18 column constructed from particularly purified, less acidic silica and created expressly for the separation of basic chemicals. The key ones include column dimensions, silica substrate parameters, and bonded stationary phase qualities. Due to a number of physical properties, silica-based packing is preferred in most current HPLC columns.

Buffer Selection
The preferred pH determines the buffer to use. Reversed phase on silica-based packing typically operates in the pH range of 2 to 8. Since buffers control pH best at their pKa, it is crucial that the buffer possess a pKa near to the desired pH. A general rule is to select a buffer whose pKa value is less than two units of the required mobile phase pH.
General consideration for buffer selection:
1. Phosphate is more soluble in methanol/water than in acetonitrile/water or THF/water.
2. Some salt buffers are hygroscopic and this may lead to changes in the chromatography like increased tailing of basic compounds and possibly selectivity differences.
3. Ammonium salts are generally more soluble in organic/water mobile phases·
4. Trifluoroacetic acid can degrade with time. It is volatile and absorbs at low UV wavelengths.
5. Microbial growth can quickly occur in buffered mobile phases that contain little or no organic modifier at all. The growth accumulates on column inlets and can damage chromatographic performance.
6. At pH greater than 7, phosphate buffer accelerates the dissolution of silica and severely shortens the lifetime of silica-based HPLC columns. If possible, organic buffers should be used at pH greater than 7.
7. Ammonium bicarbonate buffers usually are prone to pH changes and are usually stable for only 24 - 48 hrs. The pH of this mobile phase tends to become more basic due to the release of carbon dioxide.
8. After buffers are prepared, they should be filtered through a 0.2-µm filter.
9. Mobile phases should be degassed.

Buffer Concentration

For tiny compounds, a buffer concentration of 10–50 mM is usually sufficient. Generally speaking, a buffer shouldn't contain more than 50% organic material. This will be influenced by the particular buffer and its concentration. The most popular buffer systems for reversed-phase HPLC use phosphoric acid and its sodium- or potassium-based salts. When studying organophosphate chemicals, sulfonate buffers can take the place of phosphonate buffers.

Isocratic and Gradient Separations:

Constant eluent composition, which means equilibrium conditions in the column and the actual velocity of compounds flowing through the column are constant, is a feature of the isocratic mode of separation. The peak capacity is small, and the resulting peak widens the longer the component is kept on the column. The separation power of a system is greatly increased by gradient method of separation, primarily due to an increase in perceived efficiency (decrease of the peak width). The rate of the eluent composition change affects peak width. The ratio between the entire gradient time and the difference in the gradient time between the first and last component are computed to determine if a gradient or isocratic would be necessary.The calculate ratio is 0.25 gradient would be adequate.

Internal Diameter

An essential factor that affects the gradient elution's detection sensitivity and separation selectivity is an HPLC column's internal diameter (ID). Additionally, it establishes how much analyte can be placed onto a column.

Particle size

The stationary phase is often applied to the exterior of tiny, spherical silica particles in classical HPLC. There are numerous different sizes of these silica particles, with 5 m beads being the most popular. The pressure needed to achieve the best linear velocity is proportional to the square root of the particle diameter, hence smaller particles typically offer more surface area and better separations. Larger particles are utilised in non-HPLC applications including solid-phase extraction as well as preparative HPLC, where column diameters range from 5 cm to >30 cm.

Pore Size

The column's pores' size determines how well analyte molecules can interact with a particle's inner surface.

Selection of Mobile Phase

The mobile phase has an impact on efficiency, selectivity, and resolution. In RP-HPLC separation, the mobile phase composition (or solvent strength) is crucial. Tetrahydrofuran (THF), acetonitrile (ACN), and methanol (MeOH) are often used solvents in RP-HPLC with low UV cut-off wavelengths of 190, 205, and 212nm, respectively. Water and these solvents mix well. The ideal first option for the mobile phase during method development is a mixture of acetonitrile and water.

Selection of Detectors

The detector is a crucial component of HPLC. The choice of detector is influenced by the chemical makeup of the analysis, any potential interference, the desired limit of detection, and the detector's cost and/or availability. The dual wavelength UVvisible detector is a flexible absorbance detector for HPLC. This detector provides the high sensitivity necessary for routine UV-based applications to identify and quantify low-level impurities. Photographic Array (PDA). For solutions involving analytical HPLC, preparative HPLC, or LC/MS systems from Waters, Detector delivers superior optical detection. High chromatographic and spectral sensitivity is provided by its integrated software and optics improvements. This detector is the best choice for analysis of components with little to no UV absorption because to its high chromatographic and spectral sensitivity, stability, and reproducibility.

1. **Developing the approach for analysis:**

   The selection of several chromatographic parameters, such as the mobile phase, column, flow rate, and pH of the mobile phase, is the initial step in the development of an analytical technique for RP-HPLC. These parameters are all chosen through testing, and the system suitability parameters are taken into account after that. Retention time should be greater than 5 minutes, theoretical plates should be more than 2000, the tailing factor should be less than 2, resolution between 2 peaks should be greater than 5, and percent R.S.D. of the area of analyte peaks in standard chromatograms should not be greater than 2.0 percent, among other parameters that indicate a system is suitable.When two components are estimated simultaneously, the detection wavelength is often at its isobestic point. The drug's linearity is next investigated in order to determine the range of concentrations at which it exhibits a linear pattern. To determine the viability of the established method for simultaneous estimation, analysis of the laboratory combination is also done. After that, the marketed formulation is diluted till it is within the linearity range before being analysed.

2. **Sample Preparation:**

   The goal of sample preparation, a crucial step in HPLC analysis, is to create a consistent, homogenous solution that is appropriate for injection onto the column. A sample aliquot that is largely free of interferences, won't harm the column, and is compatible with the desired HPLC method is the goal of sample preparation. To achieve this, the sample solvent must dissolve in the mobile phase without impacting sample retention or resolution. Beginning at the time of collection, sample preparation continues through sample injection into the HPLC column.

3. **Method optimization:**

   Determine the method's "weaknesses" and use experimental design to improve the method. Recognize how the approach performs under various circumstances, instrument configurations, and sample types.

4. **Method Validation:**

Validation is the process of examining something and providing unbiased proof that it satisfies the requirements for a certain intended usage. a procedure for assessing a method's performance and proving that it satisfies a specific condition. In essence, it is aware of the potential of your method, especially at low concentrations.

Types of Analytical Procedures to be validated

The discussion of the validation of analytical procedures is directed to the four most common types of analytical procedures:

- Identification tests
- Quantitative tests for impurities' content
- Limit tests for the control of impurities
- Quantitative tests of the active moiety in samples of drug substance or drug product or other selected component(s) in the drug product.

Conclusion

The general methodology of HPLC method development and validated method is described in this paper. The general strategy for developing a method for separating medicinal substances was outlined. Prior to the construction of the HPLC process, it is crucial to have knowledge of the primary compound's pKa, pH, and solubility. The other contaminants in the combination, such as synthetic byproducts, metabolites, degradation products, etc., may be ionizable or not depending on the pH. The makeup of the mobile phase (organic and pH) and the choice of buffer have a significant impact on the selectivity of the separation. Changes in temperature, gradient slope, flow velocity, and the kind and concentration of mobile-phase modifiers can all be made before the final optimization.

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