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A REVIEW ON HPLC METHOD DEVELOPMENT AND VALIDATION

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ABSTRACT
HPLC is the dominant separation technique to detect, separate and quantify the drug. A number of chromatographic parameters were analyzed to optimize the method like sample pretreatment, choosing mobile phase, column, detector selection. The objective of this article is to review the method development, optimization and validation. HPLC method development depends on chemical structure of the molecules, synthetic route, solubility, polarity, pH and pKa values, and functional groups activity etc. Validation of HPLC method as per ICH Guidelines gives information regarding various stages and knowing characteristics like Accuracy, specificity, linearity limit of detection, limit of quantification.
KEYWORDS: *High Pressure Liquid Chromatography (HPLC), Method validation, Method development*

1. INTRODUCTION

High Performance Liquid Chromatography is now one of the most powerful tools in analytical chemistry. It has the ability to separate, identify, and quantify the compounds that are present in any sample that can be dissolved in a liquid. High performance liquid chromatography (HPLC) is the most accurate analytical methods widely used for the quantitative as well as qualitative analysis of drug product. The principle is that a solution of the sample is injected into a column of a porous material (stationary phase) and a liquid (mobile phase) is pumped at high pressure through the column. The separation of sample is based on the differences in the rates of migration through the column arising from different partition of the sample between the stationary and mobile phase. Depending upon the partition behaviour of different components, elution at different time takes place. The sample compound with the greater affinity to the stationary layer will travel slower and for a shorter distance in comparison to compounds with less affinity which travel faster and for a longer distance. The High Performance Liquid Chromatography is more versatile than gas chromatography since (a) it is not limited to volatile and thermally stable samples, and (b) the choice of mobile and stationary phases is wider.

HPLC has numerous advantages like

- Simultaneous Analysis
- High Resolution
- High Sensitivity
- Good repeatability
- Small sample size
- Moderate analysis condition.

Classification of HPLC can be done as

- 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 modesof operation).

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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 Method optimization
5 Method validation

1. Understanding the Physicochemical properties of drug molecule.

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. The selection of mobile phase or diluents is based on the solubility of analyte. 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.

1. Selection of chromatographic conditions:

Selection of column: Selection of the stationary phase/column is the first and the most important step in method development. The development of a rugged and reproducible method is impossible without the availability of a stable, high performance column. To avoid problems from irreproducible sample retention during method development, it is important that columns be stable and reproducible.

Buffer Selection

Choice of buffer is governed by the pH that is desired. The typical pH range for reversed phase on silica based packing is pH 2 to 8. It is important that the buffer has a pKa close to the desired pH since buffer controls pH best at their pKa. A rule is to choose a buffer with a pKa value <2 units of the desired mobile phase pH.

Buffer Concentration

Generally, a buffer concentration of 10-50 mM is adequate for small molecules. Generally, no more than 50% organic should be used with a buffer. This will depend on the specific buffer as well as its concentration. Phosphoric acid and its sodium or potassium salts are the most common buffer systems for reversed-phase HPLC. Sulfonate buffers can replace phosphonate buffers when analyzing organophosphate compounds.

Selection of Mobile Phase: The mobile phase effects resolution, selectivity and efficiency. Mobile phase composition (or solvent strength) plays an important role in RP-HPLC separation. Acetonitrile (ACN), methanol (MeOH) and tetrahydrofuran (THF) are commonly used solvents in RP-HPLC having low UV cut-off of 190, 205 and 212nm respectively. These solvents are miscible with water. Mixture of acetonitrile and water is the best initial choice for the mobile phase during method development.

Selection of Detectors

Detector	Type of compound can be detected
UV-Visible &Photodiode array	Compounds with chromophores, such as aromatic rings or multiple alternating double bonds.
Fluorescence detector	Fluorescent compounds, usually with fused rings or highly conjugated planer system.
Conductivity detector	Charged compounds, such as inorganic ions and organic acid.
Electrochemical detector	For easily oxidized compounds like quinines or amines
Refractive Index detector & Evaporative light scattering detector	Compounds that do not show characteristics usable by the other detectors, eg.polymers, saccharides.

3. Developing the approach for analysis

While developing the analytical method on RP-HPLC the first step which is followed is 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

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system suitability parameters.

4. Sample preparation

Sample preparation is an essential part of HPLC analysis, intended to provide a reproducible and homogenous solution that is suitable for injection onto the column. The aim of sample preparation is a sample aliquot that, Is relatively free of interferences, Will not damage the column, and Is compatible with the intended HPLC method that is, the sample solvent will dissolve in the mobile phase without affecting sample retention or resolution. Sample preparation begins at the point of collection, extends to sample injection onto the HPLC column.

5. Method optimization

Identify the "weaknesses" of the method and optimize the method through experimental design. Understand the method performance with different conditions, different instrument set ups and different samples.

6. Method Validation

Validation is the confirmation by examination and the provision of objective evidence that the particular requirements for a specific intended use are fulfilled. A process of evaluating method performance and demonstrating that it meets a particular requirement. In essence, it knows what your method is capable of delivering, particularly 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;

Components of method validation: The following are typical analytical performance characterstics which may be tested during methods validation:

1. Accuracy
2. Precision
3. Linearity
4. Detection limit
5. Quantitation limit
6. Specificity
7. Range
8. Robustness

1) Accuracy

Accuracy is the closeness of understanding between the worth which is acknowledged either as an ordinary genuine quality or an acknowledged reference quality, and the quality discovered.

2) Precision

It communicates closeness of understanding (level of diffuse) between a progression of estimations acquired from various testing of the same homogeneous specimen under the recommended conditions. Accuracy may be considered at three levels: repeatability, transitional exactness and reproducibility.

3) Linearity

The linearity of a systematic strategy is its capacity (inside an offered extent) to acquire test outcomes that are specifically relative to the convergance of analyte in the specimen. Linearity is dictated by a progression of three to six infusions of five or more norms.

4) Detection limit

The detection limit of an individual explanatory method is the most minimal measure of analyte in an example which can be recognized yet not so much quantitated as an accurate quality.

5) Quantitation limit

The quantitation limit of an individual expository system is the least measure of analyte in an example which can be quantitatively decided with suitable accuracy and exactness. The quantitation limit is a parameter of quantitative tests for low levels of mixes in test lattices, and is utilized especially for the determination of polluting influences and/or corruption items

6) Specificity

Selectivity and specificity are once in a while utilized conversely to depict the same idea in strategy approval. Specificity is the capacity to evaluate unequivocally the analyte in the vicinity of parts that may be required to be exhibit. The specificity of a test system is controlled by contrasting test results from an investigation of tests containing contaminations, debasement items, or placebo fixings with those got from an examination of tests without debasements, corruption items, or placebo fixing.

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7) Range

The Range is the interim between the upper and lower convergance of analyte in the example (counting these focuses) for which it has been shown that the systematic method has a suitable level of exactness, precision and linearity

8) 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

This review describes about RP-HPLC Technique. The method development and validation are continuous and interrelated processes that measure a parameter as intended and establish the performance limits of the measurement. The selection of Column, buffer, detector and wavelength and another conditions composition (organic and pH) plays a dramatic role on the separation selectivity The advantages of HPLC technique were high selectivity, sensitivity, economic, less time consuming and low limit of detection. Final optimization can be performed by changing the gradient slope, temperature and flow rate as well as the type and concentration of mobile-phase modifiers. Optimized method is validated with various parameters (e.g. specificity, precision, accuracy, detection limit, linearity, etc.) as per ICH guidelines.

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