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Review Article

Review on High Performance Liquid Chromatography Method of Development, Public Health Importance and Validation

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Summary

Chromatography is the backbone of separation science and is being used in all research laboratories and pharmaceutical industries universally. This review article summarizes method of development, public health importance and validation of High Performance Liquid Chromatography in diagnostic techniques. High Performance Liquid Chromatography methods development and validation play important roles in new discovery, development, manufacture of pharmaceutical drugs and various other studies related to humans and animals. It is an analytical technique used to identify the components in a mixture and separate mixture of very similar compounds. High Performance Liquid Chromatography has played a significance role in clinical laboratory for separation and quantitation of biomarkers in different body fluids. The development of HPLC is involve four basic steps; scouting, optimization, robustness testing, and validation. The technique is used to analyses drug and medicines for their purity, to maintain the highest standards for pharmaceutical products with end goal of helping patient with medical issues. Method of validation is the process used to confirm that the analytical procedure employed for specific test is suitable for its intended used. Validation High Performance Liquid Chromatography method as per ICH guidelines cover all the performance characteristics of validation, like Accuracy, precision, specificity, linearity, range and limit of detection, limit of quantification, robustness and system suitability the testing. The limitation of High Performance Liquid Chromatography methods of development, public health importance and validation is the automated process becomes complicated, low separation power and it is expensive but the High Performance Liquid Chromatography is the modern diagnostic technique is used in all sectors.

Keywords: High performance liquid; Chromatography; Method validation

Abbreviations: ID: Internal Diameter; HPLC: High Performance Liquid Chromatography; L1: Frist Stage Larvae; LOD: Limit of Detection; LOQ: Limit of Quantitation; PDA: Photodiode Array; RP-HPLC: Reversed-Phase High Performance Liquid Chromatography

Introduction

Today chromatography is the backbone of separation science and is being used in all research laboratories and pharmaceutical industries universally [1]. According to Eijkel, "chromatography" refers to the procedures used to separate different species of a mixture based on how they are distributed between a stationary and mobile phase [2]. One of the most extensively used separation techniques and one of the most well-established analytical methods is High Performance Liquid Chromatography (HPLC). It has been used in laboratories worldwide over the past 40-plus years for pharmaceutical sciences, clinical chemistry, food and environmental analyses, synthetic chemistry [3].

High Performance Liquid Chromatography has played a significance role in clinical laboratory for separation and quantitation of biomarkers in different body fluids [4]. High Performance Liquid Chromatography is now one of the most powerful tools

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in analytical chemistry. It is also known as high pressure liquid chromatography. The measurement of concentrations of certain biogenic substance helps in diagnosis [5]. The diagnostic laboratories currently often operate according to standard quality management procedures such as ISO/IEC 17025.

High Performance Liquid Chromatography (HPLC) is the most accurate analytical methods widely used for the quantitative as well as qualitative analysis of drug product [6]. The quantitative measurement of drug and metabolite plasma levels is done using the HPLC. This is especially crucial for the development of novel medications and for the administration of therapy [7]. High Performance Liquid Chromatography method development depends on chemical structure of the molecules, synthetic route, solubility, polarity, pH and pKa values, and functional groups activity [8].

The development of HPLC is involve four basic steps; scouting, optimization, robustness testing, and validation. In the method of scouting for Reversed –Phase HPLC, the mobile phase conditions, such as PH of buffer (solutions) other than organic solvents, affect the separation results [9]. Method of validation is the process used to confirm that the analytical procedure employed for specific test is suitable for its intended used. The validation procedures help to document that a particular protocol used by the accredited laboratory has guaranteed performance in that particular laboratory [10].

Validation of High Performance Liquid Chromatography method Guidelines gives information regarding various stages and knowing characteristics like Accuracy, specificity, linearity limit of detection, limit of quantification [11]. The validation procedures help to document that a particular protocol used by the accredited laboratory has guaranteed performance in that particular laboratory [12]. The objective of this review is to summarize method of development, public health importance and validation of High Performance Liquid Chromatography in diagnostic techniques.

Literature of Review

Historical Background of High Performance Liquid Chromatography

One of the most well-established analytical procedures and by far the most extensively used separation method is High Performance Liquid Chromatography (HPLC). It has been used in laboratories worldwide over the past 40-plus years for pharmaceutical sciences, clinical chemistry, food and environmental analyses, synthetic chemistry [3].

Mikhail S. Tswett a Russian botanist in 1930 coined the name chromatography from the Greek words chroma meaning color, and graphe in meaning to write. Prior to the 1970's, few reliable chromatographic methods were commercially available to the laboratory scientist. During 1970's, most chemical separations were carried out using a variety of techniques including open-column chromatography, paper chromatography, and thin-layer chromatography reagents [13].

Midway through the 1970s, high pressure liquid chromatography was invented. With the advent of column packing materials and the added ease of online detectors, it quickly advanced. In the late 1970s, enhanced separation of chemically identical molecules was made possible by new techniques, such as reverse phase liquid chromatography. By the 1980s, chemical compound separation was frequently accomplished using HPLC. New methods far outperformed older ones in terms of separation, identification, purification, and quantification.

HPLC Method Development

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 degrades [14]. Steps involved in Method of development are:

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 [15]. 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 [14].

To get the first "scouting" chromatograms of the sample, a set of basic settings (detector, column, mobile phase) are chosen during initial technique development. On reversed-phase separations on a C18 column with UV detection, they are typically based. At this point, a choice should be taken regarding whether to develop an isocratic or a gradient methodology.

Selection of Column

The heart of a HPLC system is the column [16]. Changing a column will have the greatest effect on the resolution of analytes during method development. The stationary phase chemistry, retention capability, particle size, and column dimensions must all be taken into account when selecting the optimum column for an application.

The three main components of an HPLC column are the hardware, the matrix, and the stationary phase. The stationary phase can be supported by a variety of matrices, including silica, polymers, alumina, and zirconium. The most typical matrix for HPLC columns is silica. A C8 or C18 column built from carefully purified, less acidic silica and intended exclusively for the separation of basic chemicals is generally acceptable for all samples [15]. The primary ones are column diameters, silica substrate parameters, and bonded stationary phase characteristics. The use of silica-based packing is favored in most of the present HPLC columns due to several physical characteristics [16].

Selection of Chromatographic Mode

Chromatographic modes based on the analyte's molecular weight and polarity. All case studies will focus on Reversed Phase Chromatography (RPC), the most common mode for small organic molecules. Ionizable compounds (acids and bases) are often separated by RPC with buffered mobile phases (to keep the analytes in a non-ionized state) or with ion-pairing reagents [13].

Optimization of Mobile Phase

The mobile phase and stationary phase compositions need to be taken into account. Mobile phase parameter optimization is always prioritized since it is more practical and straightforward than stationary phase optimisation. HPLC principle is the solution of sample is injected into a column of porous material (stationary phase) and liquid phase (mobile phase) is pumped at higher pressure through the column [13].

Buffer Selection: Different buffers such as potassium phosphate, sodium phosphate and acetate were evaluated for system suitability parameters and overall chromatographic performance [17].

Effect of ph: If analytes are ionisable, the proper mobile phase pH must be chosen based on the analyte p*Ka* so the target analyte is in one predominate ionization state, ionized or neutral. Alteration of the mobile-phase pH is one of the greatest tools in the "chromatographer's toolbox" allowing simultaneous change in retention and selectivity between critical pair of components [8].

Effect of organic modifier: Selection of the organic modifier type is relatively simple in reverse phase PLC [18]. The usual choice is between acetonitrile and methanol (rarely THF). Gradient elution is usually employed with complex multicomponent samples since it may not be possible to get all components eluted between k *(*retention factor*)* 1 and 10 using a single solvent strength under isocratic conditions [8].

Selection of Detector and Wavelength

After the chromatographic separation, the analyte of interest is detected by using suitable detectors. Some commercial detectors used in LC are: Ultraviolet (UV) detectors, fluorescence detectors, electrochemical detectors, Refractive Index (RI) detectors and Mass Spectrometry (MS) detectors. The choice of detector depends on the sample and the purpose of the analysis. In case of multicomponent analysis the absorption spectra may have been shifted to longer or shorter wavelengths compared to the parent compound. Therefore the UV spectra of target analyte and impurities must be taken and overlaid with each other, and the spectra should be normalized due to different amounts present in the mixture. A wavelength must be chosen such that adequate response is for most of the analytes can be obtained [8**,**19].

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 surface [20]. 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 [15].

Sample Preparation

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 [8]. For example, the analyst should investigate if centrifugation (determining the optimal rpm and time) shaking and/or filtration of the sample are needed, especially if there are insoluble components in the sample. Sample preparation is a critical step of method development that the analyst must investigate [9].

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 prepared sample should be an aliquot relatively free of interferences that is compatible with the HPLC method and that will not damage the column [19,21].

Method Optimization

Most of the optimization of HPLC method development has been focused on the optimization of PLC. Conditions [22]. It is necessary to consider the compositions of the fixed and mobile phases. Mobile phase parameter optimisation is always prioritised since it is more practical and straightforward than stationary phase optimisation. Only the parameters that are likely to have a major impact on selectivity in the optimisation must be looked at in order to reduce the amount of trial chromatograms required. 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. Following satisfactory selectivity, this is utilised to identify the ideal balance between resolution and analysis time. The variables include flow rate, column packing particle size, and column dimensions. These parameters may be changed without affecting capacity factor or selectivity [23].

Method of Validation

An analytical method is validated when it has been proven through laboratory tests that its performance characteristics are appropriate for the intended analytical application. Any new or modified method needs to be validated to make sure it can produce repeatable and reliable results when applied by various operators using the same equipment in the same or other laboratories. The specific approach and the applications it is intended for determine exactly what kind of validation programme is necessary. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice [19]. Use of equipment that is within specification, working correctly and adequately calibrates is fundamental to the method validation process. Analytical methods need to be validated or revalidated. Typical parameters recommended by FDA, USP, and ICH are as follow [24,9].

Specificity: An analytical technique's selectivity is defined as its capacity to measure an analyte accurately in the presence of interference, such as synthetic precursors, excipients, enantiomers, and known (or likely) degradation products that may be anticipated to be present in the sample matrix [25].

Linearity and range: The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration of analyte in the sample. A linear relationship should be evaluated across the range of the analytical procedure. It is demonstrated directly on the drug substance by dilution of a standard stock solution of the drug product components, using the proposed procedure. Linearity is usually expressed as the confidence limit around the slope of the regression line. For the establishment of linearity, minimum of five concentrations are recommended by ICH guideline [24,9]. The range of an analytical method is the interval between the upper and lower levels that have been demonstrated to be determined with precision, accuracy and linearity using the method [26,25].

Precision: The degree of scatter between a set of measurements obtained from multiple sampling of the same homogenous sample under the specified conditions is expressed as the precision of an analytical method. There are three types of accuracy: intermediate precision, reproducibility, and repeatability. Typically, the standard deviation or relative standard deviation of a sequence of data is used to express the precision of an analytical approach [26]. Precision may be either the degree of reproducibility or of the repeatability of the analytical procedure under normal conditions. Intermediate precision (also known as ruggedness) expresses within laboratories variations, as on different days, or with different analysts or equipment within same laboratory. Precision of an analytical procedure is determined by assaying a sufficient number of aliquots of a homogeneous sample to be able to calculate statistically valid estimates of standard deviation or relative standard deviation [27].

Accuracy (Recovery): 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. Applying the procedure to samples that have known amounts of analyte added allows for the determination of Lt. To make sure there is no interference, these should be compared to both standard and blank solutions. The accuracy is then computed as a percentage of the analyte recovered by the assay using the test findings. It may often be expressed as the recovery by the assay of known, added amounts of analyte [26,9].

Solution Stability: The stability of standards and samples is established during validation under typical conditions, during regular storage conditions, and occasionally inside the instrument to ascertain whether extra storage conditions, such as refrigeration or light protection, are required [9].

Limit of Detection (LOD): The lowest amount of analyte in a sample that can be identified but not necessarily quantitated as an accurate value is known as the Limit of Detection (LOD) of a specific method. The LOD can be predicated on a Signal-to-Noise (S/N) ratio (3:1), which is typically reported as the concentration of analyte in the sample, in analytical techniques that exhibit baseline noise. As s = H/h, the signal-to-noise ratio can be calculated. Where H is the height of the component-related peak. H is the absolute value of the biggest noise fluctuation from the chromatogram of a blank solution's baseline [9,27].

Limit of Quantification (LOQ): The smallest amount of analyte in a sample that can be quantitatively identified with adequate precision and accuracy is known as the Limit of Quantitation (LOQ) or quantitation limit of a specific analytical process. The LOQ is typically estimated from a determination of S/N ratio (10:1) for analytical procedures like HPLC that exhibit baseline noise, and is typically confirmed by injecting standards that give this S/N ratio and have an acceptable percent relative standard deviation as well [26,27].

Robustness: The Limit of Quantitation (LOQ) or quantitation limit of a certain analytical technique is the smallest amount of analyte in a sample that can be quantitatively recognized with sufficient precision and accuracy. For analytical techniques like HPLC that exhibit baseline noise, the LOQ is typically estimated from a determination of S/N ratio (10:1) and is typically confirmed by injecting standards that give this S/N ratio and have an acceptable percent relative standard deviation as well [26].

System Suitability: System suitability tests are an integral part of liquid chromatographic methods. They are used to confirm that the chromatographic system's detection sensitivity, resolution, and reproducibility are sufficient for the intended analysis. The tests are founded on the idea that the tools, electronics, analytical processes, and test samples make up a whole system that may be assessed as such. Peak resolution, theoretical plate count, peak tailing, and capacity are just a few examples of factors that have been examined to assess the applicability of the employed method [27,24].

Public Health Importance of -Hplc Technique

The chromatography has been preferred in the medical methods used for the identification and quantification of a drug and its metabolites [28]. The HPLC is possibly best known for its use with pharmaceuticals. The technique is used to analyses drug and medicines for their purity, to maintain the highest standards for pharmaceutical products with end goal of helping patient with medical issues. The HPLC method was developed by which not only immunoreactive but also immunounreactive albumin is measured [29]. Using this method, more patients are found to have an albumin excretion in the micro albuminuric range [9].

The purpose high performance liquid chromatography analysis of any drug is to confirm the identity of a drug and provide quantitative results and also to monitor the progress of the therapy of a disease. Whether patients who are detected as having micro albuminuria by HPLC are equally at risk for progressive renal and cardiovascular disease as those who are detected by the traditional antibody-based methods has yet to be determined. Whichever method is chosen, it is preferable to measure albumin in fresh samples [30]. These methods all require laboratory facilities. Antibody based dipstick tests for micro albuminuria also are available [31,32]. Although only semi quantitative, these tests have the advantage that they can be used easily by the general practitioner or the patient at home. Quantitative tests of the active moiety in samples of drug substance or drug product or other selected component(s) in the drug product.

Conclusion and Recommendations

HPLC techniques are continuous and interrelated processes that measure a parameter as intended and establish the performance limits of the measurement. The technique is used to analyses drug and medicines for their purity, to maintain the highest standards for pharmaceutical products with end goal of helping patient with medical issues. The chromatography has been preferred in the medical methods used for the identification and quantification of a drug and its metabolites in recent years. The choice of column, buffer, detector, wavelength, and other conditions (organic and pH) composition has a significant impact on the selectivity of the separation. The validation procedures help to document that a particular protocol used by the accredited laboratory has guaranteed performance in that particular laboratory. An analytical method is validated when it has been proven through laboratory tests that its performance characteristics are appropriate for the intended analytical application. Any new or modified method needs to be validated to make sure it can produce repeatable and reliable results when applied by various operators using the same equipment in the same or other laboratories. In terms of accuracy, specificity, linearity, limit of detection, and limit of quantification, high performance liquid chromatography methods offer good validation properties.

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