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HIGH PRESSURE LIQUID CHROMATOGRAPHY: A REVIEW

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ABSTRACT

High performance liquid chromatography (HPLC) is an essential analytical tool in assessing drug product. HPLC methods should be able to separate, detect, and quantify the various and drug related degradants that can from on storage or manufacturing, detect and quantify any drugs and drug-related impurities that may be introduced during synthesis. Validation is the process of establishing the performance characteristics and limitations of a method and identification of the influences which may change these characteristics and to what extent. This article discusses the strategies and the issue pertinent to designing HPLC method development and validation.

INTRODUCTION:

Analytical chemistry is used to determining the qualitative and quantitative composition of material under study. Both these aspects are necessary to understand the sample material. Analytical chemistry is divided into two branches quantitative and qualitative. A qualitative analysis gives us the information about the nature of sample by knowing about the presence or absence of certain components. A quantitative analysis provides numerical information as to the relative amount of one or more of this component. For analyzing the drug samples in bulk, pharmaceutical formulations and biological fluids, different analytical methods are routinely being used.

In non-instrumental, the conventional and physicochemical property are use to analyze the sample. The instrumental methods of analysis are based upon the measurements of some physical property of substance using instrument to determine its chemical composition. The instrumental methods are simple, precise and reproducible as compared to classical methods. Therefore, analytical methods developed using sophisticated instruments such as spectrophotometer, HPLC, GC and HPTLC have wide applicants in assuring the quality and quantity of raw materials and finished products.

Chromatography can be described as a mass transfer process involving adsorption using a non polar stationary phase and a mobile molar phase titrating through the column. The active component of the column, the sorbent or the stationary phase, is typically a granular material made of solid particles (e.g. silica, polymers etc.), 2-50 in size. The components of the sample mixture are separated from each other by means of mobile phase and different degrees of interaction with the sorbent particles based on their relative polarity. The pressurized liquid is typically a mixture of solvents (e.g. water, acetonitrile and/or methanol). Its composition and temperature plays a major role in the separation process by influencing the interactions taking place between sample components and sorbent. These interactions are physical in nature, such as hydrophobic, dipole-dipole or ionic.

High performance liquid chromatography (HPLC) is a chromatographic technique used to separate a mixture of compounds in analytical chemistry and biochemistry with the purpose of identifying, quantifying or purifying the individual components of the mixture. Before the invention of HPLC, chemists had column chromatography at their disposal, and column chromatography was time consuming.

To speed up a classic column chromatography, chemists would have to use a short column for separation, however this lead to poor separation of molecular components held within solution. The basic setup of a classic column chromatography would include the column that varied in I.D. from 10 to 50nm and column lengths of 50-500cm.

CHROMATOGRAPHY

Chromatography is a technique used for separation of the components of mixture by continuous distribution of the component between two phases. One phase moves (mobile phase) over the other phase (stationary phase) in a continuous manner. Chromatography according to USP can be defined as a procedure by which solute are separated by a differential migration process in a system consisting of two or more phases, one of which move continuously in a given direction.

PRINCIPLE OF CHROMATOGRAPHY

ADSORPTION CHROMATOGRAPHY: When the stationary phase is a solid and mobile phase is liquid or gaseous phase, it is called Adsorption chromatography.

Examples: Thin layer chromatography, column chromatography, Gas-solid chromatography.

Partition chromatography: When the stationary phase and mobile phase are liquid, it is called Partition Chromatography.

Example: Paper partition chromatography, Gas-liquid chromatography.

Theory of Chromatography

Two theoretical approaches have been developed to describe the processes involved in the passage of solutes through a chromatographic system.

The Plate Theory

According to Martin and Synge, a chromatographic system consists of discrete layers of theoretical plates. At each of these, equilibration of the solute between the mobile and stationary phases occurs. The movement of solute is considered as a series of stepwise transfers from plate to plate.

The Rate Theory

This theory considered the dynamics of the solute particles as it passes through the void space between the stationary phase particles in the system as well its kinetic as it is transferred to and from the stationary phase.

Phases of Chromatography

Normal Phase Chromatography: In Normal Phase mode the stationary phase is polar and the mobile phase is non polar in nature. In this technique, non polar compounds and the stationary phase. Polar compounds are retained for longer times because of their higher affinity with the stationary phase. These compounds, therefore take more times to elute. Normal phase mode of separation is therefore, not generally used for pharmaceutical applications because most of the drug molecules are polar in nature and hence take longer time to elute.

Reversed Phase Chromatography: It is the most popular mode for analytical and preparative separations of compound of interest in chemical, biological, pharmaceutical, food and biomedical sciences. In this mode, the stationary phase is non polar hydrophobic packing with octyl or octa decyl functional group bonded to silica gel and the mobile phase is polar solvent. The polar compound gets eluted first in this mode and non polar compounds are retained for longer time. As most of the drugs and pharmaceuticals are polar in nature, they are not retained for longer times and hence elute faster. The different columns used are Octa Decyl Silane (ODS) or C_{18}, C_8, C_4 (in the order of increasing polarity of the stationary phase.). An aqueous mobile phase allows the use of secondary solute chemical equilibrium (such as ionization control, ion suppression, ion pairing and complexation) to control retention and selectivity.

Ion Exchange Chromatography: the stationary phase contains ionic groups like NR_3^+, SO_3^- which interact with the ionic groups of the sample molecules. This is suitable for the separation of charged molecules only. Changing the pH and salt concentration can modulate the retention.

Ion Pair Chromatography: This technique is also referred to as Reversed Phase Ion Pair Chromatography or Soap Chromatogaphy. It may be used for the separation of ionic compounds and this method can also substitute for Ion Exchange Chromatography, Strong acidic and basic compounds may be separated by reversed phase mode by forming ion pairs (coulumbic association species formed between two ions of opposite electric charge) with suitable counter ions.

Affinity Chromatography: this technique uses highly specific biochemical interactions for separation. The stationary phase contains specific groups of molecules which can absorb the sample if certain steric and charge related conditions are satisfied. This technique can be used to isolate proteins, enzymes as well as antibodies from complex mixtures.

Size Exclusion Chromatography: It separates molecules according to their molecular mass. Largest molecules are eluted first and the smallest molecules last. This method is generally used when mixture contains compounds with a molecular mass difference of at least 10%. This mode can be further subdivided into gel permeation chromatography (with organic solvents) and gel filtration chromatography (with aqueous solvents).

Method Development

Analytical method development and validation play important roles in the discovery development and manufacture of pharmaceuticals. These methods used to ensure the identity, purity, potency, & performance of drug products. There are many factors to consider when developing methods. The initially collect the information about the analytic's physiochemical properties (pKa, log P, solubility) and determining which mode of detection would be suitable for analysis in case of UV detection). The majority of the analytical development efforts go into validating a stability indicating HPLC-method. 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 degradant [2-4].

There are many steps involve in method development which are:

- Physicochemical properties of drug.
- Set up HPLC conditions.
- Sample preparation.
- Method optimization.
- Validation of developed method.

Your desired solvent mixture travels through capillary tubes, from the solvent reservoir to the pump, where it is becomes highly pressurized. The pump is also used to control the flow rate of the mobile phase substance, which is typically measured in mL/minute. The prepared sample is then injected into the line, where it travels with the solvent into the HPLC column. There are many different columns you can choose from, depending on the sample will stick to the silica in the column and detach at different times, making them distinguishable from one another. The detector detects when these molecules detach from the silica and reports the data in the form of a chromatogram. Various types of detectors can be used such a UV-VIS, fluorescence, or an evaporative-light scattering detector (ELSD). Once the solvent has traveled through the column it goes into a waste continer, or can be collected if desired.

The parameters of the HPLC, like any instrument, are important and are dependant on your sample. The solvent mixture, containing a strong solvent and a weak solvent, will depend on whether your sample is polar in nature-or not. Common solvents include water, methanol and acetonitrile. Two different solvent methods can be use, isocratic or gradient. With start with a 100:0 ratio of weak solvent: strong solvent and increase in increments over time to the final mixture ratio. It's important to flush the system before running your samples in order to insure that the solvents used for the previous sample does not interfere with your samples. The mobile phase flow rate is important and can range from 1-10 mL/min, though 1 mL/min is a good place to start with most experiments. It's important to monitor pressure when adjusting the flow rate, as the pressure should not exceed 400 bar. The injection can also vary in volume, anywhere from 0.1-100.0 . For concentrated samples, 3-5 is appropriate and 25 for dilute samples. Starting with an injection of 10 is typical. The temperature can be adjusted but for most samples 25 ^{o}C is adequate. The temperature setting should never exceed 50-60 ^{o}C. You also need to know what wavelengths in the UV-VIS spectrum you want to monitor. A diode array detector has a range of 210-400 nm for samples, the default program setting are fine. The HPLC at UAF is an Agilent 1100 Series and is located in downstairs instrument room.

Physicochemical properties of drug:

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. In a non-polar covalent bond, the electrons are shared equally between two atoms. A polar covalent bond in one in which one atom has a greater attraction for the electrons than the other atom. The solubility of molecules can be explained on the basis of the polarity of molecules can be explained on the basis of the polarity of molecules. Polar, e.g. water, and non-polar, 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 analyte must be soluble in the diluents and must not react with any of the diluents components. The diluents should match to the starting eluent composition of the assay to ensure that no peak distortion will occur, especially for early eluting components. pH and pK_a 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= - $\log_{10}$ (H_3O^+). The acidity or basicity of a substance is defined moist typically by the pH value. Selecting a proper pH for ionizable analytes often leads to symmetrical and sharp peaks in HPLC.

Buffering Capacity is the ability of the buffer to resist changes in pH.

- Buffering Capacity increases as the molar concentration (molarity) of the buffer salt/acid solution increases.
- The closer the buffered pH is to the pKa, the greater the buffering Capacity.
- Buffering Capacity is expressed as the molarity of Sodium Hydroxide required to increase pH by 1.0.

Consideration of the affect of pH on analyte retention, type of buffer to use, and its concentration, solubility in the organic modifier and its affect on detection are important in reversed- phase chromatography (RPC) method development of ionic analytes. An improper choice of buffer, in terms of buffering species, ionic strength and pH, can result in poor or irreproducible retention and tailing in reverse-phase separation of polar and ionizable compounds [10-12].

Buffer concentration

Generally, a buffer concentration of 10-50 mM is adequate for small molecules. Generally, no more than 50% organic should be use 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. Phosphate buffers can be replaced with sulfate buffers when analyzing organophosphate compounds [13].

Selection of detector

Detector is a very important part of HPLC. Selection of detector depends on the chemical nature of analytes, potential interference, limit of detection required, availability and/or cost of detector. UV-Visible detector is versatile, dual-wavelength absorbance detector for HPLC. This detector offers the high sensitivity required for routine UV-based applications to low-level impurity identification and quantitative analysis. Photodiode Array (PDA) Detector offers advanced optical detection for Waters analytical HPLC, preparative HPLC, or LC/MS system solutions. Its integrated softeware and optics innovations deliver high chromatographic and spectral sensitivity. Refractive Index (RI) Detector offers high sensitivity, stability and reproducibility, which make this detector the ideal solution for analysis of

components with limited or no UV absorption. Multi-Wavelength Fluorescence Detector offers high sensitivity and selectivity fluorescence detection for quantitating low concentrations of target compounds [14-15].

Column Selection

The heart of a HPLC system is the column. Changing a column will have the greatest effect on the resolution of analytes during method development. Generally, modern reverse phase HPLC columns are made by packing the column housing with spherical silica gel beads which are coated with the hydrophobic stationary phase. The stationary phase is introduced to the matrix by reached a chlorosilane with the hydroxyl groups present on the silica gel surface. In general, the nature of stationary phase has the greatest effect on capacity factor, selectivity, efficiency and elution. There are several types of matrices for support of the stationary phase, including silica, polymers, and alumina. Silica is the most common matrix for HPLC columns. Silica matrices are robust, easily derivatized, manufactured to consistent sphere size, and does not tend to compress under pressure. Silica is chemically stable to most organic solvents and to low pH systems. One shortcoming of a silica solid support is that it will dissolve above pH 7. In recent years, silica supported columns have been developed for use at high pH. The nature, shape and particle size of the silica support effects, separation. Smaller particle results in a greater number of theoretical plates, or increased separation efficiency. However, the use of smaller particles also results in increased backpressure during chromatography and the column more easily becomes plugged.

CONCLUSION: This review describes the general technique of HPLC method development and validation of optimized method. The general approach for the method development for the separation of pharmaceutical compounds was discussed.

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