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HIGH PERFORMANCE LIQUID CHROMATOGRAPHY: A SHORT REVIEW

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Abstract: High performance liquid chromatography (HPLC) is an important qualitative and quantitative technique, generally used for the estimation of pharmaceutical and biological samples. It is the most versatile, safest, dependable and fastest chromatographic technique for the quality control of drug components. This article was prepared with an aim to review different aspects of HPLC, such as principle, types, instrumentation and application.

Keywords: High performance liquid chromatography, instrumentation, elution, applications, mobile phase.

INTRODUCTION

High-performance liquid chromatography (or High pressure liquid chromatography, HPLC) is a specific form of column chromatography generally used in biochemistry and analysis to separate, identify, and quantify the active compounds. [1] HPLC mainly utilizes a column that holds packing material (stationary phase), a pump that moves the mobile phase(s) through the column, and a detector that shows the retention times of the molecules. Retention time varies depending on the interactions between the stationary phase, the molecules being analyzed, and the solvent(s) used. [2] The sample to be analyzed is introduced in small volume to the stream of mobile phase and is retarded by specific chemical or physical interactions with the stationary phase. The amount of retardation depends on the nature of the analyte and composition of both stationary and mobile phase. The time at which a specific analyte elutes (comes out of the end of the column) is called the retention time. Common solvents used include any

miscible combinations of water or organic liquids (the most common are methanol and acetonitrile). [2, 3] Separation has been done to vary the mobile phase composition during the analysis; this is known as gradient elution. [3] The gradient separates the analyte mixtures as a function of the affinity of the analyte for the current mobile phase. The choice of solvents, additives and gradient depend on the nature of the stationary phase and the analyte.

TYPES OF HPLC

Types of HPLC generally depend on phase system used in the process. [3, 4] Following types of HPLC generally used in analysis-

Normal phase chromatography: Also known Normal phase HPLC (NP-HPLC), this method separates analytes based on polarity. NP-HPLC uses a polar stationary phase and a non-polar mobile phase. The polar analyte interacted with and is retained by the polar stationary phase. Adsorption strengths increase with

increased analyte polarity, and the interaction between the polar analyte and the polar stationary phase increases the elution time.

Reversed phase chromatography:

Reversed phase HPLC (RP-HPLC or RPC) has a non-polar stationary phase and an aqueous, moderately polar mobile phase. RPC operates on the principle of hydrophobic interactions, which result from repulsive forces between a polar eluent, the relatively non-polar analyte, and the non-polar stationary phase. The binding of the analyte to the stationary phase is proportional to the contact surface area around the non-polar segment of the analyte molecule upon association with the ligand in the aqueous eluent.

Size exclusion chromatography: Size exclusion chromatography (SEC), also called as gel permeation chromatography or gel filtration chromatography mainly separates particles on the basis of size. It is also useful for determining the tertiary structure and quaternary structure of proteins and amino acids. This technique is widely used for the molecular weight determination of polysaccharides.

Ion exchange chromatography: In Ion-exchange chromatography, retention is based on the attraction between solute ions and charged sites bound to the stationary phase. Ions of the same charge are excluded. This form of chromatography is widely used in purifying water, Ligand-exchange chromatography, Ion-exchange chromatography of proteins, High-pH anion-exchange chromatography of carbohydrates and oligosaccharides, etc. [3, 4]

Bio-affinity chromatography: Separation based on specific reversible interaction of proteins with ligands. Ligands are covalently attached to solid support on a bio-affinity matrix, retains proteins with interaction to the column-bound ligands.

Proteins bound to a bioaffinity column can be eluted in two ways:

- Biospecific elution: inclusion of free ligand in elution buffer which competes with column bound ligand.
- Aspecific elution: change in pH, salt, etc. which weakens interaction protein with column-bound substrate.

Because of specificity of the interaction, bioaffinity chromatography can result in very high purification in a single step (10 - 1000-fold).

PARAMETERS

For the accurate analysis of a compound, there are some parameters which are used as a standard for a particular compound. If there is a change occurs in the parameters the result may be affected greatly. The most commonly used parameters are internal diameter, particle size, pore size, pump pressure. For different compounds the parameters can be changed according to their nature and chemical properties.

Internal diameter: The internal diameter (ID) of an HPLC column is a critical aspect that determines quantity of analyte that can be loaded onto the column and also influences sensitivity. Larger columns are usually seen in industrial applications such as the purification of a drug product for later use. Low ID columns have improved sensitivity and lower solvent consumption at the expense of loading capacity.

Particle size: Most traditional HPLC is performed with the stationary phase attached to the outside of small spherical silica particles (very small beads). Smaller particles generally provide more surface area and better separations, but the pressure required for optimum linear velocity increases by the inverse of the particle diameter squared.

Pore size: Many stationary phases are porous to provide greater surface area.

Small pores provide greater surface area while larger pore size has better kinetics especially for larger analytes. Pore size defines an ability of the analyte molecules to penetrate inside the particle and interact with its inner surface. This is especially important because the ratio of the outer particle surface to its inner one is about 1:1000. The surface molecular interaction mainly occurs on the inner particle surface.

Pump pressure: Pumps vary in pressure capacity, but their performance is measured on their ability to yield a consistent and reproducible flow rate. Modern HPLC systems have been improved to work at much higher pressures, and therefore be able to use much smaller particle sizes in the columns (< 2 micrometres).

INSTRUMENTATION

Injection of the sample: Septum injectors are available; using which sample solution is injected. Sample can be injected when the mobile phase is flowing or it is stopped. A new advanced rotary valve and loop injector can be used to produce reproducible results.

The detector: There are several ways of detecting when a substance has passed through the column. Generally UV spectroscopy is attached, which detect the specific compounds. Many organic compounds absorb UV light of various wavelengths. The amount of light absorbed will depend on the amount of a particular compound that is passing through the beam at the time.

Interpreting the output from the detector: The output is recorded as a series of peaks, each one representing a compound in the mixture passing through the detector and absorbing UV light. The area under the peak is proportional to the amount of substance, which is passed through detector, and this area can be

calculated automatically by the computer linked to the display.

APPLICATION

The information that can be obtained using HPLC includes identification, quantification, and resolution of a compound. Preparative HPLC refers to the process of isolation and purification of compounds. This differs from analytical HPLC, where the focus is to obtain information about the sample compound.

Chemical Separations It is based on the fact that certain compounds have different migration rates given a particular column and mobile phase, the extent or degree of separation is mostly determined by the choice of stationary phase and mobile phase.

Purification: Purification is defined as the process of separating or extracting the target compound from a mixture of compounds or contaminants. Each compound showed a characteristic peak under certain chromatographic conditions. The migration of the compounds and contaminants through the column need to differ enough so that the pure desired compound can be collected or extracted without incurring any other undesired compound.

Identification Generally assay of compounds are carried using HPLC. The parameters of this assay should be such that a clean peak of the known sample is observed from the chromatograph. The identifying peak should have a reasonable retention time and should be well separated from extraneous peaks at the detection levels which the assay will be performed.

Other applications of HPLC: Other applications of HPLC includes

Pharmaceutical applications [5-8]

- Tablet dissolution study of pharmaceutical dosages form.
- Shelf-life determinations of pharmaceutical products
- Identification of active ingredients of dosage forms
- Pharmaceutical quality control

Environmental applications [9-12]

- Detection of phenolic compounds in Drinking Water
- Identification of diphenhydramine in sedimented samples
- Bio-monitoring of pollutant

Forensics [13-15]

- Quantification of the drug in biological samples.
- Identification of anabolic steroids in serum, urine, sweat, and hair
- Forensic analysis of textile dyes.
- Determination of cocaine and metabolites in blood

Clinical [16-19]

- Quantification of ions in human urine
- Analysis of antibiotics in blood plasma.
- Estimation of bilirubin and bilivirdin in blood plasma in case of hepatic disorders.
- Detection of endogenous neuropeptides in extracellular fluids of brain.

Food and Flavor [20]

- Ensuring the quality of soft drink and drinking water.
- Analysis of beer.
- Sugar analysis in fruit juices.
- Analysis of polycyclic compounds in vegetables.
- Trace analysis of military high explosives in agricultural crops.

CONCLUSION

It can be concluded from the entire review that HPLC is a versatile, reproducible chromatographic technique for the estimation of drug products. It has wide applications in different fields in term of quantitative and qualitative estimation of active molecules.

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