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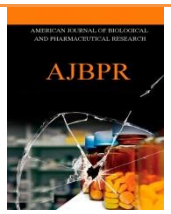

A OVERVIEW ON HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

High Performance Liquid Chromatography (HPLC) has been used for the analysis of natural and synthetic compounds. It is also employed to separate manufactured drugs from drug related impurities, to detect and quantify synthesized drug and to reduce other impurities at the time of separation. 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.

INTRODUCTION

Chromatography is probably the most powerful analytical technique available to the modern chemist. Its power arises from its capacity to determine quantitatively many individual components present in mixture by single analytical procedure [1-2]. 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 [3]. Now a day reversed-phase chromatographyis the most commonly used separation technique in HPLC due to its broad application range. It is estimated that over 65% (possibly up to 90%) of all HPLC separations are carried out in the reversed phase mode. The reasons for this include the simplicity, versatility, and scope of the reversed-phase method as it is able to handle compounds of a diverse polarity and molecular mass [4-6].

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TYPES OF HPLC

The Following types of HPLC generally used in analysis

Reverse Phase HPLC

Reversed phase chromatography has found both analytical and preparative applications in the area of biochemical separation and purification. Molecules that possess some degree of hydrophobic character can be separated by reversed phase chromatography with excellent recovery and resolution [7]. Uses water-organic as mobile phase, columns may be C18 (ODS), C8, phenyl, Trimethyl Silane (TMS), cyano as a stationary phase. It is first choice for most samples especially neutral or non ionized compounds, that dissolve in water organic mixtures.

Normal Phase HPLC

In this the mixtures of organic solvents for mobile phase and columns i.e. cyano, diol and amino silica can be used as stationary phase. It is first choice for mixtures of

isomers and for preparative scale HPLC and second choice for lipophilic samples that cannot dissolve well in water-organic mixtures [8].

Ion-exchange chromatography

In ion-exchange chromatography the stationary bed has an ionically charged surface of opposite charge to the sample ions. This technique is used almost exclusively with ionic or ionizable samples. The stronger the charge on the sample, the stronger it will be attracted to the ionic surface and thus, the longer it will take to elute. The mobile phase is an aqueous buffer, where both pH and ionic strength are used to control elution time [9].

Size exclusion chromatography

In size exclusion chromatography the column is filled with material having precisely controlled pore sizes, and the sample is simply screened or filtered according to its solvated molecular size. Larger molecules are rapidly washed through the column; smaller molecules penetrate inside the porous of the packing particles and elute later. Mainly for historical reasons, this technique is also called gel filtration or gel permeation chromatography although, today, the stationary phase is not restricted to a gel [9].

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:

1. Biospecific elution: inclusion of free ligand in elution buffer which competes with column bound ligand.
2. 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) [10].

Parameters to describe a HPLC column

The parameters used to describe a HPLC column refer to the nature, type and size of its contents, the dimensions of the column and the materials used in its construction [11].

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 [10].

Detection

It is essential to use reagents and solvents of high purity to ensure minimum detection limits for optimum sensitivity. All organic solvents and many additives, such as ion pairing agents, absorb in the UV range and the detection limit is related to the wavelength[12]. A large number of LC detectors have been developed over the past thirty years based on a variety of different sensing principles for detecting the analytes after the chromatographic separations.

However, only about twelve of them can be used effectively for LC analysis and, of those twelve, only four are in common use. The four dominant detectors used in LC analysis are the UV detector (fixed and variable wavelength), the electrical conductivity detector, the fluorescence detector and the refractive index detector. These detectors are employed in over 95% of all LC analytical applications. The choice of detector depends on the sample and the purpose of the analysis [12].

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 [10].

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.

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 [10].

Other applications of HPLC

Other applications of HPLC includes

Pharmaceutical applications

1.Tablet dissolution study of pharmaceutical dosage form [13-17].
2.Shelf-life determinations of pharmaceutical products.
3.Identification of active ingredients of dosage forms.
4.Pharmaceutical quality control.

Clinical applications

1.Detection of endogenous neuropeptides in extracellular fluids of brain.
2.Quantification of ions in human urine Analysis of antibiotics in blood plasma.
3.Estimation of bilirubin and bilivirdin in blood plasma in case of hepatic disorders [18-21].

Table 1. Parameters used to describe a HPLC column

Parameter	Description
Packing/matrix	The finely divided material with which the column is packed, usually silica. It can be used as the stationary phase in adsorption chromatography or a bonded phase is attached for use in partition chromatography.
Bonded Phase	The stationary phase is chemically bonded to the packing/matrix.
Particle size	The size of the particles in the column (if applicable), usually measured in microns.
Pore Size	The size of the pores in the particles/monolith, usually measured in angstroms.
Length	The length of the column, usually measured in cm or mm.
Diameter	The internal diameter of the column, usually measured in mm.
Hardware	The material used to construct the external tubing,end fittings of the column
Manufacturer	The name of the manufacturer of the column.

CONCLUSION

HPLC applications can be used effectively for screening analysis as well as quality evaluation of natural as well as synthetic compounds. Owing to the simplicity and efficiency of HPLC specific and rapid determination of various natural and synthetic compounds can be carried out. HPLC can be employed for the routine analysis of natural and synthetic compounds in pharmaceutical formulations and in bulk drug preparations as well as for the quality assurance of related extracts and market samples.

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