WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 12, Issue 7, 409-414. Review Article ISSN 2277– 7105

A REVIEW ON HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

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Article Received on
12 March 2023,

Revised on 02 April 2023,
Accepted on 23 April 2023

DOI: 10.20959/wjpr20237-28003

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ABSTRACT

Chromatography is defined as a set of techniques which is used for the separation of constituents in a mixture. Chromatography is the backbone of separation science and is being used in all research laboratories and pharmaceutical industries universally. It is an analytical procedure which is developed to test a defined characteristic of the drug substance or drug product against established acceptance criteria for that characteristic.

INTRODUCTION

The term "Chromatography" refers those processes aimed at the separation of the various species of a mixture on the basis of their distribution characteristics between a stationary and a mobile phase. Mikhail S. Tswett, a Russian botanist in 1930 coined the name chromatography from the Greek words chroma meaning colour, and graphein meaning to write. Chromatography comprises a group of methods for separating molecular mixtures that depends on the differential affinities of the solutes between two immiscible phases. The fixed phase is called the stationary phase, and the other is termed as mobile phase. The stationary phase may be a porous or finely divided solid, or a liquid that has been coated in a thin layer on an inert supporting material. It is necessary that the stationary phase particles be as small and homogenous as possible to provide a large surface area so that sorption and desorption of the solute will occur frequently. Depending on the type of chromatography employed, the mobile phase may be a pure liquid or a mixture of solutions or it may be a gas.

Chromatographic methods are classified according to the nature of the stationary and mobile phases. If the stationary phase is solid, the process is called adsorption chromatography. If the mobile phase is a liquid, the process is called liquid - solid chromatography (LSC) where as if

the mobile phase is a gas the method is called gas – solid chromatography (GSC). When the stationary phase is a liquid, it is termed as partition chromatography. If the mobile phase is a liquid, this is called liquid - liquid chromatography (LLC). If the mobile phase is gas, it is termed as gas – liquid chromatography (GLC). LSC and GSC have the unique nature of their separation processes. These are ion – exchange, size – exclusion and affinity chromatography.

When liquid chromatography is carried out in a single plane it is called as planar liquid chromatography. When the liquid mobile phase passes through a solid stationary phase (strip of paper) it is known as paper chromatography. When silica gel is immobilized on a glass slide that is known as thin layer chromatography (TLC). Column chromatography refers to liquid chromatography which is performed on a three dimensional stationary phase packed inside of a glass, plastic, metal column can be used for both preparative and analytical purpose.

High - performance liquid chromatography (HPLC) may be defined as a technique in an analytical chemistry used to separate, identify, quantify each component in a mixture. The solvent reservoirs are glass containers which consist of pure organic solvent or aqueous solutions of salts or buffers. The particles that are used to pack HPLC columns are small enough less than 50 micro meter to prevent solvent flow by gravity, pumps that develop pressure up to 5000 psi are needed to force the mobile phase through the column. The next components are solvent – conditioning column, is used only under special circumstances. Most HPLC column – packing materials are prepared from silica gel, which will dissolve slowly in solvents whose pH value are below 2 or above 7. This results in a shrinkage of the packing materials, giving rise to void spaces in which separated solutes remix or diluted, which leads to a loss of resolution. Therefore, to minimize this occurrence and to protect the expensive silica – based packing materials, are small column (5 to 10 cm) packed with HPLC grade silica gel is inserted into the liquid streams after the pump but before the injector. The solute mixture is introduced into the chromatography by means of a suitable injection device. The guard column is packed with a stationary phase identical to that in the main column, except that it's particle size may be larger.

The analytical column, in which the actual separation takes place, is a stainless steel tube, usually 5 to 25 cm in length, it's internal diameter is of 2 to 4.6 mm. The materials used to pack the column are of two types superficially porous, or pellicular, and totally porous. The

pellicular substances consist of a layer of porous stationary phase coated on a solid core, usually are glass bead. The totally porous materials are microparticulate and are available in sizes of 3,5,10 and 20 micrometre with the particle – size distribution closely controlled. Their average pore diameter is of about 8 nm. Packings also are available with chiral stationary phases for the separation of optical isomers. The most frequently used instrument is an ultraviolet visible spectrometer that has been fitted with a flow cell of very small (8 microlitre). Fixed wavelength models are also available at 280 nm, where the aromatic amino acid of protein and peptides absorb, or at 240 nm, where isolated double bond such as the carbonyl group absorb.

PRINCIPLE Of HPLC

The specific intermolecular interaction between the molecules of a sample and the packing material define their time "on – column. HPLC is a separation technique that involves the injection of a small volume of liquid sample into a column packed with tiny particles 3 to 5 micron in diameter called stationary phase where individual components of the sample are moved through the packed column with a liquid mobile phase forced through the column by high pressure delivered by a pump. These components are separated from one another by the column packing that involves various chemical or physical interactions between their molecules and packing particles. These separated components are detected at the exit of this tube by a flow – through device (detector) that measure their amount. An output from this detector is called a liquid chromatogram.

INSTRUMENTATION

The HPLC instrumentation involves pump, injector, column, detector, integrator and display system. In the column the separation occurs. The part include:

a) Solvent Reservoir: The contents of mobile phase are present in glass container. In HPLC the mobile phase or solvent is a mixture of polar and non polar liquid components. Depending on the composition of sample, the polar and non polar solvents will be varied.
b) Pump: The pump suctions the mobile phase from solvent reservoir and forces it to column and then passes to detector 42000 KPa is the operating pressure of the pump. This operating pressure depends on column dimensions, particle size, flow rate and composition of mobile phase.
c) Sample Injector: The injector can be solitary infusion or a computerized infusion framework. An injector for a HPLC framework should give infusion of the fluid specimen

inside the scope of 0.1 ml to 100 ml of volume with high reproducibility and under high pressure.

d) Columns: Columns are typically made of cleaned stainless steel, are somewhere around 50 mm and 300 mm long and have an inward distance across of somewhere around 2 and 5 mm. They are generally loaded with stationary phase. Preferably the temperature of the molecule phase and the column should be kept consistent during investigation.

e) Detector: The HPLC detector, situated toward the end of the column distinguishes the analytes as they elute from the chromatographic column. Regularly utilized detectors are UV-spectroscopy, fluorescence, mass-spectrometric and electrochemical identifiers.

f) Data Collection Devices or Integrator: Signals from the detector might be gathered on graph recorders or electronic integrators that fluctuate in many sided quality and in their capacity to process chromatographic information. The PC coordinates the reaction of the indicator to every part and places it into a chromatographic that is anything but difficult to interpret.

TYPES OF HPLC

Depending on the substrate used i.e. stationary phase used, the HPLC is divided into following types.

a) Normal Phase HPLC: In this method the separation is based on polarity. The stationary phase is polar, mostly silica is used and the non-polar phase used is hexane, chloroform and diethyl ether. The polar samples are retained on column.

b) Reverse Phase HPLC: It is reverse to normal phase HPLC. The mobile phase is polar and the stationary phase is non polar or hydrophobic. The more is the non-polar nature the more it will be retained.

c) Size – exclusion HPLC: The column will be incorporating with precisely controlled substrate molecules. Based on the difference in molecular sizes the separation of constituents will occur.

d) Ion – exchange HPLC: The stationary phase is having ionically charged surface opposite to the sample charge. The mobile phase used is aqueous buffer which will control pH and ionic strength.

APPLICATIONS OF HPLC

HPLC has several application applications in the fields of pharmacy, forensic, environment The and clinical. It also helps in the separation and purification of compound.

a) **Pharmaceutical Applications:** The pharmaceutical applications include controlling of drug stability, dissolution studies and quality control.
b) **Environmental Applications:** Monitoring of pollutants and detecting components of drinking water.
c) **Forensic Applications:** Analysis of textile dyes, quantification of drugs and steroids in biological samples.
d) **Food and Flavour Applications:** Sugar analysis in fruit juices, detecting polycyclic compounds in vegetables, analysis of preservatives.
e) **Clinical Applications:** Detecting endogenous neuropeptides, analysis of biological samples like blood and urine.

CONCLUSION

The HPLC is mostly used in analytical technique. It is having several advantages. With the use of HPLC one can produce extremely pure compounds. It can be used in both laboratory and clinical science. With the use of HPLC the accuracy, precision and specificity can be increased. The only disadvantage of HPLC is its high cost.

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