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A Review on High Performance Liquid Chromatography

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Review Article

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ABSTRACT

Several liquid chromatographic methods are developed for a systematic analysis of various degradation products. High-performance liquid chromatography is the most widely used analytical separation technique because of its reproducibility sensitivity and suitability for separating nonvolatile species which makes the method ideal for accurate quantitative determinations. A reversed-phase HPLC method with UV detection is developed for simultaneous separation and quantitation of organic acids and neutral degradation products present in the corn stover hydrolysate. On the other hand inorganic ions and some organic anions which are present in the water extractive from corn stover are separated and quantitated by a developed ion chromatographic method with conductivity detection. Sugars and alditols are also determined using high-performance anion chromatography with pulsed amperometric detection.

INTRODUCTION

High Performance Liquid Chromatography

Technique which has gained large popularity during the last decade is **High performance liquid chromatography** [1,2]. The technique is very much useful to pharmaceutical analysts in analysing complex formulation containing number of ingredients as it permits simultaneous separation and determination of components of mixture.

High Performance Liquid Chromatography (HPLC) is one mode of widely used analytical techniques in chromatography. Chromatographic process can be defined as separation technique involving mass-transfer between stationary and mobile phase. Only about 20% of known compounds lend themselves to analysis by Gas chromatography both because they are insufficiently volatile and cannot pass through the column or because they are thermally unstable and decompose under the conditions of separation [3-5]. HPLC is not limited by sample volatility or Thermal stability. HPLC is able to separate macromolecules and ionic species labile natural products polymeric materials and interactive mobile phase.

Types of Hplc Techniques

Normal phase chromatography

In Normal phase chromatography (NPC) the stationary phase is more polar than the mobile phase the opposite of Reverse phase chromatography (RPC). usually the mobile phase is a mixture of Organic solvents without added water (isopropanol+Hexane) and the column packing is either an inorganic adsorbent (silica or alumina) or a polar

bonded phase (Cyano diol or amino) on a silica support. Regardless of the mobile or stationary phase used sample retention increases as the polarity of the mobile phase decreases.

Reverse phase chromatography

The Reverse phase HPLC consists of a non polar stationary phase and a polar mobile phase and was developed due to increasing interest in large non-polar bi molecules. Commonly used stationary phase is silica.

Mechanism of reverse phase chromatography

When a solute dissolves in water the strong attractive forces between water molecules become distorted or disrupted [6-9]. These attractive forces arise from the three dimensional network of intermolecular hydrogen bonds. Only highly polar or ionic solutes can interact with the water network. Non polar solutes are "Squeezed out" of the mobile phase but bind with the hydrogen moieties of the stationary phase.

Ion exchange chromatography

The ion exchange resins used as stationary phases in Ion exchange chromatography consists of synthetic cross linked polymers containing covalently bonded ionisable functional groups. Anionic groups are used in cation exchange resins and cationic groups are used in anion exchange resins. The counter ion of the bonded ion is relatively free to dissolve in the aqueous mobile phase as it flows through column; i.e the ion exchange resin behaves as a poly electrolyte.

Size exclusion chromatography

It is also called as Gel permeation chromatography or Gel filtration chromatography. It is a chromatographic technique in which separation is based on the molecular size of the sample components. The stationary phase consists of porous particles that have a fixed pore size. A sample component which is too large to enter the pores is immediately swept from the column with mobile phase [10,11]. A sample component which is small enough to enter the pores completely is held on the column longest and a component which can partially enter the pores has an intermediate retention volume. Component separation is dependent upon the ability of the components to enter the pores of the stationary phase.

Bio-affinity chromatography

This chromatographic process relies on the property of biologically active substances to form stable specific and reversible complexes [12]. The formation of these complexes involves the participation of common molecular forces such as the Vander Waals interaction electrostatic interaction dipole-dipole interaction hydrophobic interaction and the hydrogen bond. An efficient biospecific bond is formed by a simultaneous and concerted action of several of these forces in the complementary binding sites.

INSTRUMENTATION

HPLC instrumentation is made up of seven basic components they are Mobile phase reservoir solvent delivery system or high pressure pump sample inlet device column detector Data processor and Drain.

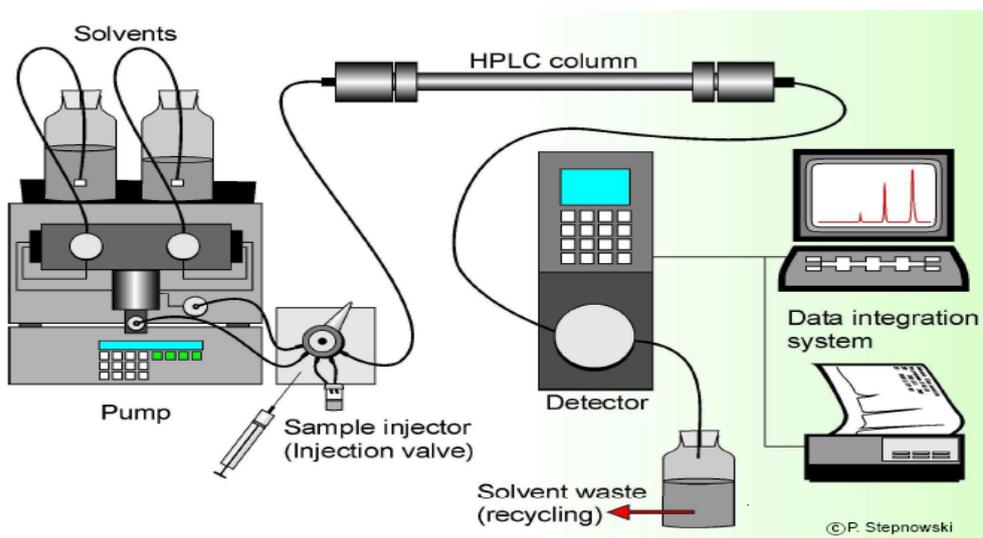


Figure 1: A Block Diagram of HPLC

Mobile phase reservoir filtering

The most common type of solvent reservoir is a glass bottle. Generally containers that are clean and inert can be used as reservoirs and they should have a capacity of 1 or 2 litres. Most of the manufacturers supply these bottles with special caps Teflon tubing and filters to connect to the pump inlet and to the purge gas (helium) used to remove dissolved air. Helium purging and storage of the solvent under helium is not sufficient for degassing aqueous solvents. It is useful to apply a vacuum for 5-10 min and then keep the solvent under a helium atmosphere (**Figure 1**).

No air bubbles should be present and small particles present should be cleaned otherwise they can be collected in a column and cause void ^[13-15]. The mobile phase should be prepared by using HPLC grade water.

Solvent delivery system (pumps)

High pressure pumps are needed to force solvents through packed stationary phase beds. Smaller bed particles require higher pressures. There are many advantages in using smaller particles but they may not be essential for all separations. The most important advantages are:

- Higher resolution
- Faster analyses and
- Increased sample load capacity.

Different types of pumps are available based on constant pressures and displacements. They are

1. Pneumatic or constant pressure pumps.
2. Constant displacement pumps.
 - (a) Syringe type pumps.
 - (b) Reciprocating pumps

Constant pressure pumps produce a heartbeat less move through the segment however any reduction in porousness of the segment will bring about lower stream rates for which the pump won't adjust ^[16-20]. These pumps work by presentation of high weight gas into pump and the gas thus constrains the dissolvable from the pump chamber into the segment. The utilization of a middle of the road dissolvable between the gas and the eluting dissolvable diminishes the odds of broke up gas straightforwardly entering the eluting dissolvable and bringing on issues amid the investigation.

Constant displacement pumps maintain a consistent flowrate through the segment regardless of changing conditions inside the section. One type of steady uprooting pump is an engine driven syringe sort pump where a settled volume of dissolvable is constrained from the pump to the section by a cylinder driven by an engine. Such pumps and in addition giving uniform dissolvable stream rates ^[21-24] likewise yields a pulseless dissolvable stream which is essential as specific locators are touchy to change in dissolvable stream rate.

Two different elution Techniques are used

- Isocratic elution
- Gradient elution

Isocratic elution

A partition in which the mobile phase arrangement stays steady all through the strategy is termed as isocratic. In isocratic elution top width increments with maintenance time directly as indicated by the condition for N the quantity of hypothetical plates. This prompts the impediment that late-eluting tops get level and expansive. Their shape and width may keep them from being perceived as crests. In isocratic elution the selectivity does not change if the section measurements (length and internal width) change – that is the tops elute in the same request.

Gradient elution

The mobile phase sythesis does not stay consistent. A slope division is one in which the mobile phase piece is changed amid the partition procedure ^[25,26]. One case is a slope beginning at 10% methanol and consummation at 90% methanol following 20 minutes. Angle elution diminishes the maintenance of the later-eluting segments with the goal that they elute quicker giving smaller (and taller) tops for generally parts. This likewise enhances the crest shape for followed tops as the expanding convergence of the natural eluent pushes the following part of a top forward. This likewise builds the top stature (the top looks "more honed") which is vital in follow examination.

Sample inlet device

Sample can be injected by using a syringe through Injection port. Two positions

1. Inject
2. Load

In inject position sample is injected into injection port and then it is loaded to send the drug sample inside for mixing with mobile phase ^[27].

Column

Typical HPLC columns are 5 10 15 and 25 cm in length and are filled with small diameter (3 5 or 10 μm) particles ^[28-33]. The internal diameter of the columns is usually 4.6 mm; this is considered the best compromise for sample capacity mobile phase consumption speed and resolution. Generally Stainless steel columns are used as they are inert to corrosion.

Detector

The detector of HPLC plays vital role in monitoring solutes that are eluted from the column. Two types of detectors

Bulk property detector

A detector that measures a property of both the solute and mobile phase ^[34-40]. Ex- Refractive index detector.

Solute property detector

A detector that measures a property of solute just. Ex-UV finder.

These are more delicate than mass and most generally utilized as a part of HPLC.

By and large a detector is chosen that will react to a specific property of the substances being isolated and preferably it ought to be delicate to atleast 10-8g/ml and give a straight reaction over a wide fixation range ^[41].

Photometric Detectors

These normally operate in the ultraviolet region of the spectrum and are most extensively used detectors in Pharmaceutical Analysis ^[42-48]. They comprise essentially a light source a dispersing device to select an appropriate wavelength for measurement a flow cell in which the absorbance of eluate is measured and a photomultiplier tube or diode to measure the intensity of transmitted light. Photometric detectors of five types:

- Single wavelength detectors
- Multi wavelength detectors
- Variable wavelength detectors
- Programmable detectors

Refractive index detector

These are differential refractometers which react to change in the mass property of the refractive list of the arrangement of the segment in the versatile dissolvable framework. The refractive list locator is the nearest way to deal with the general finder in that some dissolvable is typically accessible in which the specimen offers ascend to a quantifiable contrast in refractive file amongst dissolvable and arrangement ^[49-55]. In spite of the fact that the affectability of refractive file finder is a great deal not as much as that of particular solute property indicators they are helpful for the location of substances which don't show different properties that can be utilized as premise for particular identification ^[56-62].

Fluorescence detector

These are basically channel fluorimeters or spectrofluorimeters outfitted with uniting monochromators and miniaturized scale stream cells ^[63-78]. Their affectability relies on upon the fluorescence properties of the segments in the eluate .For the substances that are Fluorescent Fluorescence identifiers are for the most part more delicate than photometric finders especially if excitation and outflow monochromators are set at the wavelengths of greatest excitation and Fluorescence of the mixes ^[79-85].

Electrochemical Detectors

These depend on standard electrochemical standards including amperometry voltammetry and polarography ^[86-90]. The finders are extremely touchy for substances that are electroactive i.ethose that experience oxidation or diminishment at an appropriate potential and they have discovered specific test of low levels of catecholamines in organic tissues pesticides and numerous medications ^[91].

Mass Detectors

Mass Spectrometry is the most widespread of the locators since it recognizes most natural mixes and is very specific when particular ionization methods are utilized. Disconnected LC-MS where the analyte is gathered focused and examined by Mass spectrometry is a generally basic practice in the pharmaceutical Industry^[92-95]. At the point when the compound gathered part is precarious on-line LC-MS methods are favored. Fluid chromatography thermospray-mass spectrometry has been used to decide the character and immaculateness of taxol. Electrospray LC-MS has been utilized for peptide mapping of the human development hormone^[96,97].

Data processor

Since the locator sign is electronic utilizing present day information gathering systems can help the sign investigation. What's more a few frameworks can store information in a retrievable structure for exceedingly advanced PC investigation at a later time^[98-100].

CONCLUSION

HPLC is probably the most universal type of analytical procedure; its application areas include quality control process control forensic analysis environmental monitoring and clinical testing. In addition HPLC also ranks as one of the most sensitive analytical procedures and is unique in that it easily copes with multi-component mixtures. It has achieved this position as a result of the constant evolution of the equipment used in LC to provide higher and higher efficiencies at faster and faster analysis times with a constant incorporation of new highly selective column packings.

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