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Review Article

Review on Methods and Applications of High-Performance Liquid Chromatography

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ABSTRACT

High-Performance Liquid Chromatography is a technique used to separate components from the mixture. The technology consists of two phases, the stationary phase and the mobile phase. Component separation is based on differences in partition coefficients in two phases. HPLC is a form of liquid chromatography used to separate compounds dissolved in solution and qualitative and quantitative techniques used to evaluate pharmaceutical and biological samples. HPLC is the most widely used analytical separation technique for at quality control of pharmaceuticals. This mini-reviews include many aspects of HPLC, including type, instruments, and applications.

Keywords: High-performance liquid chromatography; Instrumentation; Applications

INTRODUCTION

High-Performance Liquid Chromatography (HPLC) involves separations in which the components to be separated are distributed between two immiscible phases. One of these phases is the mobile phase, and the other is a stationary phase [1,2]. There are three modes of chromatographic operation elution, frontal, and displacement. In elution, the sample components are placed or injected at the beginning of the chromatographic system. Suppose the system is composed of a column. In that case, the parts elute from the column is according to their distributions between the stationary and the mobile phases [3], the concentration distribution being most often symmetrical and Gaussian. Symmetrical peaks occur when the tiny sample size is used [4]. The highest efficiency occurs in this case, and consequently, at high speed, the liquid chromatography sample size is maintained tiny [5].

There are different types of interaction, which may occur during the separation, and these are referred to as modes of separation.

The mechanism of interactions categorized as follows:

- Adsorption
- Partition
- Bonded phase
- Ion exchange
- Size exclusion
- Affinity

INSTRUMENTATION

- Pump
- Injector
- Column
- Detector
- Recorder or data system

Pump

A pump forces the mobile phase through the column at a much higher velocity than gravity-flow columns. The pump is designed to maintain a constant flow rate and avoid pulsations even when the composition of the mobile phase changes [6].

Injectors

The injector is primarily used to inject the sample in liquid form. Two types of injectors are manual injector and automated injector [6].

Columns

The column is a significant component of HPLC, and its importance needs to be detailed. An HPLC column, which is silica-based packing's, is the most widespread and commonly used system [7].

Detector

The HPLC detector monitors the eluent to produce an electronic signal proportional to each separated component's concentration as it leaves the column [8].

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The most widely used detectors:

- Refractive Index Detector
- Fluorescence Detector
- Evaporative Light Scattering Detector
- Conductivity Detector

Data recording

When detection is complete, the detected signal is converted to an electrical signal that is then amplified by an amplifier and recorded as a chromatogram in data points. Then use the software as the display format according to the conversion requirements manually or using the software [9-11].

The development of the HPLC method mainly deals with two key stages:

- Mobile phase
- Stationary phase

APPLICATIONS

- Analyzing complex mixtures, Purifying chemical compounds
- To survey food and drug products
- To identify confiscated narcotics
- To determine the amount of chemical compounds found in new drugs in Pharmaceutics [12-14].
- Pharmaceutical applications
- Environmental applications
- Forensics
- Clinical
- Food and flavor

Chromatography involves separations due to differences in the equilibrium distribution of sample components between two immiscible phases. One of these phases is moving are the mobile phase, and the other is a stationary phase. [15-18].

There are three modes of chromatographic operation elution, frontal, and displacement. In elution, the sample components are placed or injected at the beginning of the chromatographic system. If the system is composed **of a** column, the parts elute from the column according to their distributions between the stationary and the mobile phases, the concentration distribution being most often symmetrical and Gaussian [19-21].

Symmetrical peaks occur when the tiny sample size is used. The most excellent efficiency occurs in this case, and consequently, at high speed, the liquid chromatography sample size is maintained very small [22-24].

Chromatography can be described as a mass exchange process involving adsorption. High-performance liquid chromatography relies on pumping through a pressurized fluid and an example of mixing through a portion of the adsorbent Segmentation of sample sections. The cross section's dynamic segment, the adsorbent, is usually a granular material consisting of solid particles (e.g., silica, polymers, etc.) ranging from 2 µm to 50 µm. In a part of this example, mixtures/mixtures are isolated from each other due to their unique degree of connection with the retainer particles. Pressurized fluids are usually mixtures of solvents (such as water,

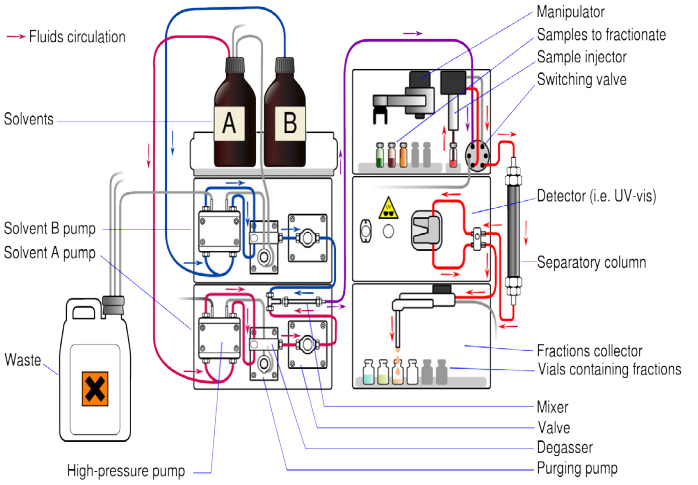

Figure 1: Graphic Illustration of HPLC [25].

acetonitrile, and methanol) and are called "mobile phases." Its structure and temperature play an essential role in the partition process by affecting the connection between the sample segment and the adsorbent [26,27]. High-performance liquid chromatography is considered conventional (low weight) liquid chromatography because operating pressures are fundamentally higher (50 bar to 350 bar).

In contrast, typical liquid chromatography periodically relies on the force of gravity to pass through portable segments [28,29]. Due to the small number of samples isolated in scientific high-performance liquid chromatography, the column section was measured in distances of 2.1 mm to 4.6 mm and lengths of 30 mm to 250 mm. Besides, HPLC fragments are made from smaller sorbent particles (2 µm to 50 µm in average molecular size). This gives HPLC high determination or resolution (ability to identify components) while isolating mixtures, which makes it an outstanding chromatographic method [30].

CONCLUSION

It is concluded that the HPLC is a multipurpose, repeatable qualitative and quantitative technique used for the evaluation of pharmaceutical and biological samples with accuracy, precision, and specificity.

CONFLICT OF INTEREST

No Conflict of Interest.

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