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REVIEW ARTICLE

A review on High Performance Liquid Chromatography

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Abstract: High-Performance Liquid Chromatography (HPLC) stands as a pivotal qualitative and quantitative analytical method extensively applied in the estimation of pharmaceutical and biological samples. Recognized as the most versatile and widely employed separation technique, this review article is dedicated to comprehensively surveying the instrumentation aspects of HPLC. Originating from the pioneering work of Csaba Horvath in 1964, the article explores the evolution, advancements, and key components of HPLC instrumentation, shedding light on its instrumental intricacies and technological innovations that have contributed to its enduring significance in analytical sciences. This review aims to offer researchers, scientists, and practitioners a valuable resource for understanding the technological intricacies that underpin the reliability and efficacy of HPLC in analytical endeavors. Through a historical lens and a contemporary viewpoint, the article showcases the instrumental landscape of HPLC, emphasizing its enduring significance and the continuous innovations that shape its trajectory in analytical sciences

Keywords: High-Performance Liquid Chromatography; Qualitative Analysis; Quantitative Analysis; Csaba Horvath; Instrumentation

1. Introduction

Chromatography technique was invented by Dr. Mikhail Semyonovich Tsvet in 1906 later in 1941 Archer John Porter Martin and Richard Laurence Millington Synge discovered Partition Column Chromatography, they have been awarded Nobel prize in 1952 for their invention. Chromatography is a Separation technique in which the mixtures of components are divided into individual components by using Stationary phase and Mobile phase. The HPLC technique was first developed by Csaba Horvath in 1964 [1]. It was the most versatile and widely used separation technique, this technique is used by chemists to separate various biological, oragnic and inorganic compounds, the pressure in HPLC is 5800-7000 psi due to this high pressure it is also called High Pressure Liquid Chromatography [2]. The main aim of this review is to cover the key aspects of HPLC instrumentation, encompassing advancements in column technologies, detector systems, and mobile phase delivery mechanisms.

2. HPLC

2.1. Principle

The Principle of Separation in HPLC is Adsorption, when sample mixture are introduced in column they travel according to their relative affinity, the compound which has more affinity towards Stationary phase travel slower and the compound which has less affinity towards stationary phase will travel faster since no two compounds have same relative affinity [3].

2.2. Instrumentation

HPLC instrumentation consists of the following main components as illustrated in the Figure 1:

- Solvent Reservoir
- Pumps
- Injectors
- Columns
- Detectors
- Recorders & Integrators

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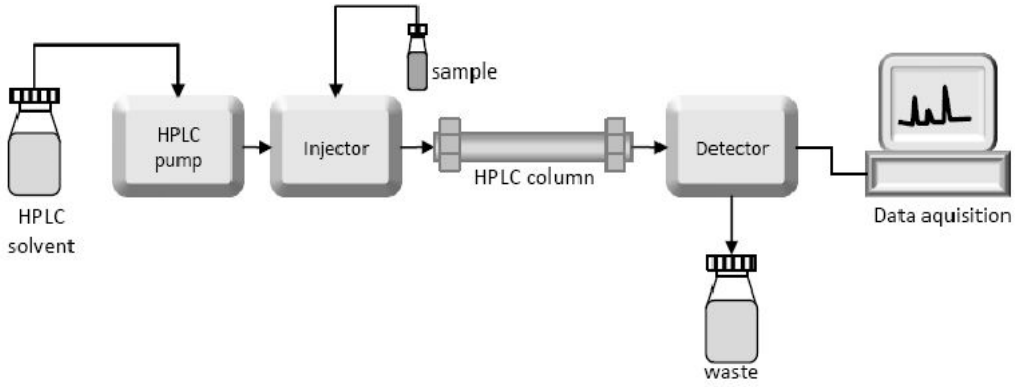

Figure 1: Instrumentation of HPLC

2.2.1. Solvent reservoir

The modern HPLC instrument is equipped with three or more glass reservoirs with capacity of 500ml or more to store the solvents. The solvents are passed from reservoir to mixing chamber to obtain homogenous mixture. A pump capable of maintaining high pressure pushes the solvent through column [4].

2.2.2. Pumps

The Mobile phase employed must passed through column at very high pressure and at controlled flow rate. The pumps are necessary to force the liquid through the column having finely packed particles, it should be noted that high pressure generated by the pumps should not lead to an explosion hazard. The particle size of stationary phase is low [5-10 μ] so the resistance will be high, hence such high pressure is employed [5]

Types of pumps

Three types of pumps are most commonly used:

Constant Displacement pump (or) Syringe pump: It consist of Large syringe like chamber equipped with a plunger that is activated by screw driven mechanism powered by a stepping motor which offers a pulse free flow.

Reciprocating pump: This is mostly used pump in HPLC. In this the solvent is drawn into small chamber and pumped out of it by back and forth motion of motor driven piston. It has many advantages like small internal volume, High output pressure (up to 10000 psi) and constant flow rate, disadvantage is that it offer pulse flow rate which produce a base line noise on chromatogram.

Constant pressure pump (or) Pneumatic pump: These pumps are operated via Gas pressure. The mobile phase is present in a collapsible container that is pressurized by compressed gas. It has many advantages like Pulse free flow and low cost, disadvantage are flow rate depend on pressure output, gradient elution not possible and limited capacity

2.2.3. Injectors

The sample is injected at head of the column with minimum disturbance to column materials. Minimum amount of sample must be injected because overloading of sample leads to band broadening. Different types of injectors used in HPLC are: Septum injectors (or) Syringe injectors, Stop Flow injectors and Rheodyne injectors [6]

Septum injectors (or) Syringe injectors

It is earliest and simplest technique. The sample is injected through self sealing elastomeric rubber septum and syringes are made to withstand pressure up to 1500 psi.

Stop Flow injectors

The Flow of solvent is stopped for a while, a fitting at column head is recovered and sample is injected at atmospheric pressure. Then the fitting is replaced and solvent flow is allowed to flow.

Rheodyne injectors

This is most widely used injector and is used for injecting sample volumes more than 10 µl. This injector has two modes (as shown in the Figure 2)

I) Load position

II) Inject mode

In the load position the sample is loaded in the loop and in inject mode the sample is injected

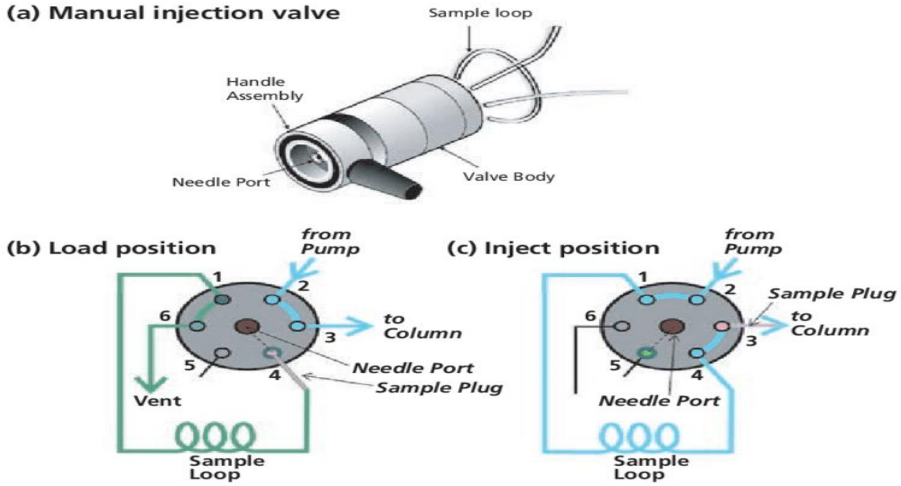

Figure 2: Load position and inject position in Rheodyne injectors

2.2.4. Columns

Columns are considered as heart of HPLC, where the separation occurs. Columns are of different categories like Glass columns, Stainless steel columns. Stainless steel columns are mostly used because they offer less fragility while compared to glass columns [7]. Columns are categorized as follows:

Guard columns: A guard column is a protective column or cartridge present between the injector and the analytical column. It has very small quantity of Adsorbent, thus helps to remove the impurities before reaching the analytical column and improves the life of analytical column but it does not involve in separation of sample [8].

Analytical columns: Analytical columns are the most important part of HPLC which decides the efficiency of separation, different types of columns are available depending on mode of separation.

There are some features that has to be considered meticulously such as

- Column Length: Varies from 5cm to 30cm
- Column Diameter: Ranges from 2mm to 50mm
- Particle size: From 1 µ to 20 µm
- Particle nature: Spherical, Porous materials.
- General dimensions of HPLC column is Length 25cm, inside diameter 4.6mm, packed with particle size 5 µm
- Macro HPLC columns having particle size of 5 µm and afford 40000 to 60000 theoretical plates per meter.
- In Micro HPLC columns the internal diameter ranges from 1 to 4.6 mm, packed with adsorbent (particle) size of 3 to 5 µm, which afford up to 100k theoretical plates per meter, these have advantage like minimal consumption of solvent and maximum desired speed attainable for analysis.

The performance of HPLC column depends exclusively on "Concentration of a band of solute" which critically decreases as it passes trough system, hence the column performance depends upon the amount of spreading that occurs eventually, the column performance can be found by using following equation [9].

$$N=16(VR/WB)^2$$

Where,

VR = Retention volume of an Analyte

WB = Volume duly occupied by an Analyte (or) peak width

Obviously for more efficient column performance WB shall remain smaller at given value of VR.

2.2.5. Detectors

The detector is required to sense the presence and amount of sample in column effluent. The output of detector is electronic signal that is proportional to some property at mobile phase or solute. A detector that measures property which is possessed by both mobile phase and solute is called bulk property detector, alternatively if the property is possessed essentially by solute, this type of detector is called as Solute property detector [10].

A Good detector should have following features

- It should respond to all components of mixture.
- It should not respond to mobile phase.
- It should remain unaffected by changes in temperature and flow rate.
- It should have high sensitivity.
- It should be non-destructive, reliable and easy to use

The commonly used detectors in HPLC are mentioned below:

Mass detector: It is ideal detector to provide molecular weight and structure of component. It has very high sensitivity and selectivity. The detection is based on the molecular fragmentation by electric fields and separation is based on the mass to charge ratio of fragmented molecules.

Fluorescence detector: This has high sensitivity for selected group of components. The component atoms are excited by the use of specific wavelength and then emit a light signal. The emitted light intensity is easy to measure the concentration of components. The compounds having fluorescence can be determined by fluorescence detector. The excitation wavelength and emitted wavelength can be selected for each compound [11].

Refractive Index detector: This is a Universal detector, it measures the refractive index of the molecule that passes through the flow cell. It works on the principle of deflection and reflection of light in solution, several types of RI detectors like Thermal lens detector, Dielectric constant detector, Christiansen effect detector, Interferometer detector are used to identify nonionic analytes.

Electrochemical HPLC detector: This detector is used to determine the molecules exhibiting oxidation-reduction reactions and measure electric current produced from these reactions. The electrochemical detector has high sensitivity and selectivity.

Electrical Conductivity HPLC detector: This detector is used to determine the conductivity. The ion in liquid can carry an electrical charge under the influence of a potential gradient and therefore if a voltage is used across two electrodes located in liquid a current travel among the solution and the electrodes. This detector is reproducible and has good sensitivity of the charged species and surfactants. The measured electronic resistance is directly proportional to the concentration of ions in sample.

Light scattering HPLC detector: This detector is used to determine the scattering light emanating from the eluent. It is useful for those molecules having large molecular weight such as surfactants, lipids and sugars

2.2.6. Detectors

Recorders are used to record the response obtained from detector. They record baseline and all the peaks obtained with respect to time [12].

Integrators are improved version of recorders with some data processing capabilities and provide more information about peaks. They can record individual peaks with retention time, height and width of peaks, peak area, Percentage of area.

3. Applications of HPLC

High-Performance Liquid Chromatography (HPLC) is a versatile analytical technique extensively employed across various scientific domains. Its applications span a wide range of fields, including environmental science, biochemical separations, forensic sciences, the chemical industry, and food analysis [13-17]. The utility of HPLC is showcased in numerous ways:

3.1. Stability Studies of Drug Formulations

HPLC is employed to assess the stability of drug formulations, providing crucial insights into the composition and longevity of pharmaceutical products.

3.2. Detection of Impurities

The technique is adept at identifying impurities within substances, ensuring the purity and safety of pharmaceutical and chemical compounds.

3.3. Analysis of Chiral Purity and Quality of Pharmaceutical Formulations

HPLC enables the precise evaluation of chiral purity, ensuring the quality and effectiveness of pharmaceutical formulations.

3.4. Isolation and Identification of Drugs

The method facilitates the isolation and identification of drugs, a fundamental aspect of pharmaceutical research and development.

3.5. Identification of Intermediates and Target Compounds

HPLC is instrumental in identifying intermediates and target compounds, crucial for understanding reaction pathways in chemical processes.

3.6. Biosynthesis Study

It plays a pivotal role in biosynthesis studies, aiding in the detection of biogenetic intermediates and enzymes involved in biological processes.

3.7. Isolation of Components of Natural or Synthetic Origin

HPLC is utilized to isolate components of both natural and synthetic origin, contributing to the study of diverse materials.

3.8. Biopharmaceutical & Pharmacokinetic Studies

The technique is crucial for studying biopharmaceuticals and conducting pharmacokinetic studies, providing valuable data on drug behavior in biological systems.

4. Conclusion

In summary, this review offers a comprehensive examination of HPLC instrumentation, tracing its evolution from Csaba Horvath's pioneering work in 1964. By dissecting key components and bridging historical perspectives with modern innovations, the article provides valuable insights into HPLC's enduring significance and ongoing advancements. Serving as a crucial resource for researchers, the review highlights the sophistication of HPLC tools and their pivotal role in qualitative and quantitative analyses across diverse scientific disciplines.

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