Libyan International Medical University

Faculty of Pharmacy

High performance liquid chromatography

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Introduction

High performance liquid chromatography (HPLC) is an important qualitative and quantitative technique, generally used for the estimation of pharmaceutical and biological samples. It is the most versatile, safest, dependable and fastest chromatographic technique for the quality control of drug components.

Chromatography : physical method which separation of components takes place between two phases- a stationary phase and a mobile phase

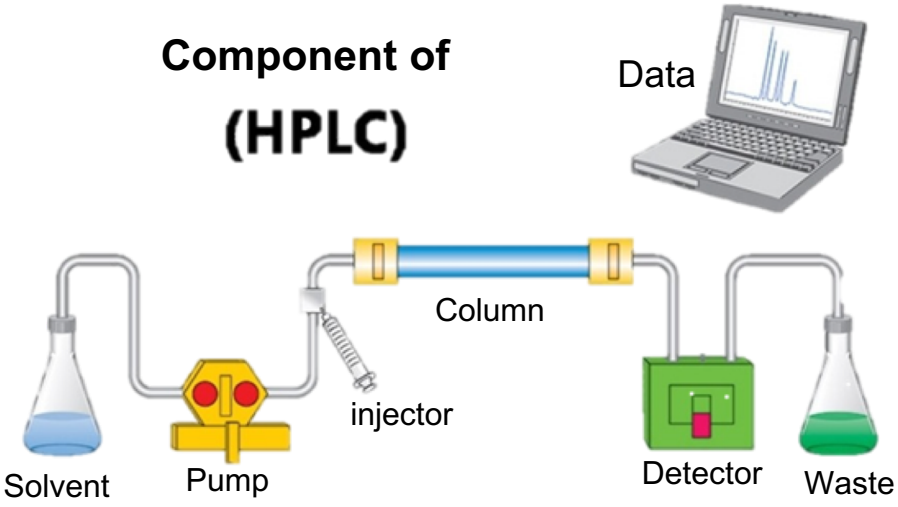
Stationary phase : The substance on which adsorption of the analyte (the substance to be separated during chromatography) takes place .

Mobile phase : solvent which carries the analyte

Principles of HPLC

In principle, LC & HPLC work the same way except the speed, efficiency, sensitivity, & ease of operation of HPLC is vastly superior .

These components are separated from one another by the column packing that involves various chemical or physical interactions between their molecules and the packing particles . These separated components are detected at the exit of this(column) by a flow through device (detector) that measures their amount.



HPLC Detectors

Fluorescence Detectors

used for separations with industry-leading sensitivity and fast sampling

Refractive Index Detector

has a high-performance, easy-to-use refractive index detector that offers excellent stability

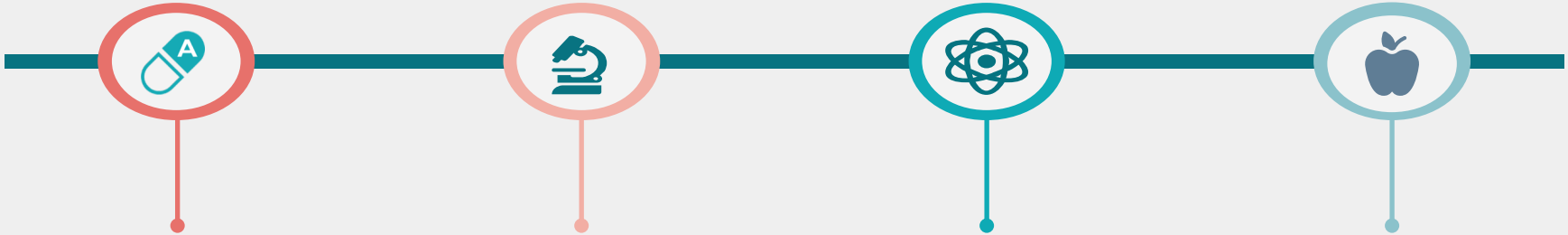
Conductivity Detector

applicable for ion chromatography or organic acid analysis

Types of HPLC

- Normal phase: Column packing is polar (e.g silica) and the mobile phase is non-polar. It is used for water-sensitive compounds, geometric isomers, cis-trans isomers, and chiral compounds.
- Reverse phase: The column packing is non-polar (e.g C18), the mobile phase is water+ miscible solvent (e.g methanol). It can be used for polar, non-polar, ionizable and ionic samples.
- Ion exchange: Column packing contains ionic groups and the mobile phase is buffer. It is used to separate anions and cations.
- Size exclusion: Molecules diffuse into pores of a porous medium and are separated according to their relative size to the pore size. Large molecules elute first and smaller molecules elute later.

Application of HPLC



In Pharmaceuticals

industries HPLC uses separations of Antibiotics, steroids, crude drugs, cosmetics etc

In biochemical

field HPLC uses separation of amino acids, proteins, carbohydrates, lipids, enzymes, hormones etc

Environmental field

Used to separate inorganic ions, organic acids, phenols etc

In Food products

HPLC is very usefull for the separation of various food products like vitamins, mycotoxins, saccharine, fatty acids, colouring agents etc

Advantage and disadvantage of HPLC

Advantage

- Rapid
- Efficiency
- Accuracy
- Versatile and extremely precise

Disadvantage

- Cost: HPLC can be costly, requiring large quantities of expensive organics.
- Complexity
- have low sensitivity for certain compounds, and some cannot be detected as they are irreversibly adsorbed.
- Volatile substances are better separated by gas chromatography.

References

https://www.shodex.com/en/kouza/f.html#

https://www.ssi.shimadzu.com/products/liquid-chromatography/hplc-detectors.html

https://microbenotes.com/high-performance-liquid-chromatography-hplc/

THANKS
ANY QUASTIONS?