

INTRODUCTION



- HPLC stands for “High-performance liquid chromatography”(sometimes referred to as High-pressure liquid chromatography).
- High performance liquid chromatography is a powerful tool in analysis, it yields high performance and high speed compared to traditional columns chromatography because of the forcibly pumped mobile phase.
- HPLC is a chromatographic technique that can separate a mixture of compounds
- It is used in biochemistry and analytical chemistry to identify, quantify and purify the individual components of a mixture.



- Chromatography** : physical method in 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 . It can be a solid, a gel, or a solid liquid combination
- Mobile phase** : solvent which carries the analyte (a liquid or a gas)

Chromatographic techniques are divided into different types based on :



The type of chromatographic bed used
i.e. column chromatography (gas chromatography) and planar chromatography (paper and thin layer)

The physical state of mobile phase
i.e. gas chromatography and liquid chromatography

The separation mechanism
i.e. ion-exchange and size exclusion

HPLC is a type of **liquid chromatography** where the sample is forced through a **column** that is packed with a stationary phase composed of irregularly or spherically shaped particles, a porous monolithic layer, or a porous membrane by a liquid (mobile phase) at high pressure.

COLUMN CHROMATOGRAPHY

Column chromatography involves the following:

1. Adsorption/retention of substance on stationary phase
2. Separation of adsorbed substance using mobile phase
3. Recovery of individual components by continuous flow of mobile phase
4. Quantitative and qualitative analysis of solute and the components which are recovered



PRINCIPLE

To understand the principle of HPLC , we must first look at the principle behind liquid chromatography

Liquid chromatography is a separation technique that involves:

- the placement (injection) of a small volume of liquid sample
- into a tube packed with porous particles (stationary phase)
- where individual components of the sample are transported along the packed tube (column) by a liquid moved by gravity.

The main principle of separation is **adsorption** .