High Performance Liquid Chromatography

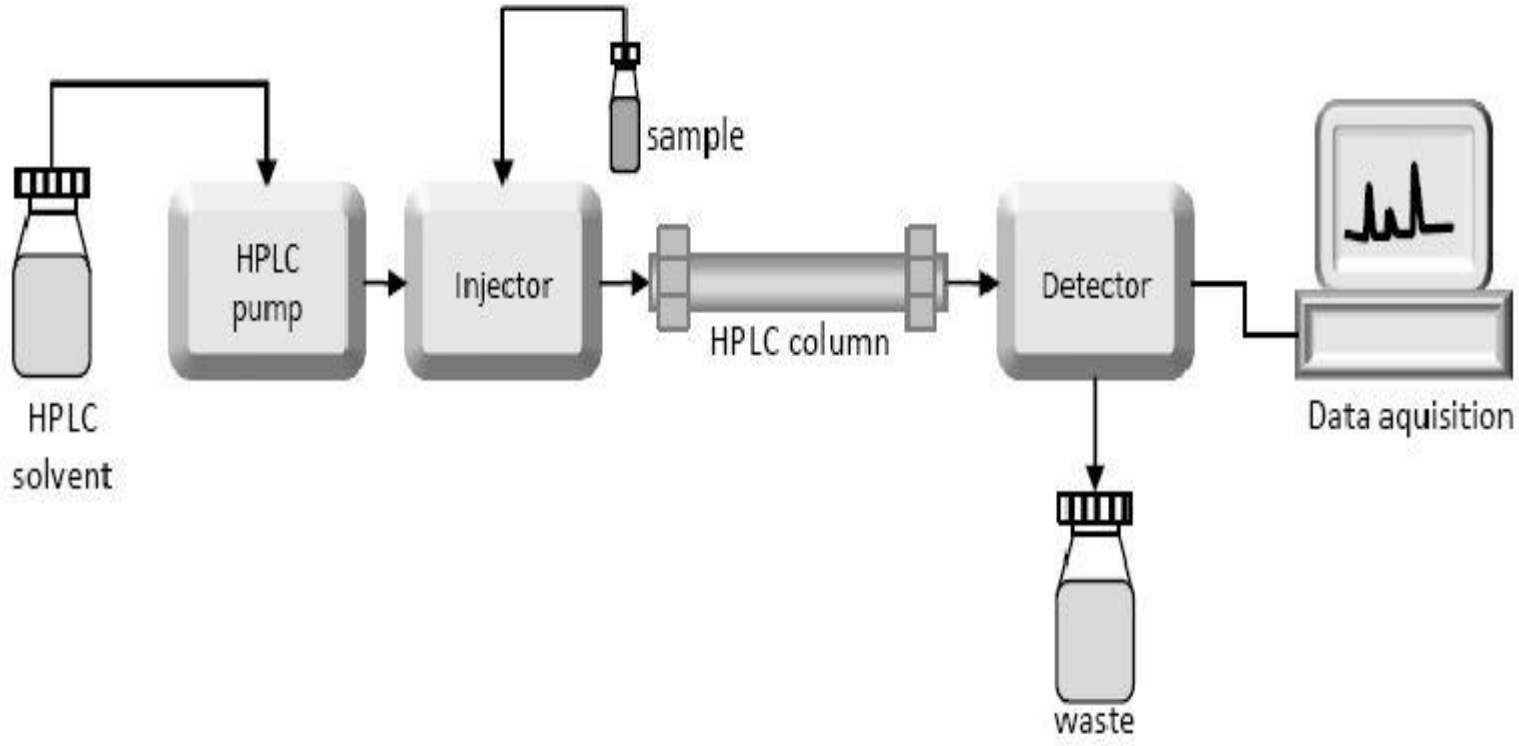
HPLC PRINCIPLE

- HPLC is a separation technique that involves:
- The injection of a small volume of liquid sample into a tube packed with tiny particles (3 to 5 micron (µm) in diameter called the stationary phase)
- Where individual components of the sample are moved down the packed tube (column) with a liquid (mobile phase) forced through the column by high pressure delivered by a pump.

HPLC PRINCIPLE

- These components are separated from one another by the column packing that involves various chemical / physical interactions between their molecules and the packing particles.
- The separated components are detected at the exit of this tube (column) by a flow-through device (detector) that measures their amount.
- An output from this detector is called a “liquid chromatogram”.

Instrumentation



COLUMN

- The heart of a HPLC system is the column.
- The column contains the particles that contains the stationary phase.
- The mobile phase is pumped through the column by a pump.
- Solvents must be degassed to eliminate formation of bubbles .

PUMP

- The role of the pump is to force a liquid(mobile phase) through the liquid chromatograph at a specific flow rate
- A pump can deliver a constant mobile phase composition.

INJECTOR

- The injector serves to introduce the liquid sample into the flow stream of the mobile phase.
- May be auto-sampler or manual

There are a wide variety of stationary phases available for HPLC :

- **Normal Phase**.
  - Polar stationary phase and non-polar solvent.

  E.g. silica gel
- **Reverse Phase.**
  - Non-polar stationary phase and a polar solvent.

  E.g. silica gel

- **Ion exchange**

  Stationary phase contains ionic groups and the mobile phase is an aqueous buffer

- **Size Exclusion**

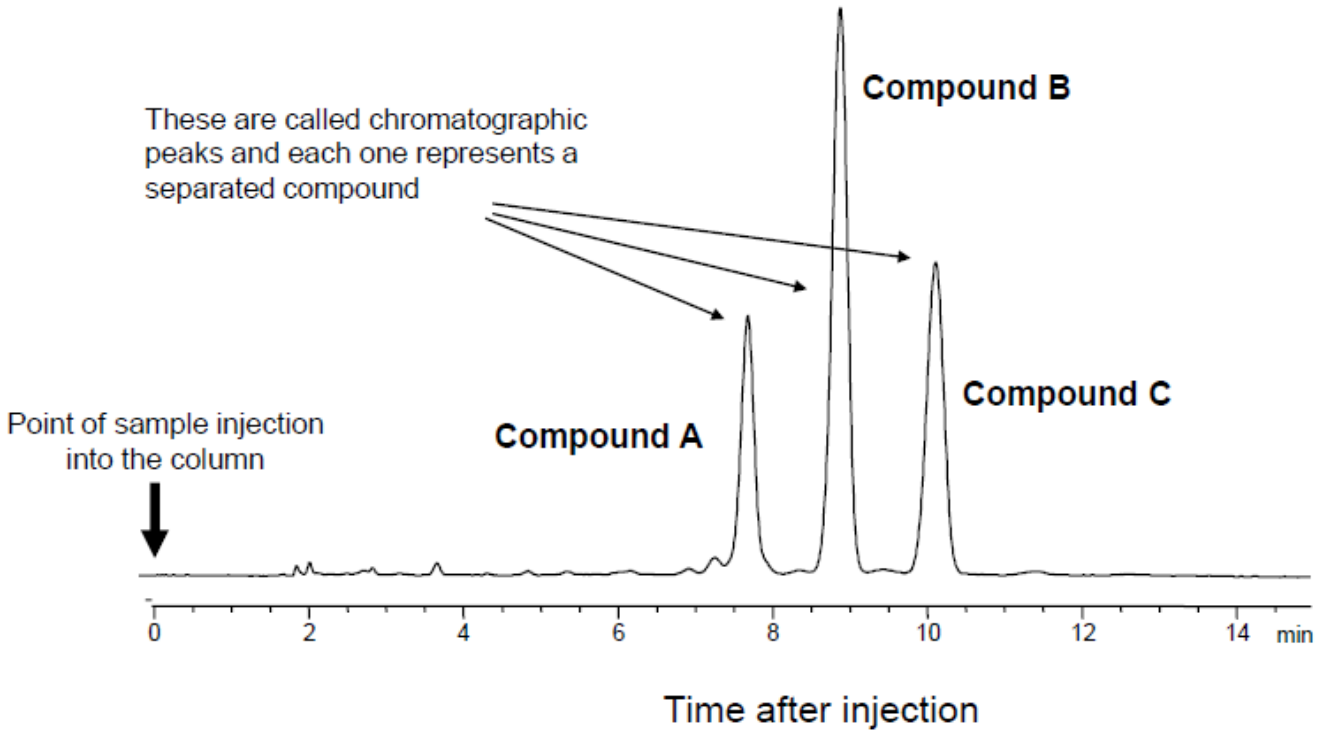
  There is no interaction between the sample compounds and the column.

  Large molecules elute first. Smaller molecules elute later

DETECTOR

- UV photometer is used to measure the concentration of the components

Chromatogram



Pharmaceutical Applications

1.To control drug stability.

2. Tablet dissolution study of pharmaceutical dosages form.

3. Pharmaceutical quality control.

Environmental Applications

1. Detection of phenolic compounds in drinking water.

2. Bio-monitoring of pollutants.

Applications in Forensics

1. Quantification of drugs in biological samples.

2. Identification of steroids in blood, urine etc.

3. Forensic analysis of textile dyes.

4. Determination of cocaine and other drugs of abuse in blood, urine etc.

- **Food and Flavour**
- Measurement of Quality of soft drinks and water.
- Sugar analysis in fruit juices.
- Analysis of polycyclic compounds in vegetables. 4. Preservative analysis.
- **Applications in Clinical Tests**
- Urine analysis, antibiotics analysis in blood.
- Analysis of bilirubin, biliverdin in hepatic disorders.
- Detection of endogenous Neuropeptides in extracellular fluid of brain etc