

HPLC

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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)

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

Basically, all chromatographic systems consists of two phases.

- **Mobile phase** - liquid or gaseous and flows over or through the stationary phase

- **Stationary phase** - solid, liquid or a solid/liquid mixture which is immobilized

Some chromatography terms

Analyte

- Substance that is to be separated during chromatography

Immobilized phase

- Stationary phase which is immobilized on the support particles or on the inner wall of the column tubing

Mobile phase

- Phase which moves in a definite direction. (liquid/gas/fluid).
- Consists of the sample being separated/analyzed and the solvent that moves the sample through the column.

Effluent

- Mobile phase leaving the column.

Different types of chromatography methods

- Paper chromatography
- Liquid chromatography
- Gas chromatography
- High performance liquid chromatography

High performance liquid chromatography

- HPLC is an extension of conventional liquid chromatography.
- Powerful tool in analytical techniques
- Columns are tightly packed, and the eluent is forced through the column under high pressure(up to 5,000 psi) by a pump.

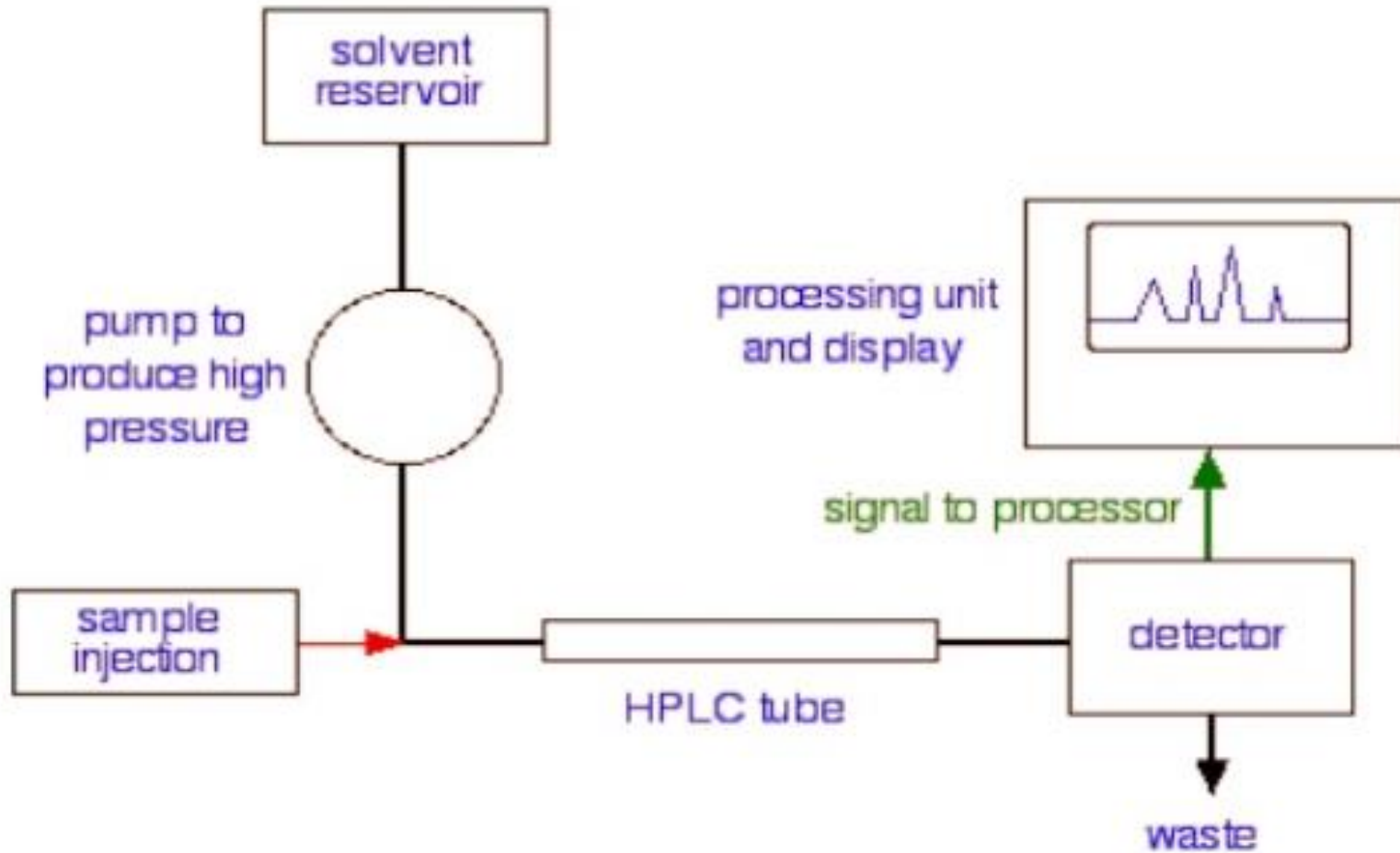
HPLC Technique

- Utilizes liquid mobile phase to separate the mixture
- Analytes are first dissolved in a solvent then through the column under high pressure of up to 400 atm
- Mixture is resolved into its components in the column

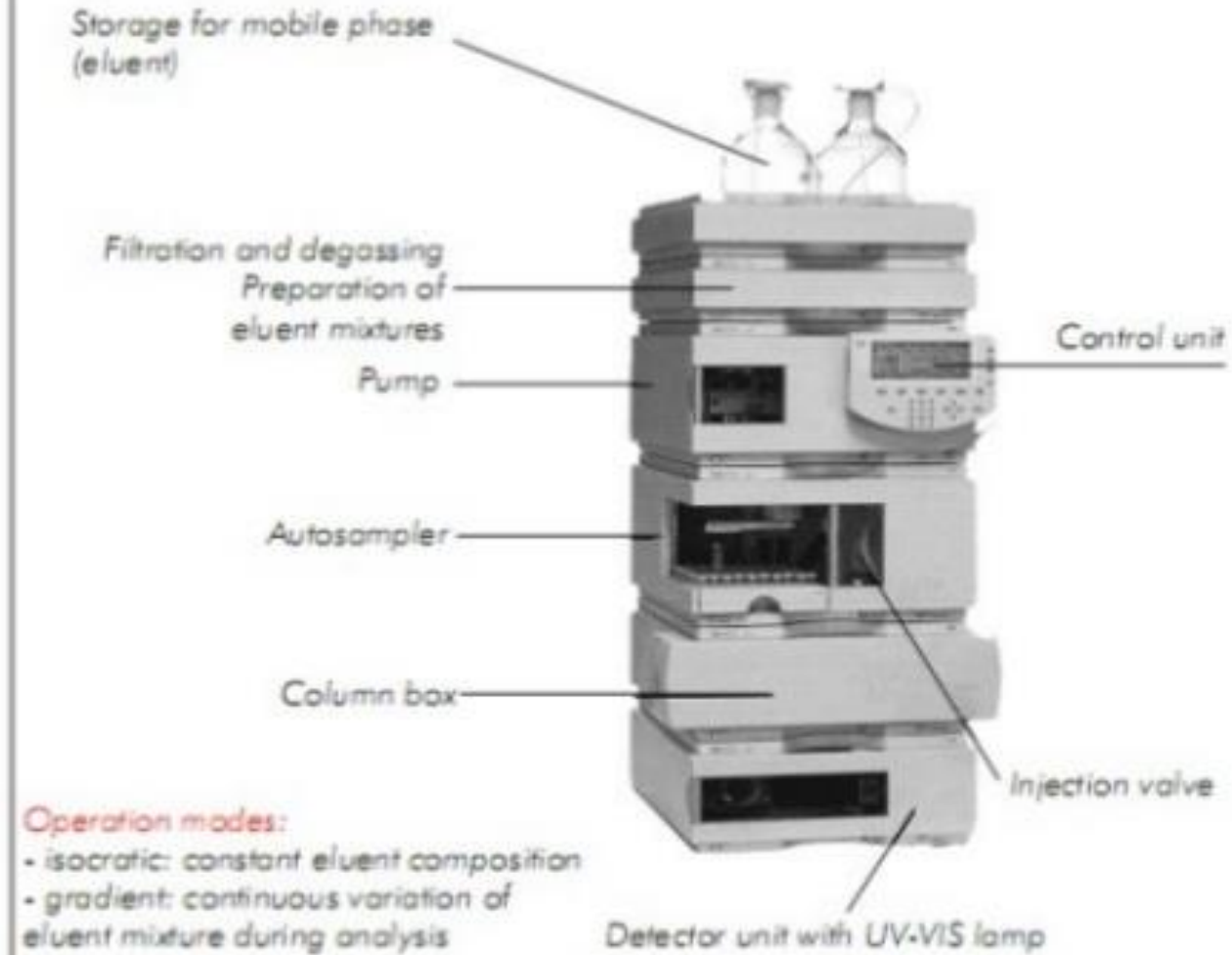
Components of HPLC

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

A Flow Scheme for HPLC



HPLC Setup



Pump

- A pump forces the mobile phase through the column at a much greater velocity than gravity-flow columns.
- The pump can be pneumatic, syringe-type, reciprocating, or hydraulic amplifier.
- Pneumatic pumps are used for preoperative purposes.
- The most widely used pump today is the multihead pump with two or more reciprocating pistons.

- Pumps are designed in order to maintain a stable flow rate, avoiding pulsations even when the composition of the mobile phase varies
- flow range – 0.01-10 ml/min

Injectors

- Inject the liquid sample within range of 0.1- 100 ml of volume under high pressure
- Produce minimum band broadening
- Produce possible flow disturbances
- Volume must be small (0.1-500 μL)

A model injector



Columns

- Smooth-bore stainless steel or heavy-walled glass tubing.
- Hundreds of packed columns differing in size and packing are available from manufacture.
- E.g. Column packing vary in size (3 to 20 μm) with the smaller particles used mostly for analytical separations and the larger ones for preparative separation.
- The most common material used for column packing is silica gel.

HPLC columns



Detector

- ▶ HPLC detectors monitor the elute as it leave the column.
- ▶ Produce an electrical signal proportional to the concentration of separated components.
- ▶ Detector crucial in trace analysis.
- ▶ High sensitivity.
- ▶ Fast response.
- ▶ simplify quantitation.
- ▶ Insensitive to changes in type of solvent.

The most widely used detection methods

- Spectrophotometers
- Fluorometers
- Electrochemical detectors
- Mass spectrometer
- Refractive index detector

Type of HPLC

Depending on the relative polarity of the solvent and stationary phase, there are two variants in use in HPLC

1. Normal phase HPLC

- Utilize polar adsorbent surface and non-polar eluent
- Polar substance in the mixture sticks to polar adsorbent than non-polar
- Non-polar ones will pass more quickly through the column

2. Reversed phase HPLC

- Utilize non-polar adsorbent surface and polar eluent
- Attraction between non-polar compound in the mixture and non-polar adsorbent

2. Reversed phase HPLC (cont.)

- Polar molecules will travel through the column more quickly because there is strong attraction between polar solvent and polar molecules when pass through the column
- Reversed phase HPLC is the most commonly used form of HPLC

Solvent and Absorbents

Solvents used in mobile phase

- hexane, heptane, cyclohexane, carbon tetrachloride, benzene, toluene, diethyl ether, chloroform etc.

Adsorbents used in stationary phase

- silica gel, alumina, celite, cellulose powder, ion-exchange, cellulose, starch

Retention time

- The time taken for a particular compound to travel through the column to the detector
- From the time at which the sample is injected to the point at which the display shows a maximum peak height for that compound.

Applications

- ▶ HPLC is used for
- ▶ Chemistry and biochemistry research analyzing complex mixtures.
- ▶ Purifying chemical compounds.
- ▶ Analyzing air and water pollutants.
- ▶ To survey food and drug products.
- ▶ To identify narcotics.

HPLC as compared with the classical techniques

- ▶ Small diameter, reusable stainless steel columns.
- ▶ Columns Packing with small particles.
- ▶ Control flow of mobile phase.
- ▶ Precise sample introduction.