HPLC

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Chromatography

Chromatography is a **method of separation** in which the components to be separated are distributed between two phases, one of these is called a **stationary phase** and the other is a **mobile phase** which moves on stationary phase in a definite direction.

Chromatography Basics

A component which is quite soluble in the stationary phase will take longer to travel through it than a component which is not very soluble in the stationary phase but very soluble in the mobile phase.

As a result of these differences in mobilities, sample components will become separated from each other as they travel through the stationary phase.

Classification of chromatography

A. On the basis of interaction of solute to the stationary phase;

- Adsorbtion Chromatography
- Partition Chromatography
- Ion Exchange Chromatography
- Size Exclusion Chromatography

Classification of chromatography

B. On the basis of physical state of mobile phase

- Liquid Chromatography (LC)

Liquid-Solid

Liquid-Liquid

- Gas Chromatography (GC)

Gas-Solid

Gas-Liquid

Classification of chromatography

C. On the basis of chromatographic bed shape;

- Two Dimensional

Paper Chromatography

Thin Layer Chromatography (TLC)

- Three Dimensional

Column Chromatgoraphy (CC)

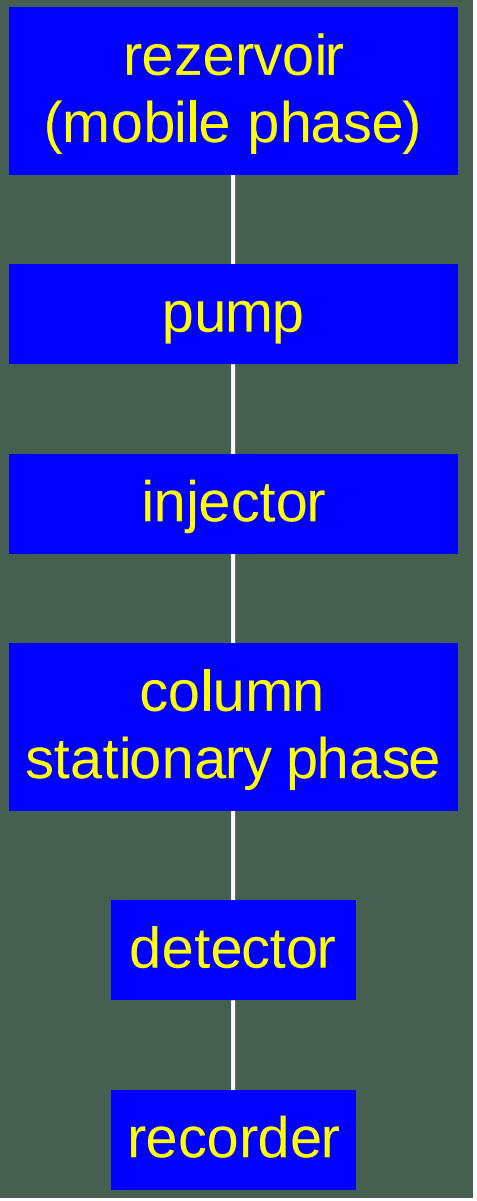
HPLC system is a mandatory tool in most of the labs involved in research.

The fields of research include

- Medical
- Biological
- Chemical
- Biochemical
- Phytochemical (plant chemical)

research

HPLC System


rezervoir
(mobile phase)
pump
injector
column
stationary phase
detector
recorder

Parts of HPLC - Pump

- The main function of the HPLC Pump is to force a liquid (which is also known as the mobile phase) via a liquid chromatograph at a specific flow rate, expressed in milliliters per min (mL/min).
- Operating pressure limits for regular HPLC systems are often in the range of 6000 – 10000 psi. An ideal pump should have solvent compatibility and corrosion resistance characteristics. For this reason, they are made of stainless steel.
- For analytical purposes, pumps with a flow rate of 0-10 ml/min are used and for preparative purposes, pumps with a flow rate of 0-10 ml/min are used.
- There are two types of pump operation:
  - ✓ isocratic pump
    delivers constant mobile phase composition
  - ✓ gradient pump
    delivers variable mobile phase composition

Parts of HPLC – Injector System

The test sample is loaded into the stationary phase via an injector system.

- Manuel injection
- Autosampler injection (Automatic)

Parts of HPLC - Column

- The separation of the compound mixture takes place in this column or HPLC tube. The HPLC tube is made up of **stainless steel**.
- Most HPLC columns are resistant to the usual HPLC pressure and also relatively inert to chemical corrosion.
- Glass tubes, tantalum tubes, and flexible polyethylene tubes are also rarely used as column tubing.
- The stationary phase is packed in the column and it is usually a solid adsorbent (silicagel, alumina, polimers).
- The mobile phase with the test sample is gone through the stationary phase. And the compounds in the test sample are separated in here. Mobile phase serves only as a carrier of the test sample. According to the solubility differences (in other words polarity differences) the mixed compounds are separated.

Column diameter

Columns, of i.d. 2-5 mm are generally used for analytical purposes. Wider columns of i.d. between 10 mm and 1 in (25.4 mm) may be used for preparative work.

Column lenght

Columns 5, 10, 15 or 25 cm long are common if microparticulate stationary phases of 10 µm or less are used.

A longer column increases the retention volume, thus decreasing the concentration of the peak in the eluate and impairing the detection limit.

Yet for preparative purposes columns up to 1 m in length are used.

Parts of HPLC - Detectors

- Detector records the relative concentrations of different components of the test sample with respect to their retention time. **Retention time** is the time taken by the compound to elute from the column. Retention time is calculated from the time of injection until the compound is eluted. Detection is based on several different physical and chemical principals.

- The detector is connected to the recorder.

- Recorder will read the output given from the detector and it will produce peaks to indicate the detected compounds as relevant.

Detectors

- UV Detectors
- -Fixed-wavelength
  -Variable-wavelength
  -Diode-Array Detectors (DAD)*
- Refractive Index Detectors
- Florescence Detectors
- Electrochemical Detectors
- Light-scattering Detectors
- Photoconductivitiy Detectors

Normal Phase (NP) HPLC

- SP: Polar (Silica)
- MP: Non-polar (hexane, isopropyl ether)

Reverse Phase (RP) HPLC

- SP: Non-polar (octadecylsilane [ODS], C-18)

Reverse Phase HPLC

- MP: Polar (Aqueous)
- Methanol
- Acetonitrile
- Ethanol
- Isopropanol
- Dimethylformamide
- Propan-1-ol
- Dioxane
- Tetrahydrofuran

Most of the HPLC analysis are reverse phase.

HPLC and Spectroscopy

- HPLC can be combined with the following spectroscopic methods:

- UV spectrometer (HPLC-UV/DAD)
- HPLC-NMR (Nuclear Magnetic Resonance)
- LC-MS (Mass Spectrometry)

Applications of HPLC

- Clinical diagnosis of diseases, disorders.
- In scientific research for discovery.
- In pharmaceutical labs for analysis.
- In food industry for quality control.
- For standards control by government.
- For separation of similar molecules

Use of HPLC in pharmacognosy

- Preparation of standardized extracts
- Qualitative and quantitative phytochemical analysis
- Determination of the purity of an isolated compound
- Stability testing of herbal products

It is given prominent importance due to its attributes like;

- High sensitivity i.e. ability to evaluate samples of **very minute concentrations** like in nano-gram and picogram
- **Detect precisely** the closely similar molecules
- **Highest accuracy** in the identification of components of complex mixtures.