

HPLC

High Performance Liquid Chromatography

HPLC

- **High Performance Liquid Chromatography or commonly known as HPLC is an analytical technique used to separate, identify or quantify each component in a mixture**
- **The mixture is separated using the basic principle of column chromatography and then identified and quantified by spectroscopy**
- **In the 1960s the column chromatography LC with its low-pressure suitable glass columns was further developed to the HPLC with its high-pressure adapted metal columns**
- **HPLC is thus basically a highly improved form of column liquid chromatography**
- **Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres**

Principle of HPLC

- **The purification takes place in a separation column between a stationary and a mobile phase**
- **The stationary phase is a granular material with very small porous particles in a separation column**
- **The mobile phase, on the other hand, is a solvent or solvent mixture which is forced at high pressure through the separation column**
- **The sample is injected into the mobile phase flow from the pump to the separation column using a syringe**
- **The individual components of the sample migrate through the column at different rates to a varying degree by interactions with the stationary phase**
- **After leaving the column, the individual substances are detected by a suitable detector**
- **At the end, a chromatogram in the HPLC software on the computer is obtained**
- **The chromatogram allows the identification and quantification of the different substances**

Instrumentation of HPLC

1. The Pump

- The pump is positioned in the most upper stream of the liquid chromatography system and generates a flow of eluent from the solvent reservoir into the system
- High-pressure generation is a “standard” requirement of pumps besides which, it should also be able to provide a consistent pressure at any condition and a controllable and reproducible flow rate
- Maximum 8000 psi pressure

2. Injector

- An injector is placed next to the pump which introduces the sample mostly through a syringe
- The use of the autosampler (auto-injector) system is also widely used that allows repeated injections in a set scheduled-timing

3. Column

- The separation is performed inside the column**
- The recent columns are often prepared in a stainless steel housing, since stainless is tolerant towards a large variety of solvents instead of glass columns**
- The packing material generally used is silica or polymer gels compared to calcium carbonate**

Types of Column packing:

- 1. Microporous column – 5-10 μm dia. Pores (Silica or Alumina)**
- 2. Pellicular column – superficially porous with central solid core**
- 3. Bonded column – stationary phase is chemically bonded on an inert support (silica polymer)**

4. Detector

- Separation of analytes is performed inside the column, whereas a detector is used to observe the obtained separation**
- The composition of the eluent is consistent when no analyte is present**
- While the presence of analyte changes the composition of the eluent, detector measure these differences and monitor as a form of an electronic signal**

5. Recorder

- The change in eluent detected by a detector is in the form of an electronic signal, and thus it is still not visible to our eyes**
- In older days, the pen (paper)-chart recorder was popularly used, nowadays, a computer-based data processor (integrator) is more common**

6. Degasser

The eluent used for LC analysis may contain gases such as oxygen that are non-visible to our eyes

When gas is present in the eluent, this is detected as noise and causes an unstable baseline

Degasser uses special polymer membrane tubing to remove gases

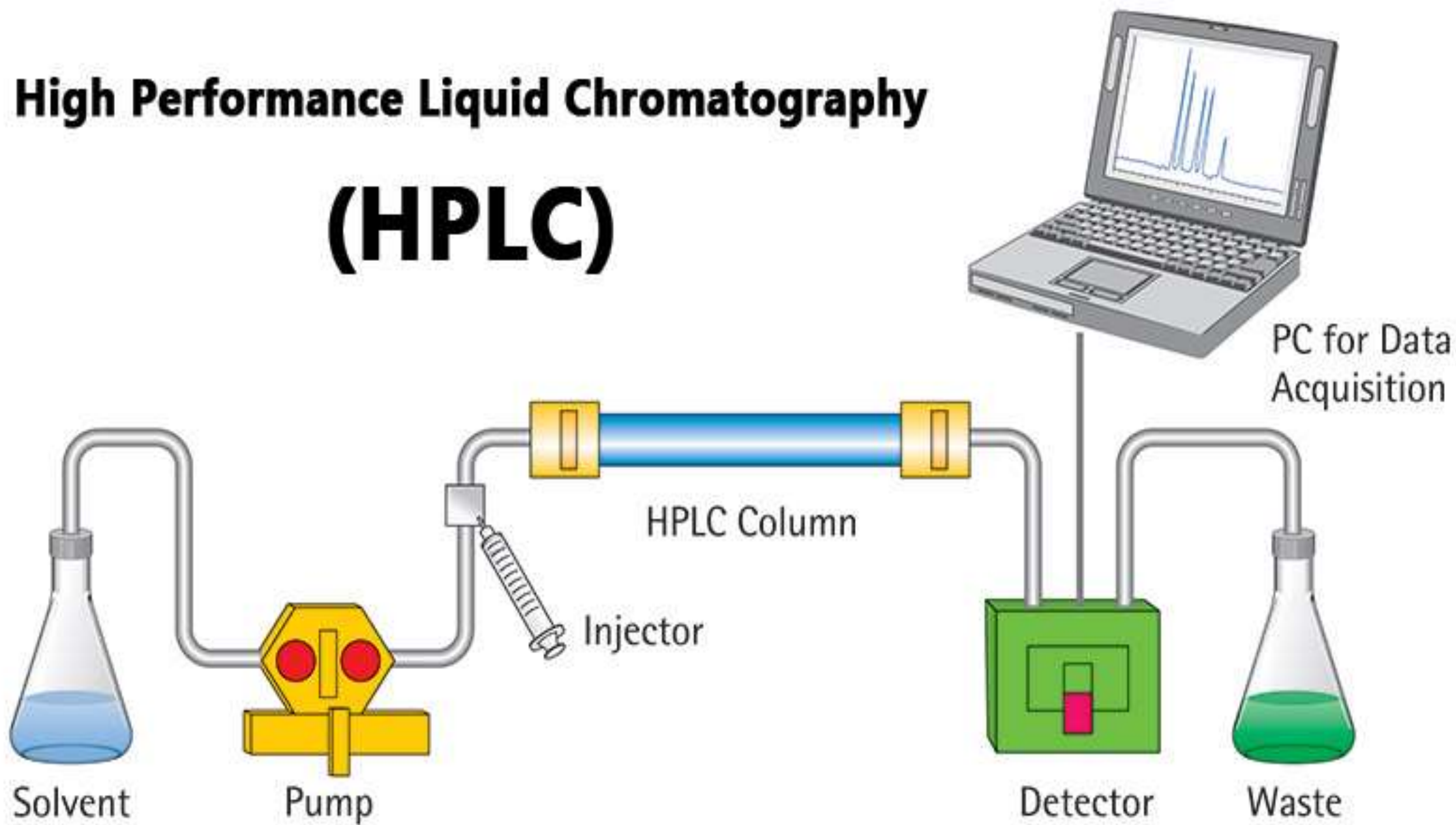
The numerous very small pores on the surface of the polymer tube allow the air to go through while preventing any liquid to go through the pore

7. Column Heater

The LC separation is often largely influenced by the temperature, and important to keep consistent temperature conditions

Thus columns are generally kept inside the column oven (column heater)

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Types of HPLC

1. Normal phase:

- Column packing is polar (e.g silica) and the mobile phase is non-polar, and used for water-sensitive compounds, geometric isomers, cis-trans isomers, and chiral compounds

2. Reverse phase:

- The column packing is non-polar and the mobile phase is water+miscible solvent (e.g methanol) mostly used for polar, non-polar, ionizable and ionic samples

3. Ion exchange:

- Column packing contains ionic groups and the mobile phase is buffer, commonly used to separate anions and cations

4. 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

Applications of HPLC

The HPLC has developed into a universally applicable method so that it finds its use in almost all areas of chemistry, biochemistry, and pharmacy

- Analysis of drugs
- Analysis of synthetic polymers
- Analysis of pollutants in environmental analytics
- Determination of drugs in biological matrices
- Product purity and quality control of industrial products and fine chemicals
- Separation and purification of biopolymers such as enzymes or nucleic acids
- Water purification
- Pre-concentration of trace components
- Ion-exchange chromatography of proteins, carbohydrates and oligosaccharides

Advantages:

- **Speed**
- **Efficiency**
- **Accuracy**
- **Versatile and extremely precise for identification and quantification of chemical components**

Limitations:

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