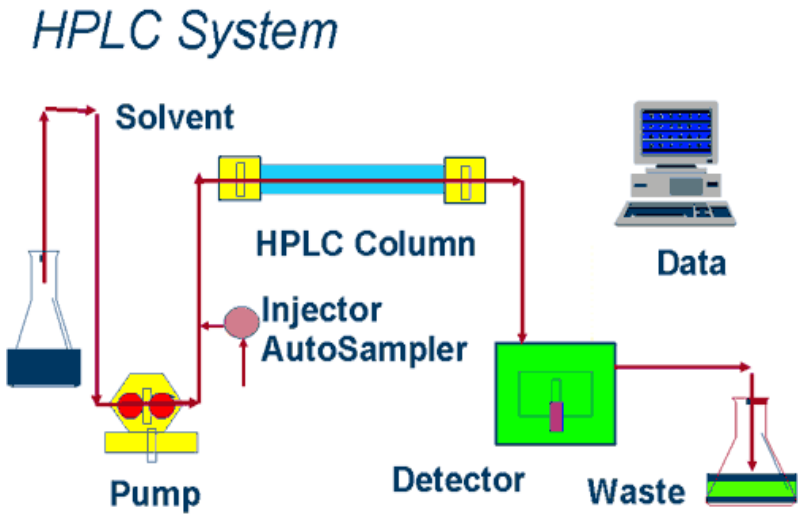
HPLC (high-pressure liquid chromatography)

HPLC (**high-pressure liquid chromatography**), is a technique in analytical chemistry used to separate, identify, and quantify each component in a mixture. It relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material . Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out the column.

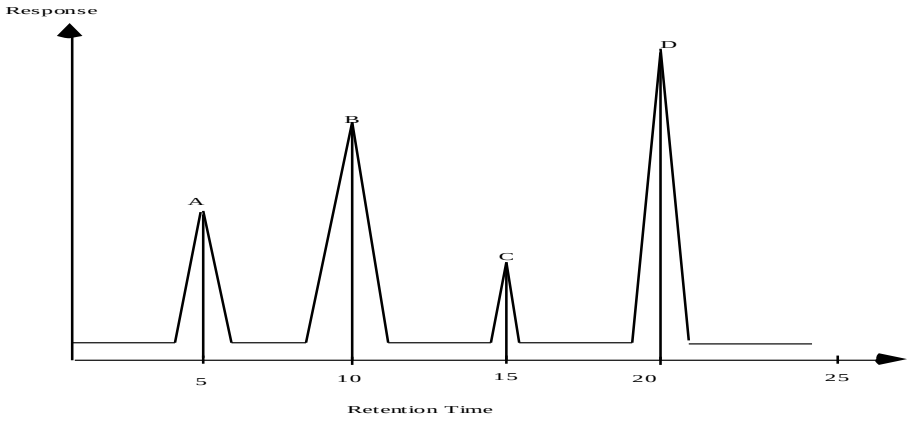
Components of HPLC

1. **Solvent Reservoir**
2. **Pumps**
3. **Sample Injection System**
4. **Columns**
5. **Detectors**
6. **Data Processing**
7. **Waste**



Retention Time

- The retention time of a solute is taken as the elapsed time between the time of injection of a solute and the time of elution of the peak maximum of that solute.
- Time required for the sample to travel from the injection port through the column to the detector.



Types of HPLC

- **Normal–phase chromatography**
- **Reversed-phase chromatography**
- Normal–phase chromatography was one of the first kinds of HPLC that chemists developed. Also known as normal-phase HPLC (NP-HPLC) this method separates analytes based on their affinity for a polar stationary surface such as silica

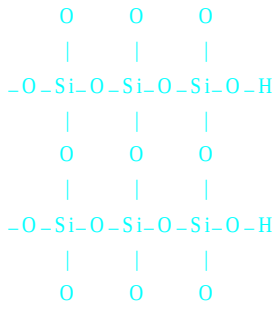
  NP-HPLC uses a non-polar, non-aqueous mobile phase (e.g. Chloroform), and works effectively for separating analytes readily soluble in non-polar solvents. The analyte associates with and is retained by the polar stationary phase

- Reversed phase HPLC (RP-HPLC) has a non-polar stationary phase , moderately polar mobile phase. One common stationary phase is a silica which has been surface-modified with RMe_2SiCl, where R is a straight chain alkyl group such as $C_{18}H_{37}$ or C_8H_{17}. With such stationary phases, retention time is longer for molecules which are less polar, while polar molecules elute more readily (early in the analysis). An investigator can increase retention times by adding more water to the mobile phase

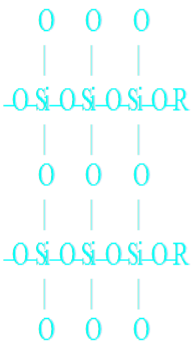
Stationary Phases

- Polar ("Normal" Phase):
  - Silica, alumina
- Non-Polar ("Reversed Phase")
  - ODS Silica gel
  - C18, C8

Silica Gel



Derivatized Silica Gel



Where R = $C_{18}H_{37}$ hydrocarbon chain (octadecylsilyl deriv. silica or "C18")

	Normal Phase	Reversed Phase
Stationary phase	Polar (silica gel)	Non-polar (C18)
Mobile phase	Non-polar (organic solvents)	Polar (aqueous/organic)
Sample movement	Non-polar fastest	Polar fastest
Separation based on	Different polarities (functionality)	Different hydrocarbon content

TLC vs. HPLC

Type of Analysis	qualitative only	qualitative & quantitative
Stationary Phase	2-dimensional thin layer plate	3-dimensional column
Instrumentation	minimal!	much! with many adjustable parameters
Sample Application	spotting (capillary)	injection (Rheodyne injector)
Mobile Phase Movement	capillary action (during development)	high pressure (solvent delivery)
Visualization of Results	UV lightbox	"on-line" detection (variable UV/Vis)
Form of Results	spots, R_f's (retention factors)	peaks, R_t's (retention times)

Application of HPLC

Pharmaceuticals industry

- To control the drug stability
- Quantity of drug determination from pharmaceutical dosage forms, ex. Paracetamol determination in panadol tablet
- Quantity of drug determination from biological fluids, ex: blood glucose level

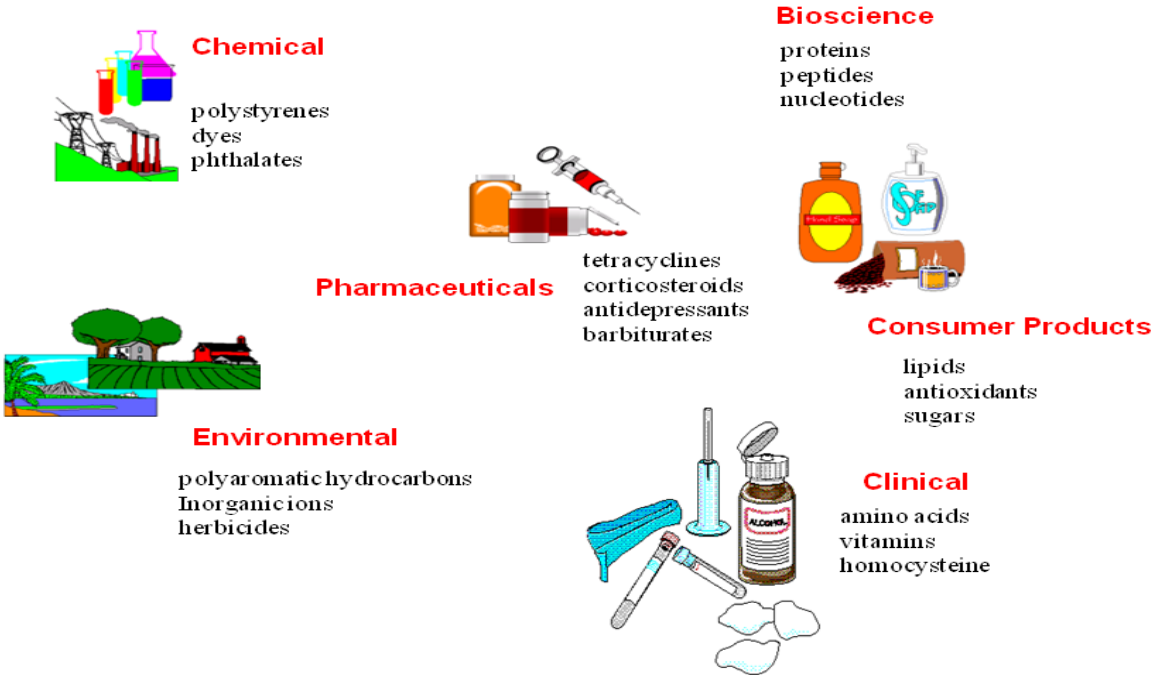
2. Analysis of natural contamination

- Phenol & Mercury from sea water

3. Forensic test

- Determination of steroid in blood, urine & sweat.

4. Clinical test



The factors which influence the HPLC performance

1. Internal diameter of column

- the smaller in diameter, the higher in sensitivity

2. Pump pressure

- the higher in pressure, the higher in separation

3. Sample size

4. The polarity sample, solvent and column

5. Temperature

- the higher in temperature, the higher in separation

Advantages

1. Needs a small sample with a high accuracy and precis
2. Non-destructed sample during operation compared to GC.

Disadvantages

- Need a skill to run the instruments
- Solvents consuming

Gas chromatography

Gas chromatography is a technique used for separation of volatile substances, or substances that can be made volatile, from one another in a gaseous mixture at high temperatures. A sample containing the materials to be separated is injected into the gas chromatograph. A mobile phase (carrier gas) moves through a column that contains a wall coated or granular solid coated stationary phase. As the carrier gas flows through the column, the components of the sample come in contact with the stationary phase. The different components of the sample have different affinities for the stationary phase, which results in differential migration of solutes, thus leading to separation

Gas chromatography can be used for both qualitative and quantitative analysis. Comparison of retention times can be used to identify materials in the sample by comparing retention times of peaks in a sample to retention times for standards. The same limitations for qualitative analysis

There are two types of Gas chromatography

Gas - Solid Chromatography (GSC)

Gas - Liquid Chromatography (GLC)

Gas - Solid Chromatography (GSC)

The stationary phase, in this case, is a solid like silica or alumina. It is the affinity of solutes towards adsorption onto the stationary phase which determines, in part, the retention time. The mobile phase is, of course, a suitable carrier gas. This gas chromatographic technique is most useful for the separation and analysis of gases like CH_4, CO_2, CO, ... etc.

Gas - Liquid Chromatography (GLC)

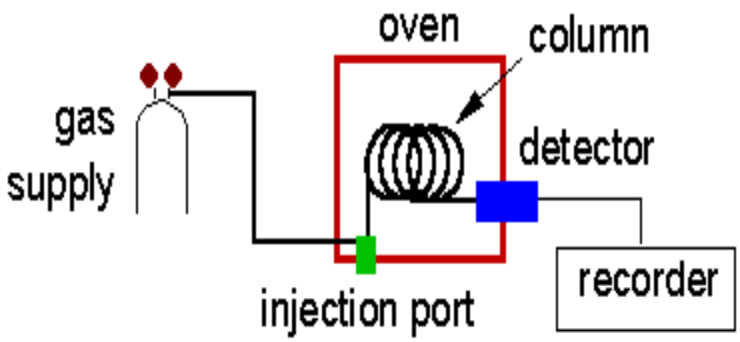
The stationary phase is a liquid with very low volatility while the mobile phase is a suitable carrier gas. GLC is the most widely used technique for separation of volatile species. The presence of a wide variety of stationary phases with contrasting selectivities and easy column preparation add to the assets of GLC or simply GC.

Gas Chromatography (GC)

Gas chromatography is a chromatographic technique that can be used to separate volatile organic compounds.

* It consists of
  - ✓ a flowing mobile phase
  - ✓ an injection port

- ✓ a separation column (the stationary phase)
- ✓ an oven
- ✓ a detector.



Principle

The organic compounds are separated due to differences in their partitioning behavior *between the mobile gas phase and the stationary phase in the column*.

- ✓ Mobile phases are generally inert gases such as helium, argon, or nitrogen.
- ✓ The injection port consists of a rubber septum through which a syringe needle is inserted to inject the sample.
- ✓ The injection port is maintained at a higher temperature than the boiling point of the least volatile component in the sample mixture.
- ✓ Since the partitioning behavior is dependent on temperature, the separation column is usually contained in a thermostat-controlled oven.
- ✓ Separating components with a wide range of boiling points is accomplished by starting at a low oven temperature and increasing the temperature over time to elute the high-boiling point components.

Gas Supply

- ✓ The mobile phase (carrier gas) should be chemically inert, dry and free from O_2 (helium, argon, nitrogen and hydrogen).
- ✓ The carrier gas should be of high purity; impurities in the carrier gas such as water vapour, air and trace gaseous hydrocarbons can cause reactions with sample and cause column deterioration and affect detector performance.
- ✓ The gas supply is associated with pressure regulator and flow controller.

Sample Injection System

Samples GC must be volatile.

Samples which are non volatile are converted into a volatile derivative.

GC Column

Most GC columns are made from high-purity fused silica capillary, the inner wall of the capillary coated with the stationary phase. GC columns vary in length from less than 2 m to 50 m or more. In order to fit into the column oven, they are usually formed as coils. The control of column's temperature is critical to attain a good separation in GC, thus the column is located inside a thermostated oven to control the temperature.

GC Detectors

- **Thermal conductivity detector (TCD)**
- **Flame ionization detector (FID)**
- **Nitrogen phosphorous detectors (NPD)**

<u>GC Applications</u>

Food Analysis

Analysis of foods is concerned with confirming the presence and determination the quantities of the analytes (lipids, proteins, carbohydrates, preservatives, flavours, colorants, and also vitamins, steroids, and pesticide residues).

Drug Analysis

GC is widely applied to identification of the active components, possible impurities as well as the metabolites.

Environmental Analysis

The environmental contaminants; e.g. (DDT) is present in the environment at very low concentrations and are found among many of other compounds. GC, with its high sensitivity and high separating power, is mostly used in the analysis of environmental samples.

Forensic Analysis

In forensic cases, very little sample is available, and the concentration of the sample components may be very low. GC is a useful due to its high sensitivity and separation efficiency.

Comparison of HPLC and GC

Sample Volatility

HPLC

- No volatility requirement , Sample must be soluble in mobile phase

GC Sample must be volatile

Sample Polarity

- HPLC Separates both polar and non polar compounds
- GC Samples are nonpolar and polar

Sample Preparation

- HPLC Sample must be filtered , Sample should be in same solvent as mobile phase
- GC Solvent must be volatile and generally lower boiling than analytes

Sample Size

- HPLC Sample size based upon column.
- GC Typically 1 - 5 μL

Separation Mechanism

- HPLC Both stationary phase and mobile phase take part
- GC Mobile phase is a sample carrier only

Detectors

- HPLC Most common UV-Vis ,Wide range of non- destructive detectors , 3-dimensional detectors
- GC Most common FID, universal to organic compounds