UNIT 3

GAS CHROMATOGRAPHY

Gas chromatography (GC) is an analytical technique used to separate and detect the chemical components of a sample mixture to determine their presence or absence and/or quantities. These chemical components are usually organic molecules or gases.

Gas chromatography working

As the name implies, GC uses a carrier gas in the separation, this plays the part of the mobile phase (Figure 1 (1)). The carrier gas transports the sample molecules through the GC system, ideally without reacting with the sample or damaging the instrument components.

- The sample is first introduced into the gas chromatograph (GC), either with a syringe or transferred from an autosampler (Figure 1 (2)) that may also extract the chemical components from solid or liquid sample matrices. The sample is injected into the GC inlet (Figure 1 (3)) through a septum which enables the injection of the sample mixture without losing the mobile phase.
- Connected to the inlet is the analytical column (Figure 1 (4)), a long (10 – 150 m), narrow (0.1 – 0.53 mm internal diameter) fused silica or metal tube which

contains the stationary phase coated on the inside walls.

- The analytical column is held in the column oven which is heated during the analysis to elute the less volatile components.
- The outlet of the column is inserted into the detector (Figure 1 (5)) which responds to the chemical components eluting from the column to produce a signal.
- The signal is recorded by the acquisition software on a computer to produce a chromatogram (Figure 1 (6)).
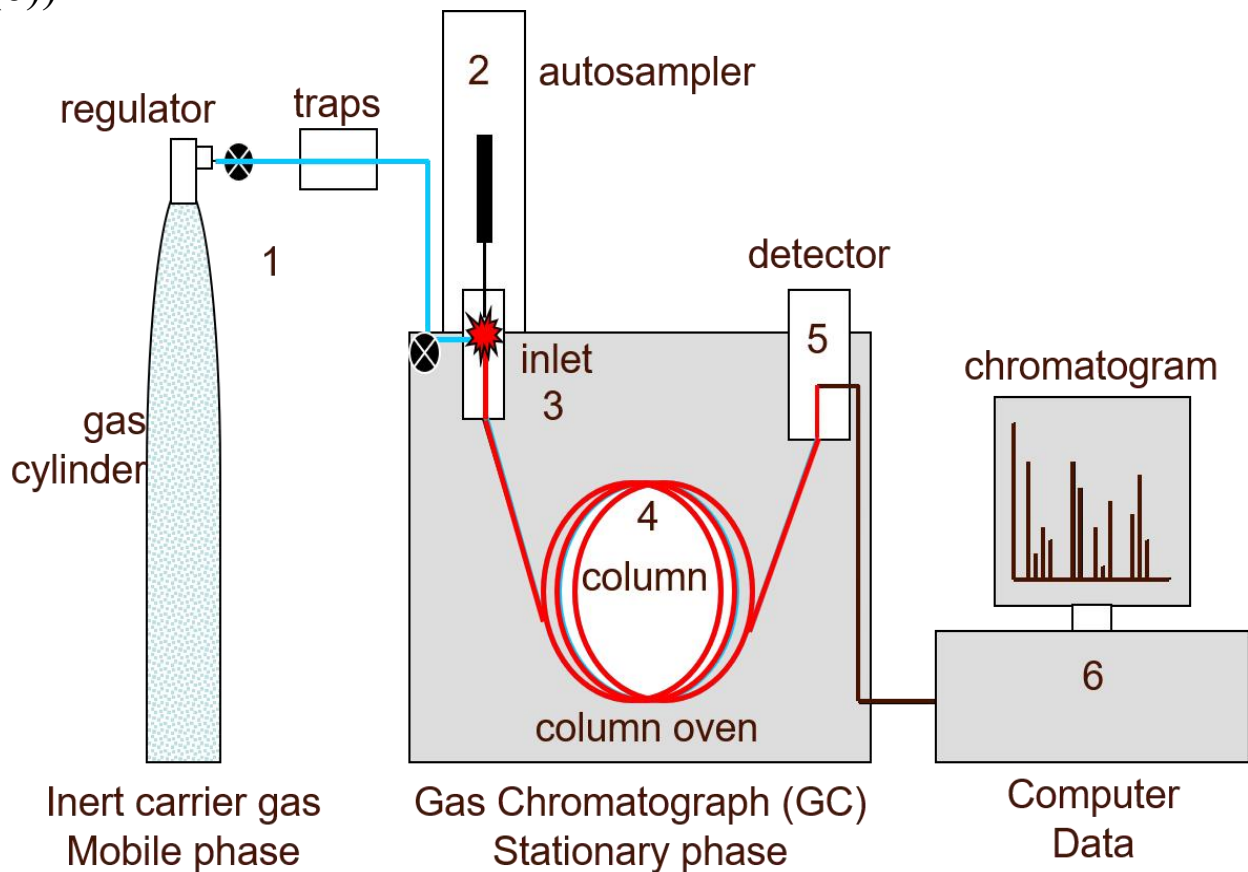
After injection into the GC inlet, the chemical components of the sample mixture are first vaporized, if they aren't already in the gas phase. For low concentration samples the whole vapour cloud is transferred into the analytical column by the carrier gas in what is known as [splitless mode](). For high concentration samples only a portion of the sample is transferred to the analytical column in split mode, the remainder is flushed from the system through the split line to prevent overloading of the analytical column.

Once in the analytical column, the sample components are separated by their different interactions with the stationary phase. Therefore, when selecting the type of column to use, the volatility and functional groups of the analytes should be considered to match them to the stationary phase. Liquid stationary phases mainly fall into two types: polyethylene glycol (PEG) or polydimethylsiloxane (PDMS) based, the latter with varying percentages of dimethyl, diphenyl or mid-polar functional groups, for example cyanopropylphenyl. Like separates like, therefore non-polar columns with dimethyl or a low percentage of diphenyl are good for separating non-polar analytes. Those molecules capable of π-π interactions can be separated on stationary phases containing phenyl groups. Those capable of hydrogen bonding, for example acids and alcohols, are best separated with PEG columns, unless they have undergone derivatization to make them less polar.

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.

High Performance Liquid Chromatography (HPLC)
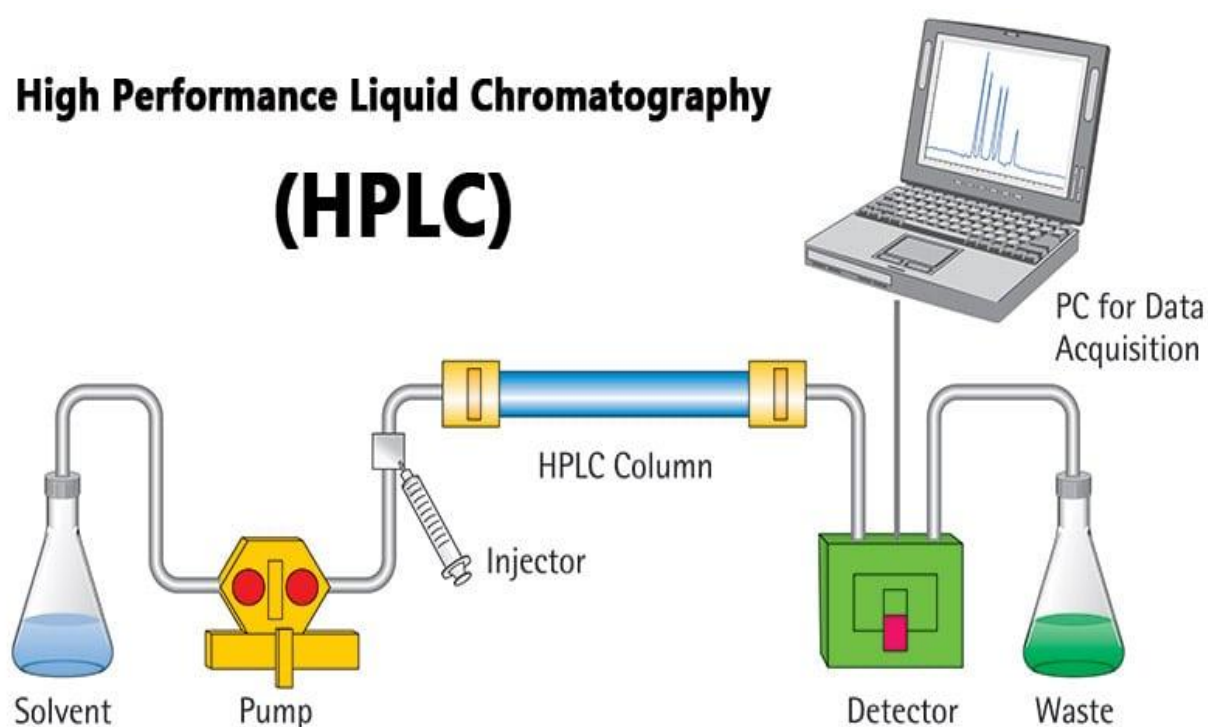
HPLC Principle

- 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.
- Via a valve with a connected sample loop, i.e. a small tube or a capillary made of stainless steel, the sample is injected into the mobile phase flow from the pump to the separation column using a syringe.
- Subsequently, the individual components of the sample migrate through the column at different rates because they are retained to a varying degree by interactions with the stationary phase.
- After leaving the column, the individual substances are detected by a suitable detector and passed on as a signal to the HPLC software on the computer.
- At the end of this operation/run, a chromatogram in the HPLC software on the computer is obtained.
- The chromatogram allows the identification and quantification of the different substances.

The Pump

- The development of HPLC led to the development of the pump system.
- 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 to be able to

provide a consistent pressure at any condition and a controllable and reproducible flow rate.
- Most pumps used in current LC systems generate the flow by back-and-forth motion of a motor-driven piston (reciprocating pumps). Because of this piston motion, it produces "pulses".

Injector

- An injector is placed next to the pump.
- The simplest method is to use a syringe, and the sample is introduced to the flow of eluent.
- The most widely used injection method is based on sampling loops.
- The use of the autosampler (auto-injector) system is also widely used that allows repeated injections in a set scheduled-timing.

Column

- The separation is performed inside the column.
- The recent columns are often prepared in a stainless steel housing, instead of glass columns.
- The packing material generally used is silica or polymer gels compared to calcium carbonate.
The eluent used for LC varies from acidic to basic solvents.
- Most column housing is made of stainless steel since stainless is tolerant towards a large variety of solvents.

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. What detector does is to measure these differences.

- This difference is monitored as a form of an electronic signal. There are different types of detectors available.

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.
- There are various types of data processors; from a simple system consisting of the in-built printer and word processor while those with software that are specifically designed for an LC system which not only data acquisition but features like peak-fitting, baseline correction, automatic concentration calculation, molecular weight determination, etc.

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.

Column Heater

- The LC separation is often largely influenced by the column temperature.

- In order to obtain repeatable results, it is important to keep consistent temperature conditions.
- Also for some analysis, such as sugar and organic acid, better resolutions can be obtained at elevated temperatures (50 to 80°C).
- Thus columns are generally kept inside the column oven (column heater).

APPLICATION OF HPLC

- Analysis of drugs
- Analysis of synthetic polymers
- Analysis of pollutants in environmental analytics
- Determination of drugs in biological matrices
- Isolation of valuable products

High-performance thin-layer chromatography

(**HPTLC**) is an enhanced form of thin-layer chromatography (TLC). A number of enhancements can be made to the basic method of thin-layer chromatography to automate the different steps, to increase the resolution achieved, and to allow more accurate quantitative measurements.

Automation is useful to overcome the uncertainty in droplet size and position when the sample is applied to the TLC plate by hand. One approach to automation has been the use of piezoelectric devices and inkjet printers for applying the sample.