UNIT 3

GAS CHROMATOGRAPHY

Gas chromatography (GC) is an analytical technique used to separate and detect the chemical componentsofasample mixture to determine their presence or absenceand/or quantities. These chemical components areusually organic molecules or gases.

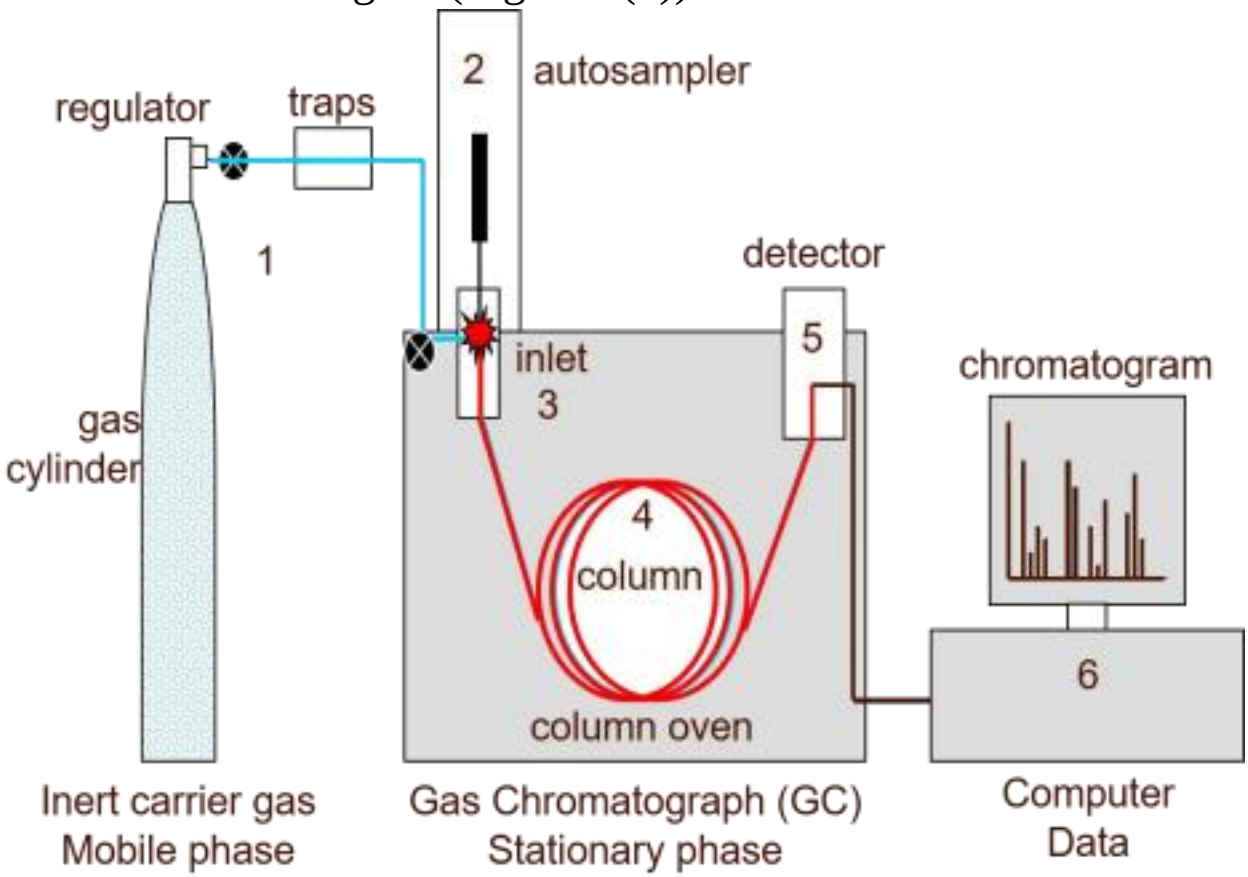
Gas chromatography working

As the name implies, GC uses a carrier gas in theseparation, this plays the part of the mobile phase(Figure1 (1)). The carrier gas transports the sample moleculesthrough the GC system, ideally without reactingwiththesample 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 fromsolidor liquid sample matrices. The sample is injectedintothe GC inlet (Figure 1 (3)) through a septumwhichenables the injection of the sample mixture without losing the mobile phase.
- Connected to the inlet is the analytical column(Figure1 (4)), a long (10 – 150 m), narrow(0.1 – 0.53mminternal diameter) fused silica or metal tube which
  contains the stationary phase coated on the insidewalls.
- The analytical column is held in the columnovenwhich is heated during the analysis to elute thelessvolatile components.
- The outlet of the column is inserted into the detector (Figure 1

(5)) which responds tothechemical components eluting fromthe columntoproduce a signal.

- The signal is recorded by the acquisition softwareonacomputer to produce a chromatogram(Figure 1(6)).



After injection into the GC inlet, the chemical components of the sample mixture are first vaporized, ifthey aren't already in the gas phase. For lowconcentration samples the whole vapour cloudis transferred into the analytical column by the carrier gasin what is known as splitless mode. For high concentration samples only a portion of the sampleistransferred to the analytical column in split mode, theremainder is flushed from the systemthrough thesplit line to prevent overloading of the analytical column.

Once in the analytical column, the sample componentsare separated by their different interactions withthestationary phase. Therefore, when selecting the typeofcolumn to use, the volatility and functional groups of theanalytes should be considered to match themtothestationary phase. Liquid stationary phases mainlyfall into two types: polyethylene glycol (PEG) or polydimethylsiloxane (PDMS) based, the latter

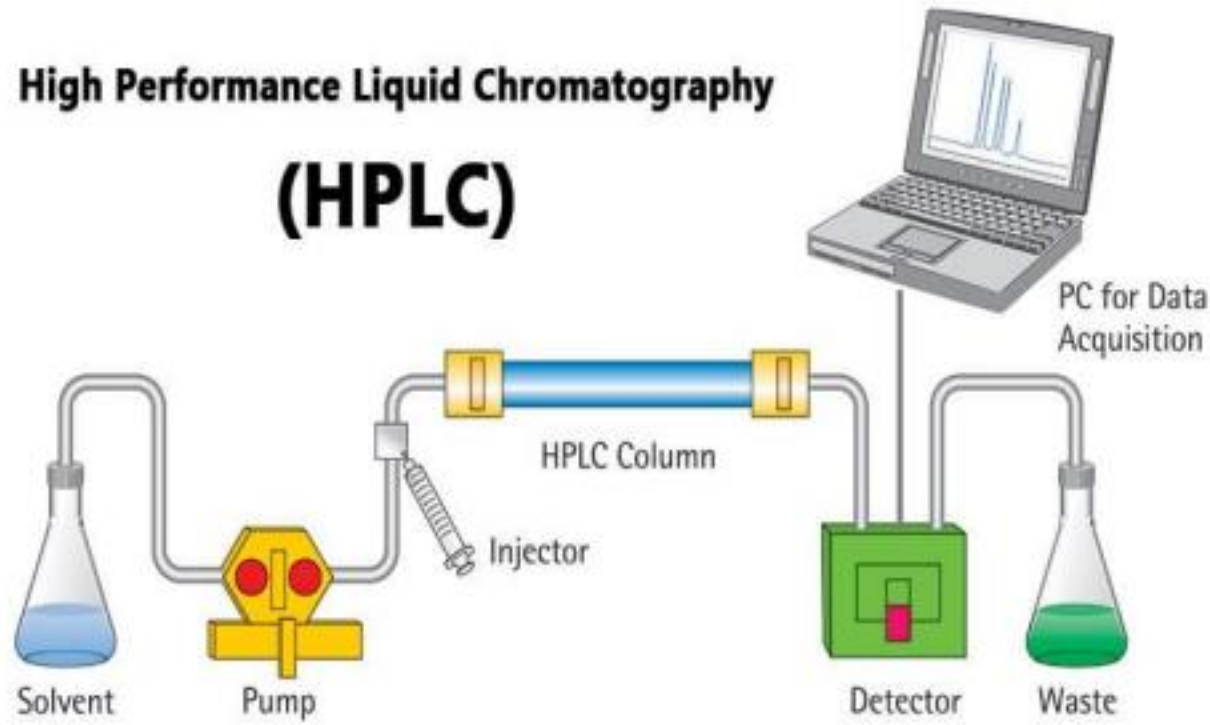
withvarying percentages of dimethyl, diphenyl or mid-polarfunctional groups, for example cyanopropylphenyl. Likeseparates like, therefore non-polar columns withdimethyl or a low percentage of diphenyl are goodfor separating non-polar analytes. Those molecules capableof π-π interactions can be separated on stationaryphasescontaining phenyl groups. Those capable of hydrogenbonding, for example acids and alcohols, are best separated with PEG columns, unless they have undergone derivatization to make themless polar. HPLC

High-performance liquid chromatography or commonly known as HPLC, is an analytical technique
used to separate, identify or quantify eachcomponentin a mixture.

The mixture is separated using the basic principleof column **chromatography** and then identified andquantified by spectroscopy.

In the 1960s, the column chromatography LCwithitslow-pressure suitable glass columns was further developed to the HPLC with its high-pressure adaptedmetal columns.

HPLC is thus basically a highly improved formof 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 to400atmospheres.



HPLC Principle

● The purification takes place in a separation columnbetween a stationary and a mobile phase. ● The stationary phase is a granular material withverysmall porous particles in a separation column. ● The mobile phase, on the other hand, is a solvent orsolvent mixture which is forced at high pressurethrough the separation column.

- Via a valve with a connected sample loop, i.e. asmall tube or a capillary made of stainless steel, the sampleis injected into the mobile phase flowfromthepumpto the separation column using a syringe.
- Subsequently, the individual components of thesample migrate through the column at different ratesbecause they are retained to a varying degree byinteractions with the stationary phase.
- After leaving the column, the individual substancesare detected by a suitable detector and passedonasasignal to the HPLC software on the computer.
- At the end of this operation/run, a chromatograminthe HPLC

software on the computer is obtained. ● The chromatogram allows the identificationandquantification of the different substances.

The Pump

- The development of HPLC led to the development ofthe pump system.
- The pump is positioned in the most upper streamofthe liquid chromatography systemand generates aflow of eluent from the solvent reservoir intothesystem.
  - High-pressure generation is a “standard” requirement of pumps besides which, it should also to be ableto provide a consistent pressure at any conditionandacontrollable and reproducible flowrate.
- Most pumps used in current LC systems generatetheflow by back-and-forth motion of a motor-drivenpiston (reciprocating pumps). Because of this pistonmotion, it produces “pulses”.

Injector

● An injector is placed next to the pump. ● The simplest method is to use a syringe, andthesample is introduced to the flow of eluent. ● The most widely used injection method is basedonsampling loops.

- The use of the autosampler (auto-injector) systemisalso widely used that allows repeated injections inaset scheduled-timing.

Column

● The separation is performed inside the column. ● The recent columns are often prepared in a stainlesssteel housing, instead of glass columns.

- The packing material generally used is silica or polymer gels

compared to calciumcarbonate. The eluent used for LC varies fromacidic tobasicsolvents.

● Most column housing is made of stainless steel sincestainless 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 obtainedseparation.

● The composition of the eluent is consistent whennoanalyte is present. While the presence of analyte changes the composition of the eluent. What detectordoes is to measure these differences.

● This difference is monitored as a formof anelectronicsignal. There are different types of detectors available. **Recorder**

● The change in eluent detected by a detector is intheform 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-baseddata processor (integrator) is more common.

● There are various types of data processors; fromasimple system consisting of the in-built printer andword processor while those with software that arespecifically designed for an LC systemwhichnot onlydata acquisition but features like peak-fitting, baselinecorrection, automatic concentration calculation, molecular weight determination, etc.

Degasser

● The eluent used for LC analysis may containgasessuch as oxygen that are non-visible to our eyes. ● When gas is present in the eluent, this is detectedasnoise and causes an unstable baseline.

● Degasser uses special polymer membrane tubingtoremove gases.

- The numerous very small pores on the surface of thepolymer tube allow the air to go through whilepreventing any liquid to go through the pore. **Column Heater**

- The LC separation is often largely influencedbythecolumn temperature.
- In order to obtain repeatable results, it is important tokeep consistent temperature conditions.
- Also for some analysis, such as sugar and organicacid,better resolutions can be obtained at elevatedtemperatures (50 to 80°C).
- Thus columns are generally kept inside the columnoven (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 enhancementscanbe made to the basic method of thin-layer chromatography to automate the different steps, toincrease the resolution achieved, and to allowmoreaccurate quantitative measurements.

Automation is useful to overcome the uncertaintyindroplet size and position when the sample is appliedtothe TLC plate by hand. One approach to automationhasbeen the use of piezoelectric devices and inkjet printers for applying the sample.