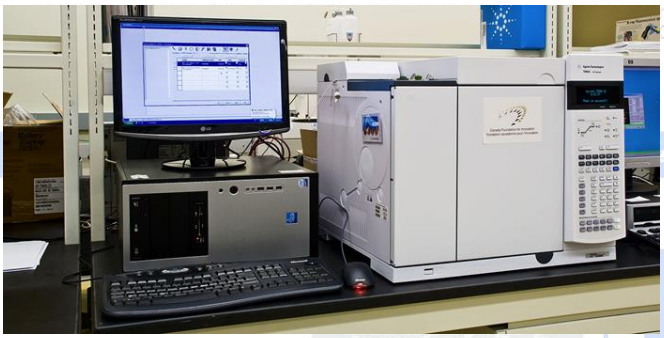
Gas Chromatography

Introduction:



Gas chromatography (GC) is an analytical technique used to separate the chemical components of a sample mixture and then detect them to determine their presence or absence and/or how much is present. These chemical components are usually organic molecules or gases. For GC to be successful in their analysis, these components need to be volatile, usually with a molecular weight below 1250 Da, and thermally stable so they don't degrade in the GC system. GC is a widely used technique across most industries: for quality control in the manufacture of many products from cars to chemicals to pharmaceuticals; for research purposes from the analysis of meteorites to natural products; and for safety from environmental to food to forensics. Gas chromatographs are frequently hyphenated to mass spectrometers (GC-MS) to enable the identification of the chemical components.

- **What is/are**:

  Chromatography?

  Chromatography Phases?

  Chromatography Types?

  Gas Chromatography?

  Gas Chromatography Types?

  Retention Time?

Response Factor?

Gas Chromatography Mechanism?

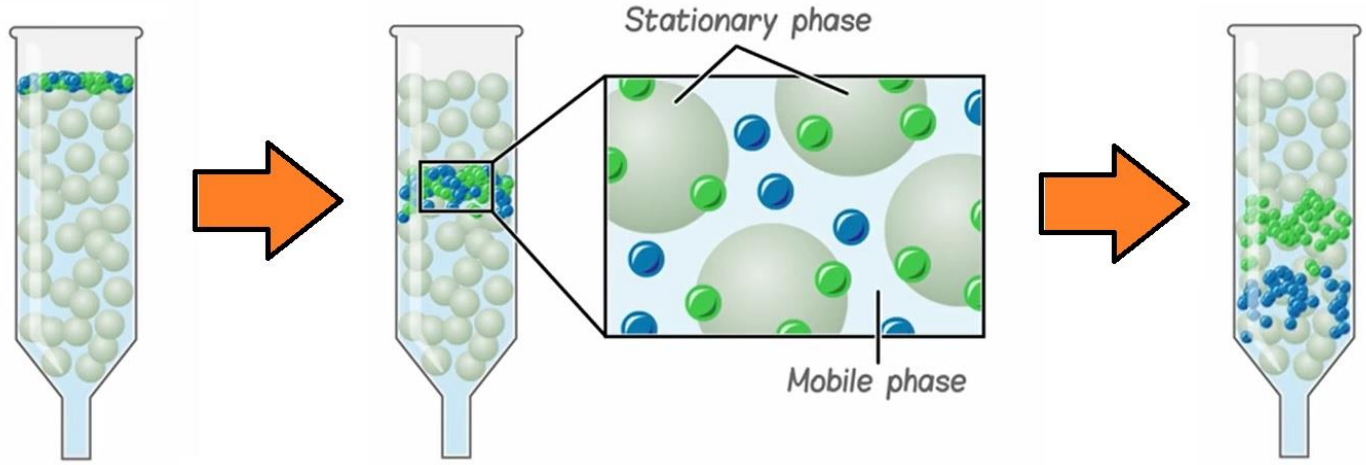
Gas Chromatography Limitations & Strengths?

Gas Chromatography Common Problems?

Chromatography:

Chromatography in Greek means to 'write with colors.' It is a versatile separation technique developed in 1903 by Mikhail Tswett, a Russian botanist. He separated colorful plant pigments using a column of calcium carbonate. Ever since its discovery, chromatography has evolved as a powerful tool in the lab for the separation and identification of different compounds in a mixture.

Chromatography Phases:



Three components thus form the basis of the chromatography technique.

1. **Stationary phase:** This phase is always composed of a "solid" phase or "a layer of a liquid adsorbed on the surface solid support".
2. **Mobile phase:** This phase is always composed of "liquid" or a "gaseous component."
3. **Separated molecules**

Chromatography Types:

Chromatography has evolved over the years based on the varying needs for molecular separation. Today several types of chromatography are being used for different purposes in labs across the world. Some important types are briefly discussed below:

I- **Paper Chromatography**: Paper soaked in a liquid is used as a stationary phase while a liquid solvent acts as the mobile phase. Separated components appear as spots on the paper once it is dried.

II- **Liquid Chromatography:** This technique uses silica and alumina as the stationary phase and organic solvents as the mobile phase.

III- **Thin layer chromatography:** Here, a plastic or glass sheet is coated with a thin layer of adsorbent such as alumina (Al_2O_3) or silica (SiO_2). Components are separated based on their affinity to the adsorbent and appear as individual spots on the sheet after chromatographic separation.

IV- **Column chromatography:** Is similar to thin layer chromatography by using the same stationary and mobile phase. The difference here is that both phases are contained within a vertical glass column and the process of separation is time consuming.

V- **Gas chromatography (GC):** An inert gas (Helium, Nitrogen, and Argon) is used as a mobile phase and a solid or liquid usually made up of silicon polymers is the stationary phase. The sample mixture is introduced to the column lined with the stationary phase and is selectively adsorbed. The separated molecules are identified using a detector as they leave the column.

VI- **High performance liquid chromatography (HPLC)**: Is an advanced form of column chromatography. In HPLC, a sample mixture is introduced into the mobile phase (often a solvent) and this is then pumped into a tightly-packed analytical column at high pressure for rapid separation of the sample molecules. This separation relies on the affinity of the molecules for both the mobile phase and particles coating the column (the stationary phase). It is also called high pressure liquid chromatography.

VII- **Ion-exchange chromatography**: This is carried out to separate ions and polar molecules on the basis of their affinity to an ion exchanger. It helps in the separation of charged molecules such as proteins, amino acids, and nucleotides. Here, the mobile phase is often a conductive solution

Gas Chromatography:

The eluent is an inert gas, often helium, hydrogen or nitrogen. The eluent actually has little effect on the separation process, which is governed more by the volatility of each sample component and its interaction with the stationary phase. Stationary phases are either solids or liquids and are contained in columns which range in internal diameter from 100 micrometers to 4 mm. The chromatographic system consists of three essential elements: an injection system, a temperature controlled column, and a detector. For a chromatograph to be used in environmental analysis, specialized injection systems, such as concentrators or thermal disrobers for air analysis, or purge and trap apparatus for water analyses are often useful. In addition, complex environmental samples often require a detector or array of detectors to assist in identification of the sample components as well as to determine their concentrations.

Gas Chromatography Types:

Two types of gas chromatography

1. Gas-solid chromatography (GSC)
2. Gas-liquid chromatography(GLC)

- **Gas-solid chromatography**

Is based upon a solid stationary phase on which retention of analytes is the consequence of physical adsorption.

- **Gas-liquid chromatography**

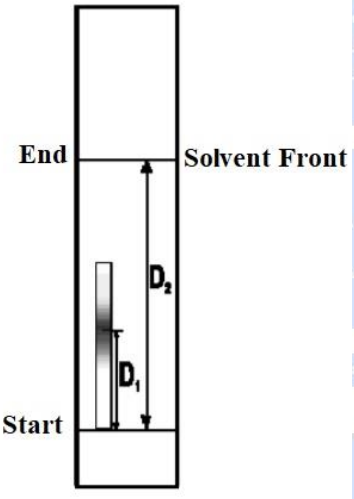
Is useful for separating ions or molecules that are dissolved in a solvent.

Retention Time:

The retention factor, R_f, is a quantitative indication of how far a particular compound travels in a particular solvent. The R_f value is a good indicator of whether an unknown compound and a known compound are similar, if not identical. If the R_f value for the unknown compound is close or the same as the R_f value for the known compound then the two compounds are most likely similar or identical. The retention factor, R_f, is defined as:

R_f = distance the solute (D_1) moves divided by the distance traveled by the solvent front (D_2).

$$R_f = \frac{D1}{D2}$$



D_1 = distance that color traveled, measured from center of the band of color to the point where the food color was applied.

D_2 = total distance that solvent traveled.

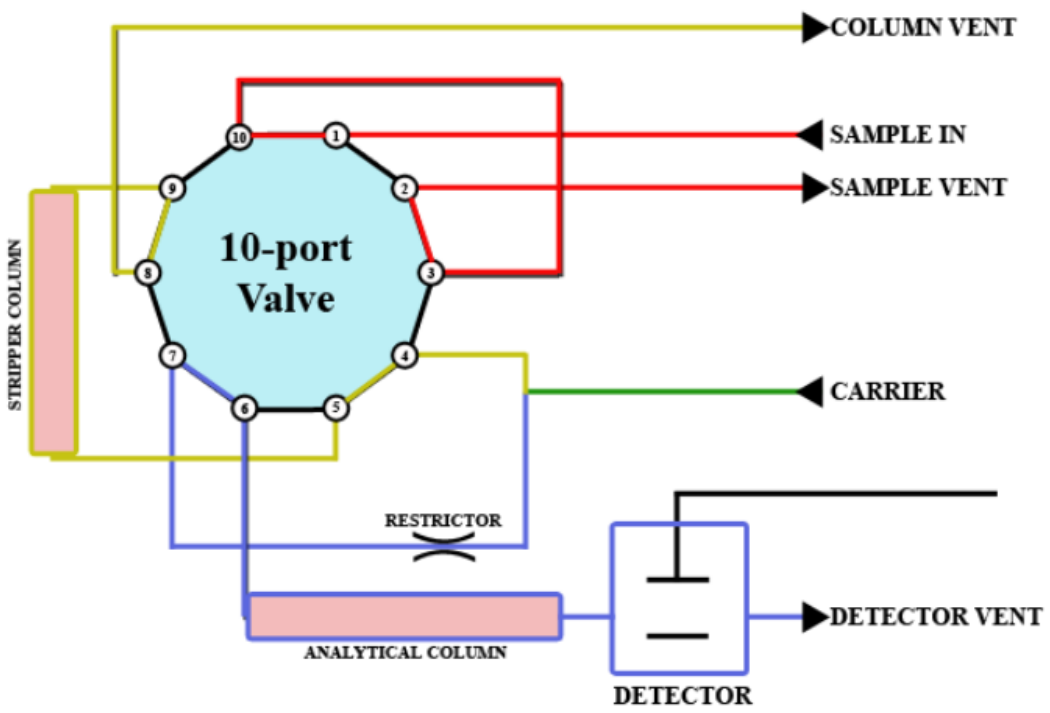
Response Factor:

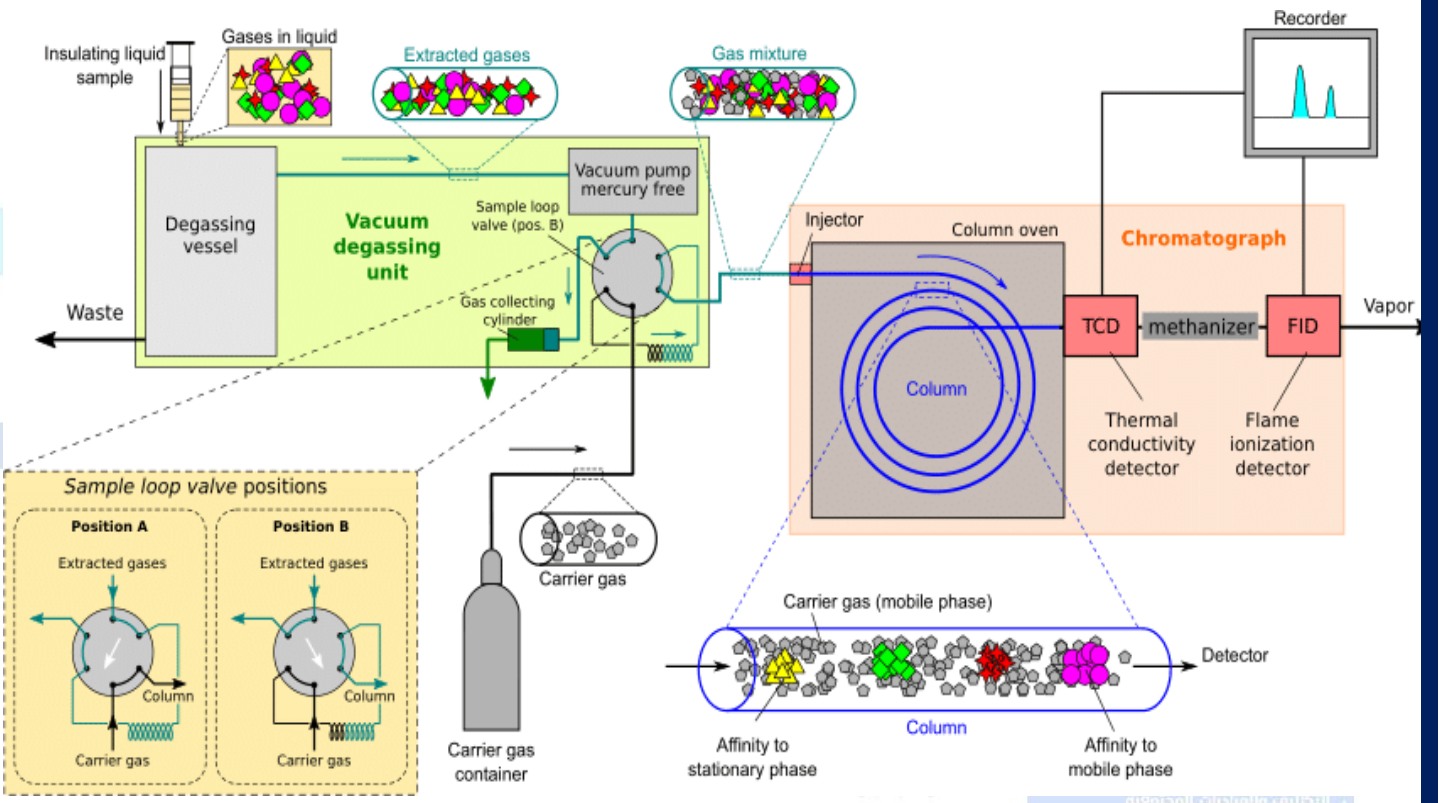
In chromatography, a response factor is defined as the ratio between the concentration of a compound being analyzed and the response of the detector to that compound. A chromatogram will show a response from a detector as a peak. While there are several ways to quantify the peak, one of the most common is peak area, thus:

$$\text{Response Factor} = \frac{\text{Peak Area (or Height)}}{\text{Sample Amount}}$$

The response factor is a correction factor allowing the calculation of the true value of an analyte's concentration when using internal standard calibration. The response factor represents differences in response between the analyte(s) and the internal standard for a particular detector. To find the accurate response factor or detector response factor (DRF), the response factor must be established experimentally for each analyte in each detector or detector system. Usually, the peak area is used in the calculation, but the peak height may be used as well.

Gas Chromatography Mechanism:





The sample solution injected into the instrument enters a gas stream which transports the sample into a separation tube known as the "column." (Helium or nitrogen is used as the so-called carrier gas.) The various components are separated inside the column. The detector measures the quantity of the components that exit the column. To measure a sample with an unknown concentration, a standard sample with known concentration is injected into the instrument. The standard sample peak retention time (appearance time) and area are compared to the test sample to calculate the concentration.

Gas Chromatography Limitations & Strengths:

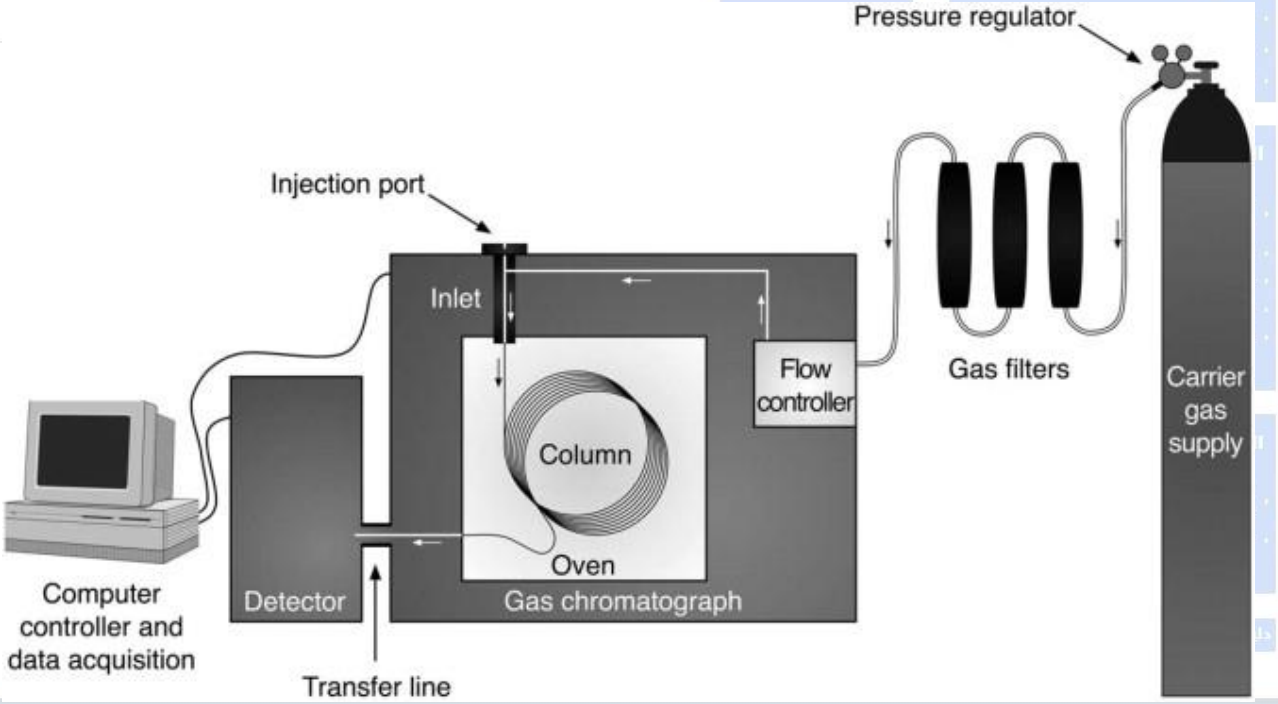
GC is limited to analyzing volatile compounds from helium/hydrogen up to molecular weights of around 1250 u. Thermally labile compounds can degrade in a hot GC, therefore cold injection techniques and low temperatures should be used to minimize this. More polar analytes can become stuck or lost in the GC, therefore the system should be deactivated and well-maintained or these analytes derivatized.

Gas Chromatography Common Problems:

The most common problem in GC is leaks. The mobile phase is a gas and flows throughout the system, therefore the correct installation of parts and consumables is important along with regular leak checking.

Activity is another issue for more polar analytes, especially those at trace levels. Silanol groups on the glass liners and column, and also a build-up of dirt in the system can cause tailing peaks, irreversible adsorption or catalytic breakdown. The inlet is the area that causes most problems as it is here the sample is injected, vaporized and transferred into the GC column. Therefore, regular inlet maintenance along with using the correct consumables, for example a deactivated inlet liner, is important to keep the instrument trouble-free.

Instrument & Instrumentation:

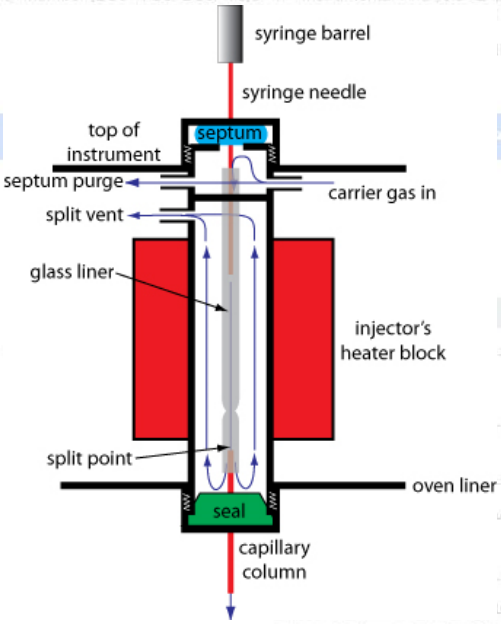


Carrier Gas:

Carrier gas is an inert gas used to carry samples. Helium (He), nitrogen (N_2), hydrogen (H_2), and argon (Ar) are often used. Helium and nitrogen are most commonly used and the use of helium is desirable when using a capillary column.

Injection Port:

It is where the liquid sample is vaporized and transported onto the column by the carrier gas. Factors which need to be taken into account are the temperature of the injection port (50+), carrier gas flow, injection technique, sample size, split ratio and the design of liner used.



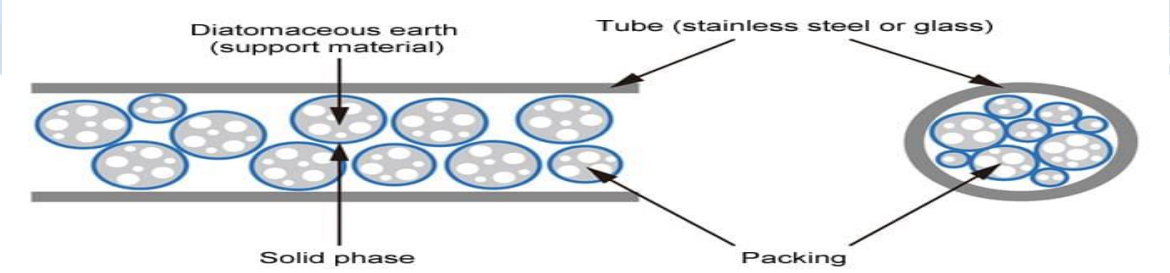
Sample:

- **Sample state**: must be either a liquid or a gas for a direct injection. The compounds off gassed by a solid can be analyzed using headspace analysis.
- **Sample size**: several micro liters for a liquid or a gas, between 0.5 - 1 gram for headspace analysis of a solid.

Column:

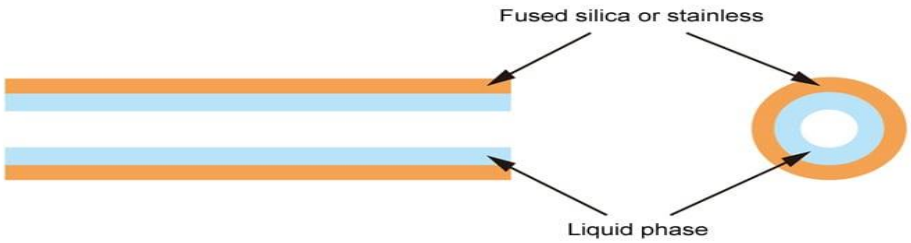
Two types of columns are used in gas chromatography:

- **Packed Column (GSC)**: Stainless steel or glass tube filled with particulate packing material (an adsorbent material, or a support material coated or impregnated with a solid phase).



- Internal Diameter: 2 to 4 mm
- Length: 0.5 to 5 m (most commonly 2 m)
- Packing: Support material with 0.5 to 25 % liquid phase (partition material) or no liquid phase (adsorbent material)
- Liquid Phase: Multiple types available

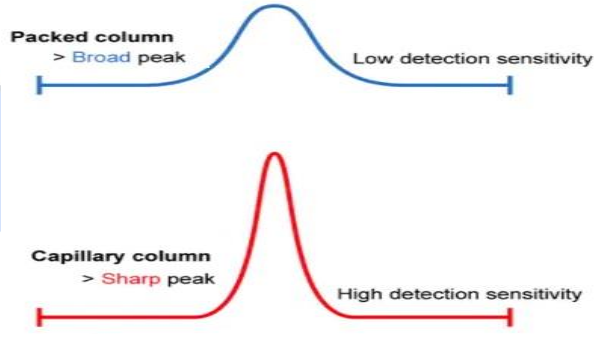
- **Capillary Column (GLC):** A typical capillary column is a thin, fused silica glass tube, lined with a liquid phase or adsorbent material or having a chemical bonding layer. Thin metal tubes are also sometimes used as capillary columns.

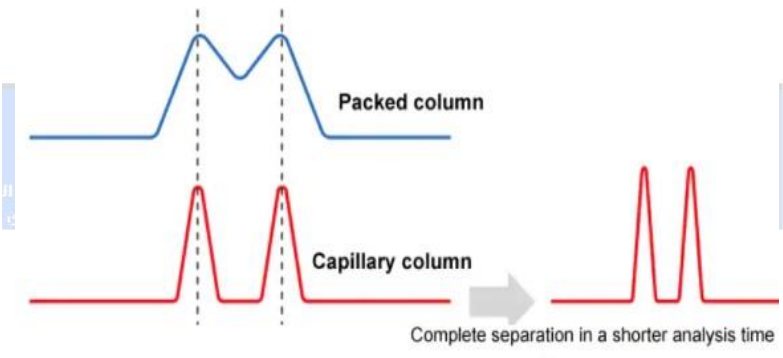


- Internal Diameter: 0.1, 0.25, 0.32, 0.53 mm
- Length: 5 to 100 m (most commonly 30 m)
- Material: Fused silica glass
- Liquid Phase: Good separation but less variety than packed columns.

Column Type and Effect on Separation

Packed columns produce broad peaks and capillary columns produce sharp peaks. In addition, capillary columns produce taller peaks, which allows the detection of lower concentrations (high detection sensitivity). This is the advantage of capillary columns.





Sharper peaks provide better separation but also shorter analysis times.

Detectors:

Detector	Detectable Compound	Detection Limit *
General-Purpose Detectors		
Flame ionization detector (FID)	Organic compounds (other than formaldehyde and formic acid)	0.1 ppm (0.1 ng)
Thermal conductivity detector (TCD)	All compounds other than the carrier gas	10 ppm (10 ng)
Barrier discharge ionization detector (BID)	All compounds other than He and Ne	0.05 ppm (0.05 ng)
Selective, High-Sensitivity Detectors		
Electron capture detector (ECD)	Organic halogen compounds Organic metal compounds	0.1 ppb (0.1 pg)
Flame thermionic detector (FTD)	Organic nitrogen compounds Inorganic and organic phosphorus compounds	1 ppb (1 pg) 0.1 ppb (0.1 pg)
Flame photometric detector (FPD)	Inorganic and organic sulfur compounds Inorganic and organic phosphorus compounds Organic tin compounds	10 ppb (10 pg)
Sulfur chemiluminescence detector (SCD)	Inorganic and organic sulfur compounds	1ppb (0.1pg)

Detector	Detector Gas	Makeup Gas (Capillary)
FID	H_2 and Air	He or N_2
TCD	Unnecessary	He or Ar or N_2 or H_2 ,etc.
BID	He	None
ECD	Mainly N_2 (The combination of gases varies by equipment model.)	
FTD	H_2 and Air	He
FPD	H_2 and Air	None (required in some models)
SCD	H_2 and O_2	N_2

– FID:

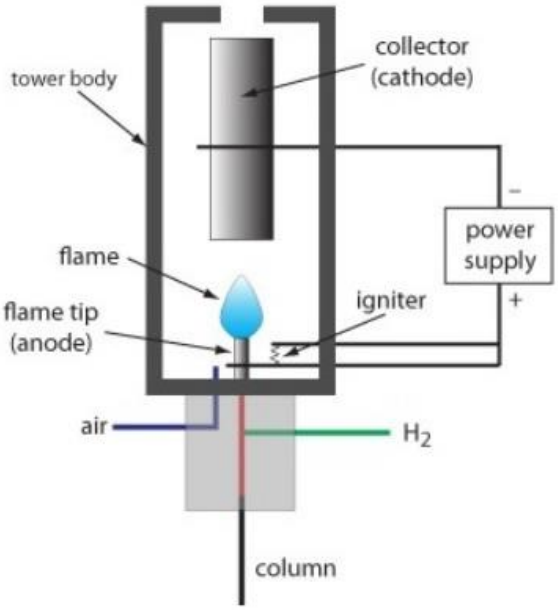
Compounds that produce ions when burned in an H_2-air flame → organic cation → releases electron → detected by collector electrode → generation of current.

Magnitude of current **α** mass of carbon material delivered to detector → used for detection & quantification of eluting solutes.

Advantages → simple, reliable, sensitive,linearity excellent.

Dis –Advantage – destroy all the sample.

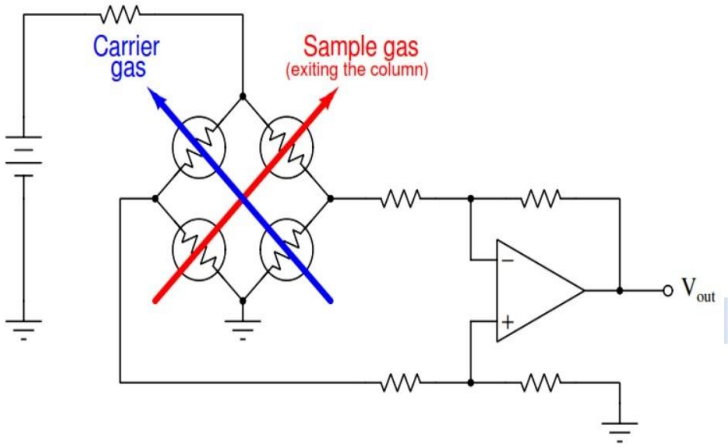
uses → detects hydrocarbon including fattyacids.



-TCD:

Universal detector → most of the analytes

Difference in thermal conductivity between the carrier gas and sample gas causes a voltage output



Applications:

- Separation & identificaton of lipids, carbohydrates & proteins.
- Separation & identificaton of aminoacids in urine by GC-MS for diagnostic purpose.
- Measurement of drugs & other metabolites in biological fluids.
- Used for toxiclogical analysis of biological fluid by using ECD detectors in GC.
- Analysis of pesticides in soil, water, food.
- Forensic analysis of blood and urine alcohol levels by using PEG-SP IN GC
- GC can be used to identify nitro-compounds in trace quantities.

Practical:

Separation of the four isomeric butyl alcohols mixture & determination of the percentage of each in an unknown mixture.