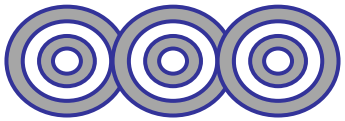
Chemical Separation and Chromatographic Methods



Gas Chromatography (GC)

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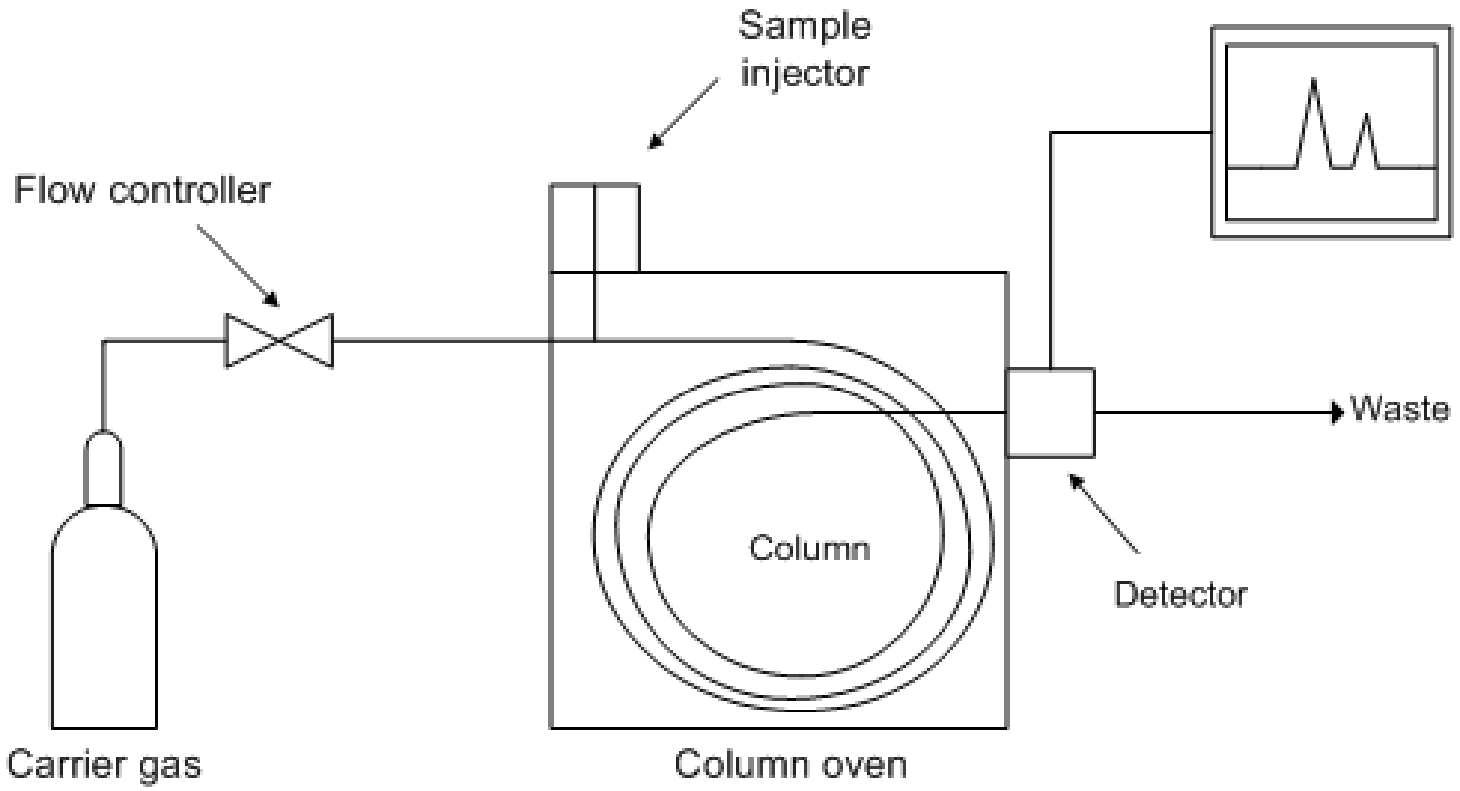
Gas Chromatography

Separation by **partition** between gaseous mobile phase and liquid stationary phase supported by inert packing **GLC**, or by **adsorption** between gaseous mobile phase and solid stationary phase **GSC**.

Separation depends on temperature. Based on a wide range of boiling points and polarity.

Differences in behaviors between the solutes and stationary phases.

GC Main Parts



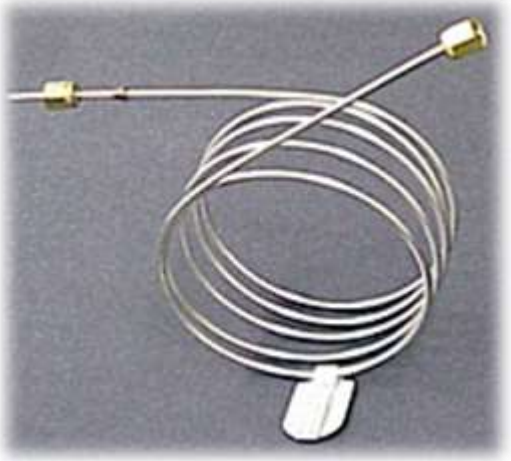
Columns

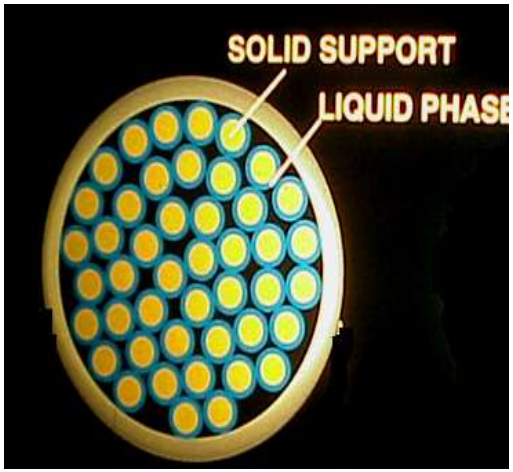
Although it's usually the smallest part, the column is considered the most important component in any column chromatographic system,

- Packed columns.
- Open tubular capillary columns.
  - Wall-coated open tubular (**WCOT**)
  - Support-coated open tubular (**SCOT**)
  - Fused silica open tubular (**FSOT**)
- Monolithic columns.

Packed Columns

- Made of stainless steel or glass.
- Diameters of 1/8 or 1/4 in.
- Length from 1 to 3 m.
- Filled with porous particles, which act as support of the stationary liquid phase.
- The internal surface of the tube is treated to avoid catalytic interactions with the sample.
- Carrier gas flow rate of typically 10 to 40 ml/min (high gas consumption).
- Although they are still used in approximately 10% of cases for routine GC work, packed columns are not well adapted to trace analyses.





Open Tubular Columns

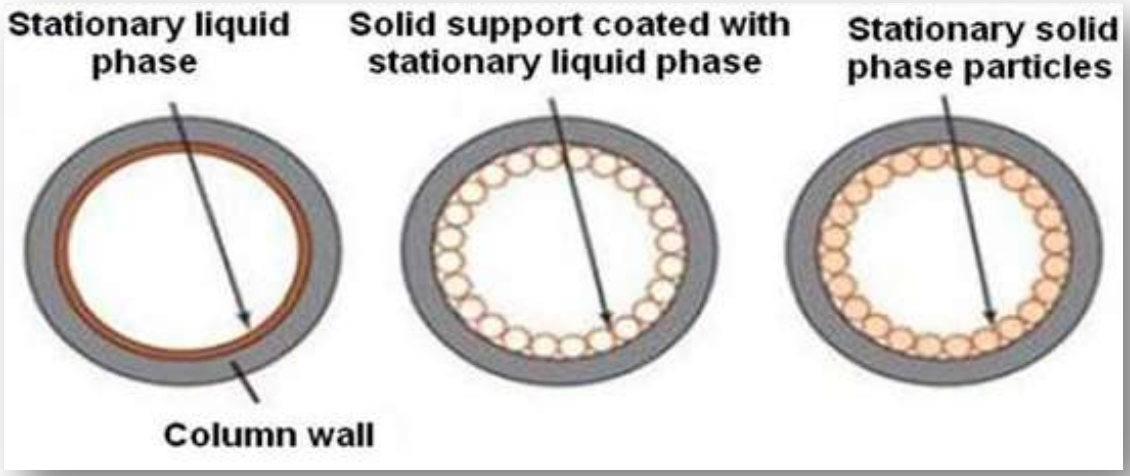
- Made of fused silica.
- The internal diameter varies from 0.1 to 0.35 mm - length from 15 to 100 m.

Open tubular columns are three classes:

- **WCOT** columns:
  Wall coated open tubular, are simply tubes coated with a thin layer of the stationary phase, which is the most popular one.

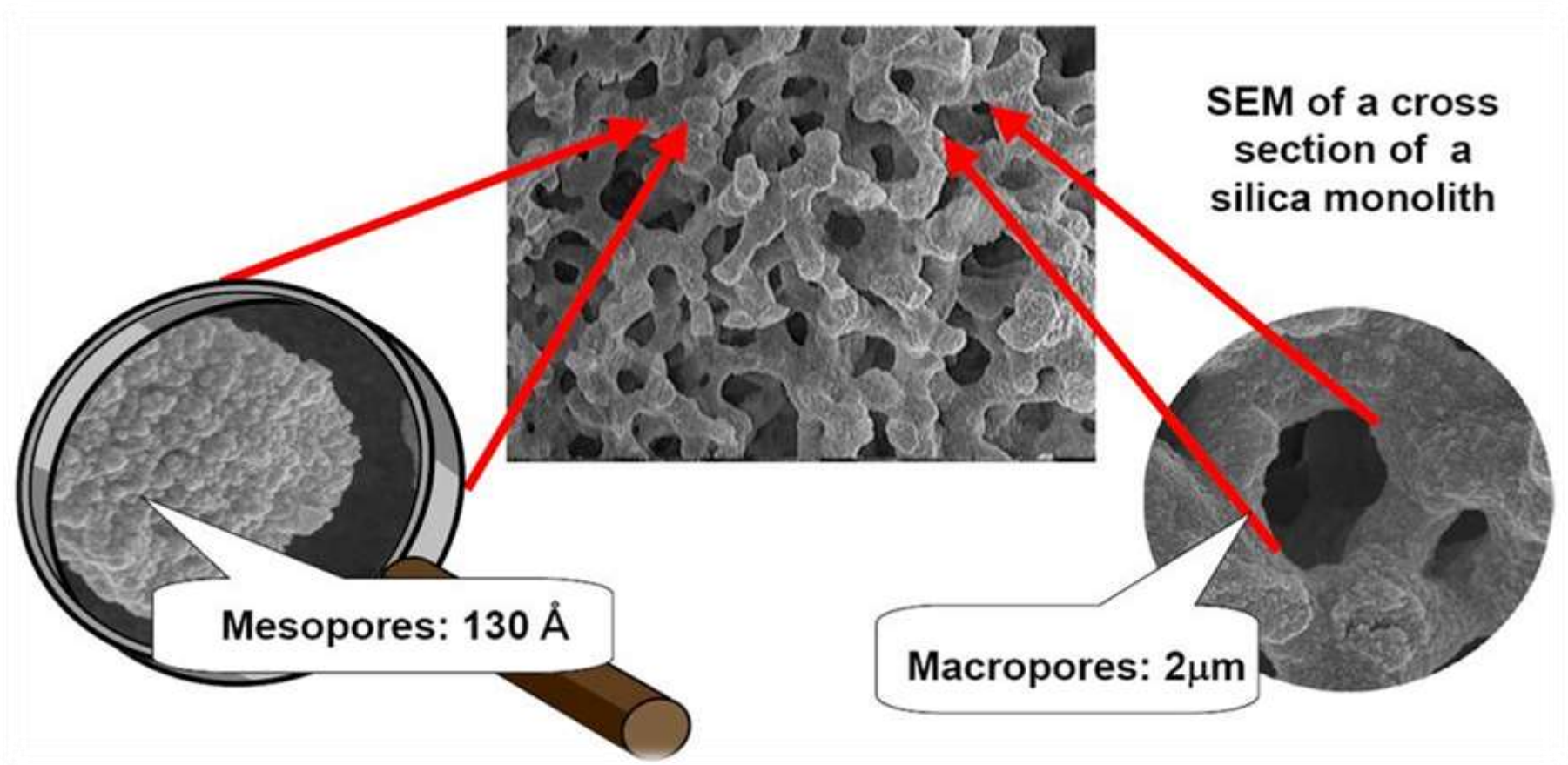
- **SCOT** columns:
  Support coated open tubular, in which a stationary phase is a solid support film coated with stationary liquid phase.

- **PLOT** columns:
  Porous layer open tubular, in which the inner surface of the capillary is lined with a thin films of a support material, that designed to increase the loading capacity of the column.



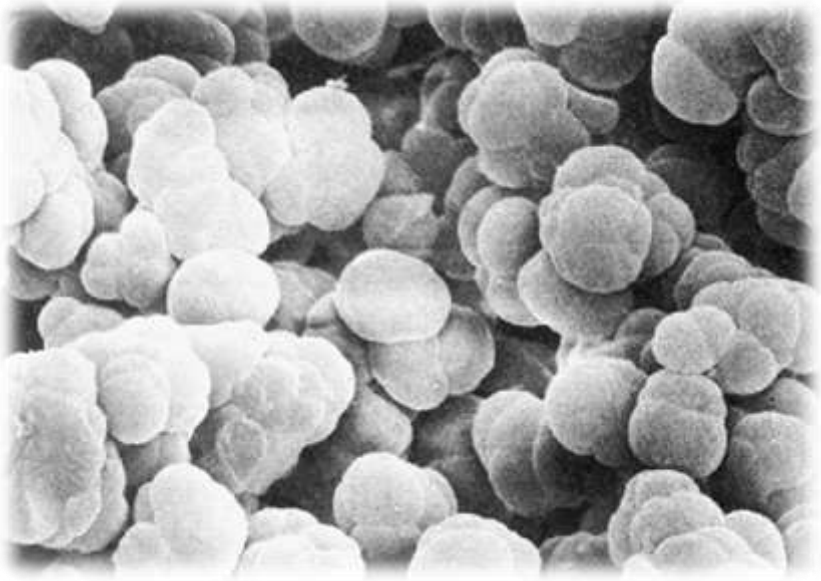
Monolithic Columns

Monoliths are a single block piece of continuous materials made of highly porous rods with two types of bimodal pore structure distribution (macropores and mesopores).

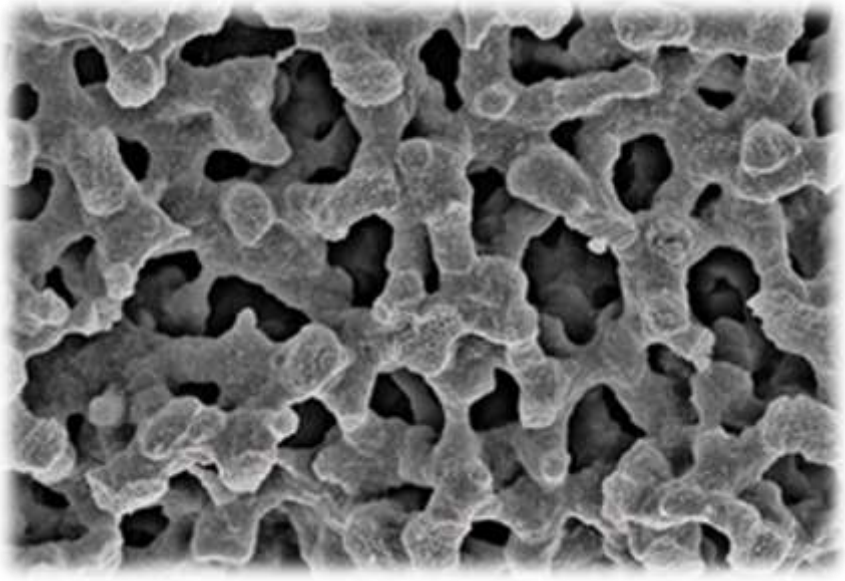


SEM of the macroporous and mesoporous structures in a monolithic silica rod. Macropores dramatically increase the column porosity, which reduce the analysis time, while mesopores form the fine porous structure and provide large active surface area for high efficiency separations.

Two types of monolithic columns have been developed for chromatography:



Organic polymers
e.g. polymethacrylates, polystyrenes or polyacrylamides



Inorganic polymers
e.g. polysilicates,

Packed vs Open tubular columns

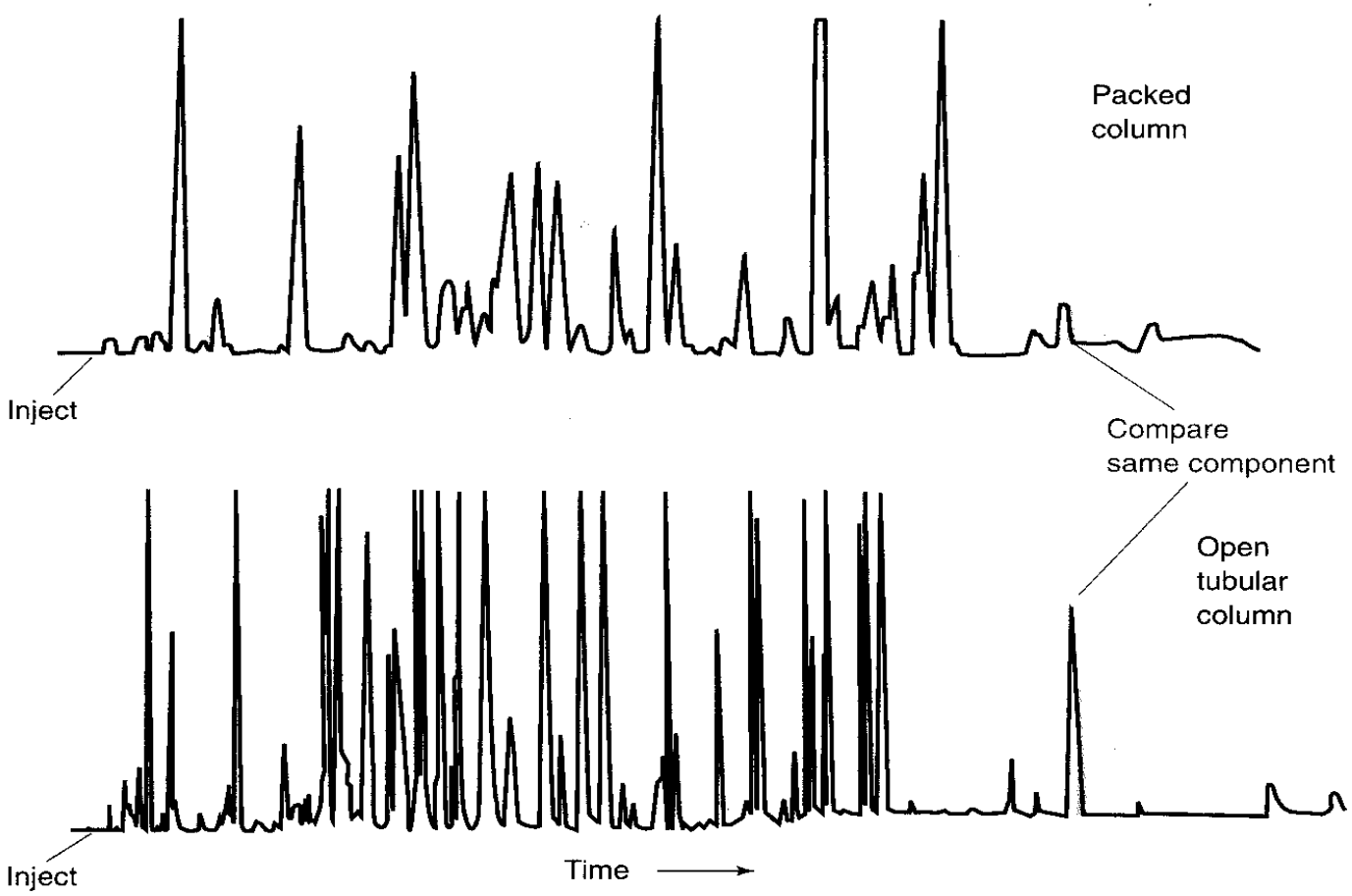


Figure 24-3 Gas chromatographic separation of a perfume oil on a 2-mm-diameter × 1.5-m-long packed column (upper trace) and a 0.25-mm-diameter × 30-m-long open

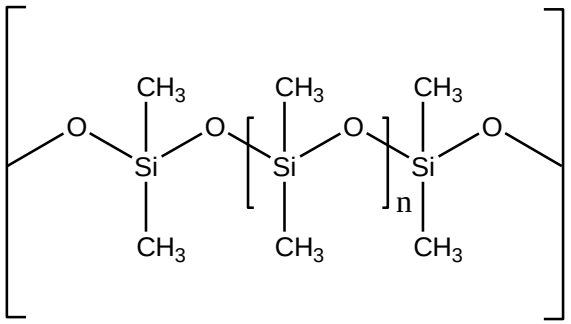
Principle of Separation

Like dissolve like (like attract like)
Non-polar stationary phases best for non-polar analytes
Polar stationary phases best for polar analytes

Stationary Phase	Common Trade Name	Maximum Temperature, °C	Common Applications
Polydimethyl siloxane	OV-1, SE-30	350	General-purpose nonpolar phase; hydrocarbons; polynuclear aromatics; drugs; steroids; PCBs
Poly(phenylmethyldimethyl) siloxane (10% phenyl)	OV-3, SE-52	350	Fatty acid methyl esters; alkaloids; drugs; halogenated compounds
Poly(phenylmethyl) siloxane (50% phenyl)	OV-17	250	Drugs; steroids; pesticides; glycols
Poly(trifluoropropyldimethyl) siloxane	OV-210	200	Chlorinated aromatics; nitroaromatics; alkyl-substituted benzenes
Polyethylene glycol	Carbowax 20M	250	Free acids; alcohols; ethers; essential oils; glycols
Poly(dicyanoallyldimethyl) siloxane	OV-275	240	Polyunsaturated fatty acids; rosin acids; free acids; alcohols

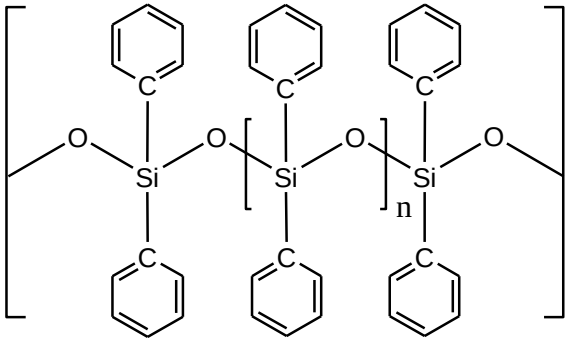
Stationary phases

100% dimethyl polysiloxane
Least polar phase



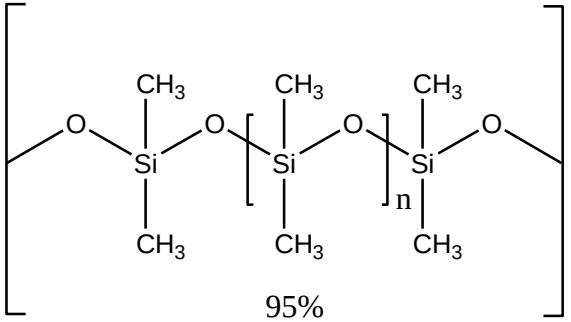
100%

5% diphenyl
95% dimethyl
polysiloxane
Non- polar phase



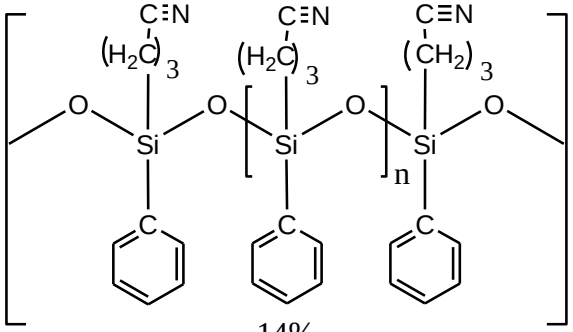
5%

+



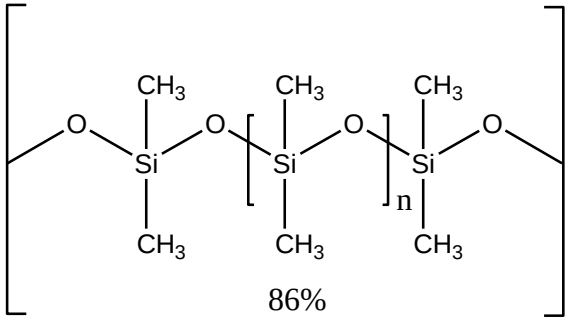
95%

14% cyanopropylphenyl
86% dimethyl
polysiloxane
polar phase

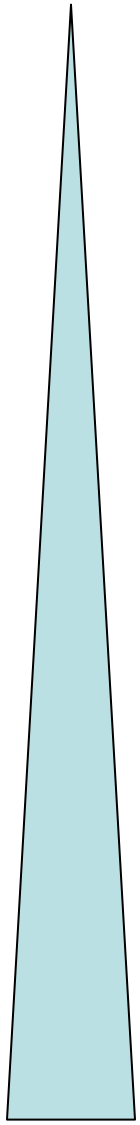


14%

+

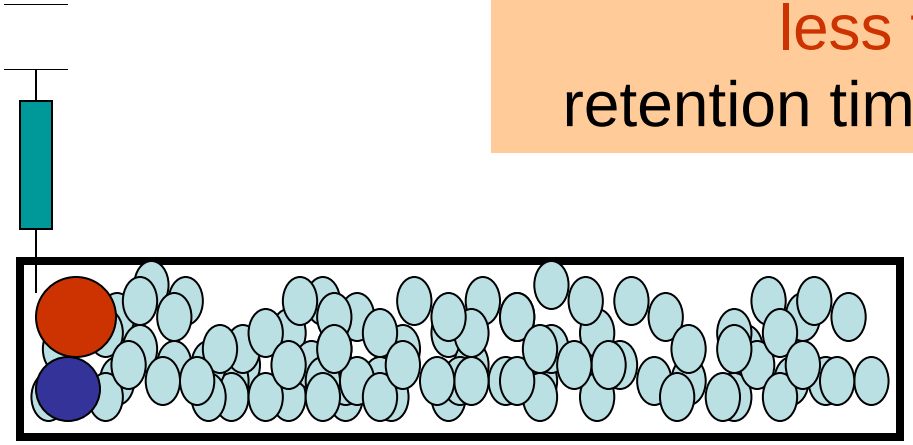


86%



Polarity

Retention time of CCl_4 is
less than
retention time of CH_3OH



polar column

CCl_4

CH_3OH

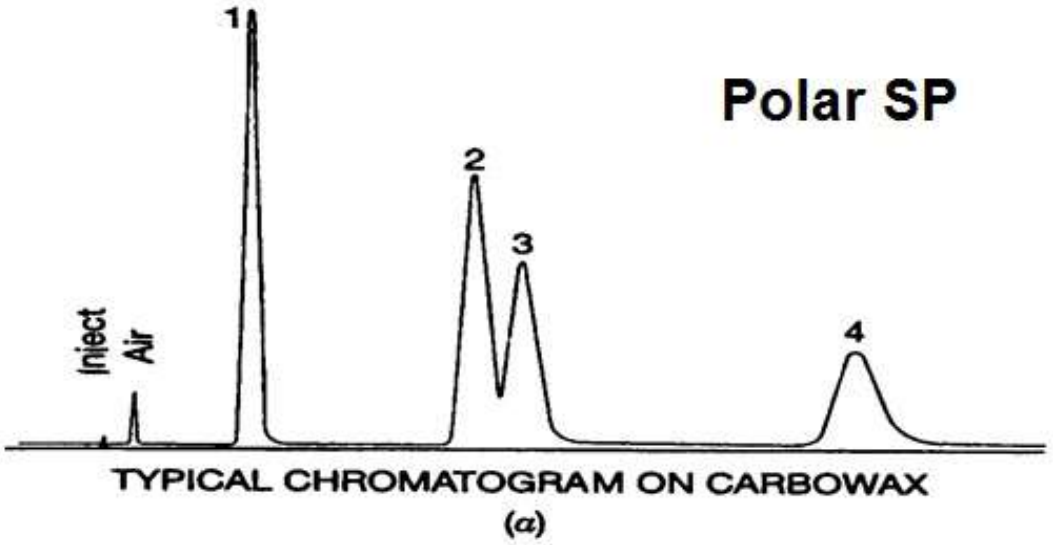
SP

Methyl alcohol (CH_3OH) is polar and is attracted to SP and therefore travels slowly through column.

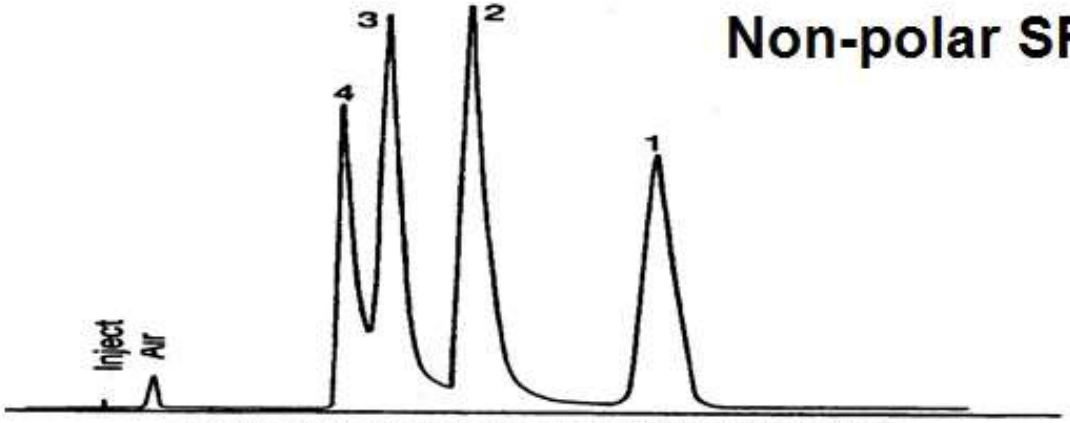
Carbon tetrachloride (CCl_4) is nonpolar and is not attracted to SP and therefore travels rapidly through column.

Effect of SP on Retention Times

(1) n-heptane
(2) tetrahydrofuran
(3) 2-butanone
(4) n-propanol



TYPICAL CHROMATOGRAM ON CARBOWAX
(a)



TYPICAL CHROMATOGRAM ON DC 200
(b)

Carrier Gas

- Chemically inert gases **(**He, H_2, N_2 & CO_2)
- High purity, 99.9995% pure or better
- Free from water and oxygen
- Detector compatibility
- Economic / safety reasons
- Efficiency / speed

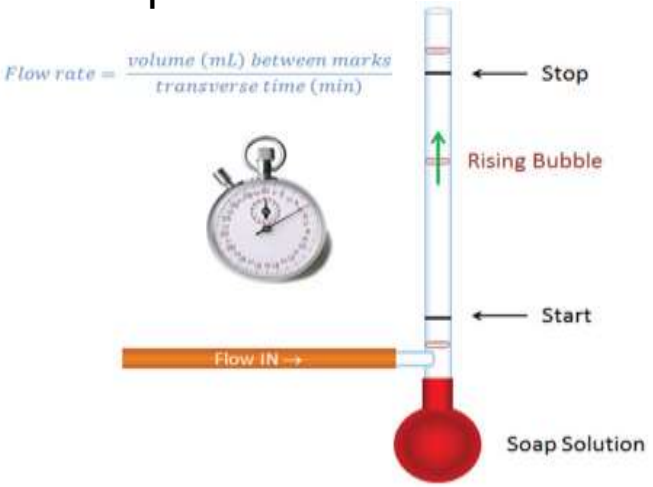
H_2: efficient, cheep and rapid but not safe.
N_2: cheep and safe but less efficient and not inert.
He: efficient, rapid, inert and safe but relatively expensive.

Carrier Gas Supply

Pressure regulators:
- Reduce pressure of gas
- Control the flow rate

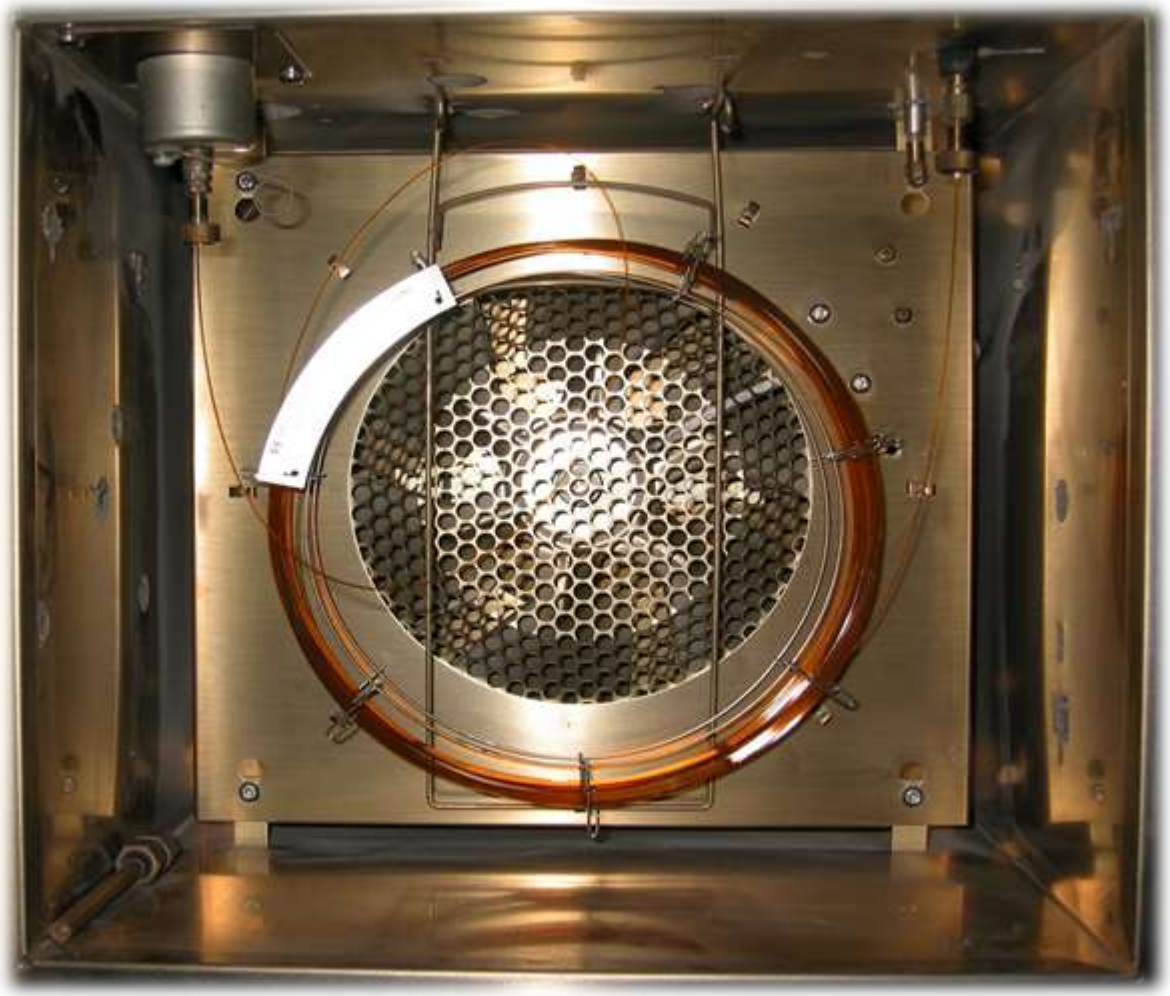
Flow meters

soap bubble flow meter



Electronic flow meter

Oven

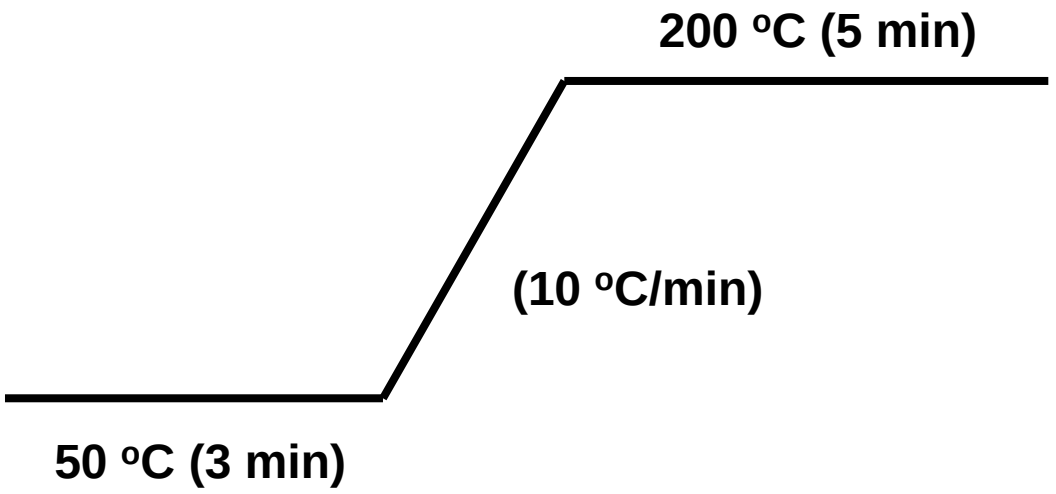


Temperature control

Isothermal

80 °C

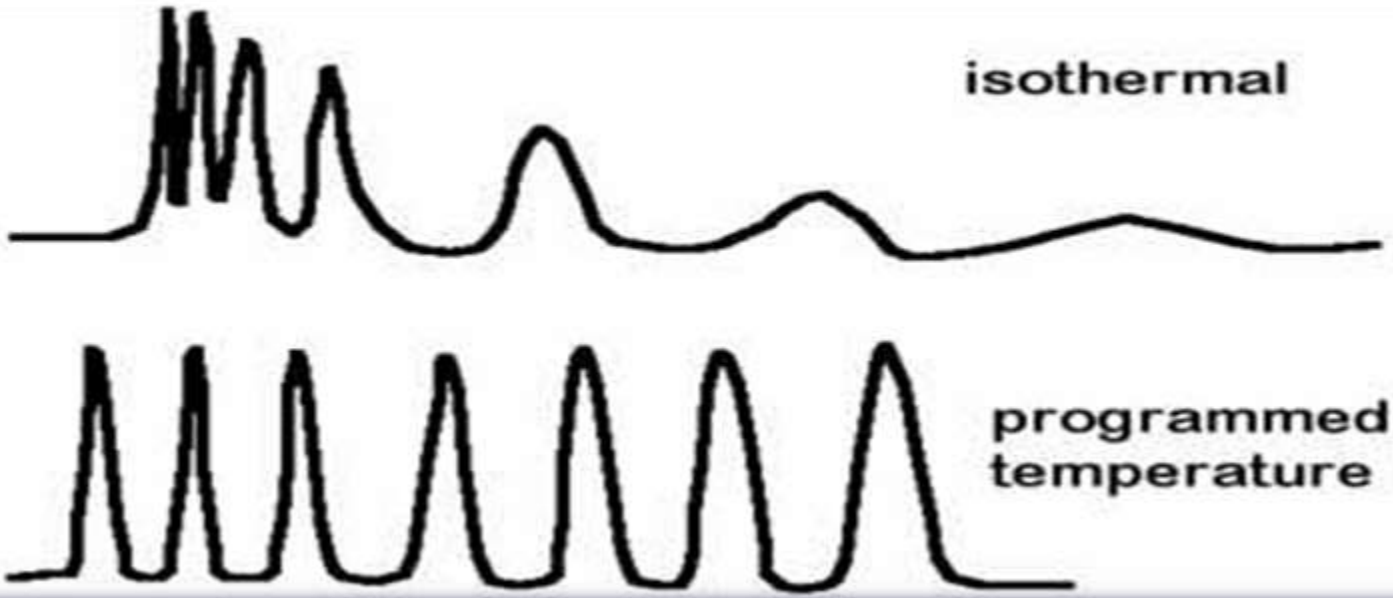
Gradient



Temperature Program

Factors to consider

- Changes in volatility of solutes.
- Stability of solutes.
- Flow rate changes.
- Stability of stationary phase.

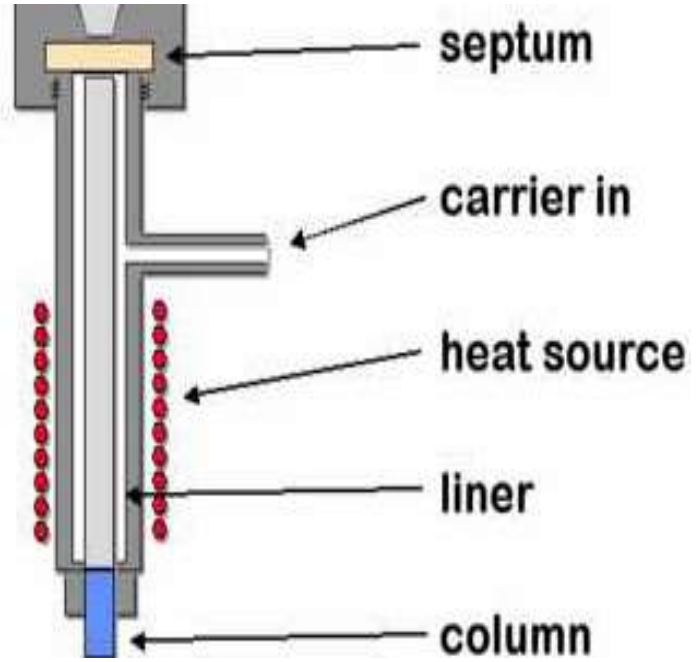


Injector

- Role of injectors
  - Works as an inlet for the sample.
  - It vaporizes and mix the sample with the carrier gas before the sample enters the head of the column.
- The injection volume has a great effect on the quality of the separation.
- The type of column used in the analysis sets the mode of injection.

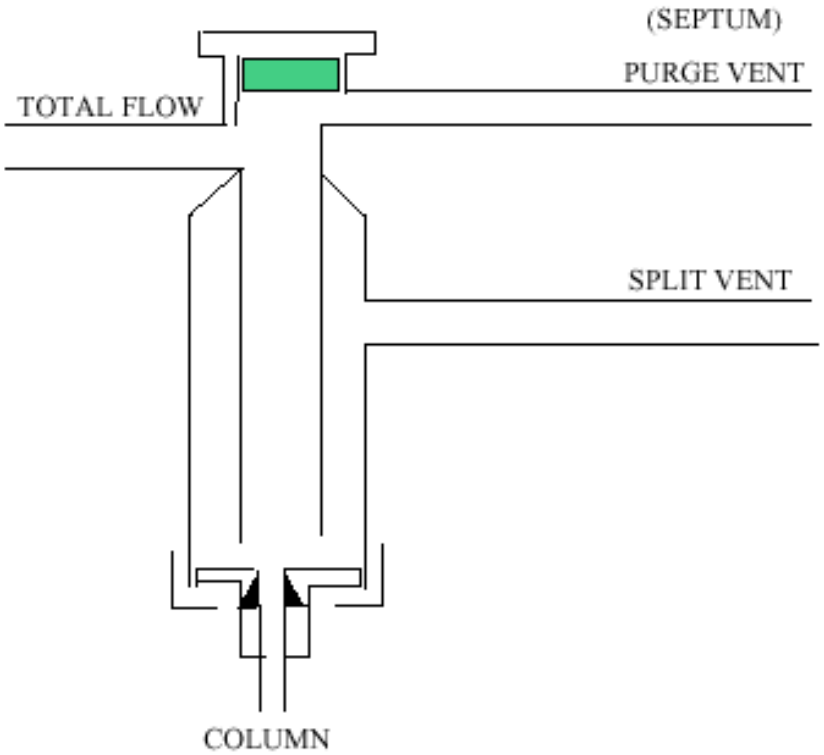
Direct Vaporization Injector

- For packed columns.
- Uses a metal tube with a glass sleeve or insert.
- The glass insert is swept by the carrier gas and heated to the vaporization temperature.
- Contains a septum made of silicone rubber that allows the syringe needle to pass through it into the system.



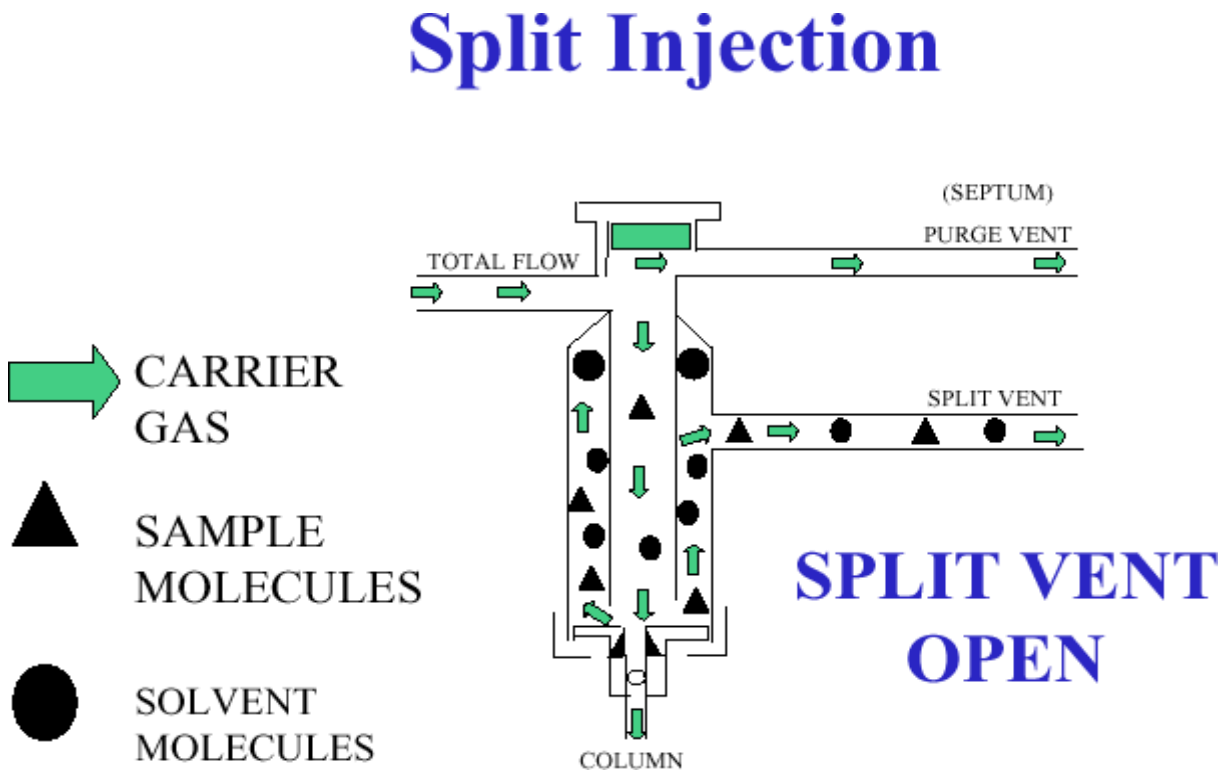
Split / Splitless Injector

- For capillary columns.
- Operate in two modes, with or without flow splitting.



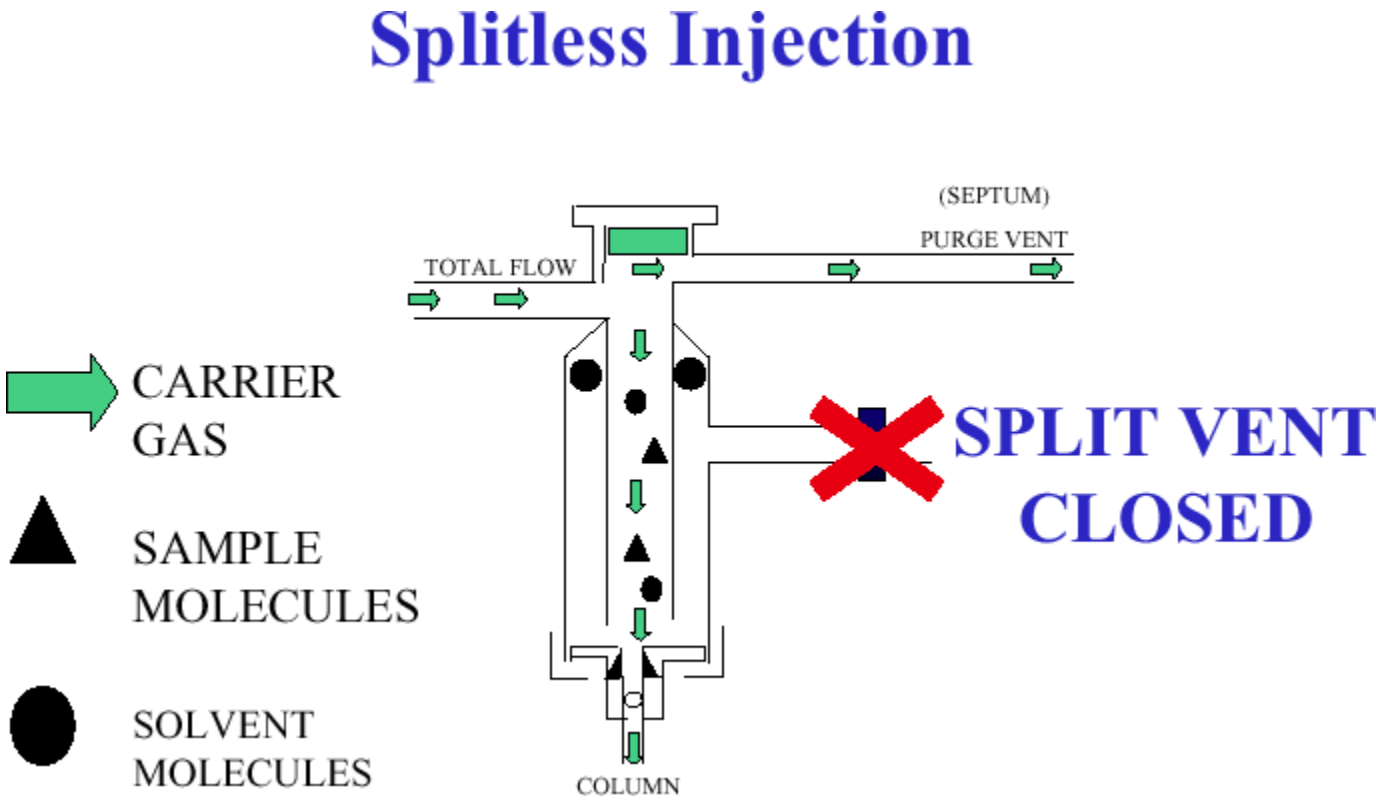
Split Mode

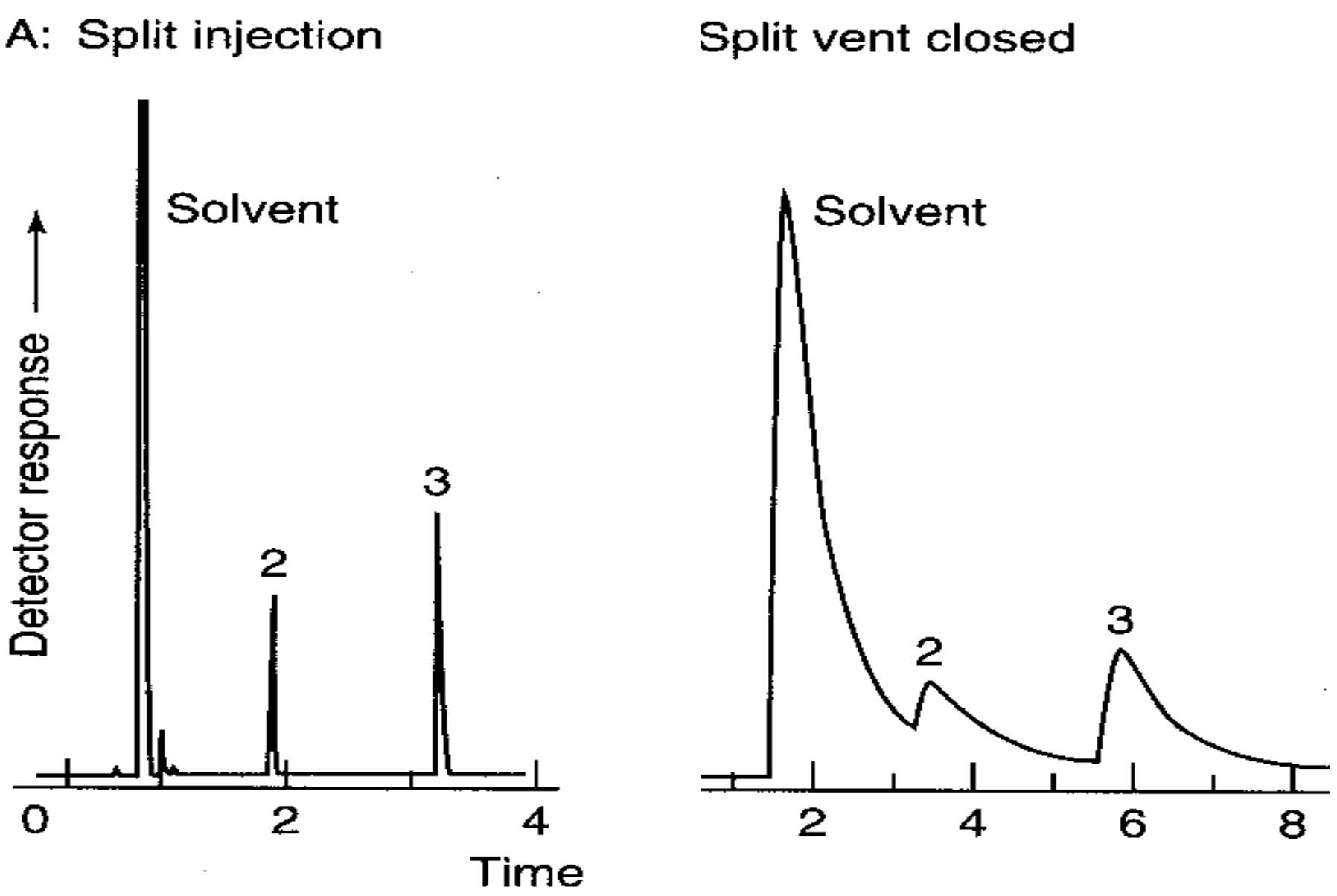
- Carrier gas arrives in the vaporization chamber with a relatively large flow.
- A vent valve separates the carrier gas flow into two parts of which the smallest enters the column.
- The split ratio varies between 1 : 20 and 1 : 500.



Splitless Mode

- All sample to column.
- Best for quantitative analysis.
- For trace analysis.




A: Split injection
Solvent
2
3
Detector response
0
2
4
Time
Split vent closed
Solvent
2
3
2
4
6
8

Injection Techniques

- Syringe injection.
- Gas sampling loop/valve.
- Purge and trap.
- Solid phase microextraction (**SPME**).

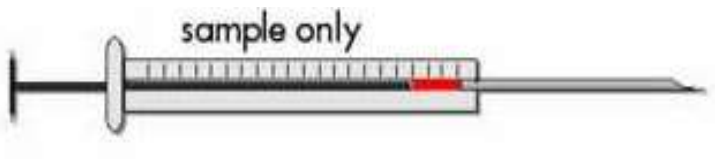
Syringe injection

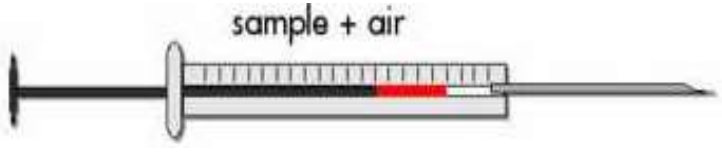
Bevel

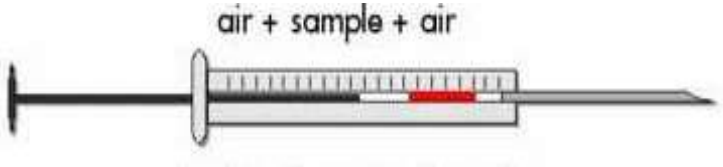
Blunt

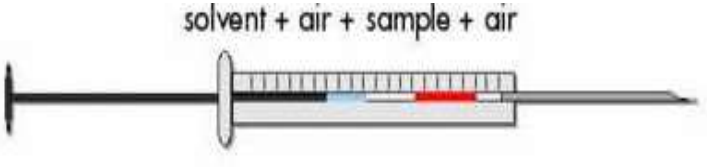
Introduce the sample with a microsyringe 0.1-10 µL.

Syringe loading methods







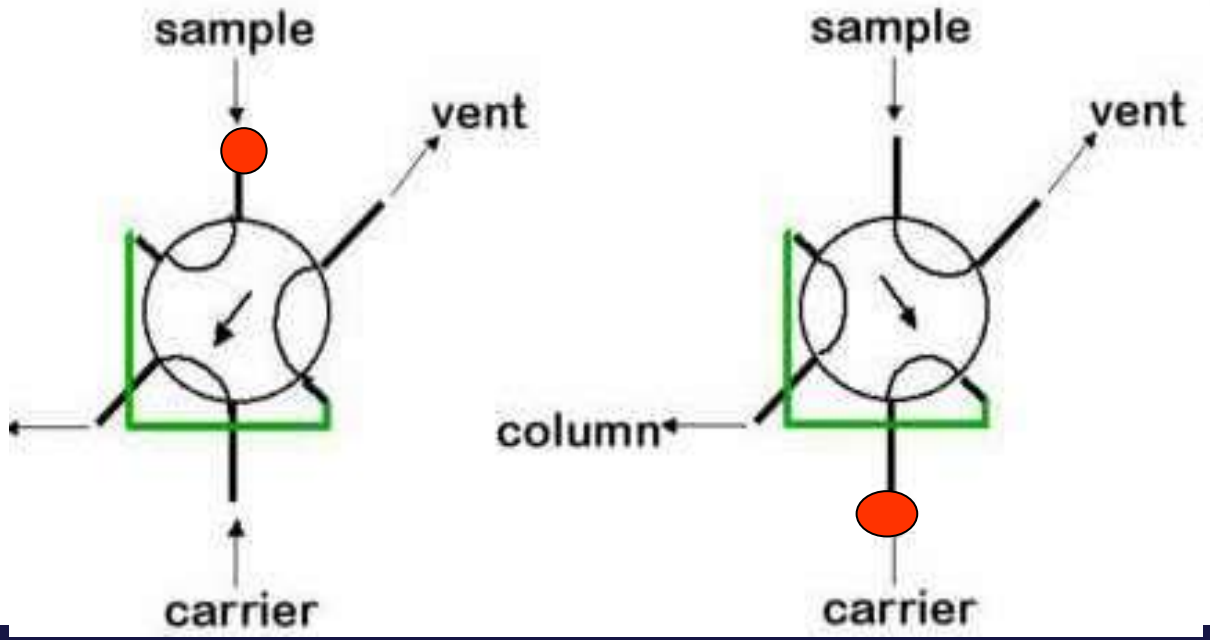


Hot needle method

- Insert needle into injection port and allow to heat for a few seconds.
- Rapidly inject sample and withdraw the needle.
- Sample should be injected as a plug.

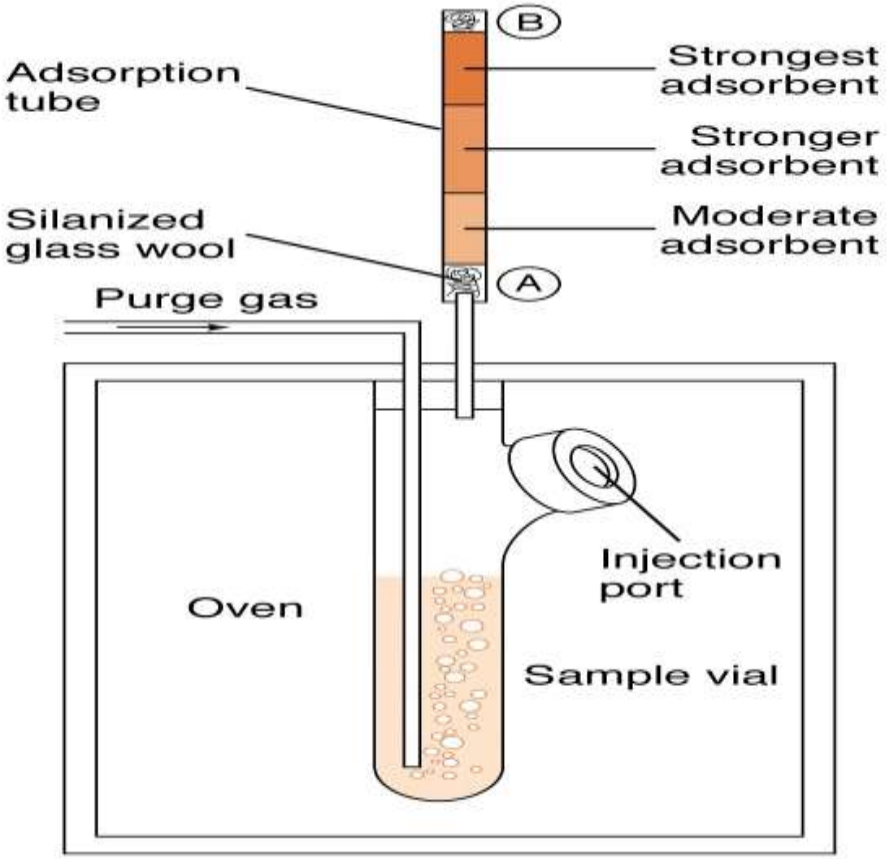
Gas Sampling Loop / Valve

- Introducing a constant amount of a gas can be difficult with a syringe.
- Valves give better reproducibility.
- Require less skill.
- Can be easily automated.

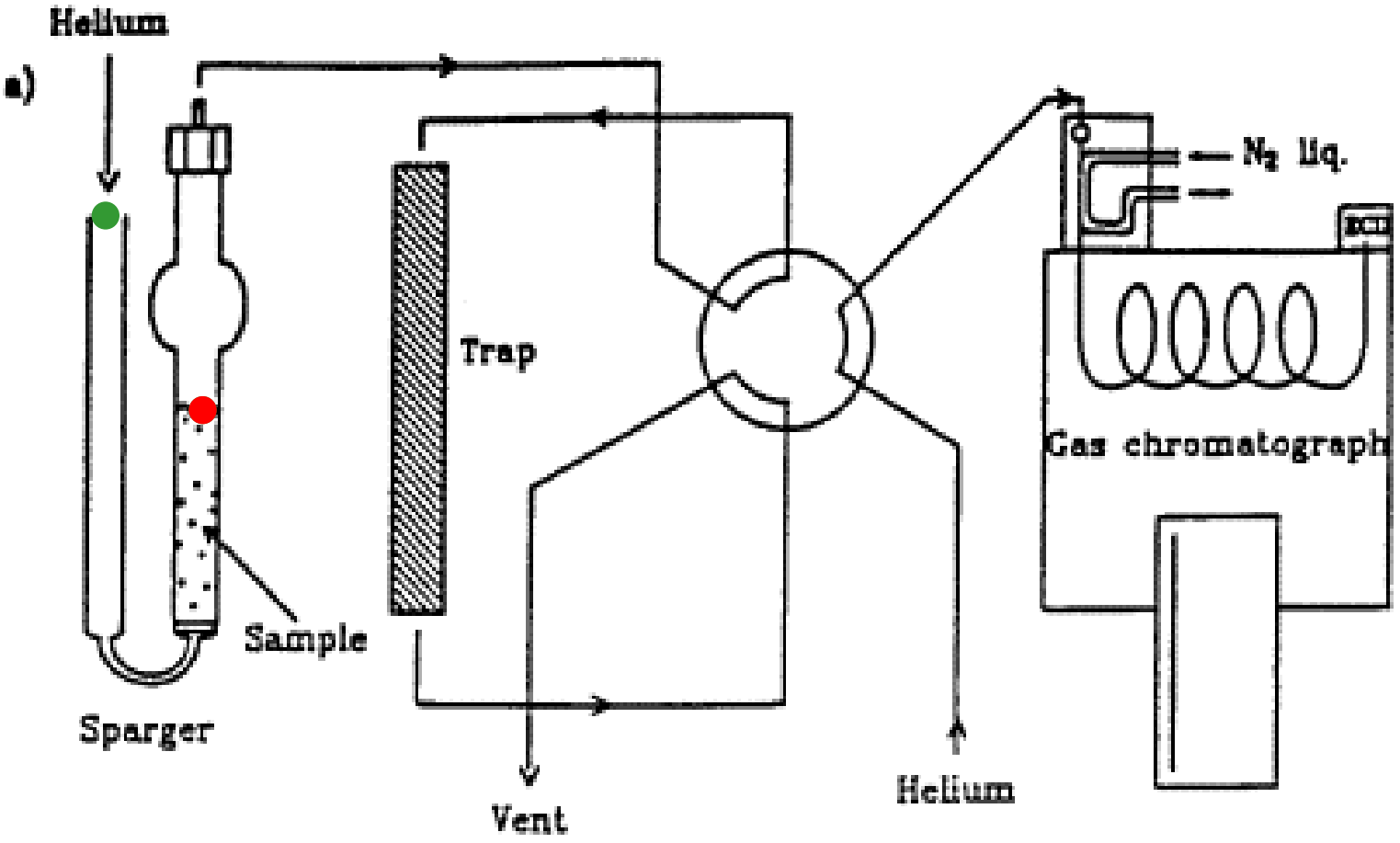


Purge and Trap

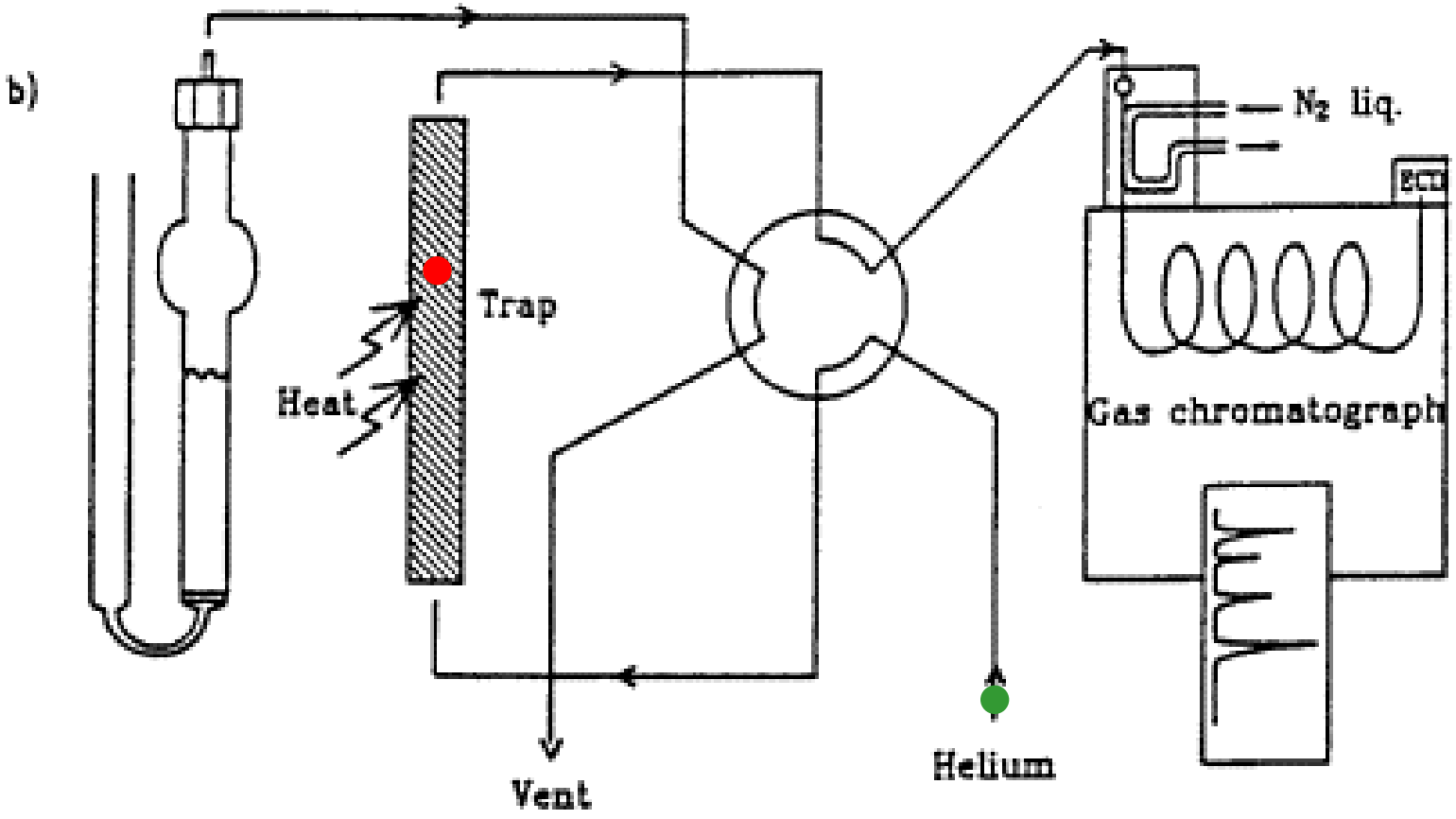
- The sample is permanently purged with carrier gas, which carries the analytes to the trapping medium.
- Lower detection limit.
- Useful for concentrating insoluble or poorly soluble volatile organic compounds (VOCs).



Purge &Trap Step



Desorption step

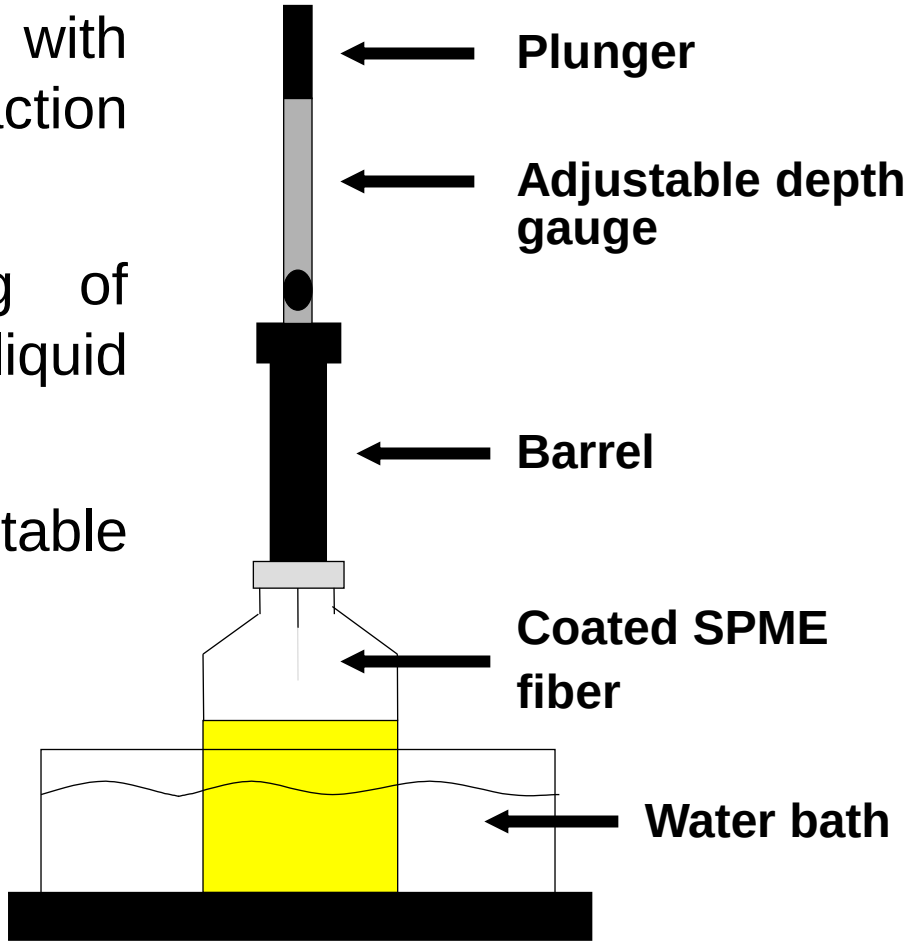


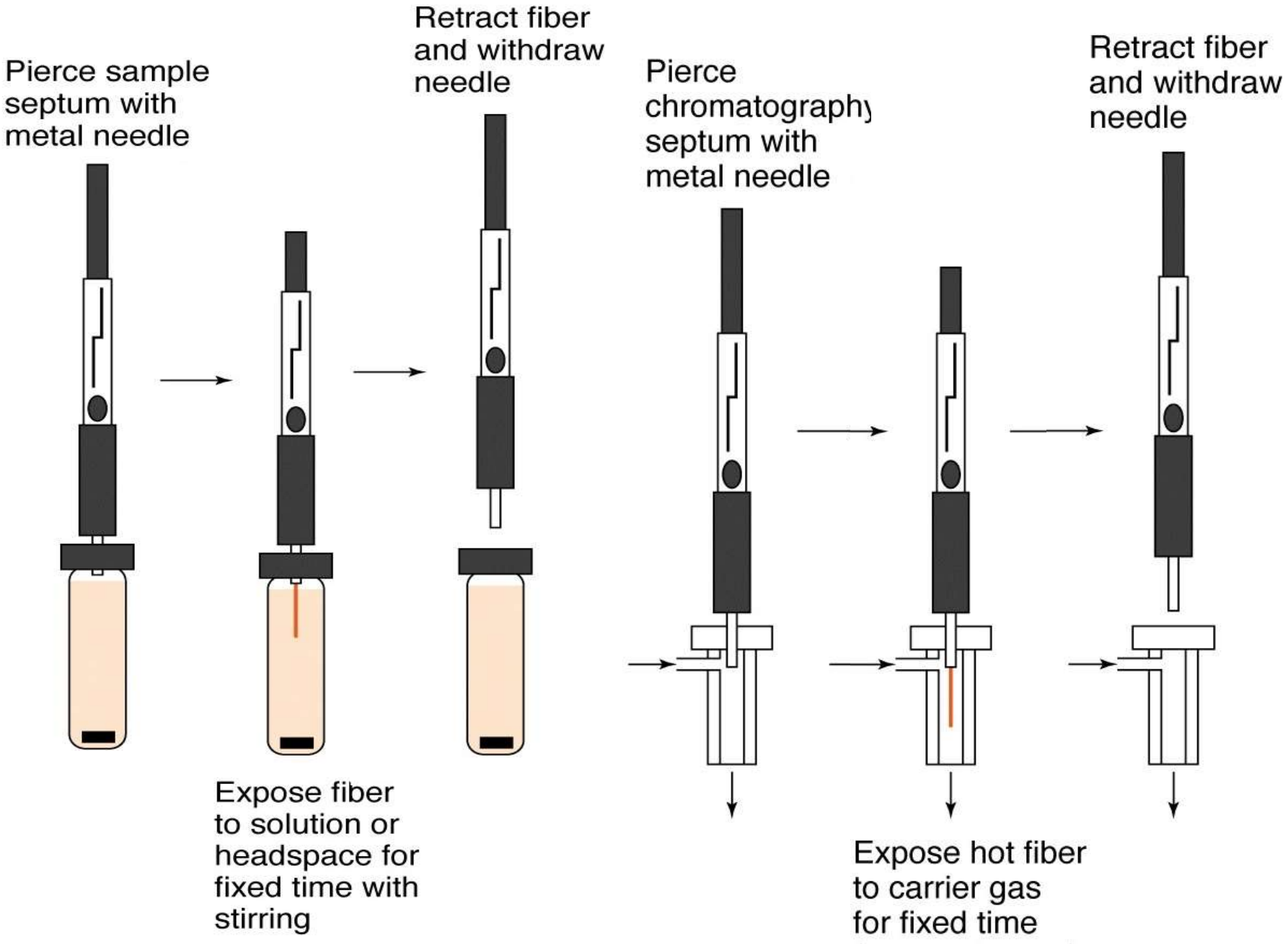
Solid Phase Microextraction (SPME)

A technique that uses a short, thin, silica fused rod which is coated with absorbent polymer (fiber) for extraction of compounds.

Principle: Equilibrium partitioning of compounds between the fiber and liquid sample.

It is fast, sensitive, inexpensive, portable and solvent-free.



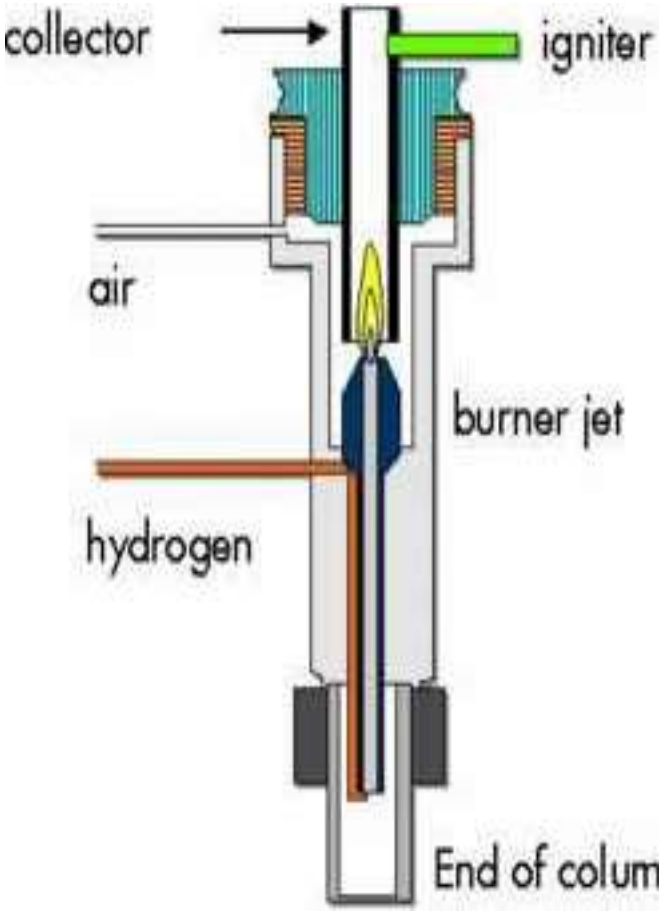

Pierce sample septum with metal needle
Retract fiber and withdraw needle
Expose fiber to solution or headspace for fixed time with stirring
Pierce chromatography septum with metal needle
Retract fiber and withdraw needle
Expose hot fiber to carrier gas for fixed time

Detectors

- Generates an electrical signal proportional to the sample concentration.
- Must be hot enough (20 to 30 ^{o}C above the column temperature).
- High sensitivity-possible selectivity.
- Rapidly respond to concentration changes.
- Large linear range.
- Low sensitivity to variation in flow, pressure and temperature.
- Produces an easily handled signal.

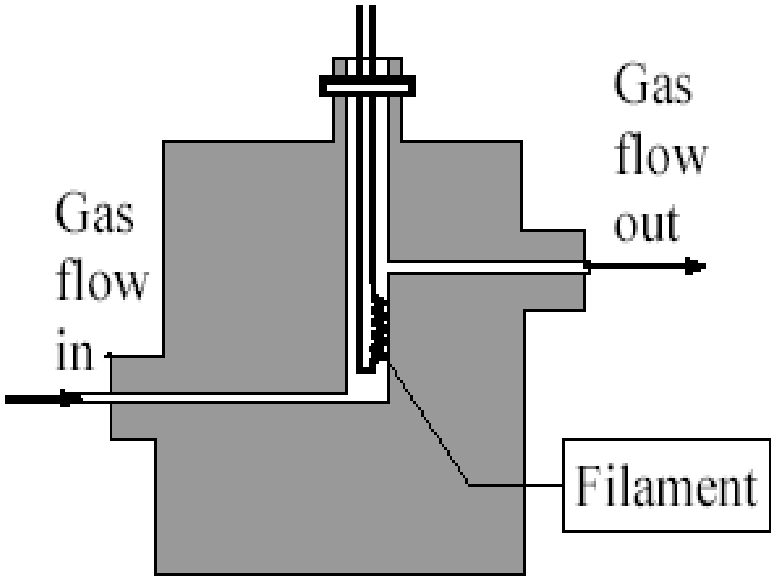
Flame Ionization Detector (FID)

- The effluent from the column is mixed with hydrogen and air, and ignited.
- Organic compounds burning in the flame produce ions and electrons which can conduct electricity through the flame.
- A large electrical potential is applied at the burner tip, and a collector electrode is located above the flame.
- The current resulting from the pyrolysis of any organic compound is measured which is proportional to the carbon content of the molecule entering.
- **FID** is a general detector for organic compounds.
- Has high sensitivity.
- Large linear response range.
- Low noise.
- Unfortunately, it destroys the sample.



Thermal Conductivity Detector (TCD)

- Compares the thermal conductivity of two gas flows-pure carrier gas (reference gas) and carrier gas plus components (column effluent).
- Response is universal and proportional to concentration.
- Best gases for TCD: H_2 or He, because of highest thermal conductivity.
- Doesn't destroy the sample.



Gas Chromatographic Detectors

Type	Applicable Samples	Typical Detection Limit
Flame ionization	Hydrocarbons	0.2 pg/s
Thermal conductivity	Universal detector	500 pg/mL
Electron capture	Halogenated compounds	5 fg/s
Mass spectrometer	Tunable for any species	0.25–100 pg
Thermionic	Nitrogen and phosphorous compounds	0.1 pg/s (P) 1 pg/s (N)
Electrolytic conductivity (Hall)	Compounds containing halogens, sulfur, or nitrogen	0.5 pg Cl/s 2 pg S/s 4 pg N/s
Photoionization	Compounds ionized by UV radiation	2 pg C/s
Fourier transform IR	Organic compounds	0.2 to 40 ng

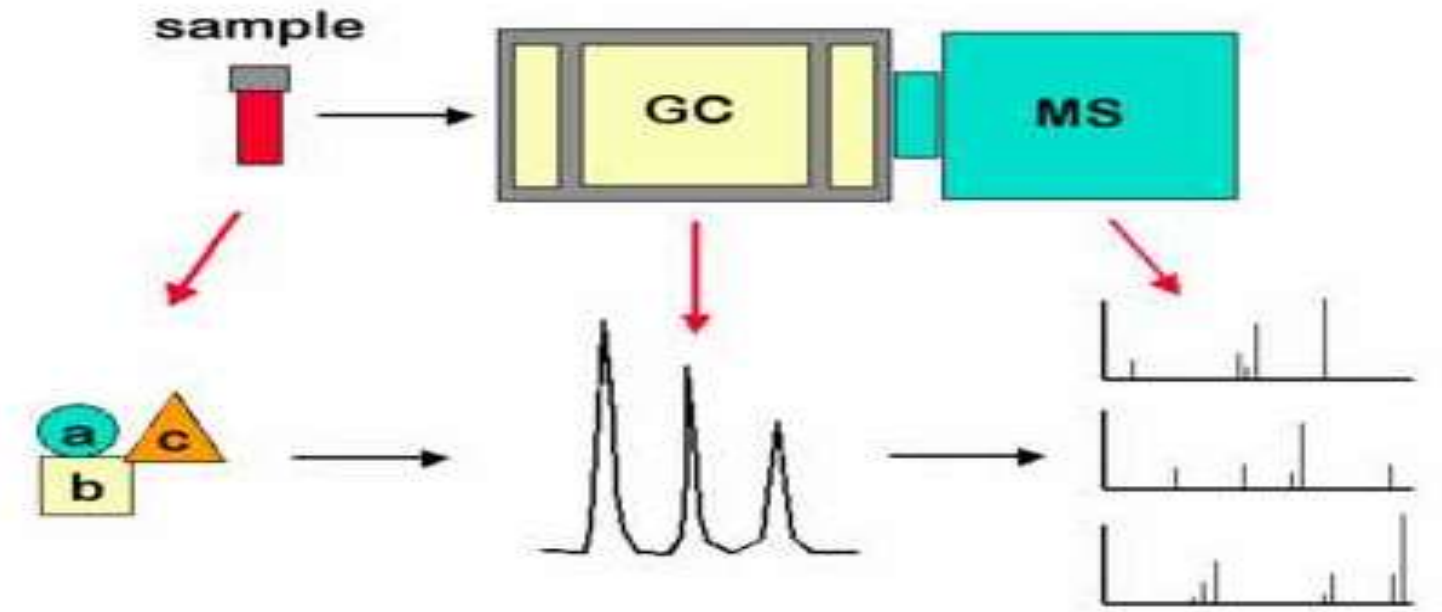
Hyphenated GC

- **GC** can be attached to a second instrument that will produce qualitative and/or quantitative data.
- The combination of a chromatographic and spectral method.
- Exploit advantage of each method.
- Chromatograph- produce pure fraction from your sample.
- Spectral method- yield qualitative information about a pure component.

E.g.,
(1) Mass spectrometry (GC-MS)
(2) Infrared spectrometry (GC-FTIR)

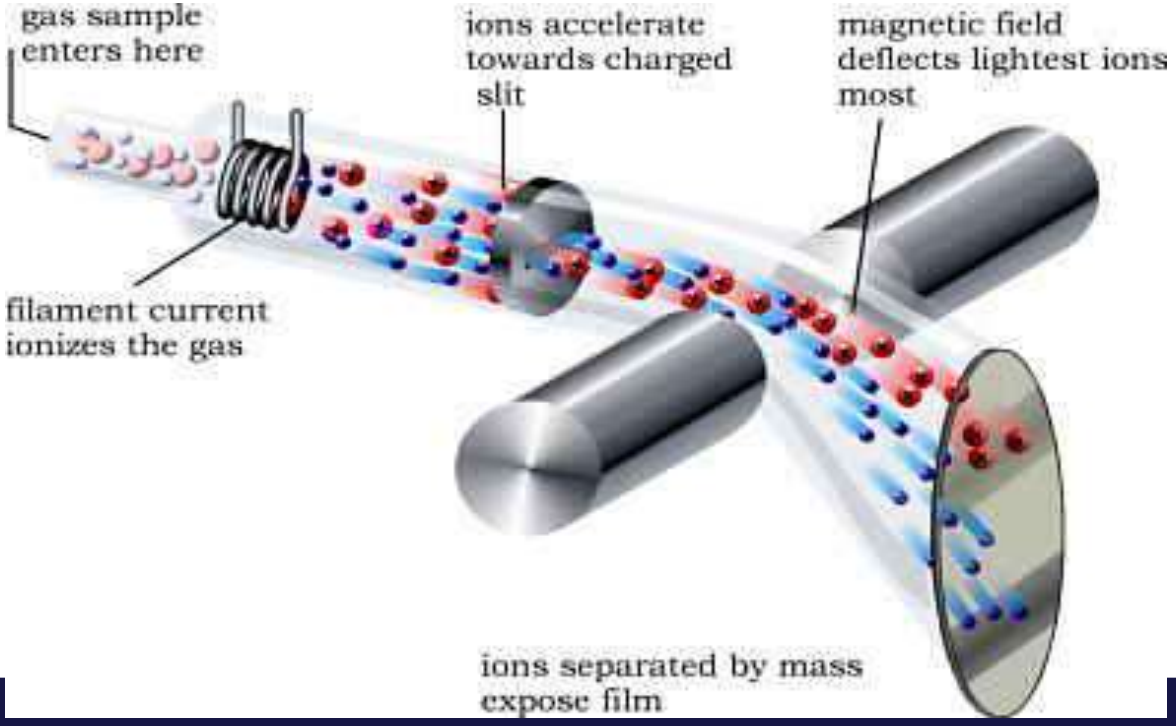
Mass Spectrometry (GC-MS)

- Synergistic combination of two powerful analytic techniques.
- The gas chromatography separates the components of a mixture in time.
- The mass spectrometer provides information that aids in the structural identification of each component.
- Uses the difference in mass-to-charge ratio (m/e) of ionized atoms or molecules to separate them from each other.



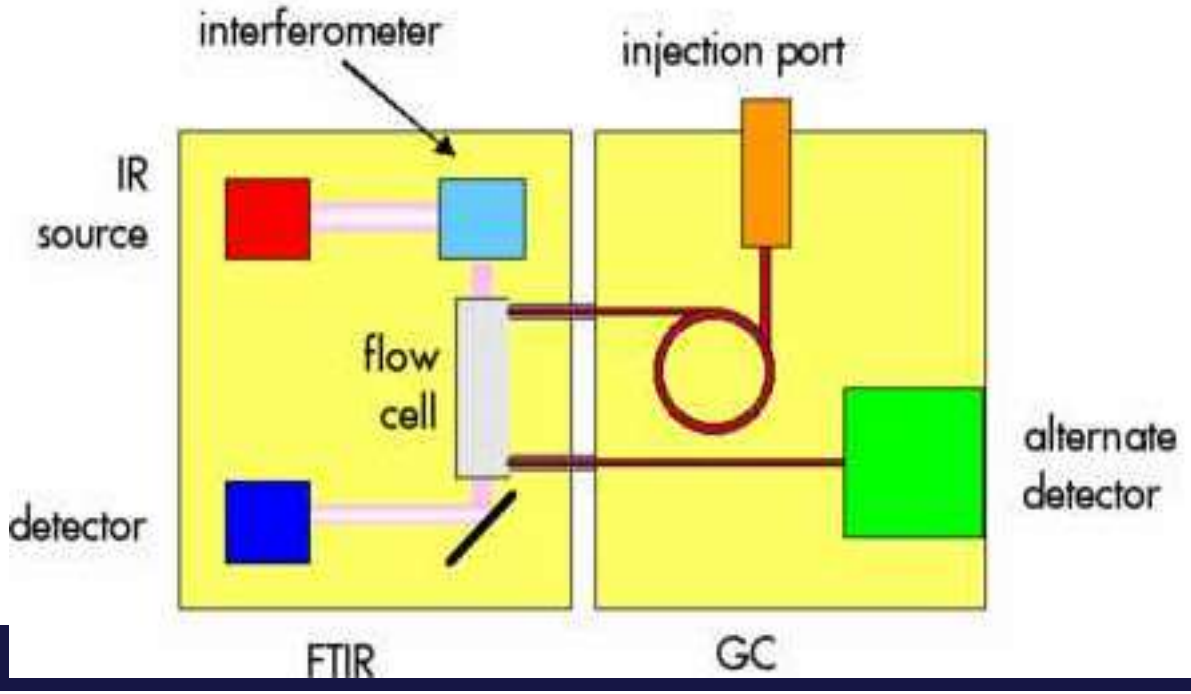
The general operation of a mass spectrometer is:

- Result of the GC goes through an ionizer where it is bombarded by a high energy electron beam.
- This beam breaks the complex molecules into a standard set of fragments.
- The ionized samples then go through magnetic field which deflects ion according to mass to charge ratio.
- A detector picks up the fragments of a certain mass.
- Each peak of a chromatogram becomes a “fingerprint” of the compound.
- The fingerprints are compared with a library to identify the compounds.



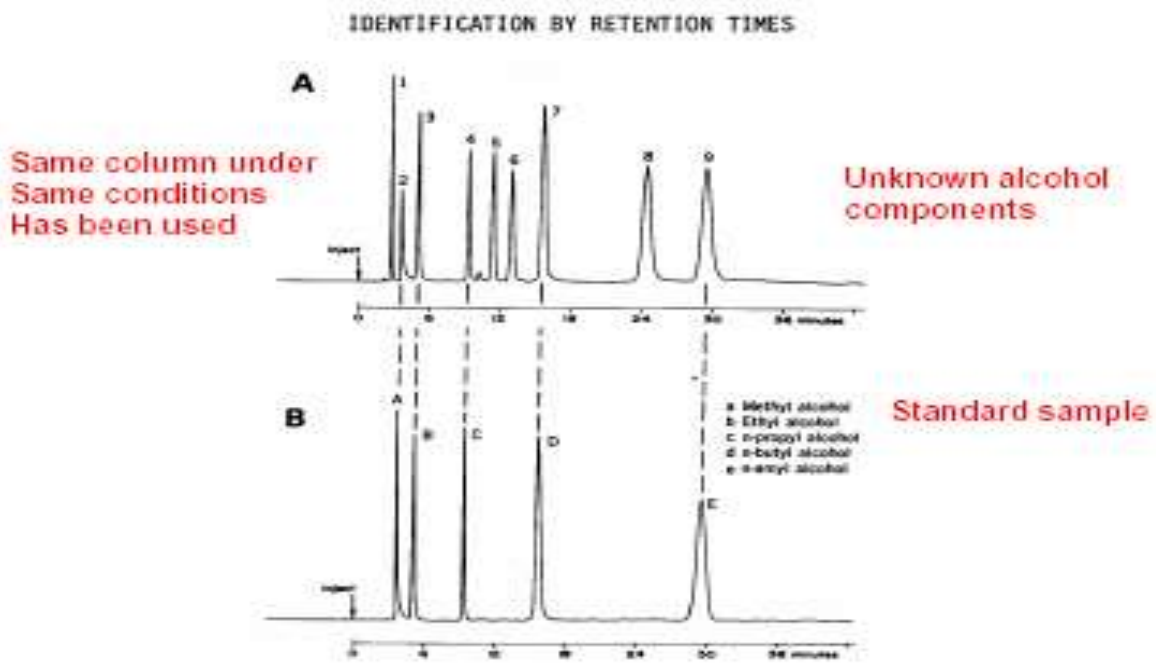
Infrared Spectrometry (GC-FTIR)

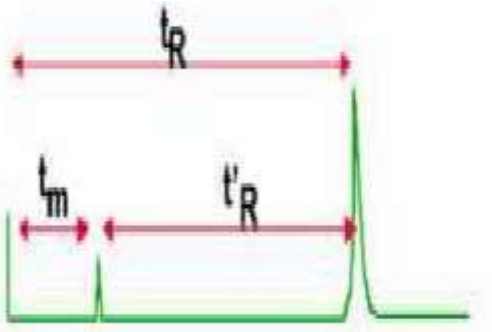
- GC with IR can enable the separation and identifying the compounds.
- Gas Chromatograph partitions the sample as it passes through the column.
- Is especially useful for qualitative analysis of functional groups and other structural features.
- Very sensitive.
- Very expensive.



Qualitative Analysis in GC

- Qualitative analysis is based on retention data.
- Retention time t_R: is characteristic of a substance, compared to a standard.
- t_R: It is the time elapsed from the point of injection to the peak maximum
- Adjusted t'_R: It is the time from the maximum of the peak of the mobile phase to the peak maximum of a certain component.
- t_M (hold up time): is the time required for the mobile phase to be eluted completely from the column.





Quantitative Analysis in GC

Three main methods

- External standard method.
- Standard addition method.
- Internal standard method.

External Standard Method

Single point calibration method

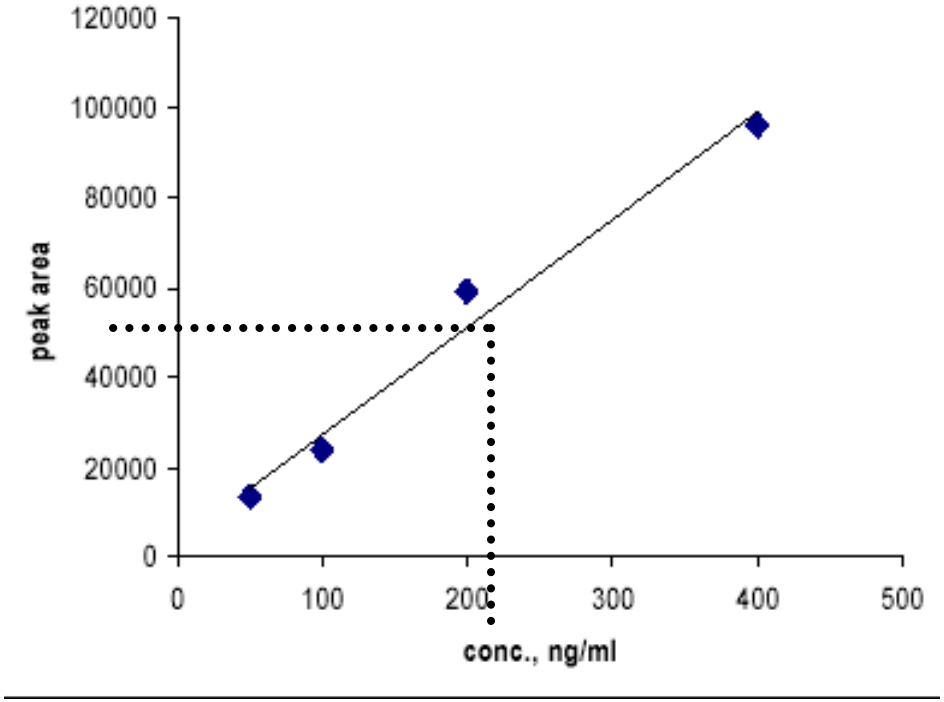
- Inject standard before and/or after analyzing the sample.
- Standard will be on a different chromatogram.

$$conc_{unknown} = \frac{Area_{unknown}}{Area_{known}} conc_{known}$$

Multilevel calibration

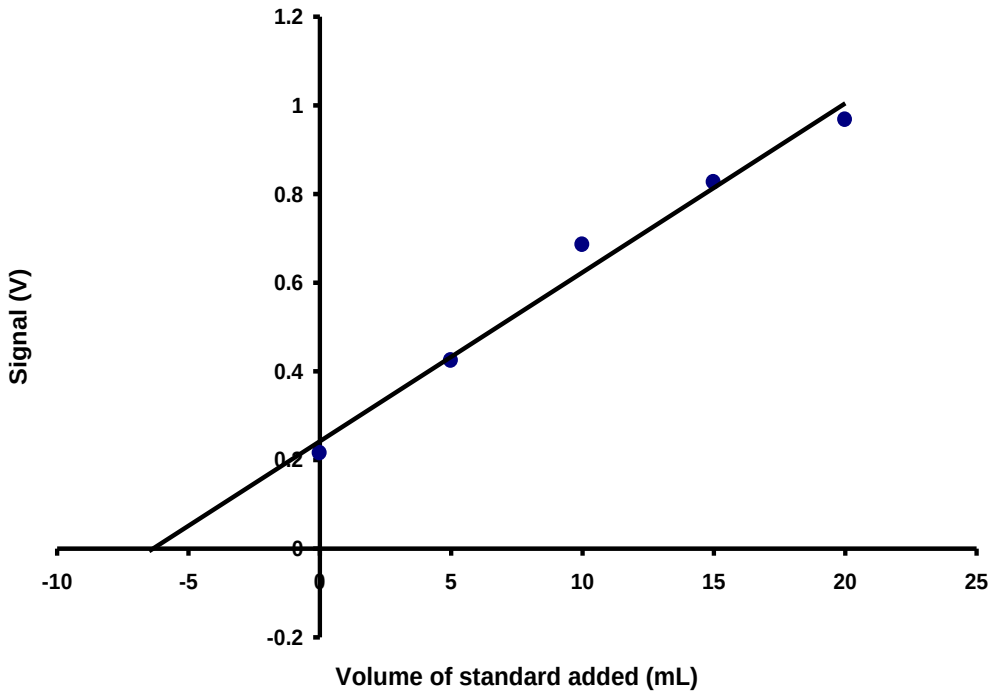
- Prepare standard solutions containing graded amount of the standard object component, and inject a constant volume of each standard solution, exactly measured.
- Depends on good injection reproducibility.

Standard	Conc., ng/ml	Area
1	50	13500
2	100	24000
3	200	59000
4	400	96000



Standard Addition Method

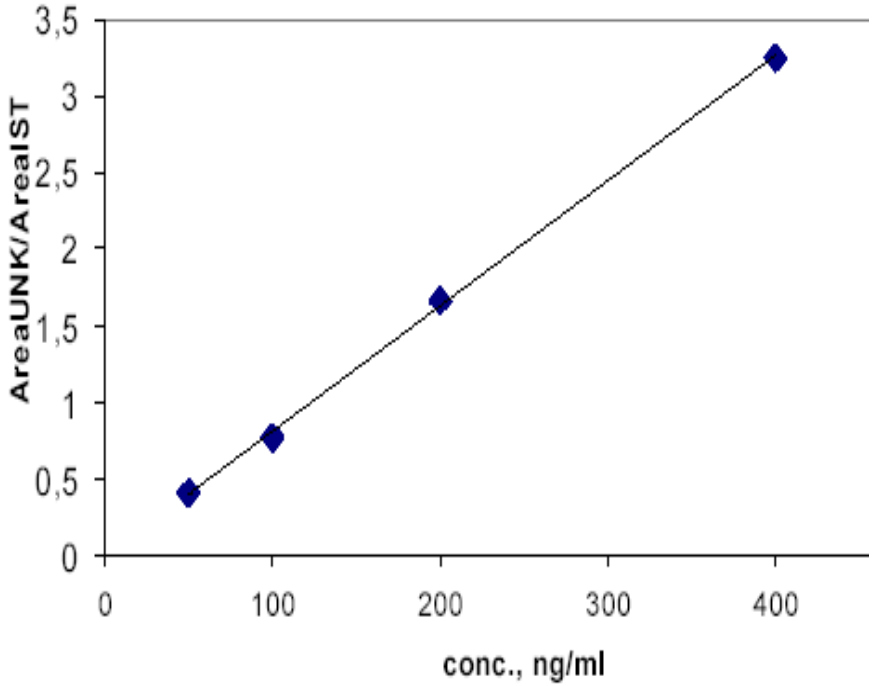
- This method addresses the influence of matrix effects.
- The standard is again the analyte itself. An analytical measurement is made on the unknown and the signal intensity noted.
- A known amount of the analyte is then added to the unknown and a second analytical measurement made.
- From the increase in analytical signal, a response factor, i.e. the signal per unit concentration, can be calculated.
- The concentration of analyte in the original sample may then be obtained by dividing the signal from the original sample by the response factor.



Internal Standard Method

- Known substance (internal standard) at a constant concentration is added to all standards and samples.
- Internal standard must be pure, inert with respect to the sample components and does not overlap with sample components.
- To create IS calibration plot, the IS concentration is maintained constant while changing the concentration of analyte.

Std	conc., ng/ml	AreaUNK	AreaIST	AreaUNK/AreaIS
1	50	13500	33200	0,407
2	100	24000	31200	0,769
3	200	59000	35400	1,667
4	400	96000	29500	3,254




Thank You!