Pharmaceutical Analysis

(Separation methods)

Chromatography

الأستاذ الدكتور جمعه الزهوري (دكتوراه صيدلة-ألمانيا 1991)

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Advantages of TLC

1- Open system (you see on your eye)

2- development all Spot even those don't absorb UV.

3- ability of Simultaneous assay

4- Simple and sheep

5- Ability to use all Solvent

6- High sensitive (HPTLC) ng

GAS CHROMATOGRAPHY

Gas Chromatography

- gas chromatography :

  A chromatographic technique in which the mobile phase is a gas .

In gas chromatography (GC) the sample, which may be a gas or liquid , is injected into a stream of an inert gaseous mobile phase (often called the carrier gas).

The sample (vaporized) is carried through a packed or capillary column where the sample s components separate based on their a ability to distribute themselves between the mobile and stationary phases.

Why the method quick separated?

1. Short time of analysis
2. Low sample amount
3. High power of separation
4. High sensetitive pg
5. Ability to automatize

اختصر 20 عام بساعة

The analysis Substance must be :

1. Gas form
2. Volatile ability
3. Stable in high temperature
4. Soluble in Garrier Gas when its in Gas form

Alpha tocopherol (Vit.E)

ASSAY

Examine by gas chromatography (*2.2.26*), using *dotriacontane R* as the internal standard.

Internal standard solution. Dissolve 0.20 g of *dotriacontane R* in *hexane R* and dilute to 100.0 ml with the same solvent.

Test solution. Dissolve 0.100 g of the substance to be examined in the internal standard solution and dilute to 50.0 ml with the internal standard solution.

Reference solution. Dissolve 0.100 g of *α-tocopherol CRS* in the internal standard solution and dilute to 50.0 ml with the same solution.

The chromatographic procedure may be carried out using:

— a silanised glass column 2.0 m to 3.0 m long and 2.2 mm to 4.0 mm in internal diameter packed with *silanised diatomaceous earth for gas chromatography* µm to 150 µm or 150 µm to 180 µm), impregnated with 1 per cent *m/m* to 5 per cent *m/m* of *polydimethylsiloxane R*; a plug of silanised glass wool is placed at ea the column,

— *nitrogen for chromatography R* as the carrier gas at a flow rate of 25 ml/min to 90 ml/min,

— a flame-ionisation detector,

maintaining the column at a constant temperature between 245°C and 280°C and the injection port and the detector each at a constant temperature between 270 320°C. Set the temperature of the column and the flow rate of the carrier gas in such a manner that the required resolution is achieved.

Make the injections directly onto the column or via an injection port (preferably glass-lined) using an automatic injection device or some other reproducible injection r Measure the peak areas by electronic integration.

Resolution. Inject 1 µl of the reference solution. Repeat this operation until the response factor (RF) determined as described below is constant to within ± 2 per cer resolution (R_s) between the dotriacontane peak and the α-tocopherol peak is at least 2.6.

Factors which affect GC separations

Efficient separation of compounds in GC is dependent on the compounds traveling through the column at different rates. The rate at which a compound travels through a particular GC system depends on the factors listed below

1- Volatility of compound:

Low boiling (volatile) components will travel faster through the column than will high boiling components

2- Polarity of compounds: Polar compounds will move more slowly, especially if the column is polar.

3- Column temperature: Raising the column temperature speeds up all the compounds in a mixture.

4- Column packing polarity: Usually, all compounds will move slower on polar columns, but polar compounds will show a larger effect.

5- Flow rate of the gas *through the column: Speeding up the carrier gas flow increases the speed with which all compounds move through the column.*

6- Length of the column*: The longer the column, the longer it will take all compounds to elute. Longer columns are employed to obtain better separation*

Development of Gas Chromatography

- 1944 Martin & Synge (first concept of GC was suggested)
- 1952 Martin & James (Separation of Fatty acids)
- 1955 the first commercial GC instrument appeared in the market.
- Until 1960 it developed as packed column techniques.
- 1979 Capillary column.
- ab 1990 :
- GC-MS (Gas chromatography –Mass Spectrometry)
- GC-FTIR-MS (GC-Fourier transform infrared-MS)

Gas chromatography

First instrumental chromatographic method developed commercially.

Reason - it is relatively easy to produce a stable flow and pressure for the mobile phase - carrier gas.

All that is really needed is a tank of compressed gas, pressure regulator and a valve.

Basic Instrumentation

- The carrier gas flows through the preheated inlet into which a very small amount of the sample is injected .The vaporized sample is transported by carrier gas into the column, where the separation of the individual components take place. The column is placed in a thermostatically controlled oven, so that the components remain in vapor form. After separation the carrier gas and the component bands pass through the detector and are recorded on the recorder or computer dada system.

Modes of GC

- **Gas-Liquid chromatography (GLC)**

in GLC a liquid substance serve as a stationary phase. (partition process , packed and capillary columns), in case of packed column, the liquid stationary phase is nonvolatile and distributed in the form of a thin film on an inert solid support, the most commonly used supports are diatomaceous earths such as kieselguhr.

either on the internal wall of the capillary (wall-coated open tubular, WCOT) or on a fine porous support material as a thin layer attached to the inside column walls(support-coated open tubular ,SCOT).

Modes of GC

- **Gas Solid Chromatography (GSC)**

  In GSC the stationary phase is an uncoated solid ,which may be a simple adsorbent, for example ,alumina and silica or a porous solid such as molecular sieve.

  The separation is based on the adsorption/ desorption process of the analytes on the stationary phase, which can occur not only in packed columns but also in capillary porous-layer open tubular (PLOT) columns. The PLOT is prepared by depositing a porous material on the inner column walls.

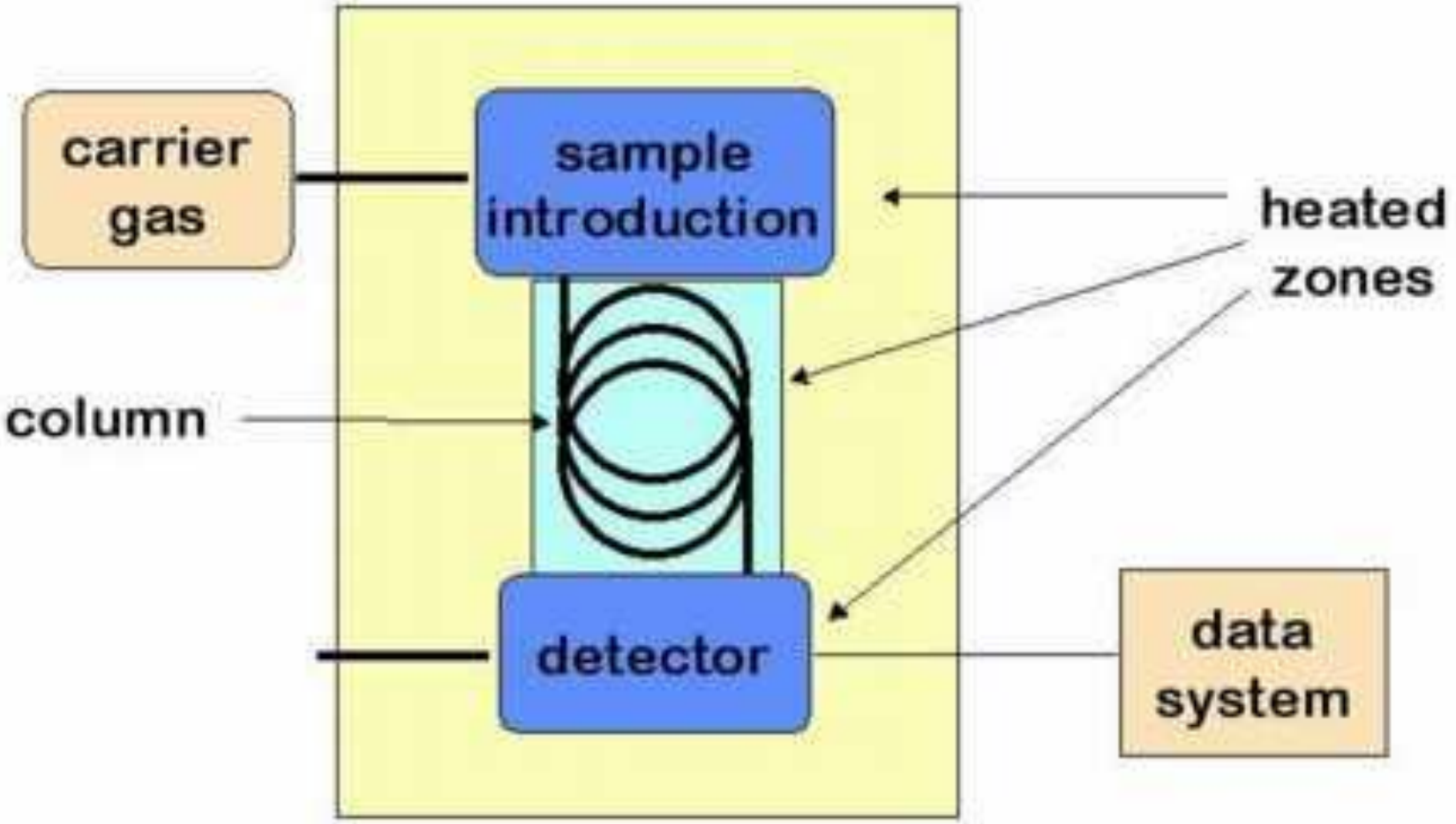
ترسيب

Basic instrumentation

The basic gas chromatography consists of :

- Mobil phase (carrier gas supply) { gas cylinder , pressure regulator, flow controller}.
- The injector (inlet).
- The Oven which include the column.
- The detector.
- The data system.

Schematic of a packed column gas chromatograph



I- GAS SUPPLY SYSTEM

In GC a supply of carrier gas is required as the mobile phase to transfer the sample from the injector , throw the column , and in to the detector .

Mobile phase

- The most commonly used carrier gases (mobile phase) for GC are Helium He , Argon Ar , Nitrogen N_2 ,Hydrogen H_2 , carbon dioxide $CO_2$and Neon Ne
- The carrier gas should be chemically inert toward both the sample and the stationary phase, dry , and pure.
- The choice of which carrier gas to use is often determinated by the instrument so detector, the purity of the gases , the availability and it cost.
- The selection of the best carrier gas is important ,because it will determine the column separation processes. (efficiency of the column separation)

Type of Gases in GC

1- H_2 as Carrier and Burner
2- He Carrier
3- Ne Carrier
4- O_2 Burner
5- N_2 Carrier
6- Air Burner
7- Ar Carrier

Mobile phase

- ***With packed columns the mobile-phase velocity is usually within the range of 25-150 ml/min, whereas flow rates for capillary columns are 1-25 ml/min***
- ***Actual flow rates are determined with a flow meter placed at the column outlet.***

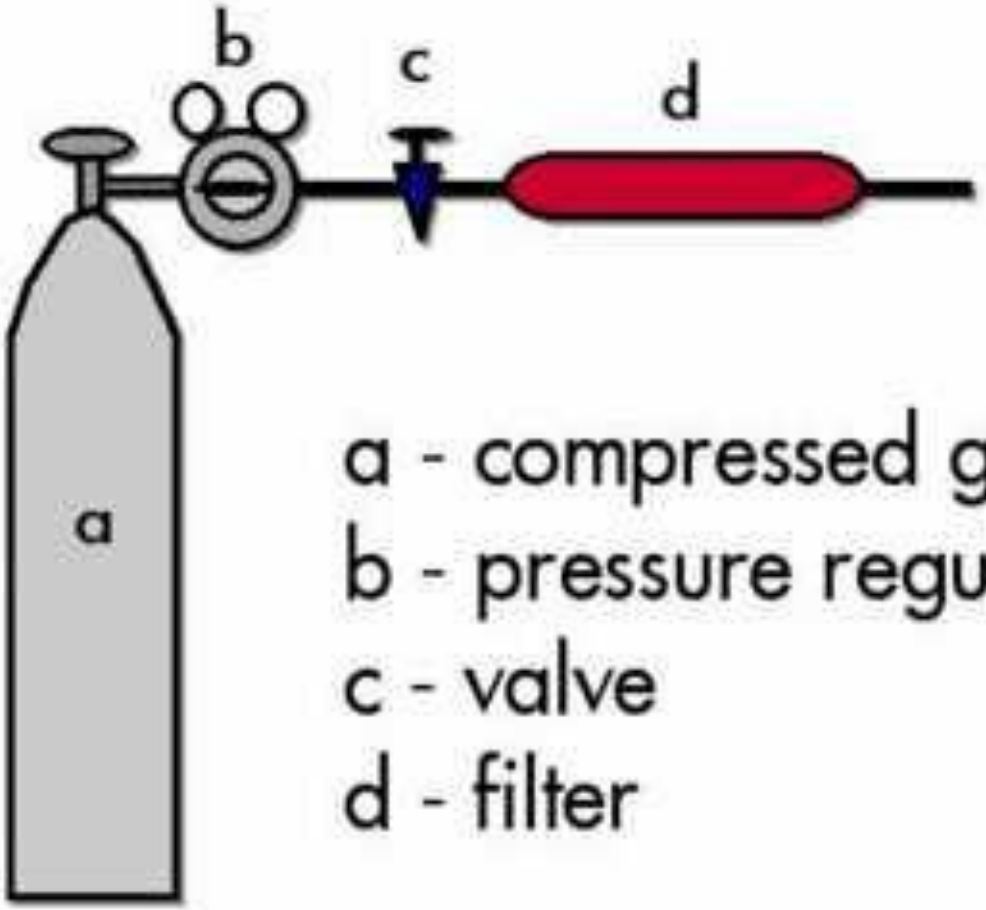
GAS SOURCES

- ***Commercial pressurized (150 – 160 atm) gas cylinder.***

- ***Throw Generator (designed specially for chromatography use).***

gas cylinder

Flow control



a - compressed gas cylinder
b - pressure regulator
c - valve
d - filter

Gas -Generator

Flow measurement

While flow is relatively easy to control, it still must be measured.

Bubble meters - post column or detector measurement of flow. Cheap and relatively accurate.

Rotameters - pre-column measurement of flow via position of ball floating in a calibrated glass tube.

Flow measurement

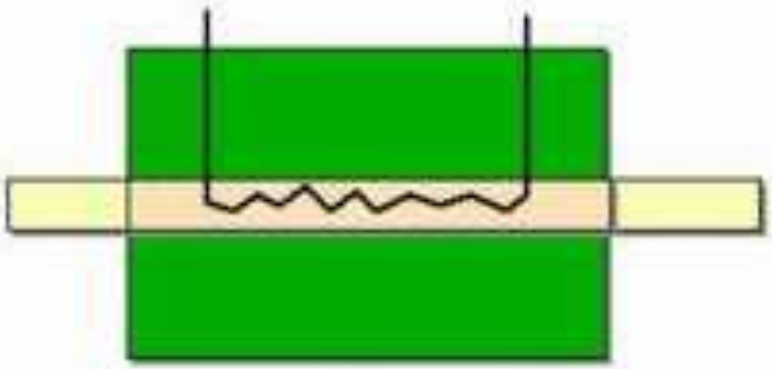
Electronic flow sensor

A modified thermal conductivity detector.

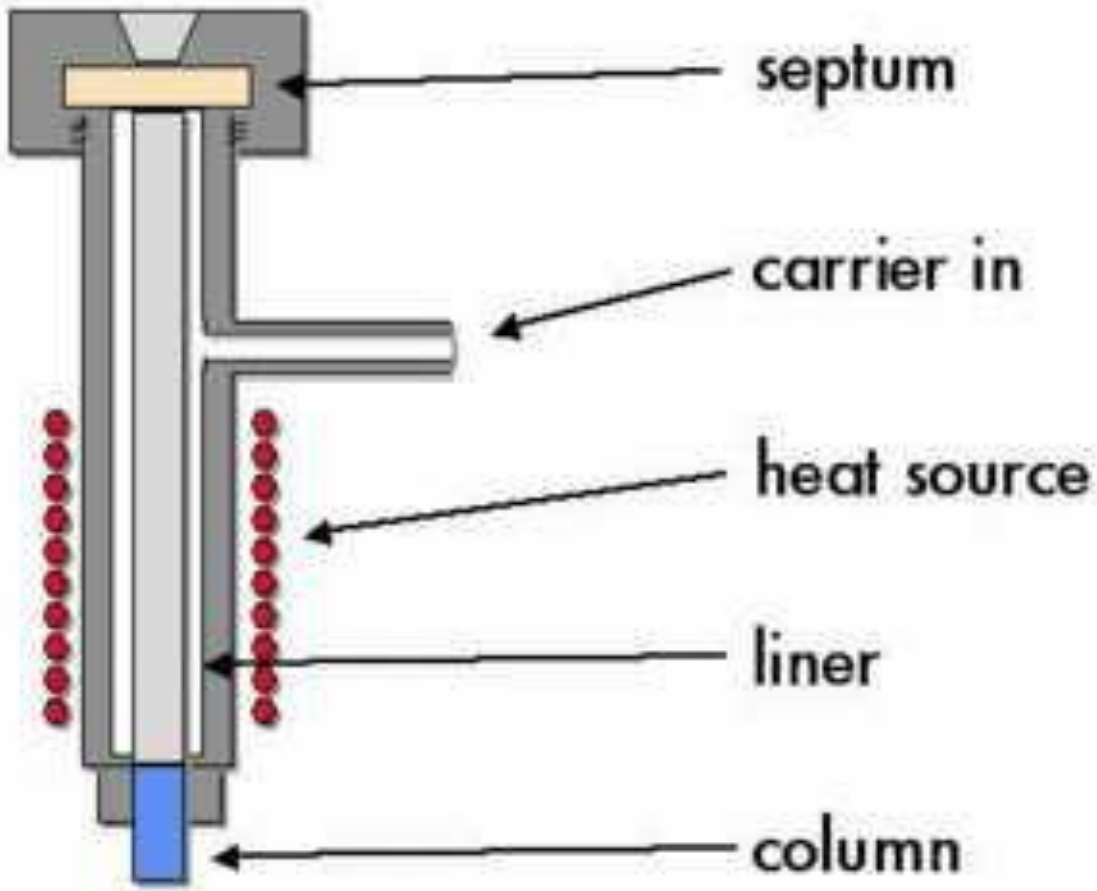
Permits continuous measurement of flow over a reasonably large range.

Must be calibrated for accurate flow measurement.

Response will also vary based on carrier gas used



II- Injection port



Liner

The vaporizer tube is usually a glass Liner, which serves to prevent the sample coming in contact with the heated metal surfaces so that the thermal decomposition can be minimized.

Injection port

Purpose of port is to flash evaporate your sample and introduce it into the column.

T_{INJ} > 50°C above T_{column}

Injection is through a septum.

Septum must be

stable at the T_{inj}

replaced regularly to maintain seal

- The Temperature of injection port is usually adjusted so as to be approximately 50 C° higher than of the column. This ensures a process termed flash Vaporization of the Liquid Sample.

SAMPLE INLETS (INJECTION)

- **Sample introduction is critical step in GC especially when WCOT columns are used.**
- **Therefore ,the inlet system must be well designed to facilitate the injection of the sample on the head of the column without degradation of the column performance and without discrimination of sample compounds.**

- In order to minimize the band spreading and to obtain the best resolution ,the sample must be injected rapidly and must be of a suitable quantity.
- Introduction of a large volume or too concentrated sample may give rise to poor resolution and distorted peaks.
- The sample Size depended on the dimension of the columns and on the sensitivity of the detector .

- Capillary columns require smaller quantity than the packet columns.
- For packed column , sample size ranges from 0.1-10 µl .
- For Capillary column need much less sample 0.01 – 1 µl .
- For quantitative purpose the sample introduction must be attained with high degree of **precision and a accuracy**.

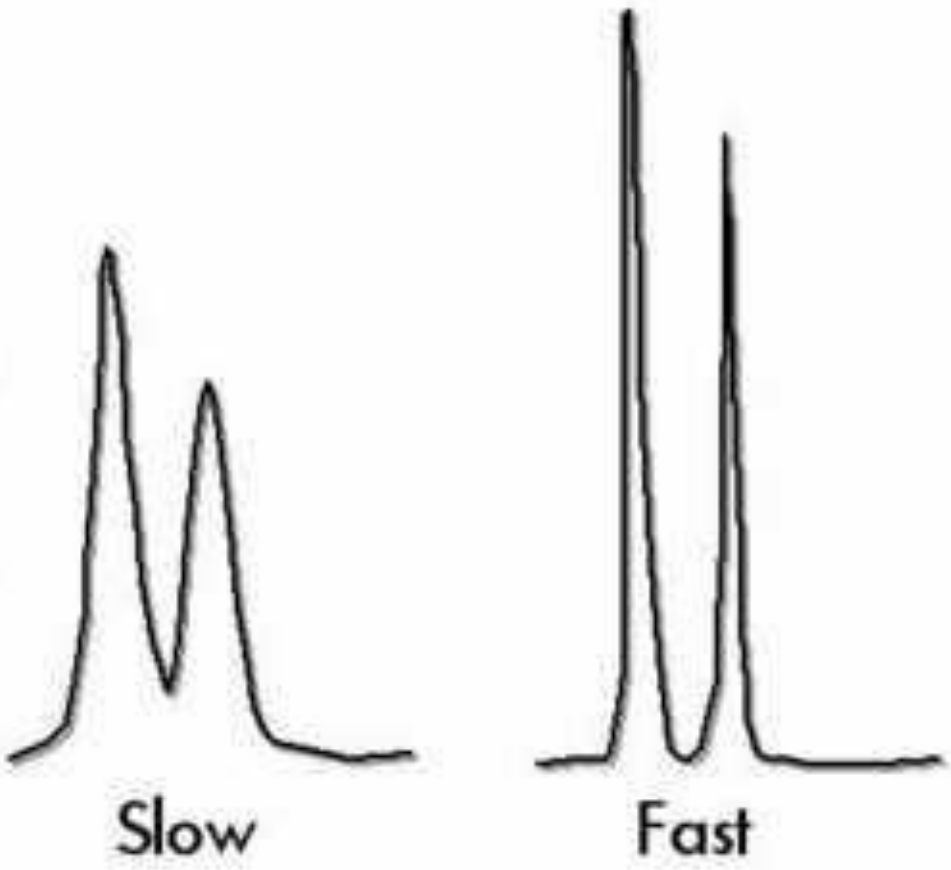
- Several techniques have been developed to introduce sample on to the GC column.
- The samples in the form of a liquid are usually injected into GC using a micro syringe throw a rubber or silicone septum. For the solid samples ,it is convenient to dissolve them in a suitable volatile solvent.
- For repetitive or perrodic injection of a large number of the same or different samples, auto – samplers may be used.
- Due to the high sample capacity , the introduction of the sample in to packed columns is usually problem free. so for capillary column several techniques for injection are developed.

Syringe injection

Major source of precision error is from poor injection technique.

Samples should be injected as a plug.

Rapid and consistent injection is necessary in order to obtain acceptable precision.



Syringe injection

Several techniques can be tried to help improve manual injection precision.

Try different, less volatile solvent

Use syringe guide

Use 'through the barrel' syringe

Measure needle volume

"Hot needle" method

Syringe loading

Hot needle method

1. Draw sample into syringe barrel.
2. Next, draw 2-3 µl air into barrel.
3. Insert needle into injection port and allow to heat for a few seconds.
4. Rapidly inject sample and withdraw the needle.

   This insures that all sample is injected and the 'hot needle' assists in solvent volatilization.

s Chromatography

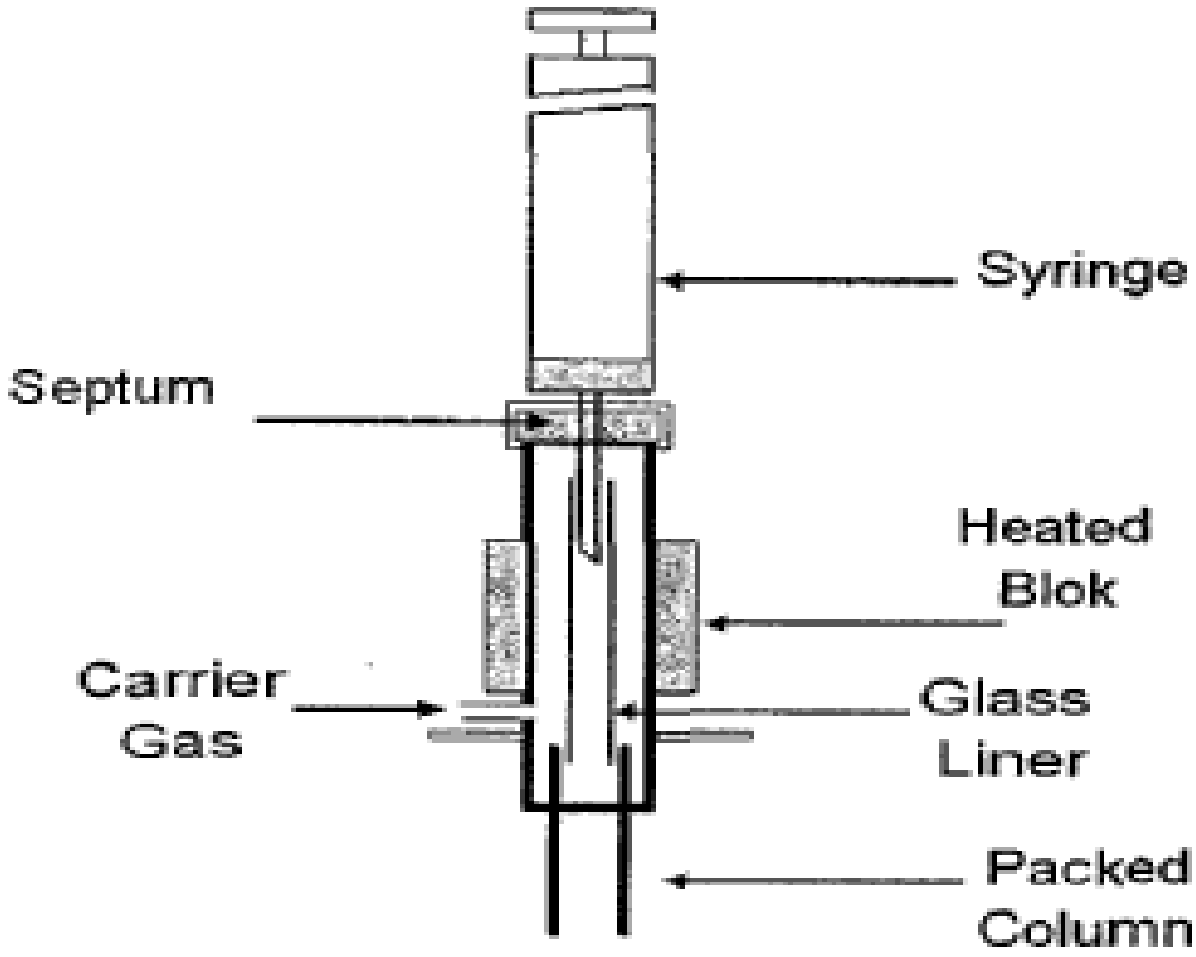


Diagram of the injection system for packed columns.

- Split Injection
- Spiltless injection
- Direct injection (Cold on –column Injectors)
- Programmable Temperature vapouriser (PTV)
- Injection for Gas Samples
- Headspace Analysis.

Split Injection

- Split injection involves injecting a liquid sample into a heated injection port ,vaporizing the sample un the injection inlet, and splitting the vaporized samples in to two parts so that small fraction of the vaporized sample enters the column and the major portion is vented to waste
- Split injection was firstly developed for open tubular columns.

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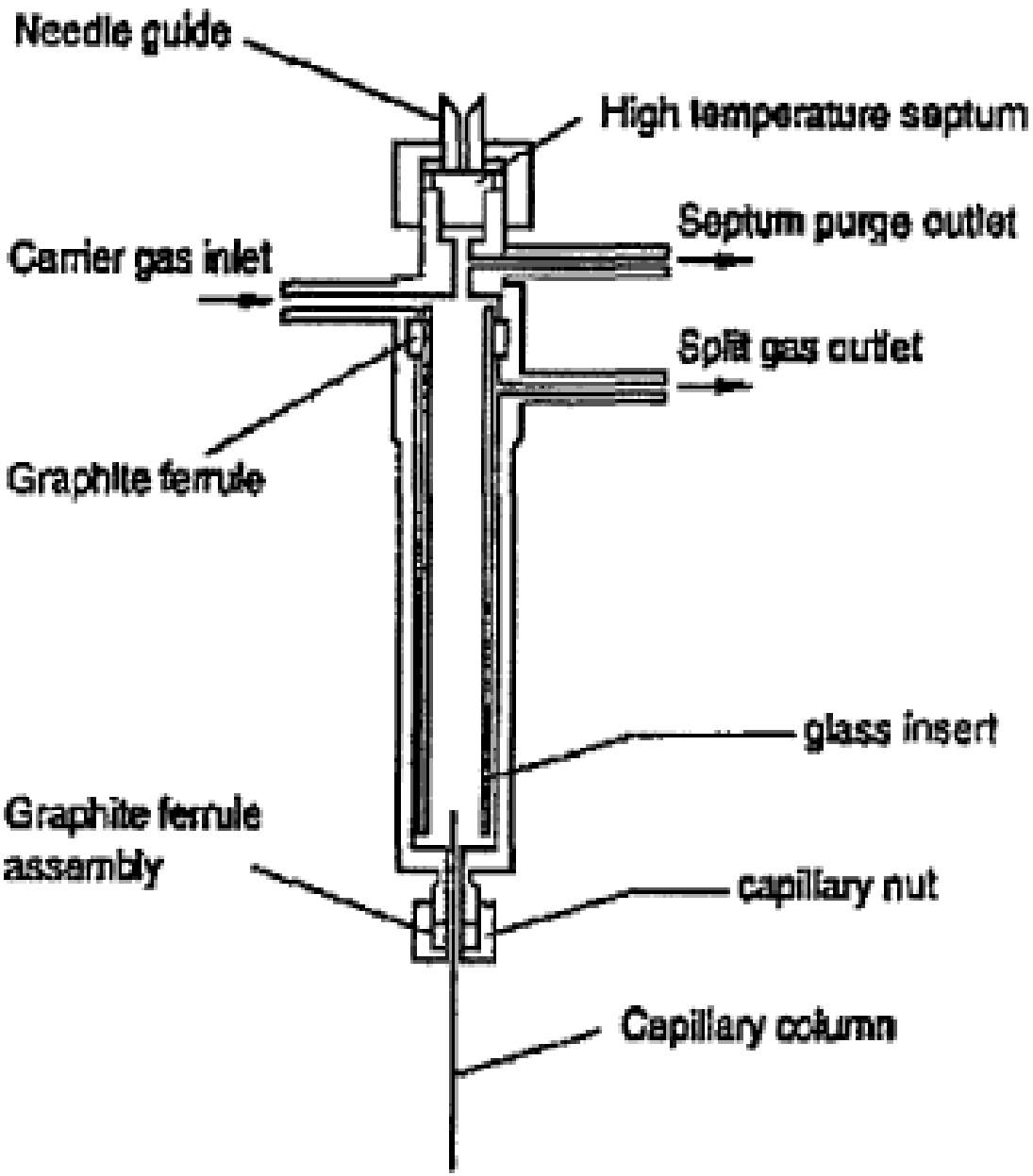
Split Injection

- Split Injection has been one of the most commonly used methods for many applications, since it offers many practical benefits when analyzing concentrated samples with little risk of band broadening. (1/10 or 1/100)
- It is easy to automate and it is compatible with both isothermal and temperature-programmed operation.
- The classical split injector is flash vaporization device.



Classical split injector.

Splitless Injectors

- The Spiltless injection is usually used for analysis of trace compounds.
- As the name indicates ,there is no split in this injection technique, which is achieved by closing the valve (in Classical injector).
- The design of the splitless injectors differs from the split injectors only in the addition of the solenoid valve downstream from the vent.

Splitless Injectors

- During the spiltless period, most of the sample and solvent enter the column.
- The residual vapor in the injector, however, may cause peak tailing, especially for the solvent peak. To avoid this problem, the inlet is switched back to the split mode after a nearly completed sample transfer, so that all remaining solvent and vapors are purged out of the split vent.

Direct Injection

(Cold On-Column Injectors)

- The cold on-column injectors allow the injection of liquid sample directly onto the column.
- During injection ,the injection zone is maintained at low temperature to avoid needle discrimination.
- A major advantage of this injection technique is that the sample is completely transferred without discrimination, and high precision and accuracy of the results can be obtained for samples with a wide range of component volatilities and thermally stabilities.

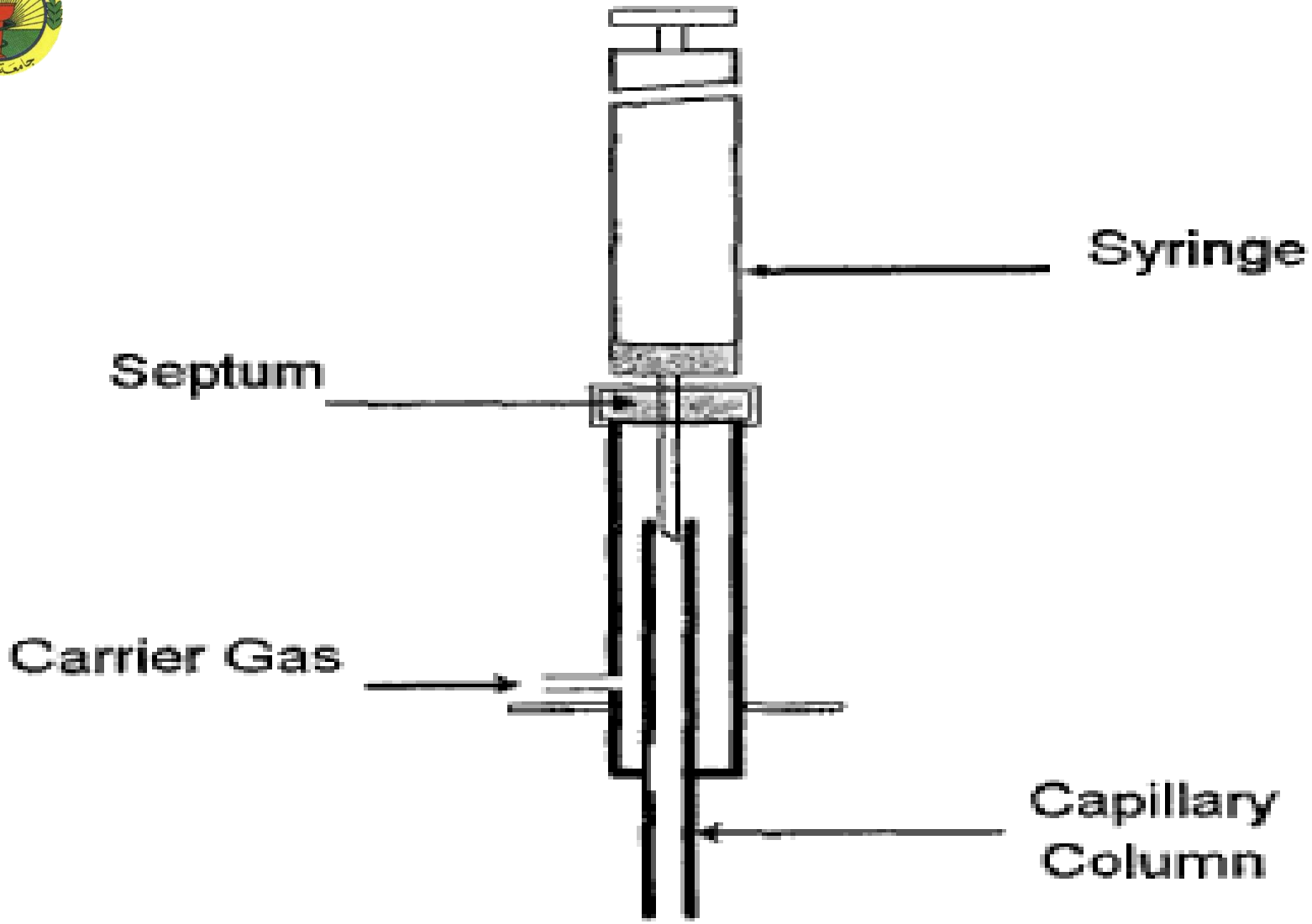


Diagram of a cold on-column injector.

programmable temperature vapouriser (PTV)

- The PTV injection is claimed as a nearly universal injector, since it can be operated in several possibilities, that is, hot or cold spilt injection, hot or cold spiltless injection, cold on column, and direct injection. In addition, this mode offers the injection of a large sample volume and in multiple parallel capillary columns.
- This mode offer the injection of a large sample volume .

Injector for Gas Samples

- For injecting gases and vapors, gas –tight syringes with Teflon-tipped plungers and syringe barrel are available.
- Many analysts favor using of gas syringes for gas samples, however ,the introduction of accurately measured volumes of a gas remains a problem.

Sample size

Liquids

0.1 - 10 µl is typical

Gases

0.5 - 5 ml is typical

Injection precision with a syringe is +/- 1%

Headspace Analysis

- The headspace injection permits the analyses of volatile components of complex samples when the matrix is of no interest.
- A major advantage of this technique is that the sample can be directly analyzed without a complicated extraction of the analytes from the samples.

Headspace Analysis

- In this case ,the sample is transferred in a sealed headspace vial and positioned in a thermo- stated bath, usually at 40-60 C^o .
- The volatile components, which have a suitably high vapor pressure above the liquid or solid sample matrix, are in the form of gases distributed in the headspace of sealed sample vial.
- The injection of the gaseous sample into the GC column is done by means of a gas-tight syringe or a specially designed valve.

Headspace Analysis

- The principles of headspace injection are based on the thermodynamic conditions of the phases. When a sample containing volatile components is placed in an airtight vial, equilibrium is reached between a liquid and its vapor.
- The vapor phase or headspace can be determined either qualitatively or quantitatively, since the vapor phase has the same composition as the liquid at a given temperature and pressure
- The concentration in the vapor phase is a related to the concentration in the original mixture.

III- OVEN

1- Convential GC-Oven

التقليدي (محدود الأمكانية برفع الحرارة وتبريدها)

2- Flash GC-Oven

استخدامه يمكن أن يختصر زمن التحليل 10 مرات بسبب تزويده بمقاومات حرارية

3- Microwave GC-Oven

يسخن العامود فقط مما يتيح لوضع أكثر من عامود بآن واحد

4-Infrared Heated GC -Oven

IV- GAS CHROMATOPHIC COLUMNS

- The column, where the actual chromatographic separation occurs ,is described as the heart of the chromatographic system.
- There are two general types of the gas chromatographic columns most commonly used, namely, packed and capillary (or open tubular).

Types of columns

- Packed
  - GSC
  - GLC
- Open tubular (Capillary)
  - WCOT
  - SCOT
  - PLOT

- The packed columns were developed first but today the majority of GC has been carried out on capillary columns.
- The principal difference between the two columns is reflected in their plate numbers.
- The packed columns are characterized by their relatively low plate number, but they have higher capacities which simplify sample introduction techniques and can accommodate a larger quantity of sample.

- On the other hand , the capillary columns have very high plate numbers which possess the high efficiencies and separating capabilities.
- The faster gas velocities can be applied on capillary columns, giving rise to shorter analysis.
- Other important features of capillary columns compared with packed columns are greater inertness, longer life, lower bleed, and more compatible with spectroscopic detectors.

1. Packed Columns :

- Packed columns consist of metal (stainless steel, copper, or aluminum) or glass tubing filled with solid material either uncoated adsorbents (GSC) or a solid support coated with a stationary liquid phase (GLC).
- The selection of tubing material is dictated from the particular analytical use.

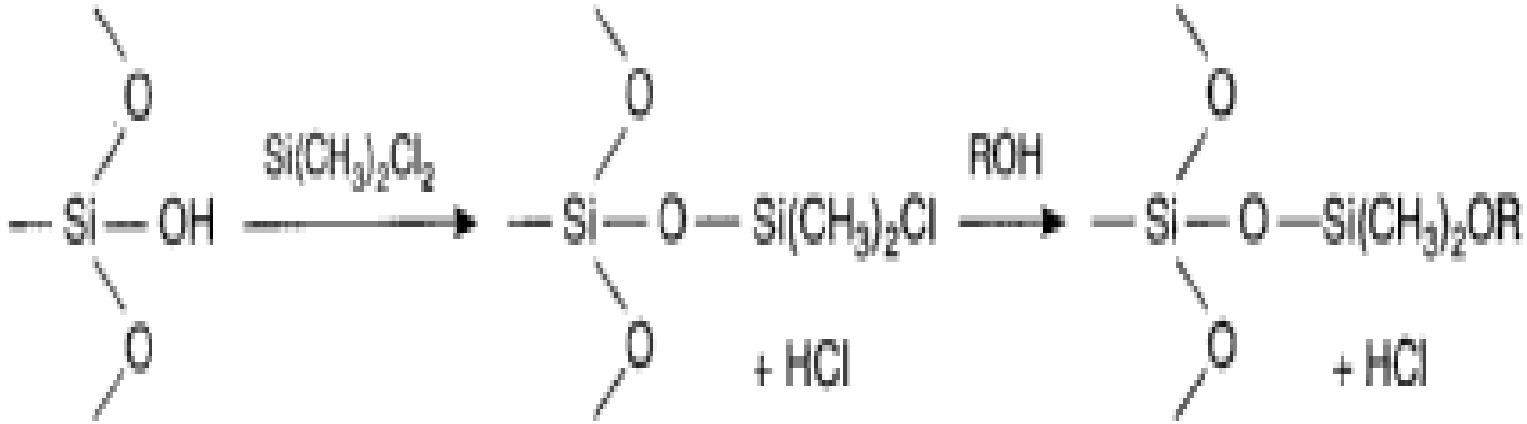
- Glass column can be used at high temperature when the metal tubing would catalyze decomposition of sample. For this reason ,metal columns are undesirable for thermally labile compounds are being analyzed, such as steroids and essential oils.
- Typically packed columns for routine analysis are 1-6 m in length and 2-4 mm in an internal diameter .In order to incorporate them to the oven for thermo-stating ,the packed columns are usually formed as cols.

- In the GLC columns ,the solid support is needed to hold the liquid stationary phase .The material should consist of inert uniformly spherical particles having a large surface area per unit volume (particle size between 80 – 120 mesh) in order to minimize the void volume and to supply a specific surface area for interaction with the analytes.In addition they should be mechanically strong over a wide temperature range.

- The most frequently used solid supports for GLC are diatomaceous earths, either kieselguhr that is sold under trade names Chromosorb W or G , or crushed firebrick, which has the trade name of chromosorb P. In GSC the most commonly used adsorbents are activated charcoal , silica gel, alumina ,and glass beads.

لتحسين الخواص الحاملة للداعم يعالج أحيانا بالحمض لأستبعاد تأثير الألمنيوم والحديد والمغنزيوم والكالسيومAW

- لتحسين الفعالية للسطح يعالج بمركبات السيليس مثل DMCS , HMDCS



- In gas –liquid chromatography (GLC) ,separation is based on the partitioning of solutes between a gaseous mobile phase and a liquid stationary phase coated on the solid packing material.
- To avoid the adsorption of solute molecules on exposed packing material, which degrades the quality of the separation ,surface silanols are deactivated by silanizing with dimethyldichlorosilane and washing with an alcohol (typically) methanol before coating with stationary phase.

packed column

A wide-bore column containing a particulate packing material (37-354 µm the particle has a surface areas of 0.5-7.5 m^2/g, It has –SiOH ,as active sites that adsorb. solute in GSC

- (L) Length 1 – 6 m
- (ID) Internal diameter 2 – 4 mm
- (N) Number of theoretical plate 1000 – 10000 (depend on L)

2. Capillary Columns

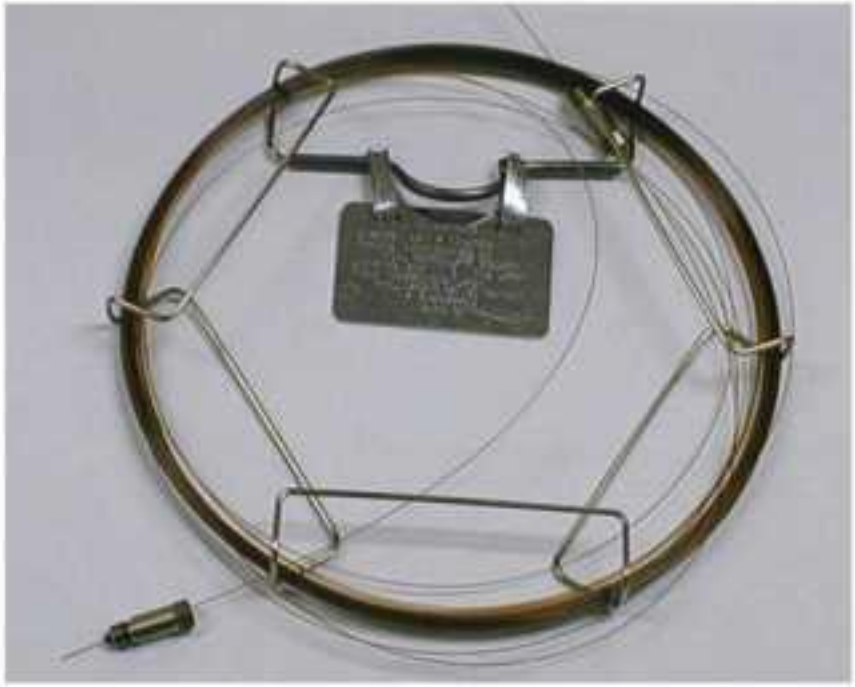
- There are three main types of Capillary columns (or open tubular columns)

I- WCOT = Wall coated open tubular are the most commonly used in GC.They are prepared by coating the inner column walls with the liquid stationary phase as a thin film. precently fused silica is the most commonly used column material , which is manufactured from synthetic quartz with very low (less than 1 ppm) metallic impurities.

II- SCOT = support coated open tubular: in this column the liquid is located on porous support as a thin – layer .It has higher sample capacity but les efficiency than WCOT.

III- PLOT = Porous layer open tubular : is similar to SCOT columns in that a porous material is deposited on the inner column wall.

WCOT = Fused silica capillary column



WCOT = wall-coated open tubular column :

An open tubular column in which the stationary phase I coated on the column s wall.

- Length = 10 – 100 m
- Internal diameter 0.20- 0.75 mm
- N = 100000– 500000 (N/m = 1000-5000)

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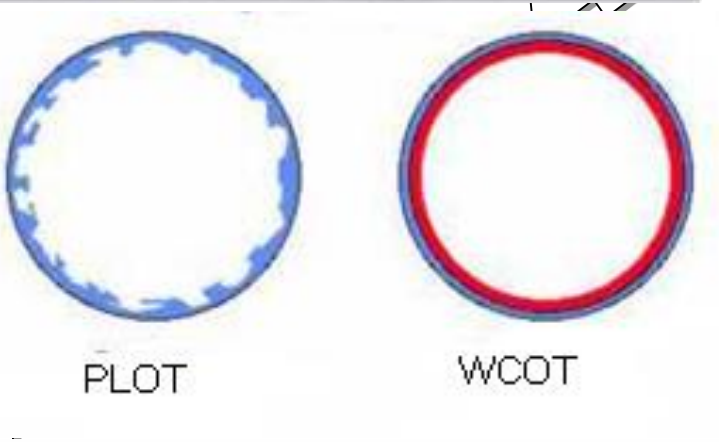

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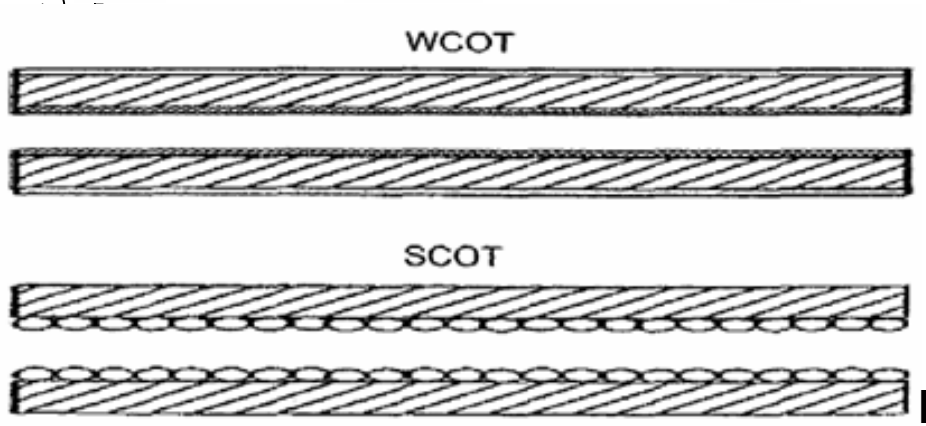

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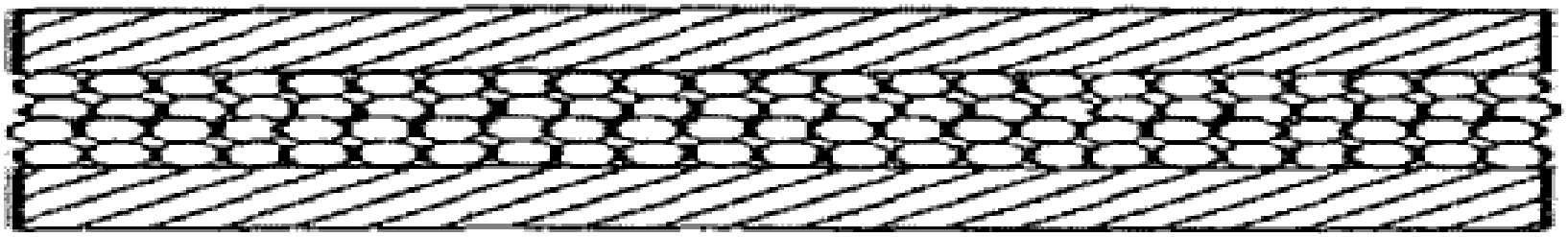
- SCOT : L = 10 – 100 m , ID= 0.5,N=60000-120000 (N/m= 600-1200)

Support-coated open tubular column :

An open tubular column in which the stationary phase is coated on a solid support that is attached to the column s walls.

- Capillary Columns are available in several standard length, 10, 25,50 .. . The 25 m is the most frequently used.

Packed column



WCOT

SCOT

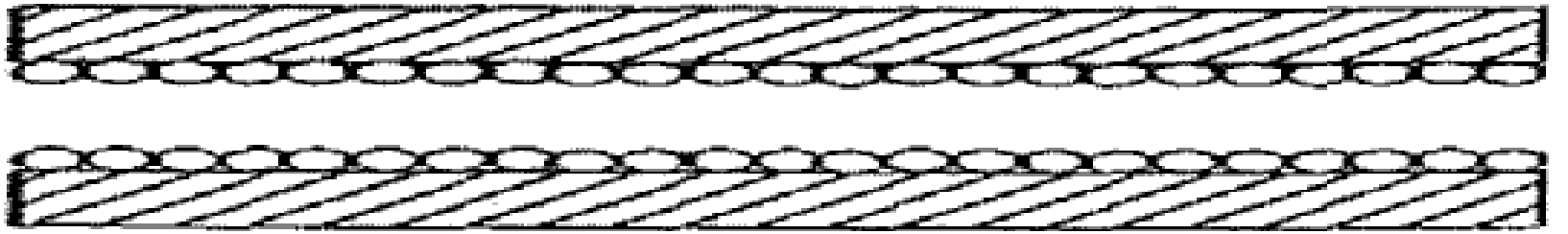


Diagram of the cross-section of the GC columns.

Stationary Phases in GC

- The stationary phases are usually liquids, which should exhibit thermal stability ,chemical inertness, and low volatility to prevent bleeding of the column. The boiling point of the separating liquid should be at least 100 C° higher than the required column temperature. The same stationary phases are employed in packed or capillary columns.

- Elution order in GLC is determined primarily by the solute s boiling point and, to a lesser degree, by the solute s interaction with the stationary phase.
- Solutes with significantly different boiling points are easily separated .On the other hand , two solutes with similar boiling points can be separated only if the stationary phase selectively interacts with one of the solutes.
- In general , non polar solutes are more easily separated with a nonpolar stationary phase, and polar solutes are easier to separate using a polar stationary phase.

- The main criteria for selection a stationary phase are that it should be:

1. Chemically inert
2. Thermally stable
3. Low volatility
4. An appropriate polarity for the solutes being separated.

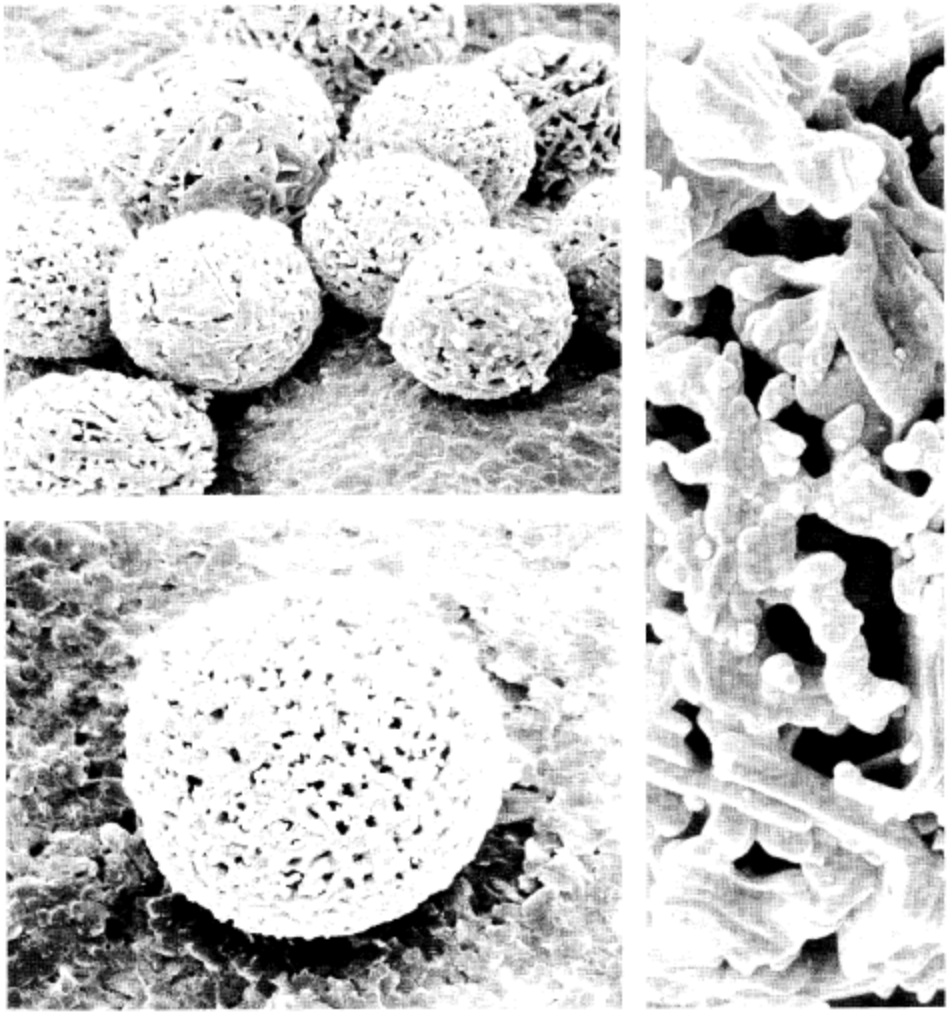
- Hundreds of stationary phases have been developed, many of which are commercially available.
- The majority of GLC separations are a accomplished with perhaps five to ten common stationary phases.

Selected Stationary Phases for Gas–Liquid Chromatography

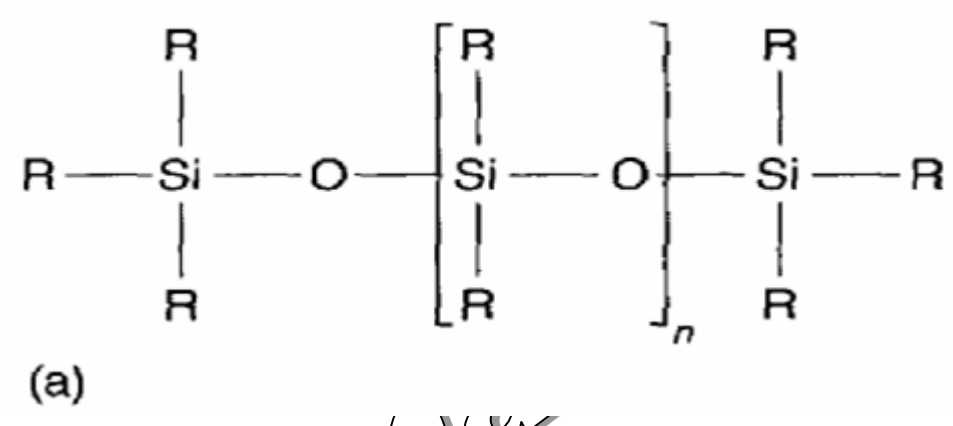
Stationary Phase	Polarity	Trade Names	Temperature Limit (°C)	Applications
squalane	nonpolar	squalane	150	low-boiling aliphatic hydrocarbons
Apezion L	nonpolar	Apezion L	300	amides fatty acid methyl esters high-boiling aliphatic hydrocarbons terpenoids
polydimethyl siloxane	slightly polar	SE-30	300–350	alkaloids amino acid derivatives drugs pesticides phenols steroids
50% methyl-50% phenyl polysiloxane	moderately polar	OV-17	375	alkaloids drugs pesticides polyaromatic hydrocarbons polychlorinated biphenyls
50% trifluoropropyl-50% methyl polysiloxane	moderately polar	OV-210	275	alkaloids amino acid derivatives drugs halogenated compounds ketones phenols
50% cyanopropyl-50% phenylmethyl polysiloxane	polar	OV-225	275	nitriles pesticides steroids
polyethylene glycol	polar	Carbowax 20M	225	aldehydes esters ethers phenols

Chose of Stationary Phase

1. To separate high polarity substance (acid, alcohol …) chose polar phase like OV225.
2. To separate moderated polarity Substances (aldehyde , Ketone ..) chose moderated phase like OV-17
3. To separate non-polar substances (aliphatic , aromatic ..) chose non-polar phase like SE-30



- General structures of common stationary phases for gas chromatography.



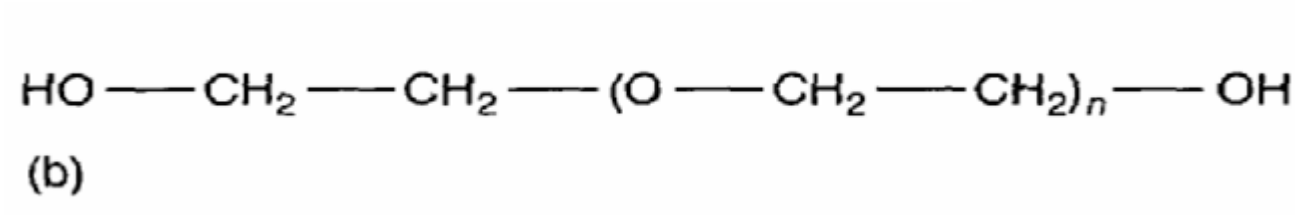
(a)

•Many stationary phases have this general structure , A stationary phase of polydimethyl siloxane , in which all the –R groups are methyl groups (-CH_3), is slightly polar (≈ nonpolar) and often makes a good first choice for a new separation.

•The order of elution when using polydimethyl siloxane usually follows the boiling point of the solutes, with lower boiling solutes eluting first.

•Replacing some of the methyl groups with other subsituents increases the polarity ,providing greater selectivity.

- Increasing polarity is provided by substituting trifluoropropyl (- $C_3H_6CF_3$)and cyanopropyl (-C_3H_6CN) functional groups or using a stationary phase based on polyethylene glycol :



(b)

- An Important problem with all liquid stationary phase is their tendency to “bleed” from the column. Therefore we must not operated above the limits temperature for each S. phase.
- The thickness of the stationary phase is very important character. Separation efficiency improves with thinner films. Thinner films are used when separating solutes of low volatility, such as steroids.
- The most common film thickness is 0.25 µm.
- The most notable are S. phases containing chiral functional groups, which can be used for separating enantiomers.

- The boiling point of the separating liquid (s. phase)should be at least 100C° higher than the required column temperature.
- Polar phase have the functional groups

CN, C=O, OH

- Recently (2002) anew S. Phase was developed ,SOP-75 have a high inertness up to 400-410 C°.

Cetostearyl Alcohol

- Gas Chromatographic assay

The Chromatographic procedure may be carried out using a column (3 m X 4 mm) packed with 3 % OV-17 on chromosorp w 125-180 mesh .....

Each 100 mesh = 0.125 mm

Phases

G1 to G48

Example :

G3 50% Phenyl-50% methylpolysiloxane.

G7 50% 3-cyanopropyl-50% phenylmethylsilicone.

G9 Methylvinylpolysiloxane.

etc

- Column Temperature:

Column temperature is the most important variable in GC, since it directly effects the retention and the selectivity of the sample compounds. Therefore , the optimum column temperature must be found to obtain a good separations, the analyses are usually performed in isothermal mode, whereby the temperature of the column is held constant throughout the run. In this case the column temperature is generally held around the average boiling point of the sample.

- However, problems arise for complex mixtures that contain compounds with widely different boiling points. At too high temperatures, the very volatile components will be eluted quickly but are not fully separated, whereas the high boiling components may be well separated.
- If the column is operated at low temperature ,all volatile components may be separated satisfactorily, however ,less volatile compounds will appear in the chromatogram as flat peaks with a very long retention time , and thus the total analysis time is extended. This problem was overcome by using the mode of temperature programming or programmed-temperature (PTGC)

Temperature programming

The column sits in an oven.

If the temperature is held constant during the entire analysis it is isothermal.

If you vary the temperature during the analysis, you typically use a temperature program.

Why bother?

- Temperature programming :

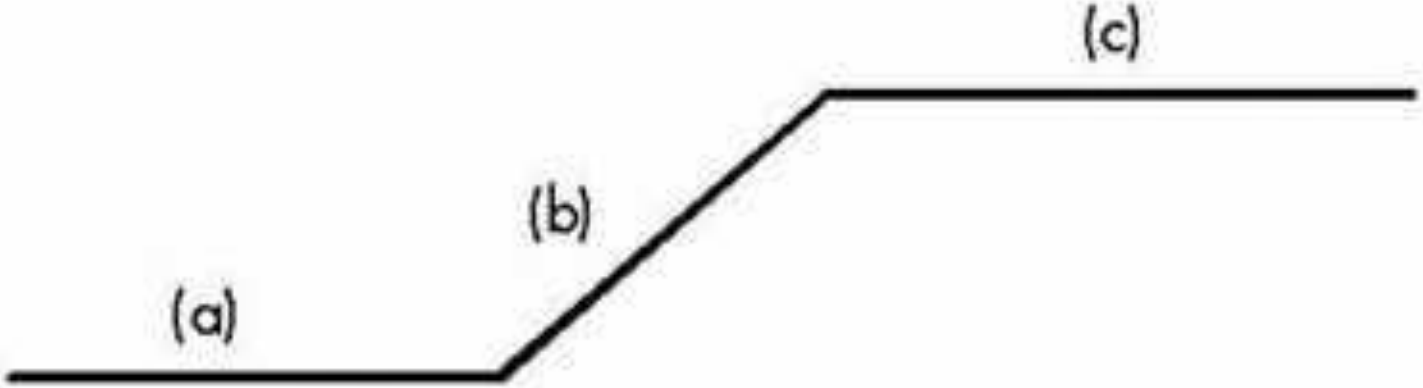
  In this method the temperature of the column is raised during analysis. this technique is usually performed as the following:

1- Initial isothermal period time

2- Column temperature increased at a constant rate. (usually 0.5-10C^{o}/min.)

3-Final isothermal hold time

A temperature program



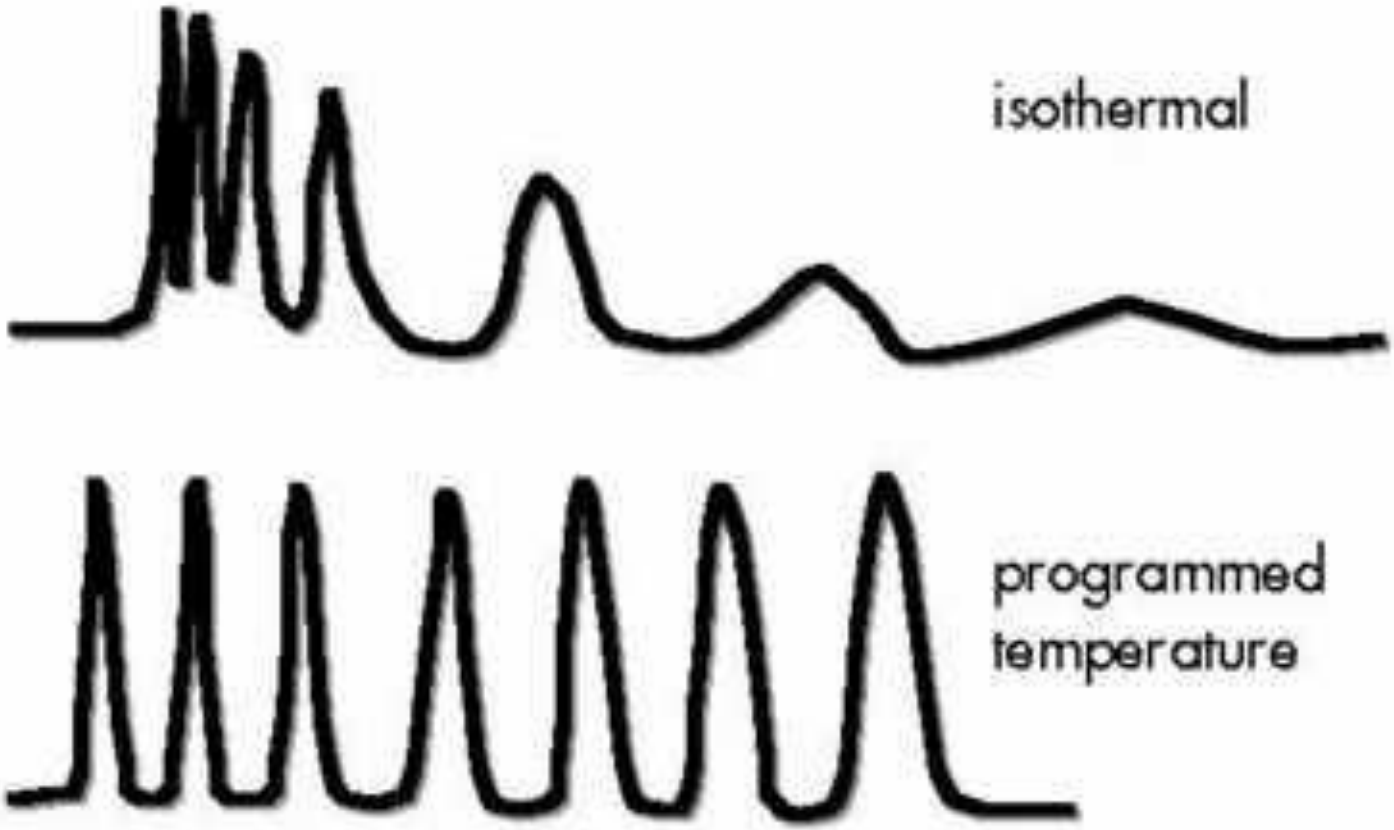
a - initial temperature and time

b - ramp (°C/min)

c - final hold time and temperature

Some GCs will allow for a more complex program.

Example



- The initial temperature should be law enough to resolve the low-boiling compounds. whereas the final temperature is selected such the least volatile compound elutes as rapidly as possible without exceeding the maximum operating limits of the stationary phase.
- An increasing baseline in the chromatogram is commonly observed due to the column bleed.

Temperature programming

- With homologues, the retention time increases exponentially with the number of carbon.
- As t_R increases, width increases and the height decreases, making detection impossible after a few peaks have eluted.
- Since solubility of a gas in a liquid decreases as temperature goes up, we can reduce the retention of a material by increasing T_{column}.

Temperature programming

Factors to consider:

- Variations in solubility of solutes
- Changes in volatility of solutes
- Stability of solutes
- Flowrate changes
- Stability of stationary phase

-اختلاف بالانحلالية
-اختلاف بالتطاير
-اختلاف بالثبات
- ثباتية الطور الثابت

Must stay within T_{min}/T_{max} of column.
Other factors are found experimentally.

V- Detectors

We need a way to measure our eluents as they evolve from the column.

Virtually every method of directly or indirectly observing eluents as been looked at.

We'll cover some of the more common types.

Detector for Gas Chromatography

- The detector in GC senses the differences in the composition of the effluent gases from the column and converts the column s separation process into an electrical signal, which is recorded.
- Their are many detectors that can be used in GC and each detector gives different types of selectivity.

Properties of a good detector

High sensitivity - possible selectivity

Rapidly respond to concentration changes

Large linear range

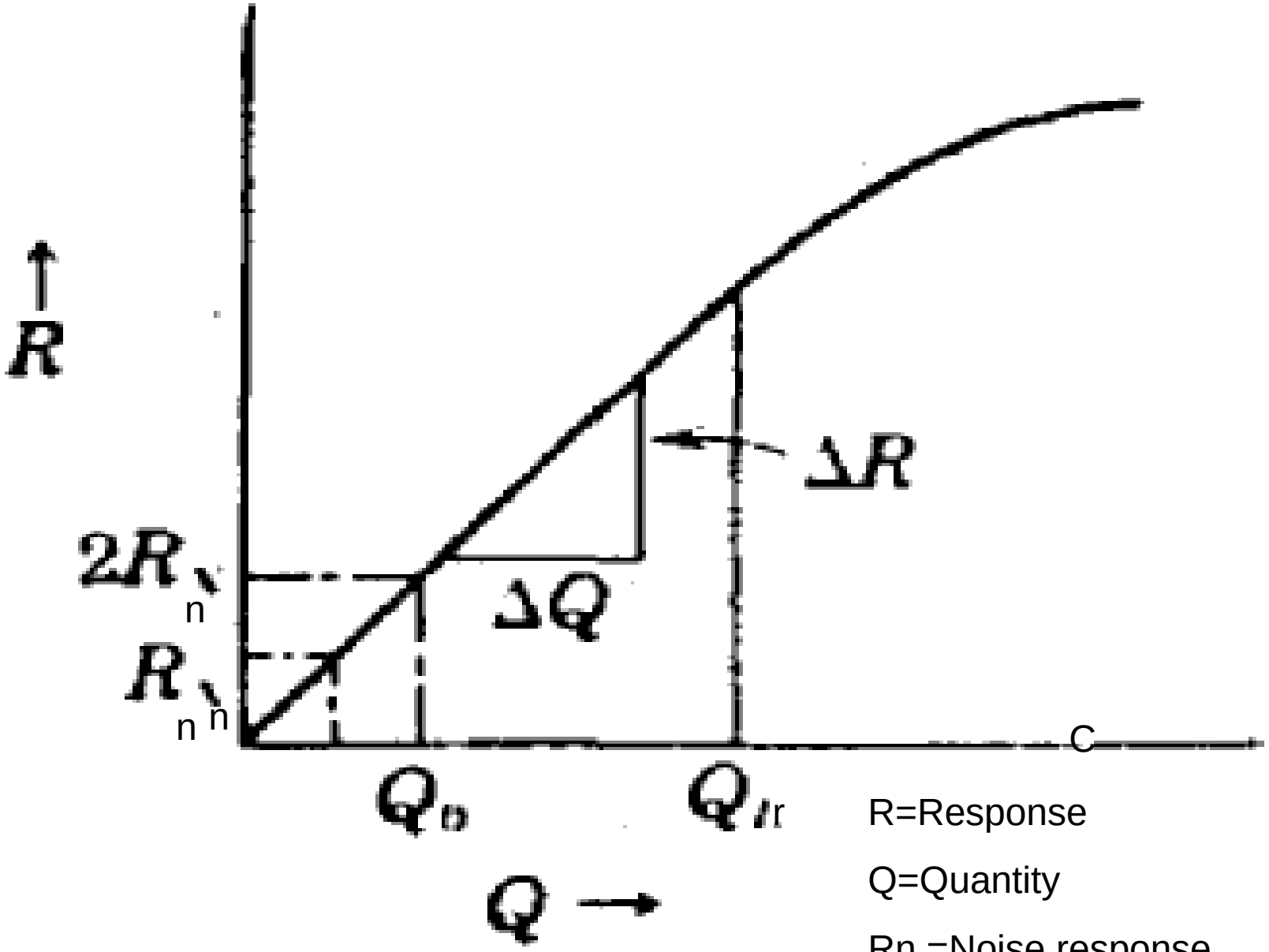
Stable with respect to noise

Low sensitivity to variations in flow, pressure and temperature

Produces an easily handled signal

Sensitivity

Tangent = opposite/adjacent = sensitivity



R=Response

Q=Quantity

Rn =Noise response

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No uniformity in the literature. We'll stick with some standard definitions.

$$\text{Sensitivity} = \frac{\Delta R}{\Delta Q} \quad \frac{\text{(change in response)}}{\text{(change in quantity)}}$$

Detection limit

$$Q_o = 2\,R_n / S \quad \text{(mg/ml), conc. limited}$$

LOW Detection Limit 3N = الحد الأدنى للكشف

Low Quantitative Limit N 10 = الحد الأدنى للمعايرة

Detectors for Gas Chromatography

1. Thermal Conductivity Detector (TCD)
2. Flame Ionization Detector (FID)
3. Electron-Capture Detector (ECD)
4. Nitrogen/Phosphorous Detector (NPD)
5. Flame Photometric Detector (FPD)
6. Photo ionization detector (PID)
7. Electrolytic conductivity detector (ELCD)
8. Atomic Emission Detection (AED)

Thermal Conductivity Detector (TCD)

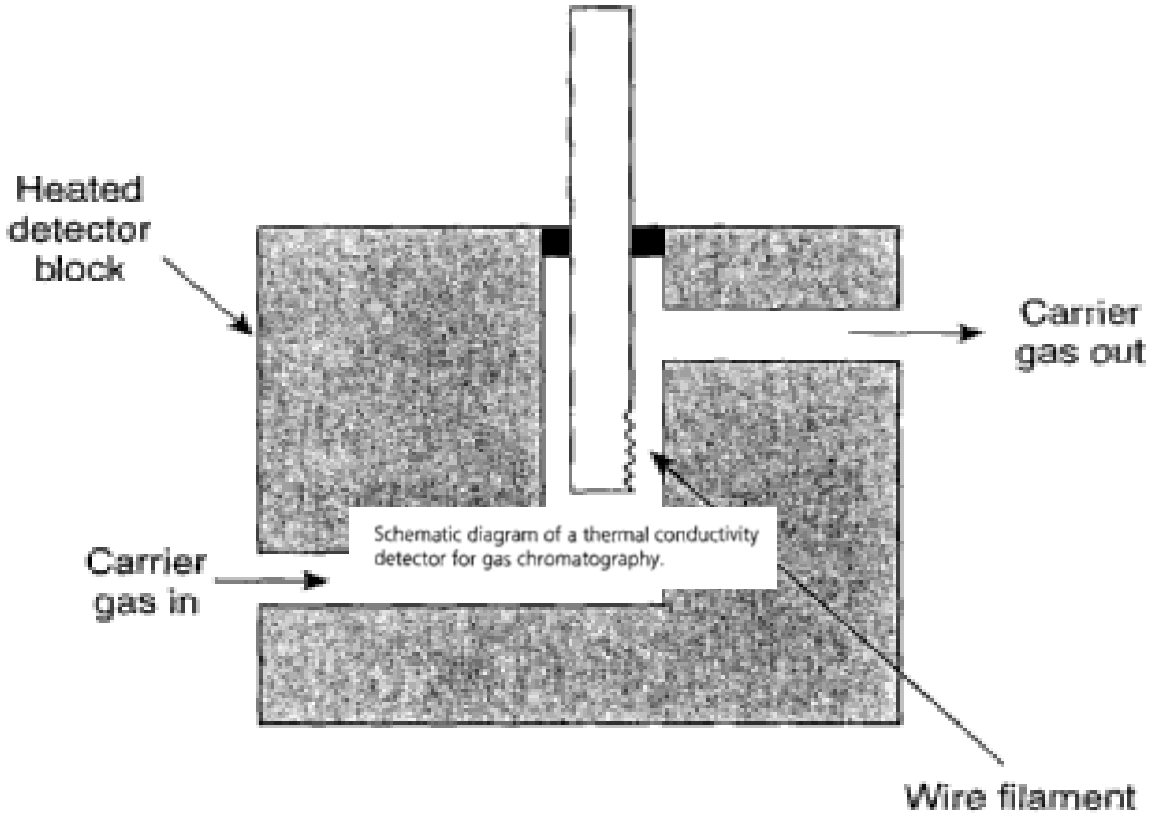
متحري الناقية الحرارية

- السلك الساخن يفقد حرارته بمعدل يعتمد على التوصيل الحراري للغاز المحيط به .
- يعتمد التوصيل الحراري للغاز على تركيبه .
- بدلا من قياس حرارة السلك للدلالة على التوصيل الحراري للغاز الحامل ، نقيس مقاومة السلك .
- مقاومة السلك تتناسب طرداً مع درجة حرارته .

1-Thermal Conductivity Detector (TCD)

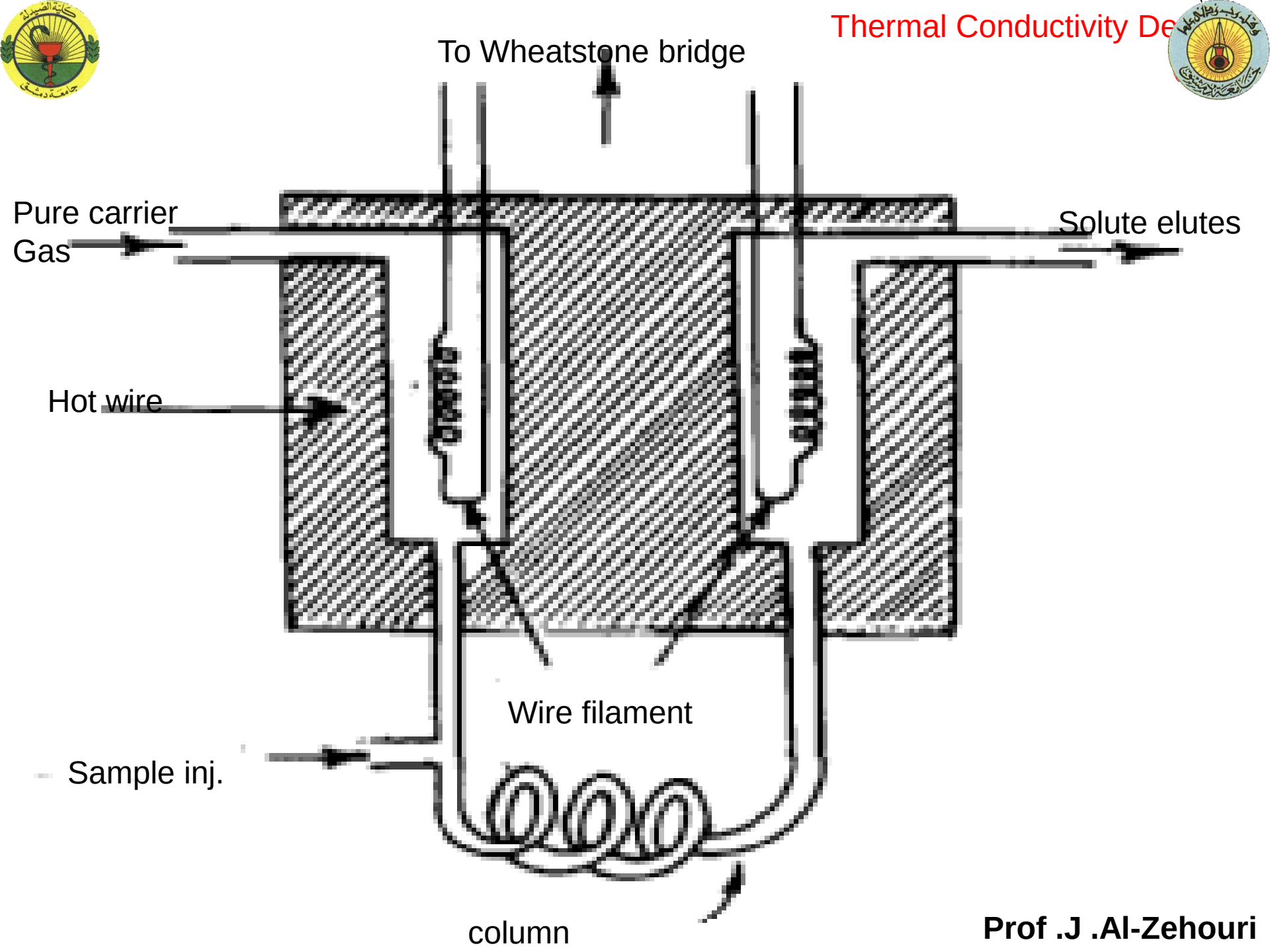
- The thermal conductivity detector ,which was one of the earliest detectors for gas chromatography, still finds wide application. This device consists of an electrically heated source whose temperature at constant electric power depends on the thermal conductivity of the surrounding gas. (Twin detector are ordinarily used)

الناقلية الحرارية للسلك تعتمد على الغاز المحيط



Schematic diagram of a thermal conductivity detector for gas chromatography.

Schematic diagram of a thermal conductivity detector for gas chromatography.



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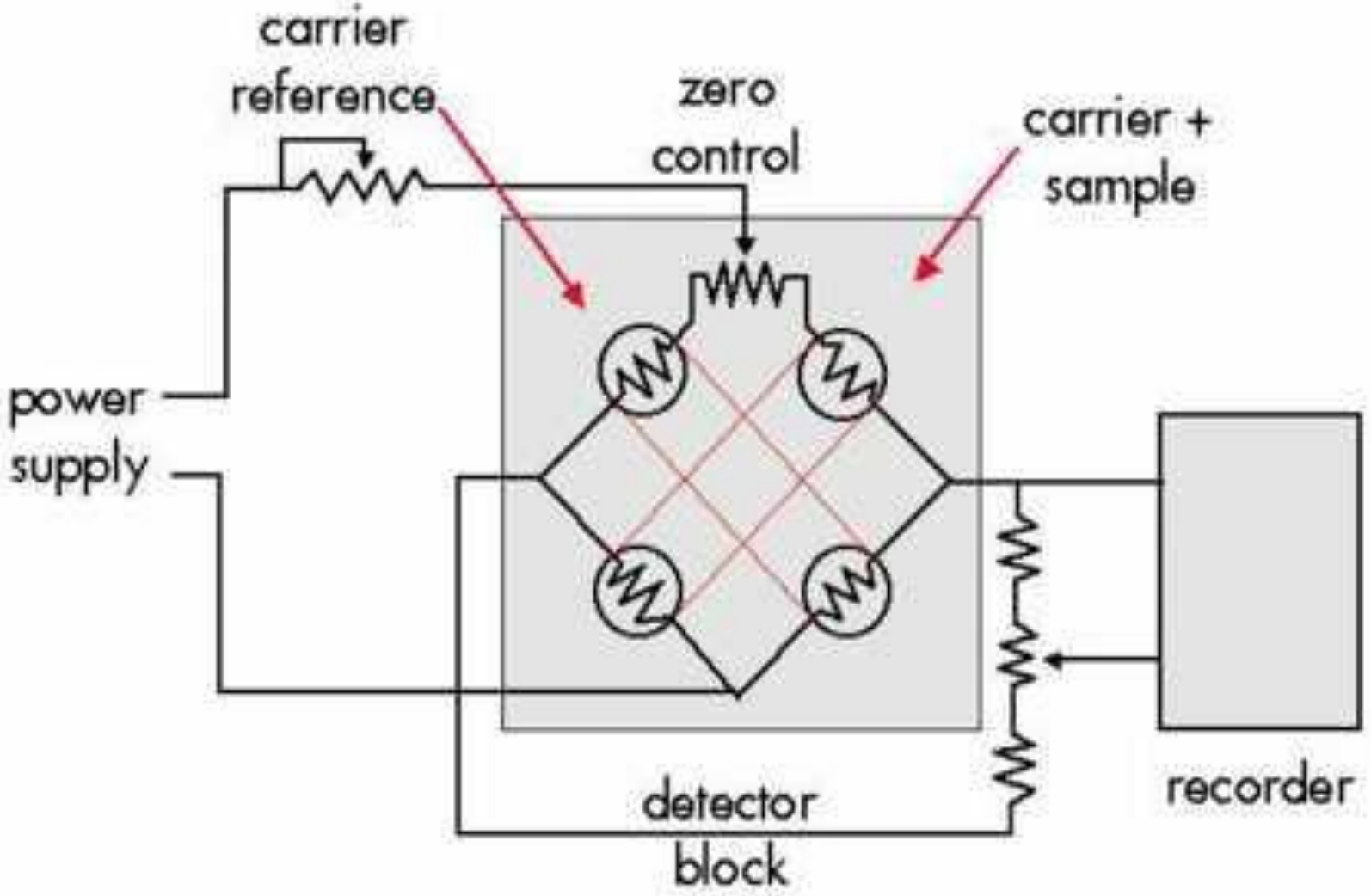
Thermal Conductivity Detector (TCD)

- A **universal** GC detector in which the signal is a change in the thermal conductivity of the mobile phase.
- As the mobile phase exits the column, it passes over a tungsten –rhenium or platinum or gold wire filament. The filament s◌ electrical resistance depends on its temperature, which in turn ,depends on the thermal conductivity of the mobile phase.
- When a solute elutes the thermal conductivity of the mobile phase decreases so the resistant of wire filament increases. (A reference cell ,through only MP pass is present)
- Because of its high thermal conductivity, **helium** and **hydrogen** are the mobile phase of choice when using a TCD(Their thermal conductivities are 10-15 times greater than most organic compounds).

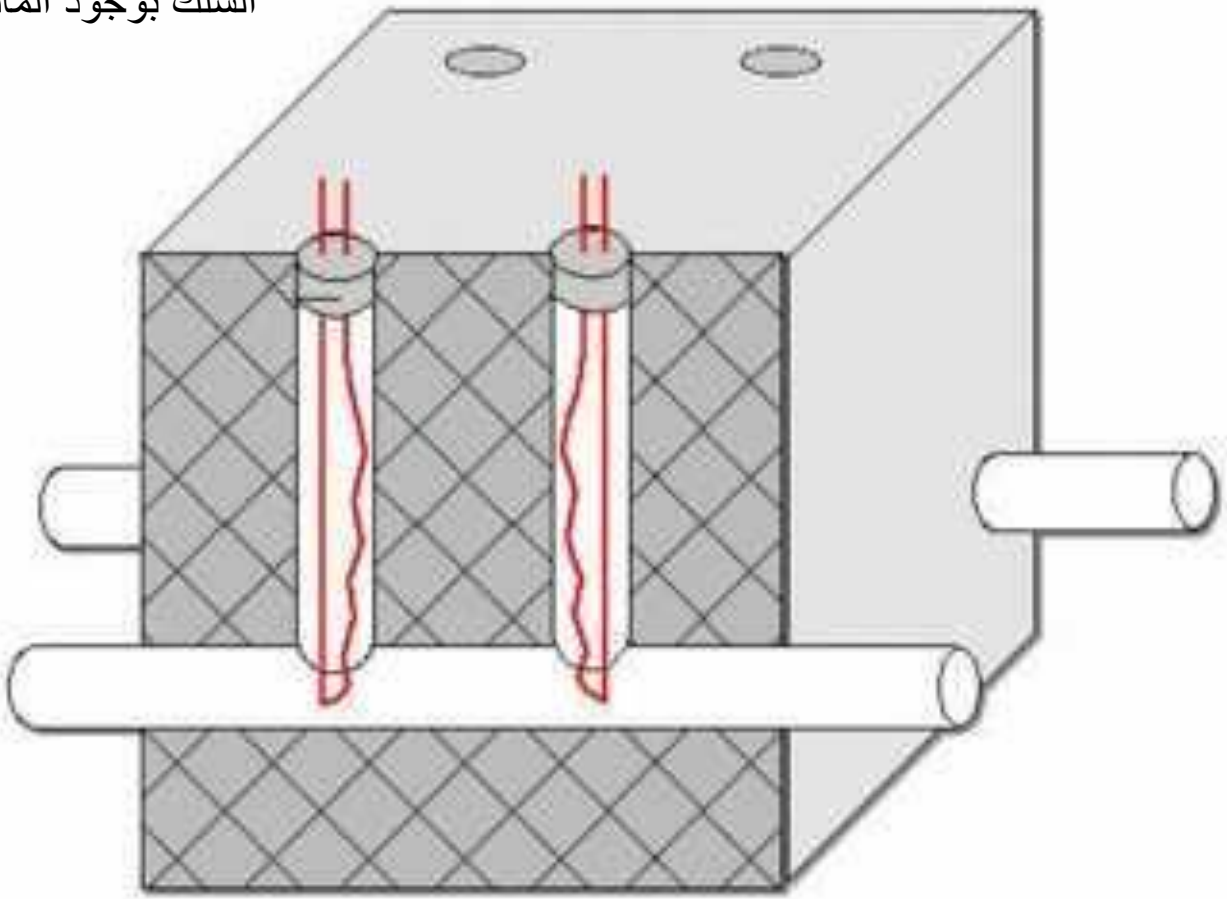
The principle of the detection is using a Wheatstone bridge

Thermal conductivity detector



Thermal conductivity detector

تقاس مقاومة السلك بوجود الغاز الحامل فقد وتقارن مع مقاومة السلك بوجود المادة



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While H_2 has the largest TC Value

He is commonly used – Less reactive

Thermal conductivity detector

- **General purpose** (response for organic and inorganic)
- **Nondestructive** and Simple
- **Limit of detection** 10^{-6} g
- Permit collection of solute after detection.

The chief limitation of the thermal conductivity detector is its relatively low sensitivity.

الذي يحد من استخدامه هو حساسيته المنخفضة

Mode of detection

Change in resistance of a wire based on variations in the thermal conductivity of the gas evolving from a column.

2-Flame Ionization Detector

المتحري اللهبي التشردي

المبدأ :

المركبات الهيدروكربونية ، عندما تمر فوق اللهب تنتج جزيئات من نمط CHO^+ وتطلق اليكترون مولدة تيار كهربائي يتناسب طرداً مع عدد الأليكترونات والتي بدورها تتناسب مع كمية المادة .

Flame Ionization Detector

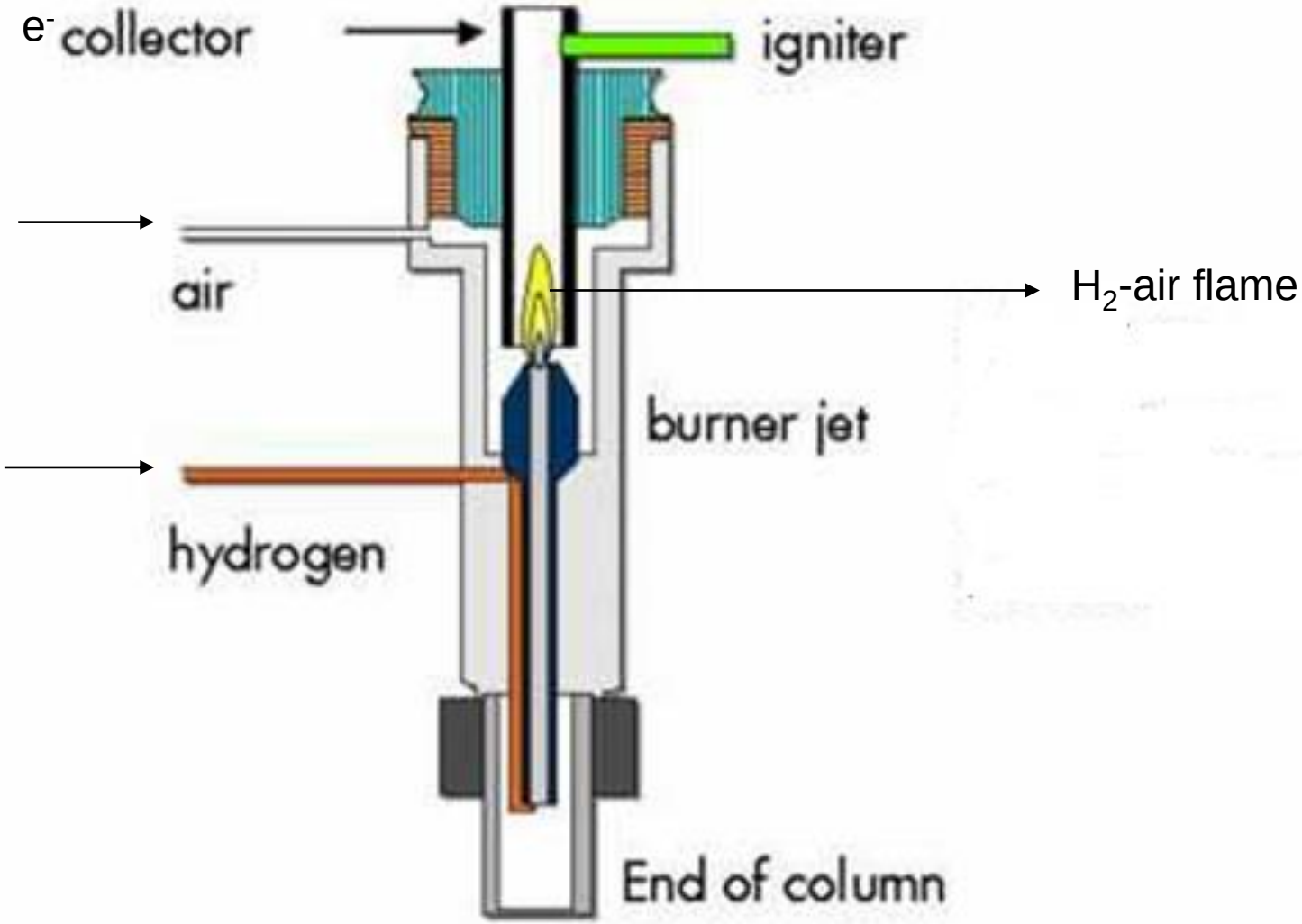
- The flam-ionization detector (FID) is the most widely used and generally applicable of all detectors for gas chromatography.
- Most organic compounds, when pyrolyzed in a hot flame, produce ionic intermediates that conduct electricity through the flame.

Flame Ionization Detector

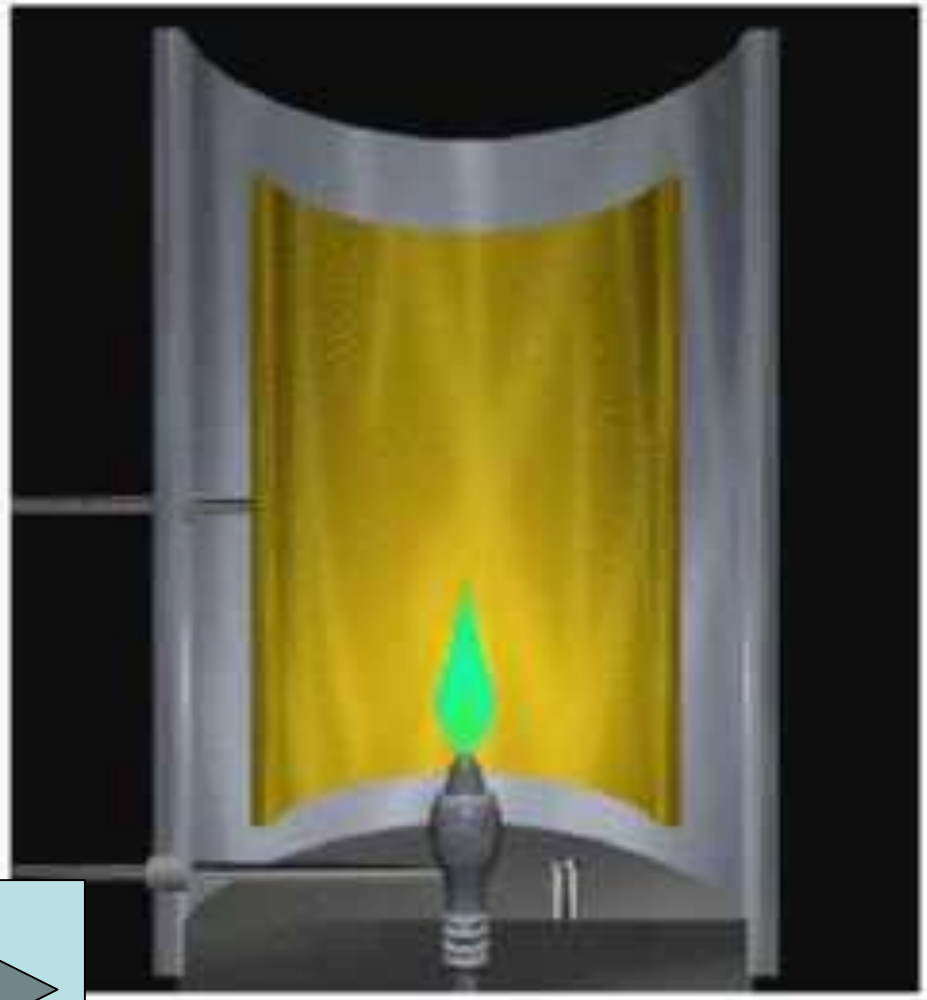
- A nearly universal GC detector in which the solutes are combusted in an H_2/air flame, producing a measurable current.
- The most commonly used in GC.
- FID consists of a hydrogen/air flame and a collector electrode plate.
- The effluent from the column passes through the flame, which oxidizes the organic molecules and produces ions. A collector electrode attracts the negative ions to the electrometer amplifier producing an analog signal, which is connected to data system.

$CH. + O \longrightarrow CHO^+ + e^-$

Flame ionization detector



Flame ionization detector



Sample components enter at the base of the detector. They mix with hydrogen and enter the flame.

Ions are produced that can be measured.

- **FID is sensitive to all compounds which contain C-H and considerably less sensitive to insensitive to certain functional groups of organic compounds, such as alcohol,amine,carbonyl,and halogen.**

- **FID destroy the sample (disadvantage)**

- **The hydrogen flow usually ranges between 20-30 ml/min.**

- **The airflow is about 120-200 ml/min for packet column.**

- **The ratio air hydrogen should about 10:1 . (packet column)**

- **For capillary columns , the flow-rate may be less than 1 ml/min.**

- **The Detection limit 10^{-9} g**

Flame ionization detector

Compounds with little or no FID response

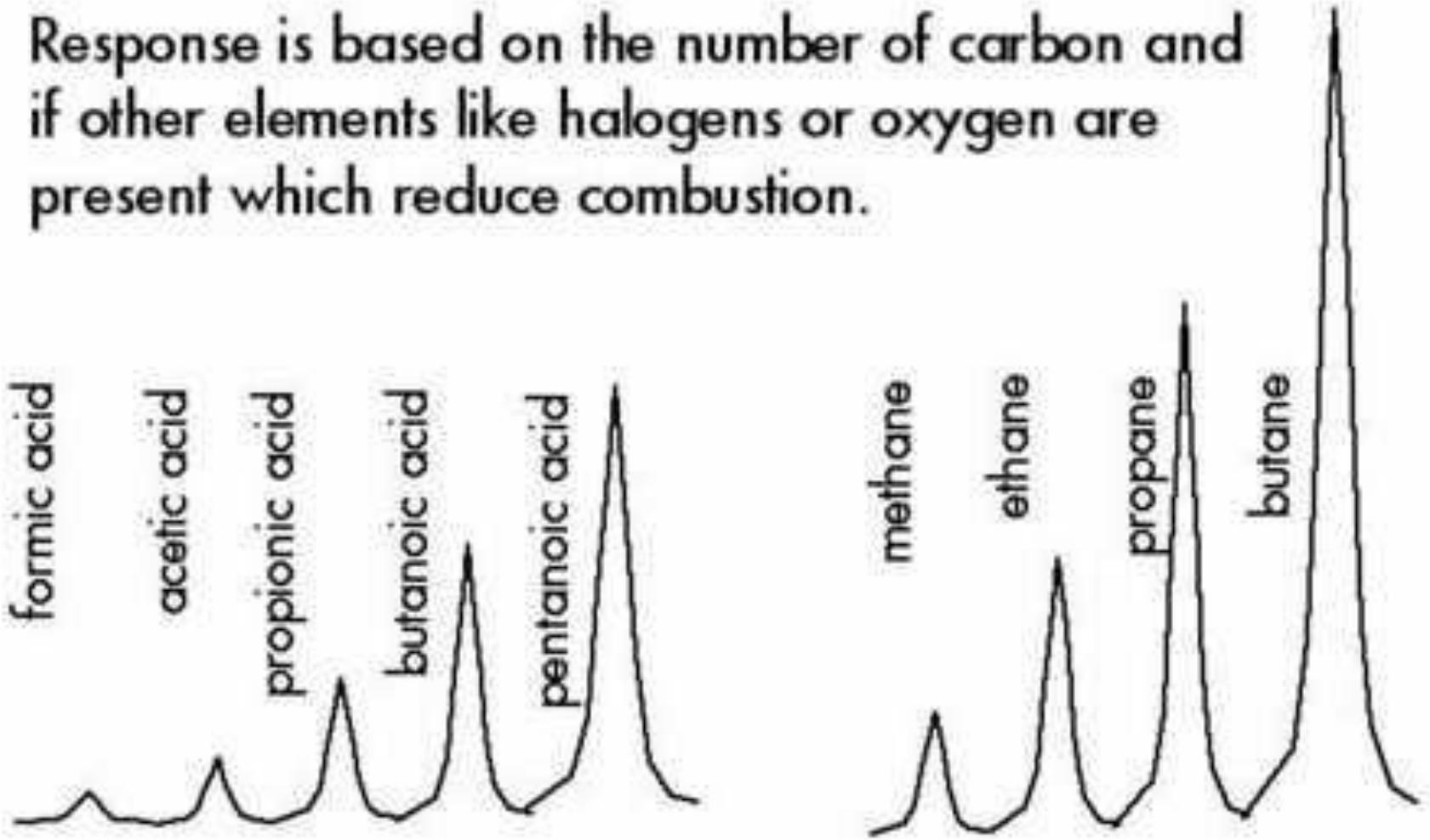
noble gases	NH_3	CS_2
NO_x	CO	O_2
H_2O	CO_2	N_2

perhalogenated compounds

FID response

كلما زاد عدد ذرات الفحم بالمادة كلما زاد زمن الاحتباس
والاستجابة (ارتفاع القمة)

Response is based on the number of carbon and if other elements like halogens or oxygen are present which reduce combustion.



نفس التركيز

Flame ionization detector

- Specific - sample must be combustible
- Destructive
- Limit of detection 10^{-9} g
- A lamination of the FID is that it destroys the sample during the combustion step.

قابلة للأحتراق

Mode of detection

Production of ions in a flame result in a current that can be measured.

3-Electron- Capture Detector (ECD)

المتحري الآسر للاليكترونات

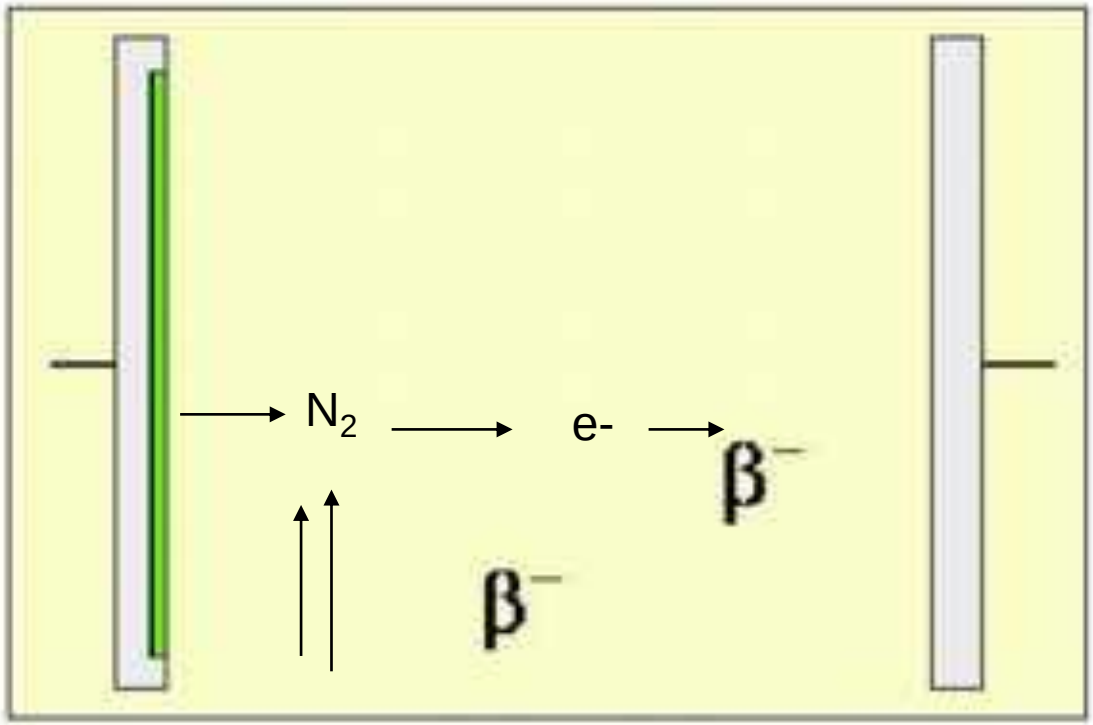
مادة مشعة تعطي أشعة بيتا (اليكترونات) باستمرار معطية تيار مستقر فعند مرور مادة لاقطة للاليكترونات فسوف تعمل على أسر كمية من هذه الأليكترونات مؤدية لأنخفاض التيار الكهربائي بمقدار يتناسب طرداً مع تركيز المادة .

3-Electron- Capture Detector (ECD)

- In this detector , the sample elute from a column is passed over a radioactive ß emitter, usually nickle-63 .The electron from the emitter bombard the carrier gas (nitrogen), resulting in ions and a burst of electrons. In the absence of an analyte, a constant standing current between a pair of electrodes results from this ionization process. The current decreases markedly , however ,in the presence of organic molecules containing electronegative functional groups that tend to capture electrons.

Electron capture

Cathode ——**e⁻**——→ **Anode**



β⁻ are emitted by an ^{63}Ni source.

Electrophores will absorb β⁻, reducing the current.

This is the basis for the response

Electron- Capture Detector (ECD)

- A detector for GC that provides selectivity for solutes with halogen and nitro functional groups.
- The ECD consist of a beta emitter (a beta particle is an electron) such as ^{63}Ni,the emitted electrons ionize the mobile phase ,which is usually N_2,resulting in the production of additional electrons that give rise to an electric current between a pair of electrodes. When a solute which a high cross section for the capture of electrons elutes from the column ,the electric current decreases .This decrease in electric current serves as the signal.

Electron capture detector

Sensitivity of ECD increases in the order of :

F< Cl < Br < I .

Provides excellent trace analysis of
- halogenated compounds
- nitro group compounds
- eluents with conjugated double bonds

Most common use is environmental analysis of organochlorine pesticides

Major problem - detector is radioactive. Requires regular area testing and must be licensed.

Electron capture

- Specific - sample must contain a gas phase electrophore (halogens, peroxides and nitro groups)
- Non-destructive
- Limit of detection 10^{-14} g Very highly sensitive

The detector is insensitive to functional groups such as amines, alcohols and hydrocarbons.

Mode of detection

Absorption of β particles by species containing halogens, nitriles, nitrates, conjugated double bonds, organometallics.

4-Nitrogen/Phosphorous Detector (NPD)

متحري الأزوت والفوسفور

المبدأ :

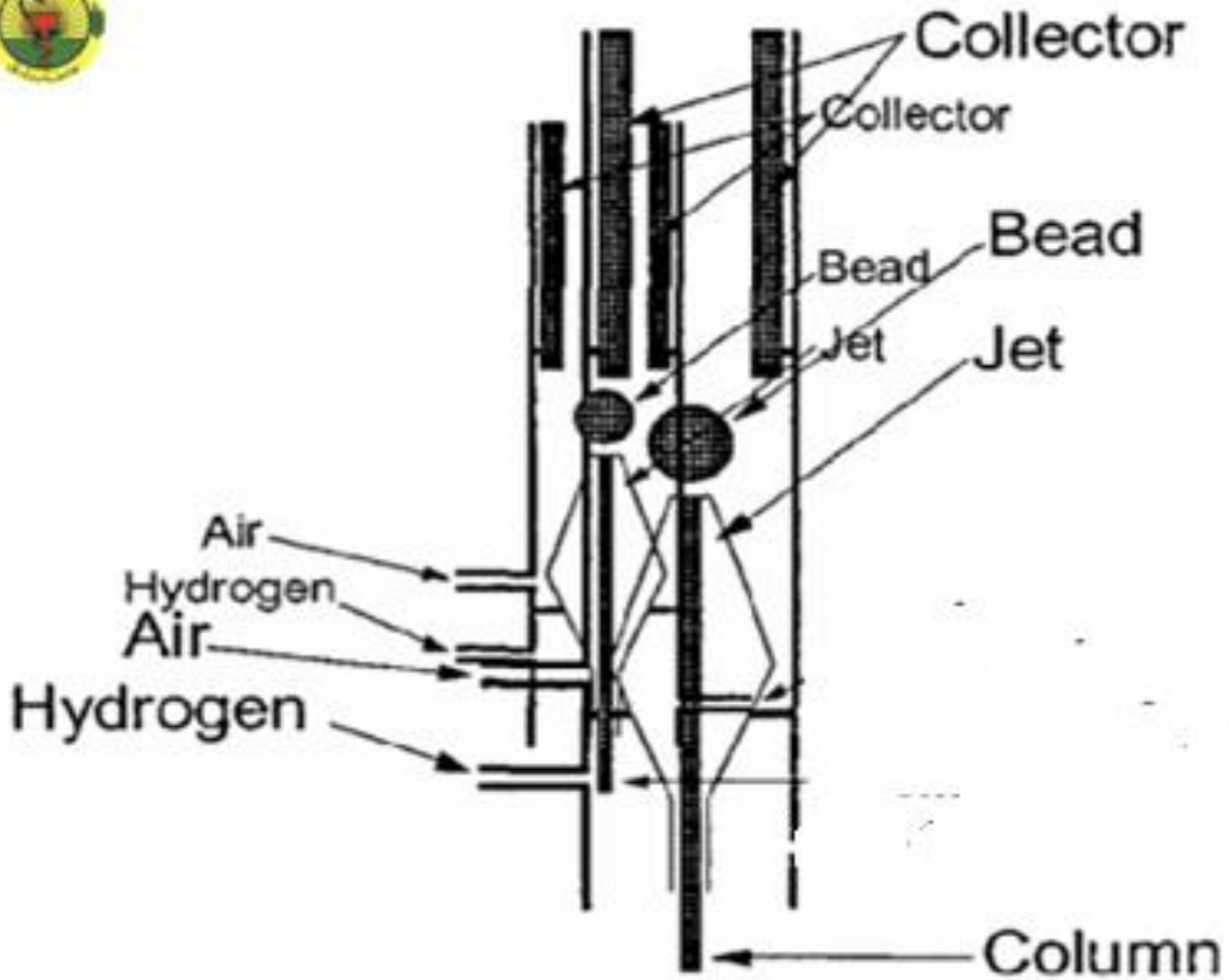
هو متحري FID معدل

يتألف من معجونة على شكل كرية تحتوي على ملح البوتاسيوم القلوي او الروبيديوم أو السيزيوم حيث تسخنً للدرجة 800 مؤدية لتأين مركبات الفوسفور والنيتروجين حصراً مطلقة اليكترونات تشكل تيار يتم قياسه وتمنع باقي المواد من التأين

4-Nitrogen/Phosphorous Detector (NPD)

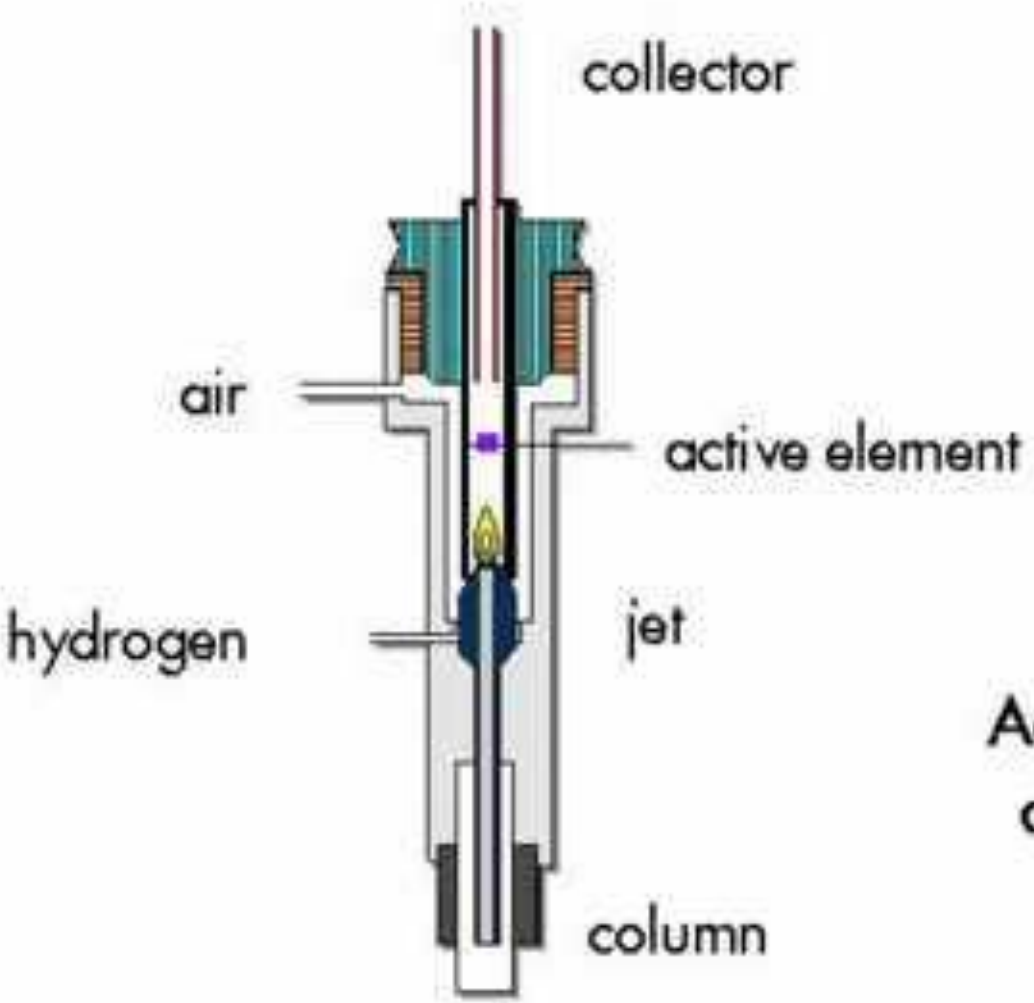
Modified FID

- The Detector is commonly used for analyzing pesticide (p). It is selectively detects organophosphates.
- Detection limit 10^{-10} g
- It is similar in design with FID,
- An electrically heated (up to 800C^{o}) thermionic bead is positioned between the jet orifice and the collector. Generally the bead consists of heated silica bead doped with an alkali Potassium, rubidium or cesium salt .N and P containing molecules will collide with the hot bead and undergo catalytic surface reaction and produce ions, and the ions will be attracted to collector electrode, amplified, and output to the data system. مصمم فقط لتأين الفوسفور وانيتروجين وتحجب الباقي



Nitrogen phosphorus detector

NPD



Active element is
a K or Rb salt.

Nitrogen-phosphorous detector

- Specific - sample must contain nitrogen or phosphorous - based on operational mode.
- Destructive
- Limit of detection 10^{-10}g

Mode of operation. Essentially a modified FID. Active element acts to block undesired species

5- Flame Photometric Detector (FPD)

المتحري اللهبي الضوئي

خاص بالكبريت والفوسفورفعند مرور المركبات الحاوية على هذين العنصرين على لهب خفيف تتشكل HPO و S_2 اللذين يصدرا الضوء عند الموجة 394 للكبريت و 520 للفوسفور حيث يتم قياس هذا الضوء مباشرة

5- Flame Photometric Detector (FPD)

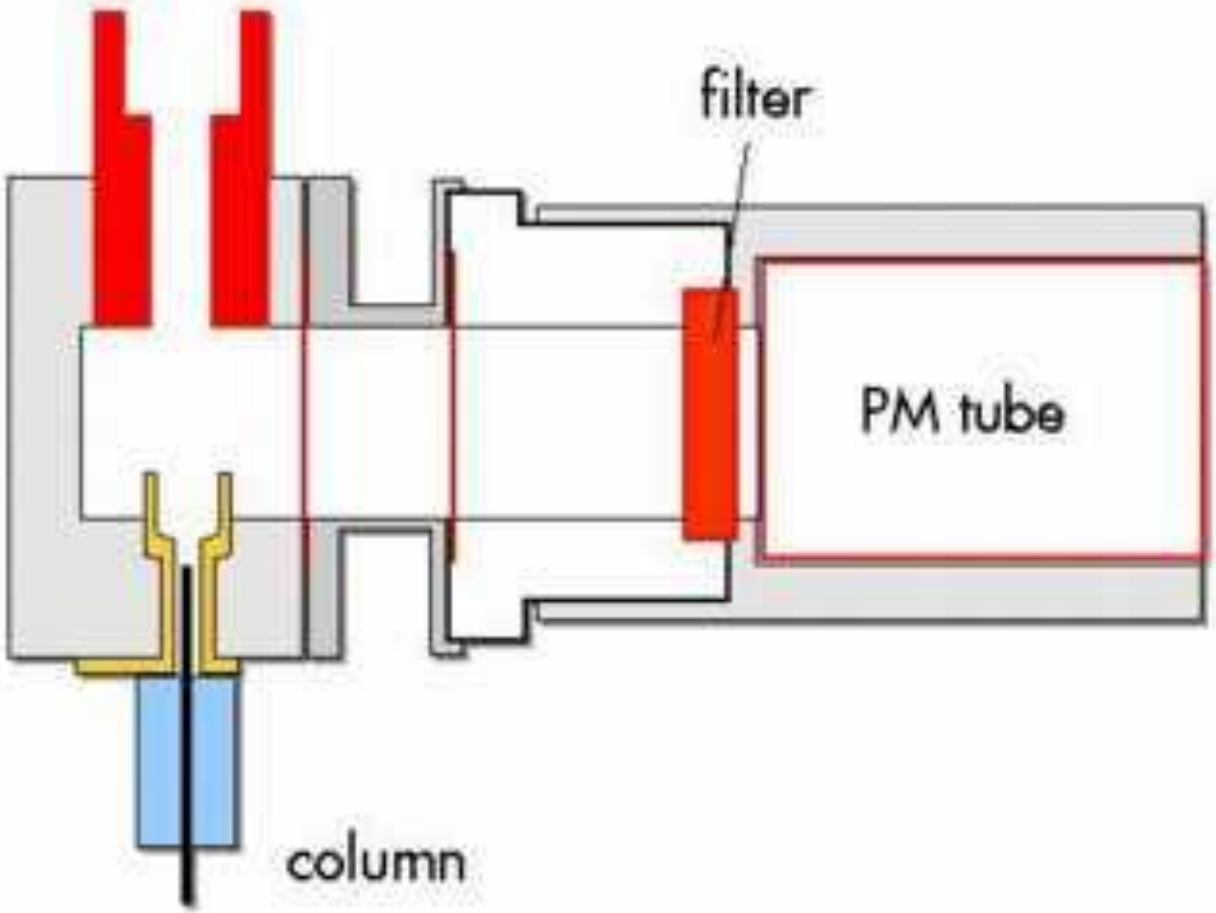
- FPD is selective for S- and P-containing molecules.
- The effluent passed in to a low temperature H_2/air flam ,which converted phosphorus and sulfur to emitting species (color). HPO and S_2, respectively.
- Sulfur has λmax at 394 nm.
- Phosphorus has λ max at 520 nm.

Flame Photometric Detector

- Specific. Phosphorous or Sulfur
- Destructive
- Limit of detection 10^{-12} g

Mode of operation. Directly measure light produced during combustion of sulfur or phosphorous containing species.

Flame photometric detector



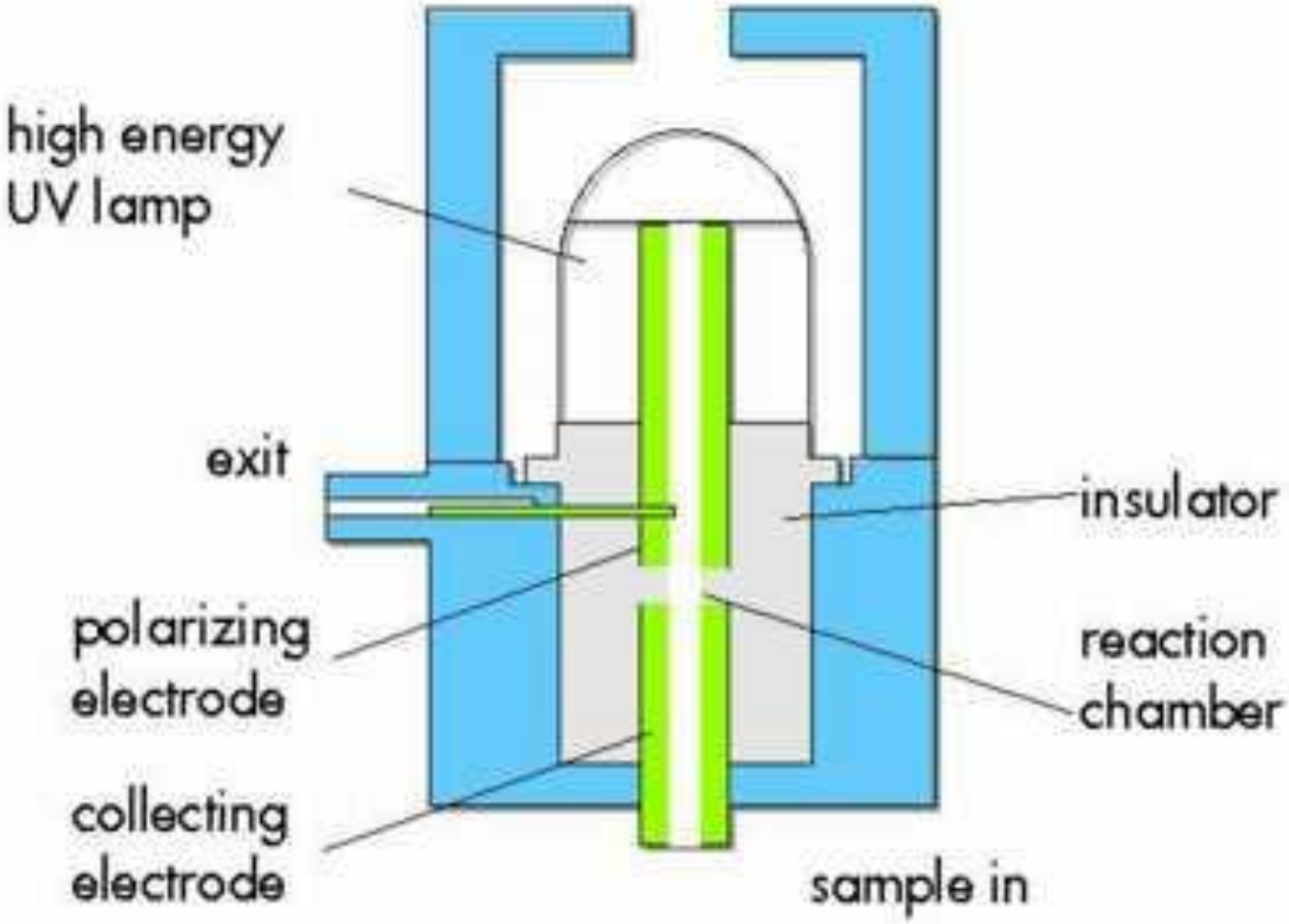
6-Photoionization detector

PID

المتحري التاييني الضوئي

تستخدم اشعة ال UV لتأين المركبات حيث ينتج تيار كهربائي يتم قياسه وتستخدم بحالة المركبات العطرية

Photoionization detector



Photoionization detector

PID

- Specific. Compounds ionized by UV
- Limit of detection 10^{-10}g
- Specific for Aromatic Compounds

Mode of operation. UV light is used to directly ionize sample components. The resulting current is measured.

Electrolytic conductivity detector
ELCD

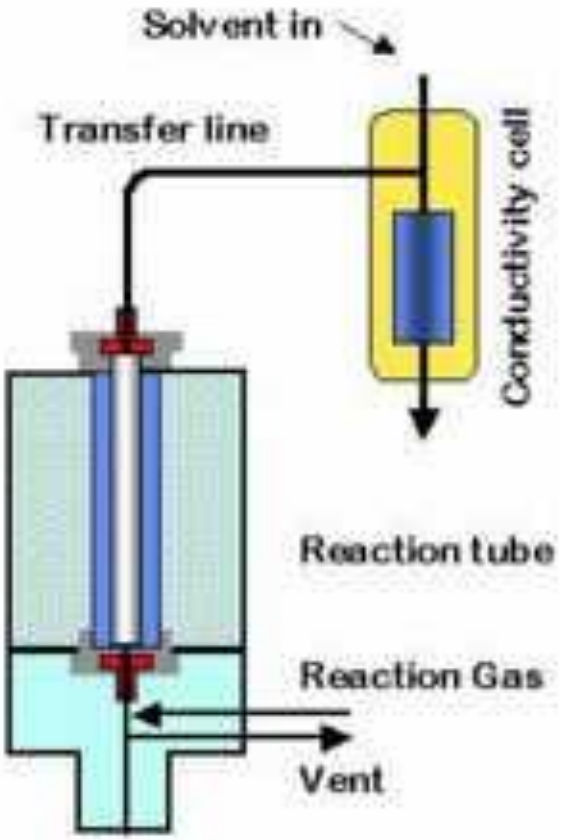
متحري الناقلية الأليكتروليتي

المبدأ :

تمتزج المركبات مع غاز التفاعل (الهيدروجين) وتمرر بأنبوب التفاعل عالي الحرارة مؤدية لأرجاعها حيث تمرر هذه المركبات عبر خلية كهرليتية حيث تذاب في محل (غالباً نظامي البروبانول) مؤدية لتغير في ناقليته الكهربائية ، حيث يقاس هذا التغير(خاص بالهالوجين كبريت وأزوت)

7- Electrolytic Conductivity Detector

ELCD



Compounds are mixed with a reaction gas and passed through a high temperature reaction tube.

Specific reaction products are created which mix with a solvent and pass through an electrolytic conductivity cell.

Change in electrolytic conductivity of is measured and a signal is generated.

Reaction tube temperature and solvent determine what compounds are detected.

Electrolytic Conductivity Detector

ELCD - Hall detector

- **Selectivity:** Halogens, sulfur or nitrogen containing species. Only one at a time.
- **Sensitivity:** 5-10 pg (halogens)
  10-20 pg (S)
  10-20 pg (N)
- **Linear range:** 10^5 -10^6 (halogens)
  10^4 -10^5 (N)
  10^3 -10^4 (S)
- **Reaction Temperature:** 800-1000°C (halogens);
  850-925°C (N); 750-825°C (S)

Electrolytic Conductivity Detector

Haloacid example.

- Catalytically reduction relies on high temperature hydrogen gas.
- The mixture is passed through a heated nickel reaction tube.
- The reduced compounds flow into the electrolytic conductivity cell via a Teflon® transfer line, where they dissolve in a solvent (typically n-propanol).
- As the n-propanol conductivity changes, the compounds are detected.

Gas Chromatography Detectors

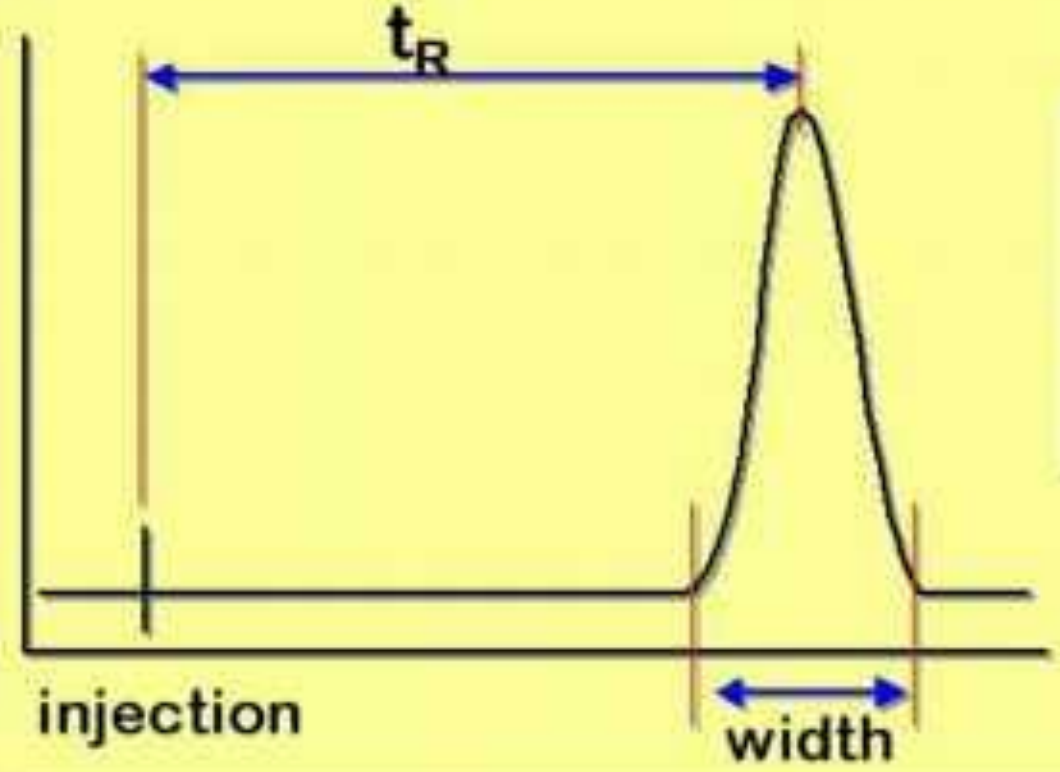
Type	Applicable Samples
Thermal conductivity	Universal detector
Flame ionization	Hydrocarbons
Electron capture	Halogenated compounds
Thermionic (Nitrogen-phosphorus)	Nitrogen and phosphorus compounds
Electrolytic conductivity (Hall)	Compounds containing halogens, sulfur, or nitrogen
Photoionization	Compounds ionized by UV radiation
Fourier transform IR (FTIR)	Organic compounds
Mass spectrometer (MS)	Tunable for any species

Theoretical plates - N

- In solvent extraction, a plate is represented by each equilibrium (extraction) we conduct.
- In a chromatographic column, we can't see these plates so they are theoretical.
- We can estimate the number of theoretical plates in our column based on peak retention times and widths.
- Both factors are important in determining if a separation will work.

Determining N

The number of plates can be determined from the retention time and peak width.



It doesn't matter what units are used as long as they are the same.

Determination of N

The number of plates (N) calculates as :

$$N = 16 (t_R / W)^2$$

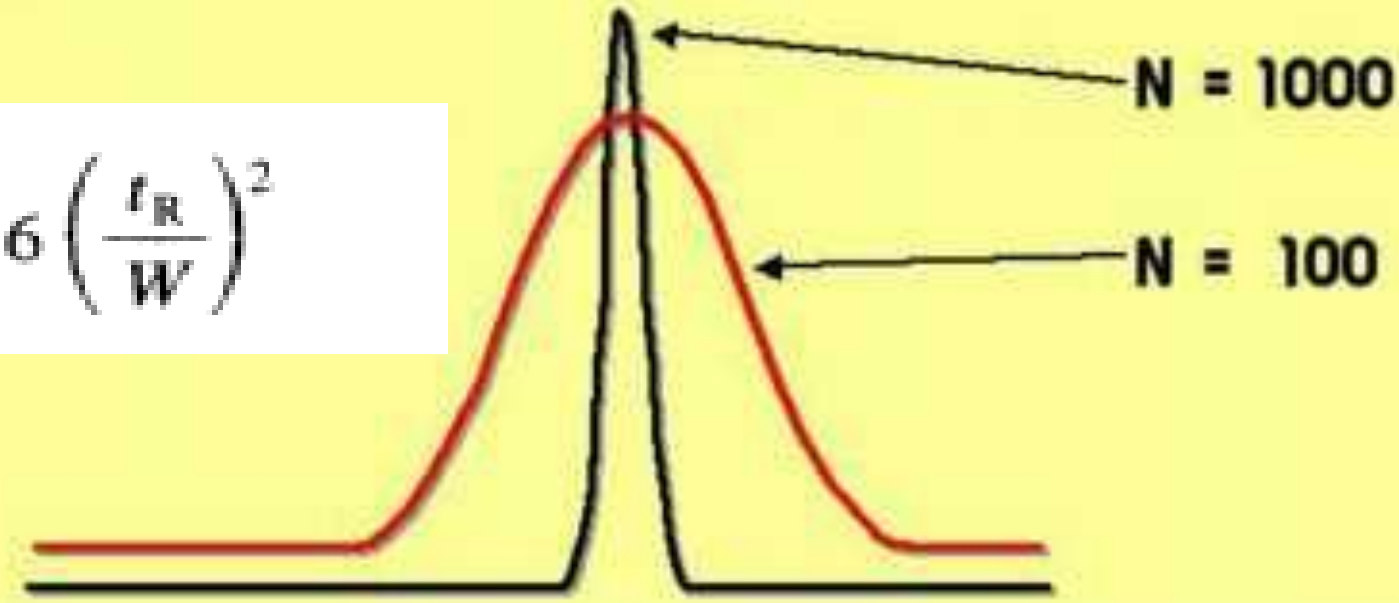
For a fixed length column , we can calculate an additional term – h (or HETP)

h= height equivalent of a theoretical plate = column length./N (L/N)

Determination of N

In this example, we have materials with the same elution time but different numbers of plates.

$$N = 16\left(\frac{t_R}{W}\right)^2$$



Example

- Substances A and B have retention times of 16.40 and 17.63 min, respectively, on a 30.0 –m column. The peak widths (at base) for A and B are 1.11 and 1.21 cm, respectively. Calculate :

1- The average number of plates in the column,

2- The plate height.

(A = 1,06, 3445, 0.0087cm respectively)

Calculation of N_{av}

$$N = 16\left(\frac{f_R}{W}\right)^2$$

$$N = 16\left(\frac{16.40}{1.11}\right)^2 = 3493 \quad \text{and} \quad N = 16\left(\frac{17.63}{1.21}\right)^2 = 3397$$

$$N_{av} = \frac{3493 + 3397}{2} = 3445 = 3.4 \times 10^3$$

Calculation of palate height

$$H = \frac{L}{N} = \frac{30.0}{3445} = 8.7 \times 10^{-3}\ \text{cm}$$

Optimization of the GC Column Parameter

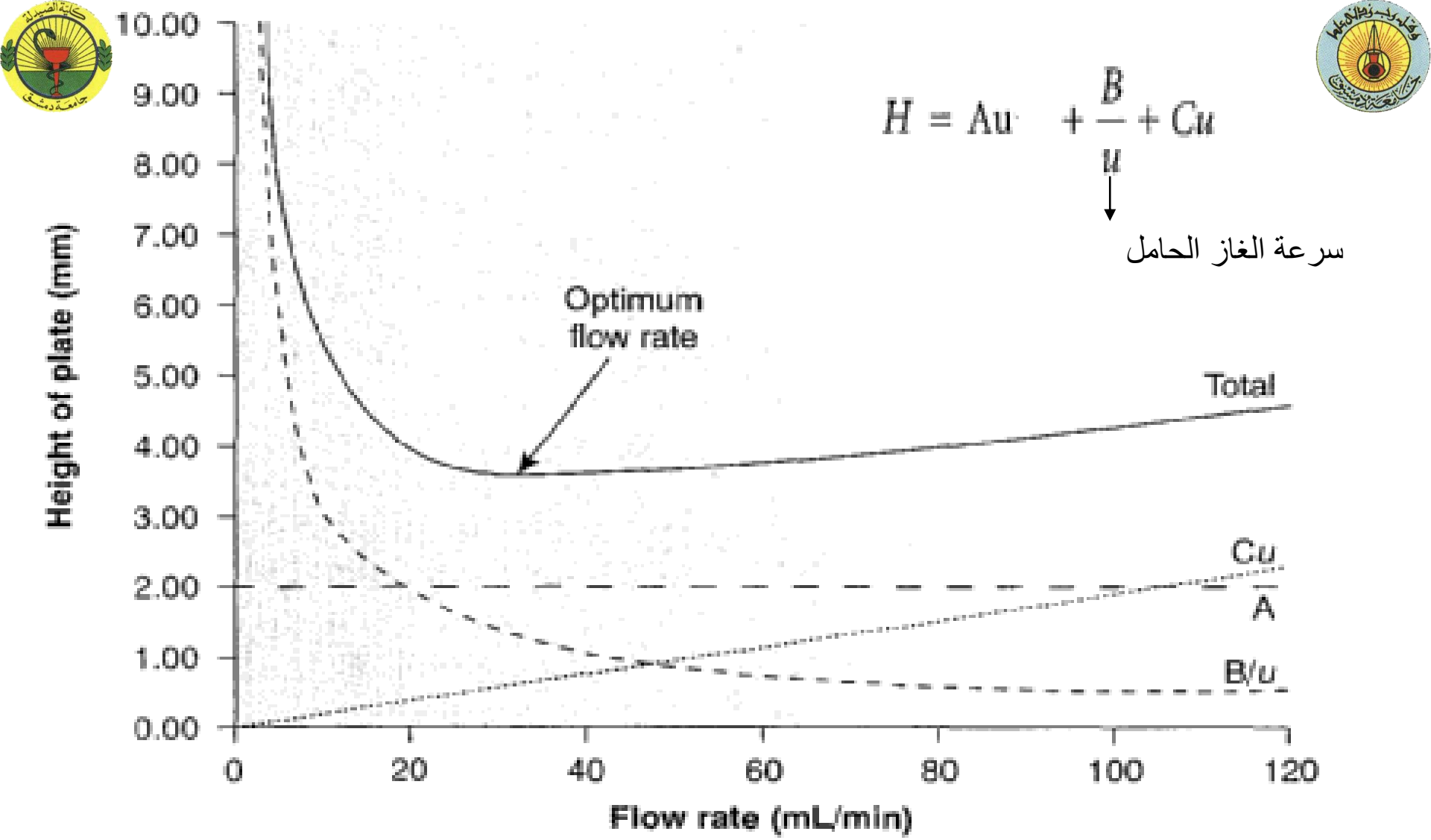
- The choice of the stationary phase for particular application in packed columns is more complicated than in open tubular columns.
- The important criteria to select the GC columns for the separation are the polarity of the sample components and the polarity of the stationary phase .This is combined with the general rule “ **Like dissolves Like**” for selecting the separating liquid.

Optimization of the GC Column Parameters

- However, this is not perfect quide so that" trial-and-error tests" are also applied to obtain the optimal separation conditions.
- Small diameter open tubular column is most excellent for separating simple mixture, on the other hand for , difficult separations, wider columns is required, This is due to the need of longer column.
- As the plate height the separation become better.

3- Carrier Gas Flow :

According to van Demeter equation (An equation showing the effect of the mobile phase sُ flow rate on the height of a theoretical plate) the theoretical plate numbers (N) of a column rely on the flow of the carrier gas used .so the minimum of curve corresponds to the maximum efficiency of the separation.



Plot of the height of a theoretical plate as a function of mobile-phase velocity using the van Deemter equation. The contributions to the terms *A*, *B*/*u*, and *Cu* also are shown.

A,B &C ثوابت خاصة بالعامود (الحشوة ، الأنتشار الجزيئي ...) ، كلما كانت H صغيرة كلما كان القصل أفضل وعندها تحدد السرعة المثلى

ecreases the theoretical plate decreases the efficiency.

•For each column ,the carrier gas velocity should be practically optimized to find out the flow-rate at which the van Deemeter plot becomes linear.

•In the modern GC ,the carrier gas flow during analysis can be changed by gradually increasing the inlet pressure. the advantages of this flow programming are the analyses can be performed in a reduced separation time and at lower temperatures of the column. Increasing the carrier gas flow rate decreases the elution time of the components and hence shortens analysis time.

Why ? Derivatization in GC

1. To separate non-volatile Substance
2. To Improve the separation of Closed Substances
3. To separate the thermal unstable Substance.
4. To improve the sensitivity.
5. Avoid Tailing

The most common method of derivatization

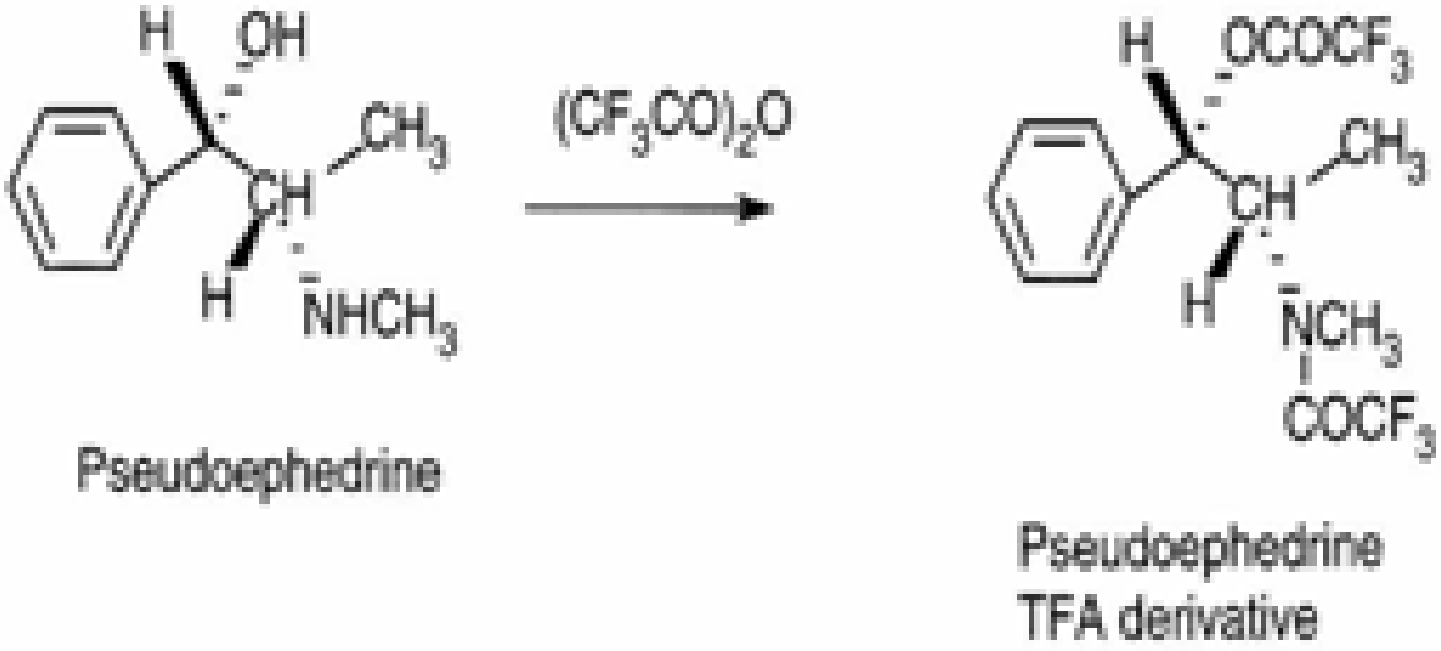
-Methylation add $-CH_3$

-Acylation add –CO-R

- Silinization -Si-R

- Trifluoroacetic anhydride (TFA)

derivatisation of pseudoephedrine with trifluoroacetic anhydride (TFA).

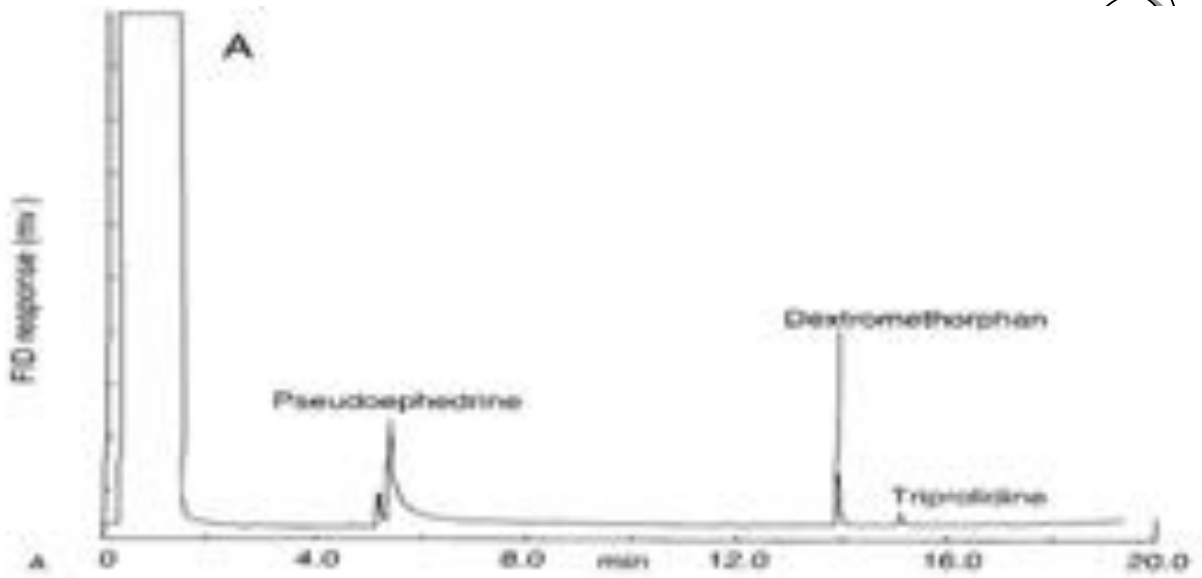


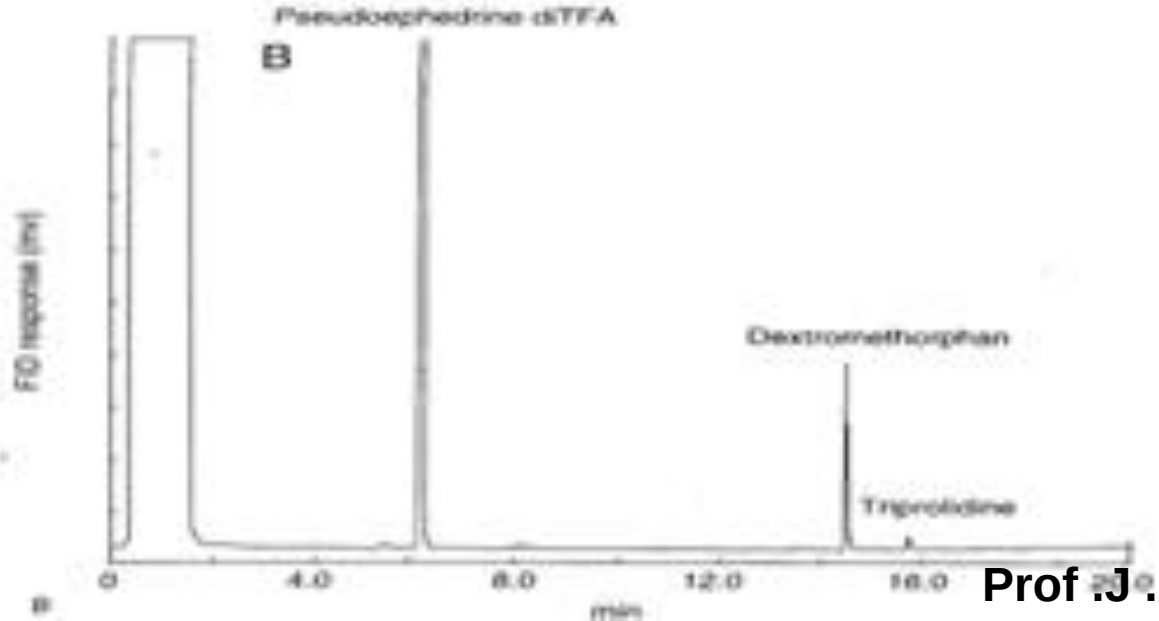
Pseudoephedrine

Pseudoephedrine TFA derivative

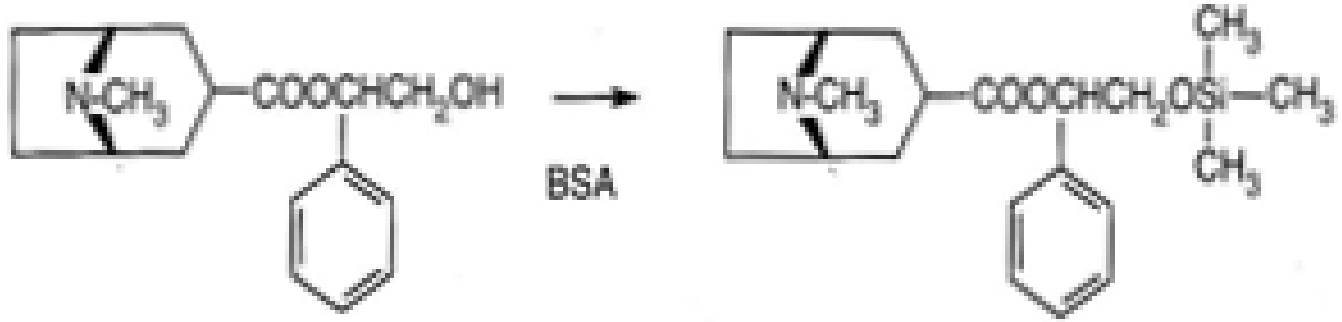
Derivatization improve the peak shape of pseudoephedrine

GC traces of an extract from a decongestant syrup. (A) Underivatised and (B) after treatment with trifluoroacetic anhydride.





Trimethylsilylation of atropine.



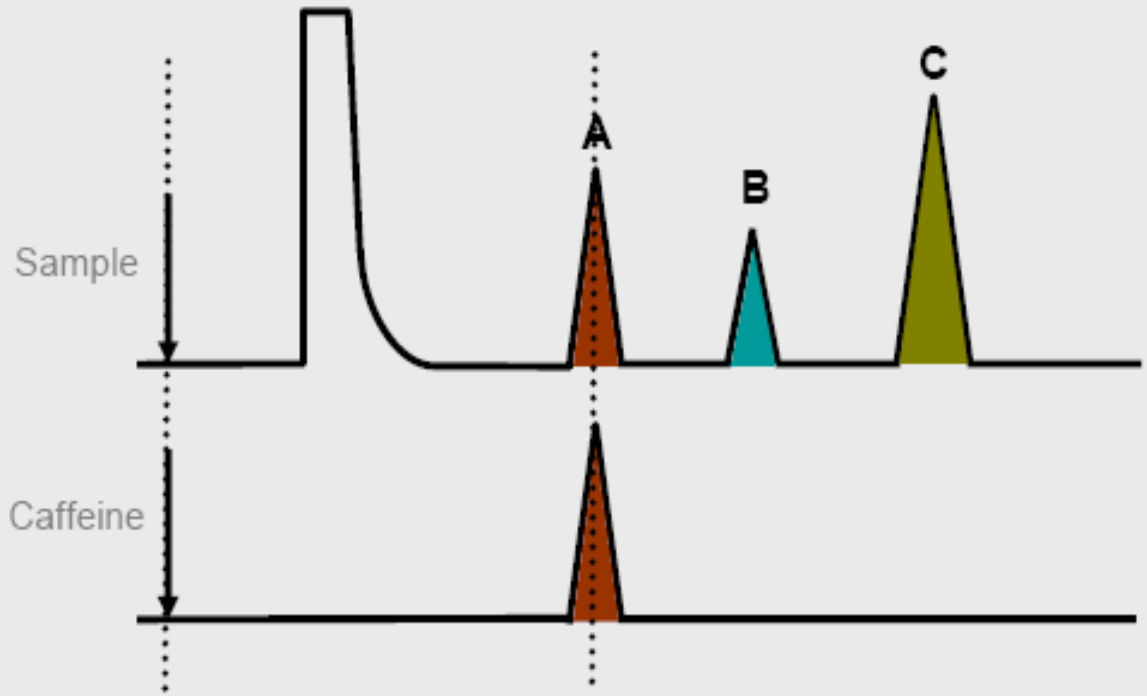
Atropine

Qualitative and Quantitative Analysis

- As other chromatography methods ,GC could be used for qualitative and quantitative analysis.

Identification

What is component A?



Component A elutes the same time as a caffeine peak.

Component A is identified as caffeine.

Qualitative Analysis

- If R_t of two substances are relatively identical ,It does not always means that the two substances are identical, but if R_t

  are not identical if means that the two substances are not identical.
- Authentic standard is necessary.
- Absence of strange peaks indicate the purity.
- For analysis of an unknown substance the combination of GC with other spectroscopic methods such as MS and or FTIR are very helpful to Identify the unknown substance.

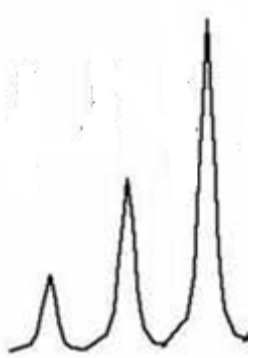
Quantitative Analysis

1. ***Normalization method.***
2. ***External Standardization method.***
3. ***Internal Standardization method.***
4. ***Standard addition method.***

1.Normalization method

- In this method, all the analyte (s) present in the sample must elute from the column. The peak area is proportional to the weight of the analyte (s) having passed through the detector.

$$\%C_i = \frac{A_i}{\Sigma A_i} \times 100$$



(A=Area)

التركيز المئوي = مساحة القمة / مجموع مساحات القمم مضروبة ب 100

Normalization Method

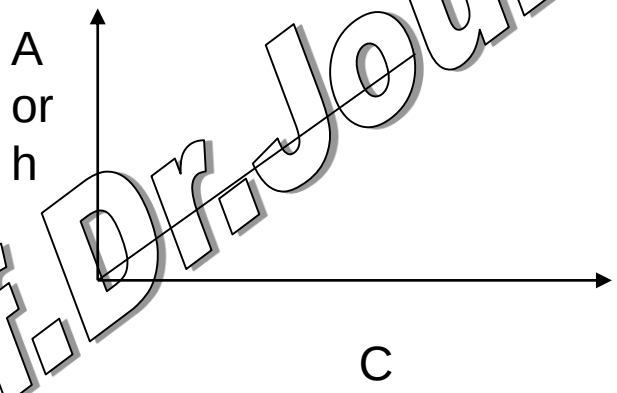
- A sample consist of Benzene , Heptan and 2- methyl Hexane gave a chromatogram with 3 peaks have the following area 35,58 and 13 respectively ,What is the % consist?

A (33 %, 54.7% and 12.26%)

$$\%= \frac{35}{(35+58+13)} X100 = 33\% \text{ (Benzene)}$$

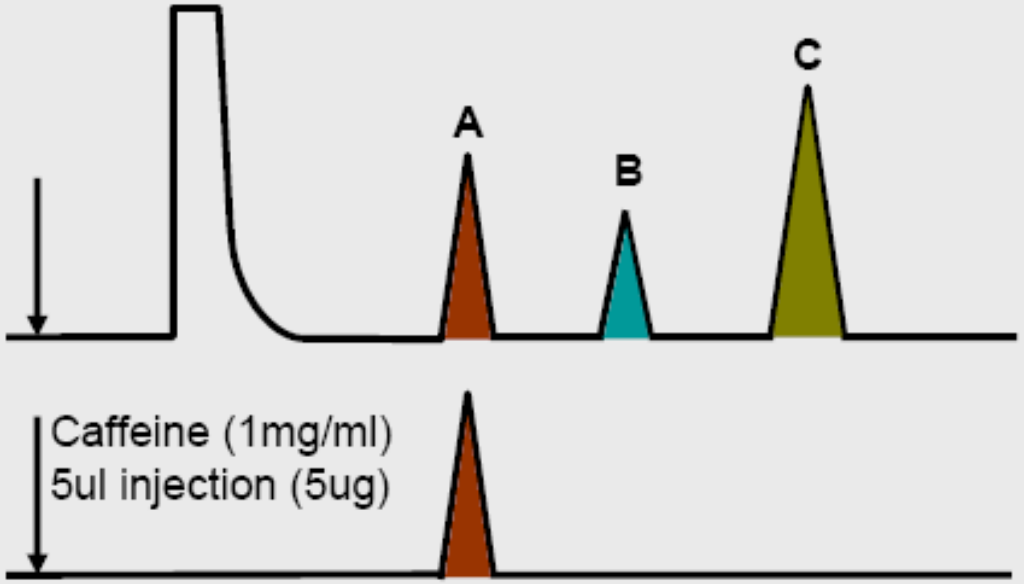
2.External Standardization method

- This method is the most general method for determining the concentration of an analyte in a samples, it involves the construction of calibrate plot (area or height vs analyte concentration)



Determination

What is the concentration of component A?



Peak area (or height) is proportional to the concentration (or amount) of the component.

The concentration of component A(caffeine) is determined by comparing the peak area with that of the standard caffeine peak.

External standard method

Requirements for proper use:

Standard solution containing all eluents to be quantified.

Standard eluents should be of similar concentration as unknowns.

The standard and sample matrix should be as similar as possible

Analysis conditions must be identical - stable instrument, same sample size ...

External standard method

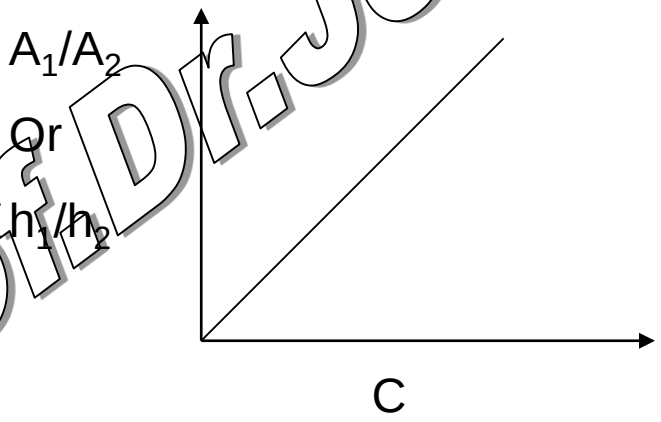
You either assume that response is linear over the entire concentration range or actually measure it. Then:

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

This is assuming that the same injection volume was used for both the unknown and standard.

3.Internal Standardization Method

- The principle of this method involves the addition of known quantity of the internal standard to the analyzed sample (s) and to the reference standards (s) .This is to compensate errors mostly by variation of the injected amounts.
- The method is not applied in auto-sample injection.
- The calibration plot is constructed by using the ratio of peak area or height of standard (s) and internal standard (s) against concentration of standard (s)



The concentration of the analyte (s) can be determined by calibration curve (s).

Internal standard method

Requirements for an internal standard.

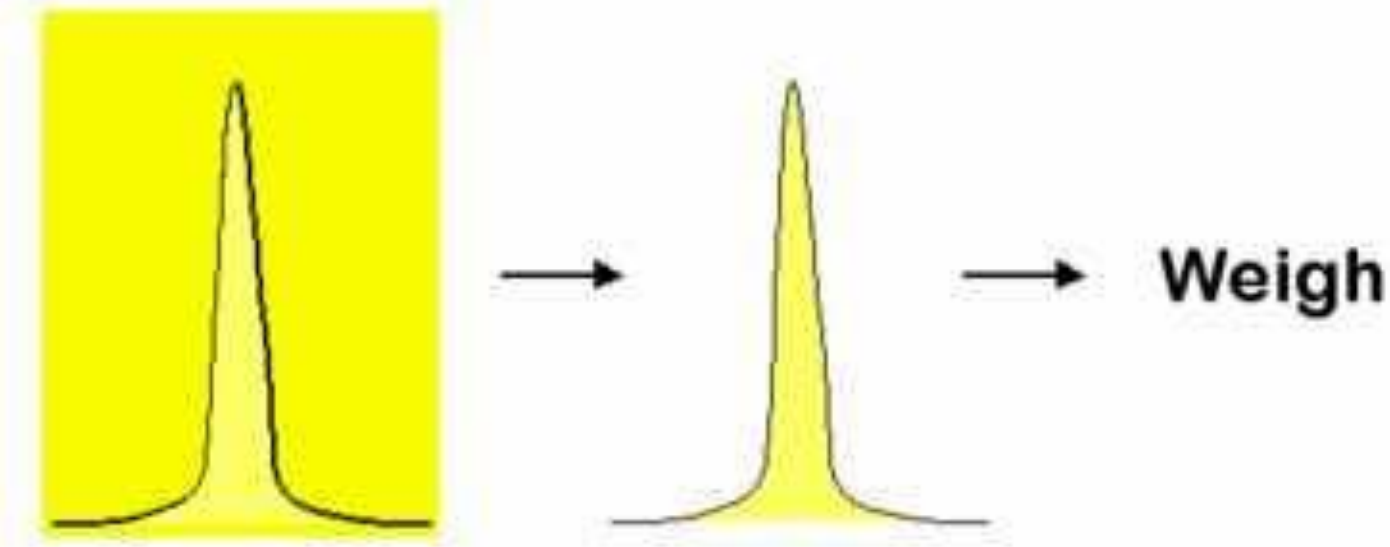
- Must be present at a constant concentration in all samples and standards.
- Must be stable and measurable under the analysis conditions.
- Must not interfere with the analysis or co-elute with sample components.

4. Standard Addition Method

- If the concentration of the analyte is below the (linear) range of the calibration curve, the external and internal standardization methods cannot be applied. In this case, one should add certain amount of the analyte in the sample(spiking). A linear regression curve is constructed of the peak area (y-axis) against the added concentration of the analyte in the sample (x-axis) . The concentration of the analyte can be calculated from the intercept of the regression curve with the x-axis.

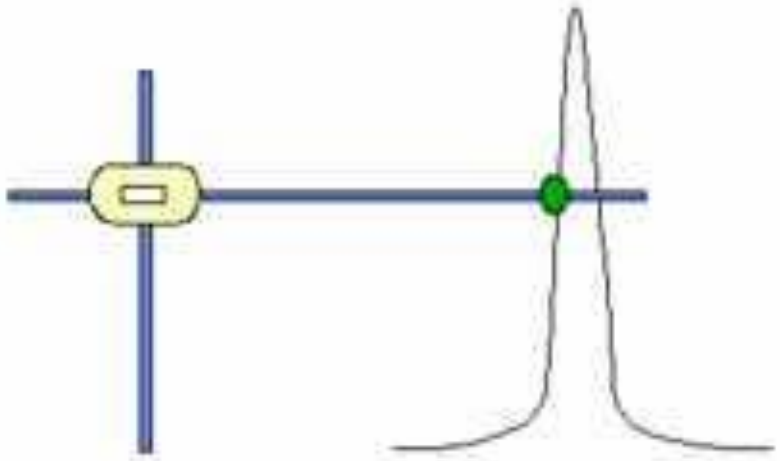
Cut and weigh

With this approach, each peak is cut from the recording paper and weighted. Weight is then considered proportional to area.



Planimeter

A device used to trace the peak. It produces a number that is proportional to peak area.



Triangulation

Main manual method.

Assumes that each peak approximates a triangle.
Area can be determined by

$$\text{area} = \text{peak height} \times W_{1/2}$$

Integrating recorders

The larger the peak response gets, the more rapidly the second pen sweeps back and forth.

The total number of zigs and zags can then be related to the peak area.

If the peak gets to large, the second pen stops moving. You must keep the peak with in range.

Computer systems

Include the same methods of peak detection and integration as integrators.

Major advantage is that the entire chromatographic run is stored prior to analysis.

This allows you to test out various methods of integration on a single run and to reanalyze data if a peak is missed.

Peak height

In some cases, you can assume that peak height is proportional to concentration.

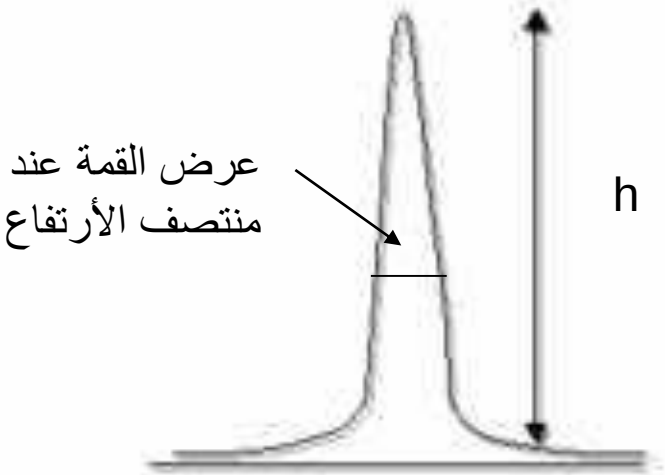
Advantages

- Simplicity
- Rapid calculations

Disadvantages

- Height is more variable than area

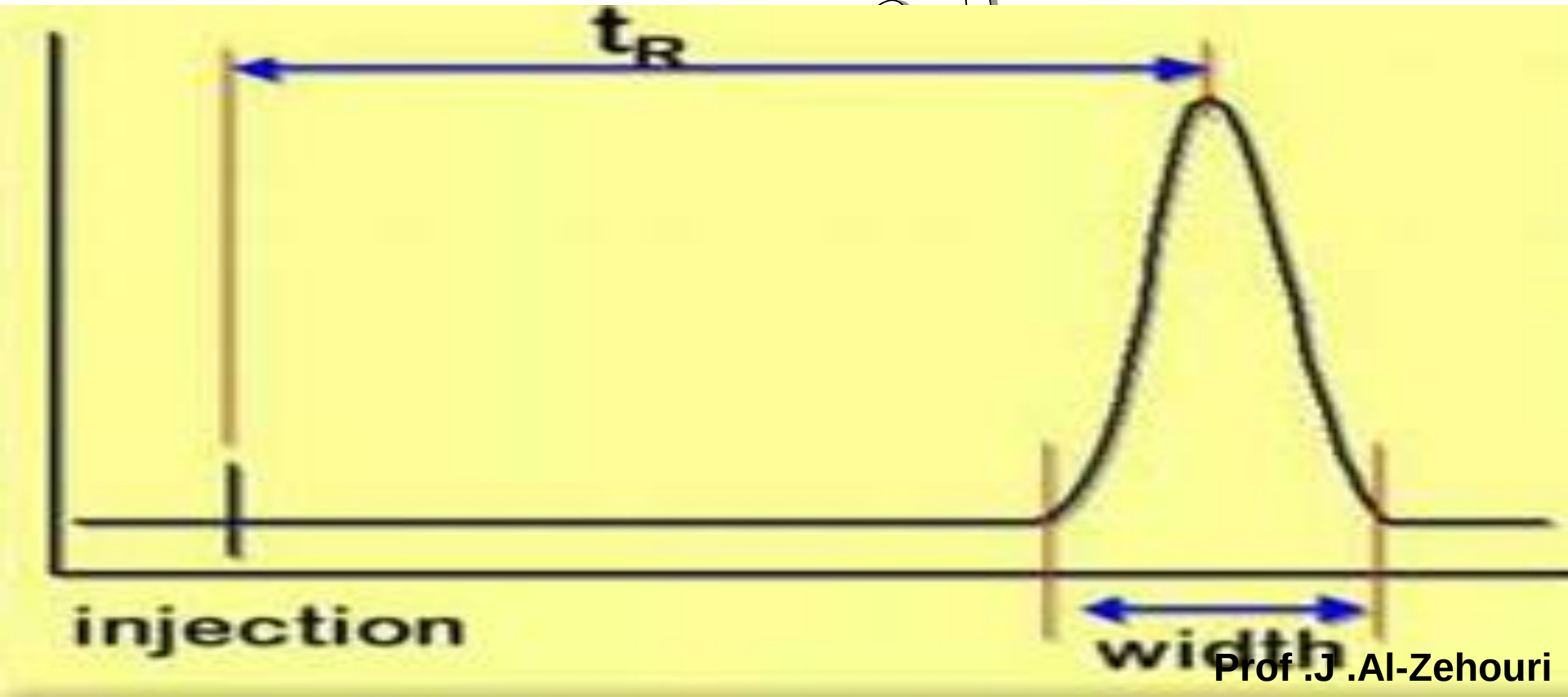
Typically used only with capillary columns



Parameters used in Chromatography (GC & HPLC)

Retention Time t_R

The time between injection and appearance of the peak maximum



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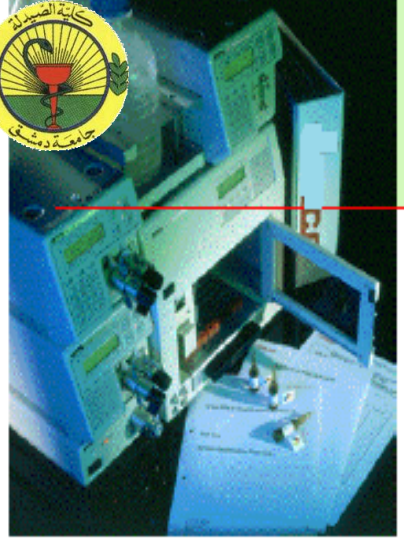
A symmetry Factor (Tailing Factor) :

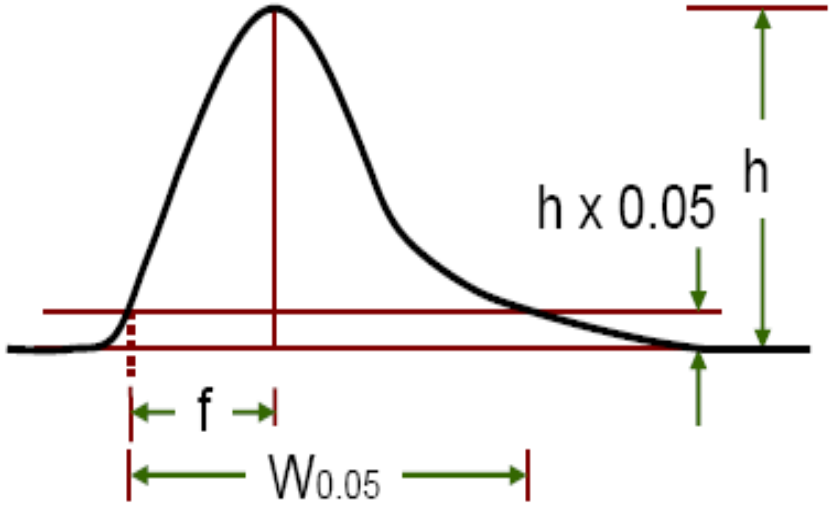
describing the shape of a chromatographic peak. Theory assumes a Gaussian shape and that peaks are symmetrical.

The peak asymmetry factor is the ratio (at 5% of the peak height) of the distance between the peak apex and the back side of the chromatographic curve to the distance between the peak apex and the front side of the chromatographic curve. A value > 1 is a tailing peak, while a value <1 is a fronting peak .(Leading)



Peak symmetry

S : Symmetry factor (T : Tailing factor)



$$S = \frac{W_{0.05}}{2f}$$

S = 1 : The peak is completely symmetric.
S > 1 : Tailing
S < 1 : Leading

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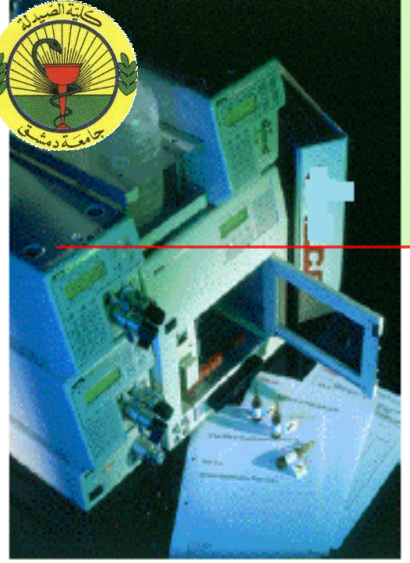
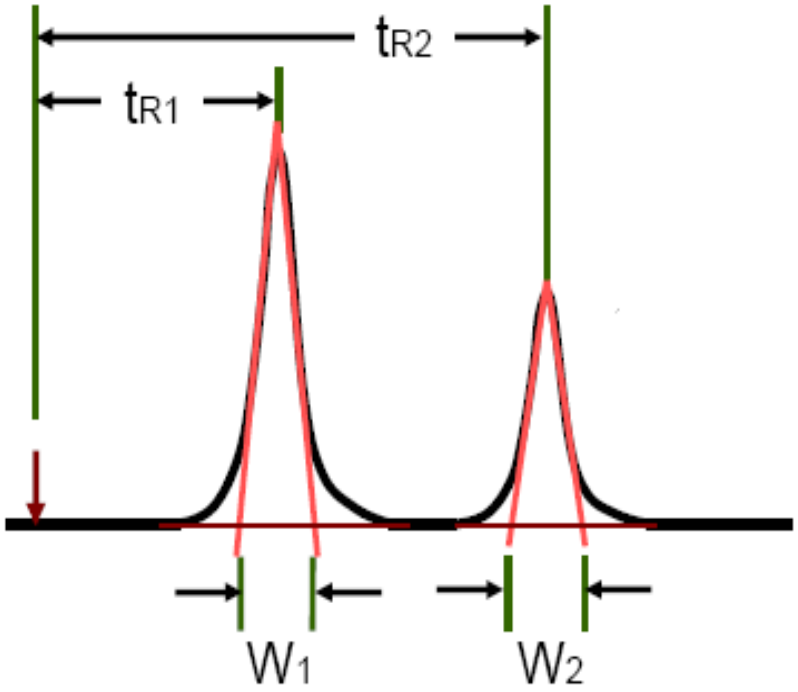
Resolution Rs

A measure of how completely two neighboring peaks are separated from each other. Also it is show the ability of a column to separate chromatographic peaks



Degree of separation



Resolution : $R_s = 2 \times \frac{t_{R2} - t_{R1}}{W_1 + W_2}$

How to calculate Relative Retention Time (RRT)

Divide the retention time of the peak of interest by the retention time of the main peak .

RRT < 1 the peak elute before the main peak.

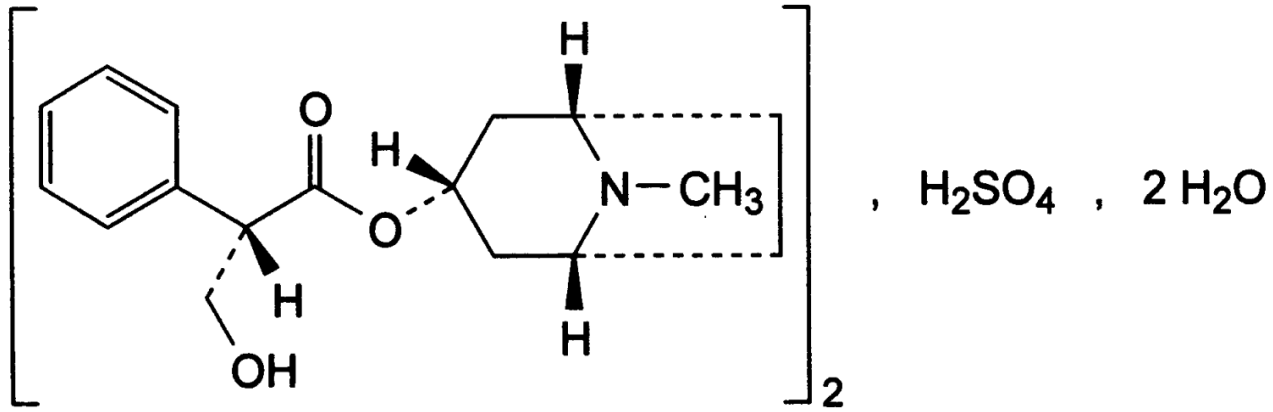
RRt >1 the peak elute after the main peak

Potassium clavulanate (USP)

Chromatographic system (see Chromatography ‹ 621 ›)— The liquid chromatograph is equipped with a 220-nm detector and a 4-mm × 30-cm column that contains 3- to 10-µm packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the column efficiency determined from the analyte peak is not less than 550 theoretical plates; the tailing factor for the analyte peak is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%. Chromatograph the *Resolution solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.5 for clavulanic acid and 1.0 for amoxicillin; and the resolution, *R,* between the amoxicillin and clavulanic acid peaks is not less than 3.5.

Pharmaceutical example

Hyoscyamine Sulphate



(C17H23NO3)2,H2SO4,2H2O 713

6835-16-1

Action and use

Anticholinergic.

Pharmaceutical example

Hyosyamine sulfate tablet 125 mg

According to USP 25 the assay depended on GC. If the average weight 200mg, the sample weight 100 mg ,the standard concentration 0.05% and A_s=0.622 ,

A_{st}=0.501.

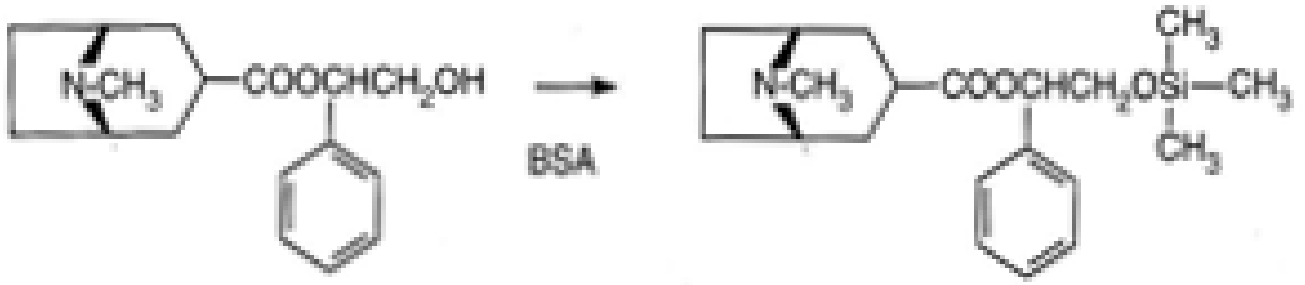
1- What is the practical tablet contain?

2- What is the % content?

II-Analysis of methyl testosterone in Tablets.

III-Analysis of Atropine in eye drops using BSA to mask polar groups (BP1993)

Trimethylsilylation of atropine.



Atropine

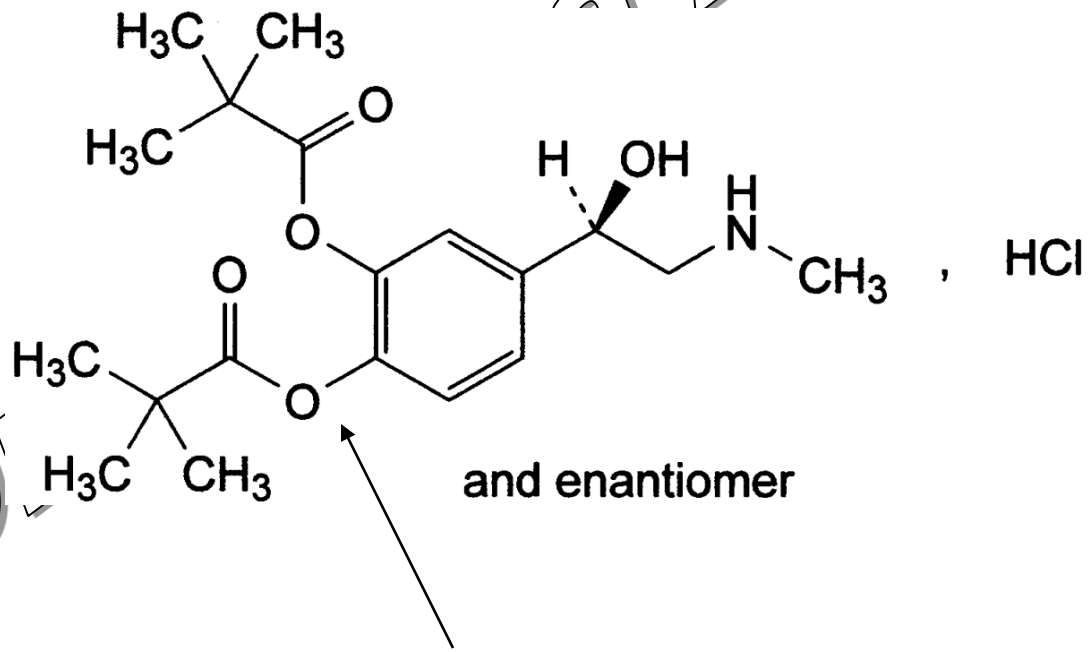
IV-Quantification of ethanol in a formulation

GC provides a useful method for quantifying very voltile materials.

Ethanol is used in the preparation of tinctures and in disinfectant solutions.

V- Determination of degradation (GC provides a useful technique for estimating voltile degradation product)

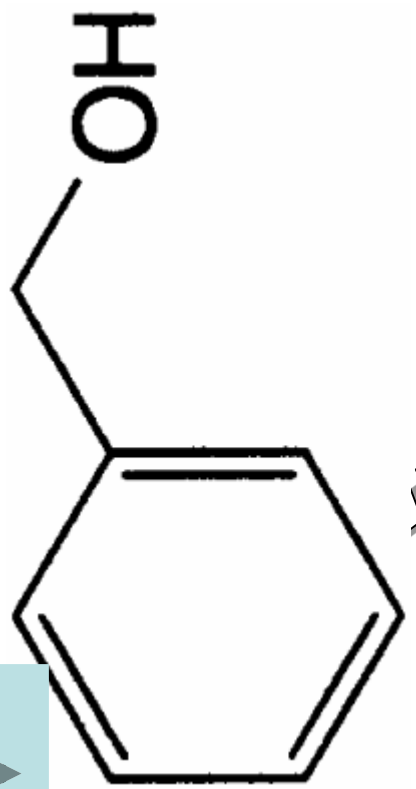
ex. 1. ***Pivalic acid in dipivefrine*** eye drops which release from the hydrolysis of dipivefrine).

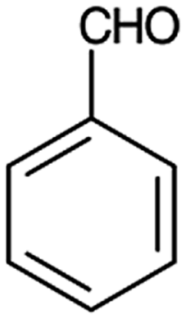


Action and use and enantiomer

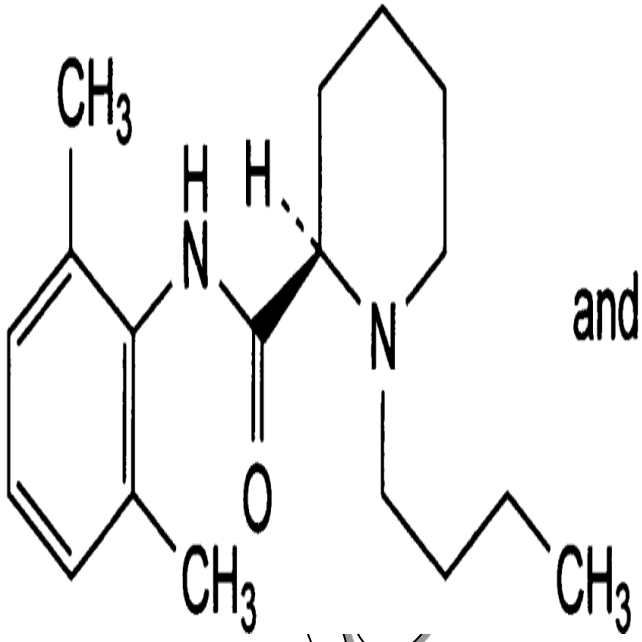
Sympathomimetic

2- Determination of ***Benzyl alcohol*** and his degradation **benzaldehyde**





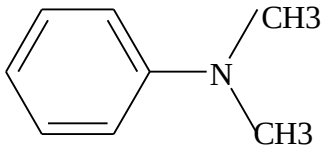
VI-Determination of dimethylaniline in bupivacaine injection

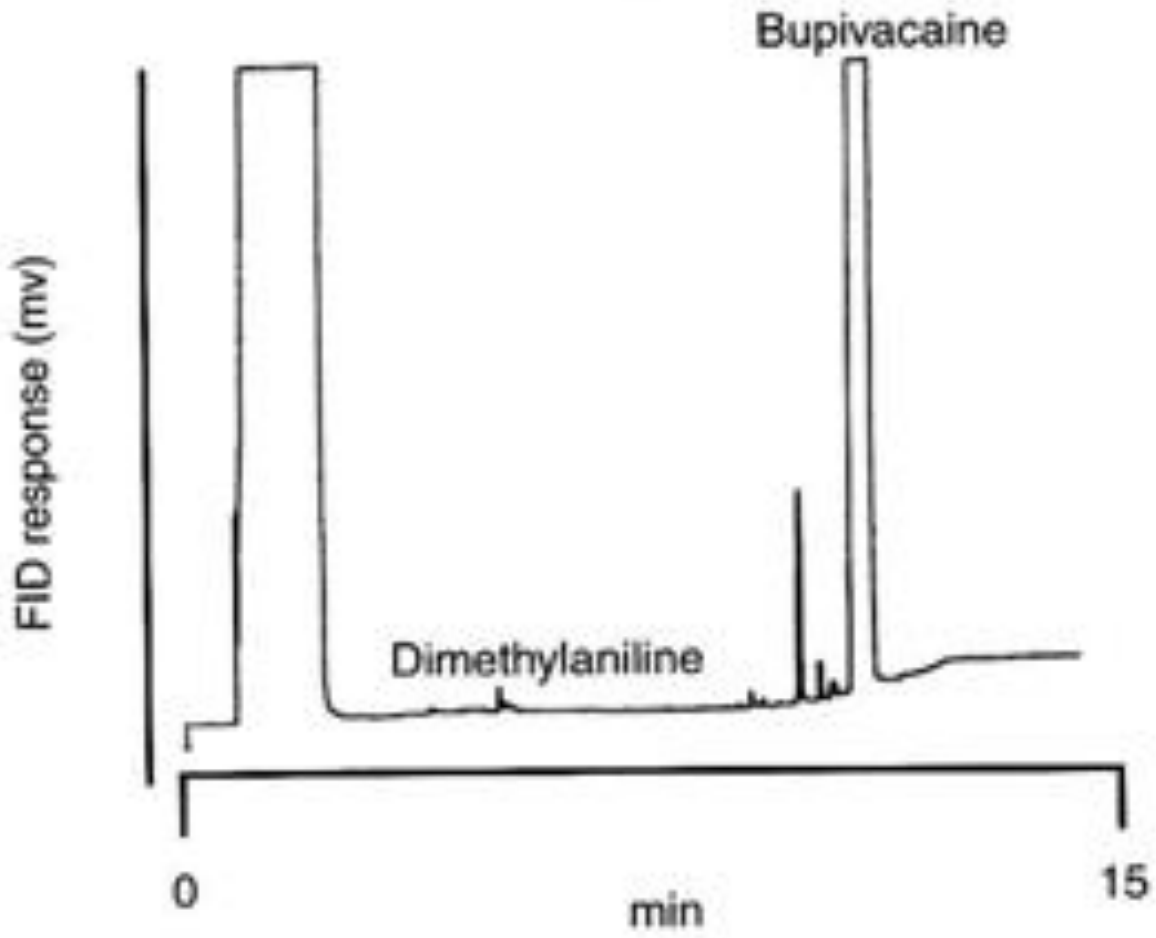


and enantiomer , HCl , H_2O

Action and use

Local anaesthetic.





GC analysis of impurity residues in bupivacaine extracted from an injection. RTX-1 column 15 m × 0.25 mm i.d. × 0.25 μm film. Programmed 70°C (3 min) then 20°C/min to 320°C (5 min).

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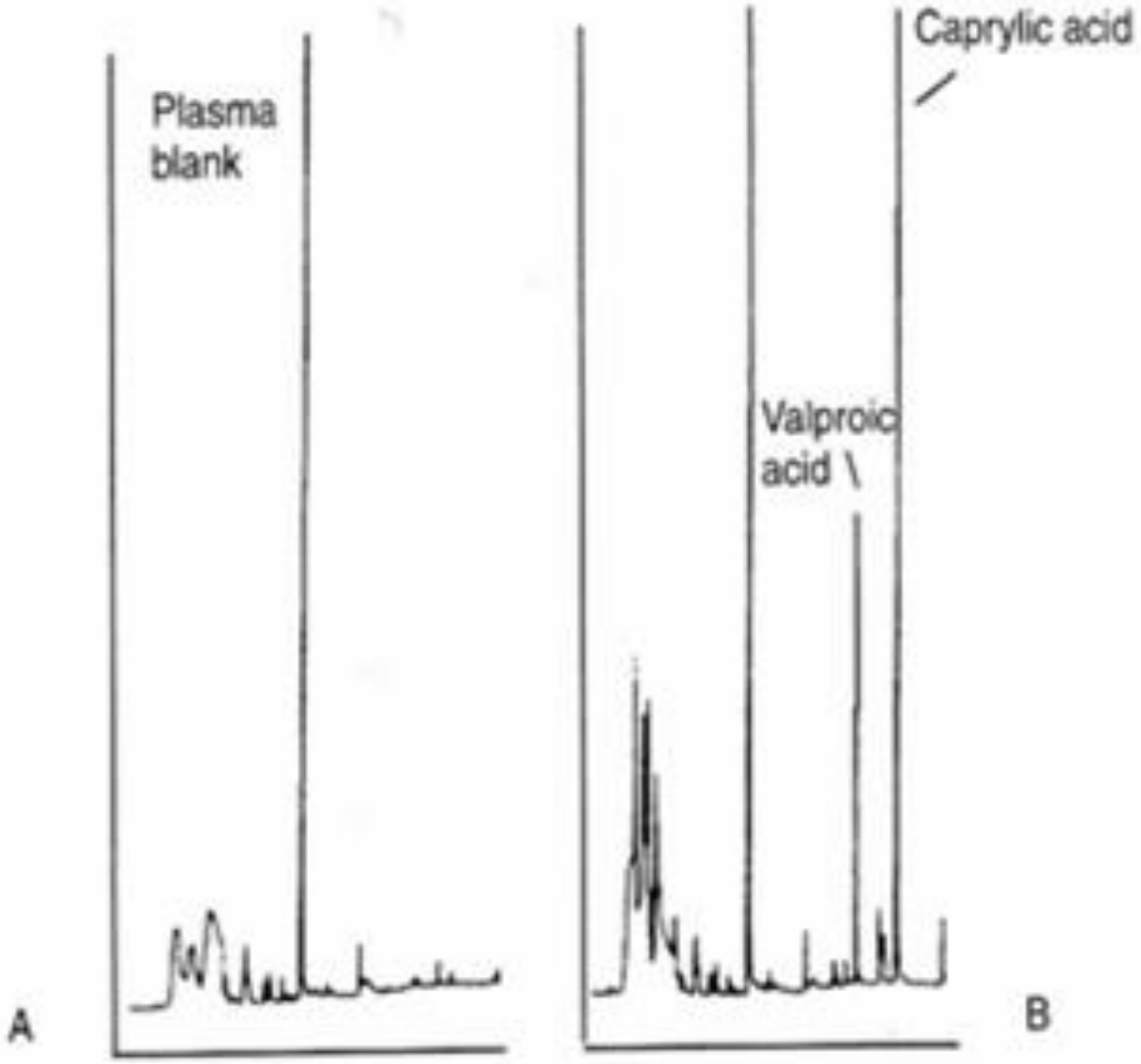
Application of GC in bioanalysis

Analysis of drug inbiological fluids and tissues by GC is quite common, although GC-MS.

Example : determination of anti-epileptic drug valproic acid in plasma by GC using FID .

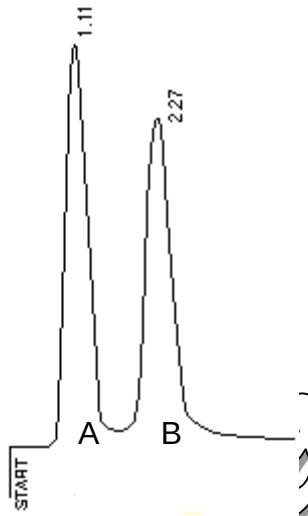


(A) The GC trace of an extract of blank plasma obtained from a patient. (B) The GC trace of an extract of plasma obtained from the same patient after treatment with valproic acid (peak 1) to which caprylic acid (peak 2) has been added as an internal standard.

Al-Zehouri,j
Martine Luther University ,Germany

1988

1) Study the chromatograph (below) of a mixture of Compounds A and B, run on the GC



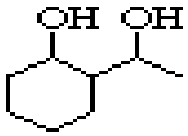
RT	AREA	TYPE	AR/HT	AREA%
1.11	XXXX	XX	XX	55.874
2.27	XXXX	XX	XX	44.117

a. What is the retention time of compound A? Compound B?
b. Which compound is present in a larger amount?
c. Which compound has the lower boiling point?
d. What would happen to the retention times of compounds A and B if the column temperature were raised?

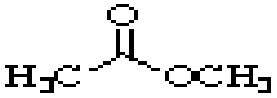
following compounds in terms of their expected retention times on GC (OV-101 column) and their R_f values

hydroxyme thyl-
cyc lohexanol
MW 114
bp 183
mp -43
d 0.93

me thylc yclohe xane
MW 98
bp 101
mp -127
d 0.77



2-hydroxyme thyl-
cyc lohexanol
MW 144
bp 270
mp (not given)
d 0.98



me thyl acetate
MW 74
bp 57
mp -98
d 0.93

In GC, on a non-polar column, they are likely to separate by boiling point. Thus the first off (and lowest RT) would be methyl acetate, then methylcyclohexane, then 2-hydroxymethylcyclohexanol and finally hydroxymethyl cyclohexanol. The strong polarity of methyl acetate might cause it to move slower than the methyl cyclohexane, so standards would need to be run.

On TLC, the most polar compound would move the slowest (and have the lowest R_f), and the least polar the fastest. The most polar is methyl acetate, then 2-hydroxymethyl cyclohexanol, then hydroxymethylcyclohexanol, and finally methylcyclohexane.

(On a very polar GC column, the 4 components would probably be in the same order as given for the TLC.)

Hyphenated Methods

The combination of a chromatographic and spectral method.

Exploits advantages of each

Chromatograph - produces 'pure' fractions from your sample.

Spectral method - yields qualitative information about a 'pure' component.

Methods we'll look at.

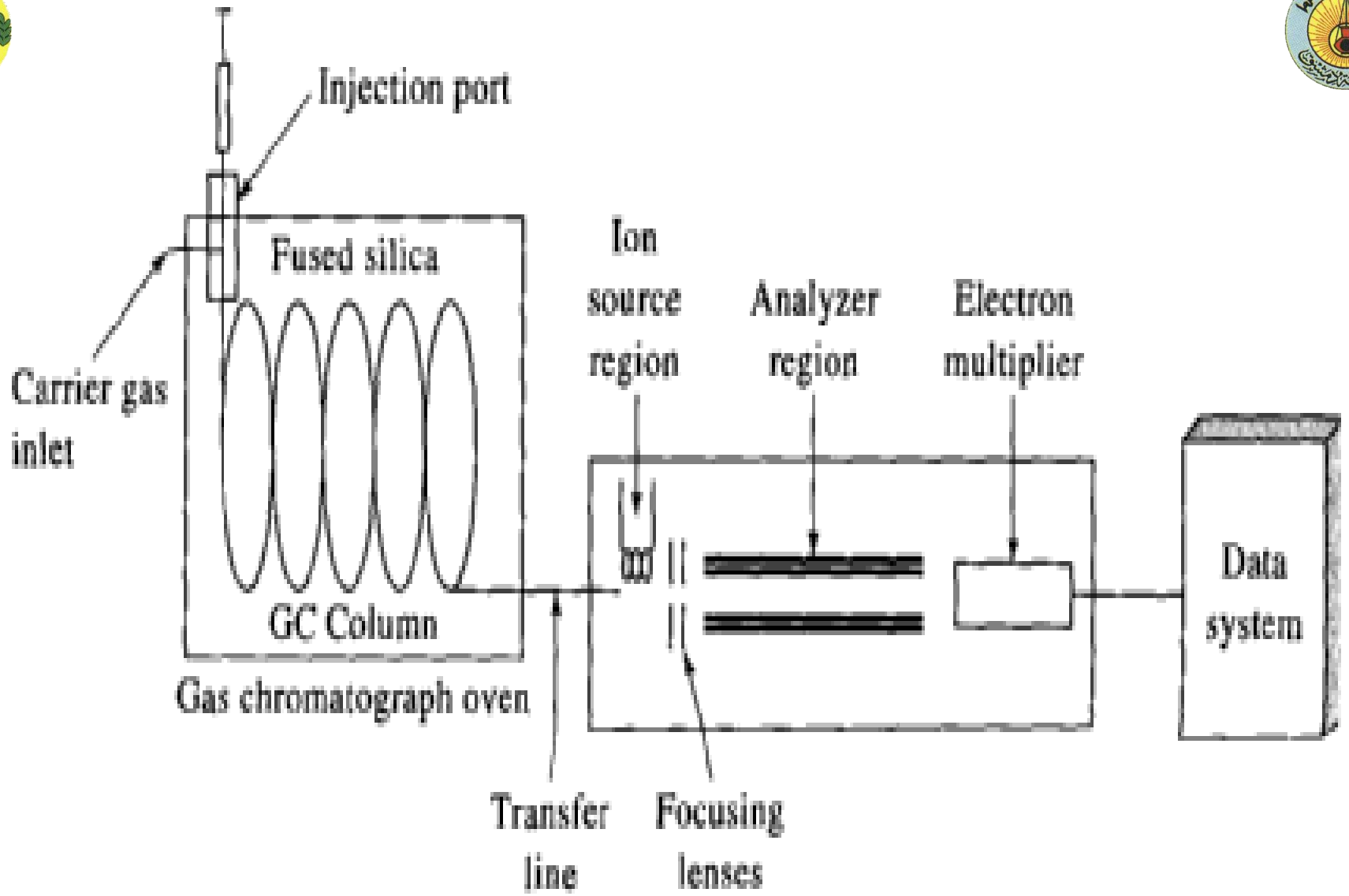
Gas Chromatography

- Mass Spectrometry - GC-MS
- Infrared Spectrometry - GC-FTIR
- Atomic Emission - GC-AES

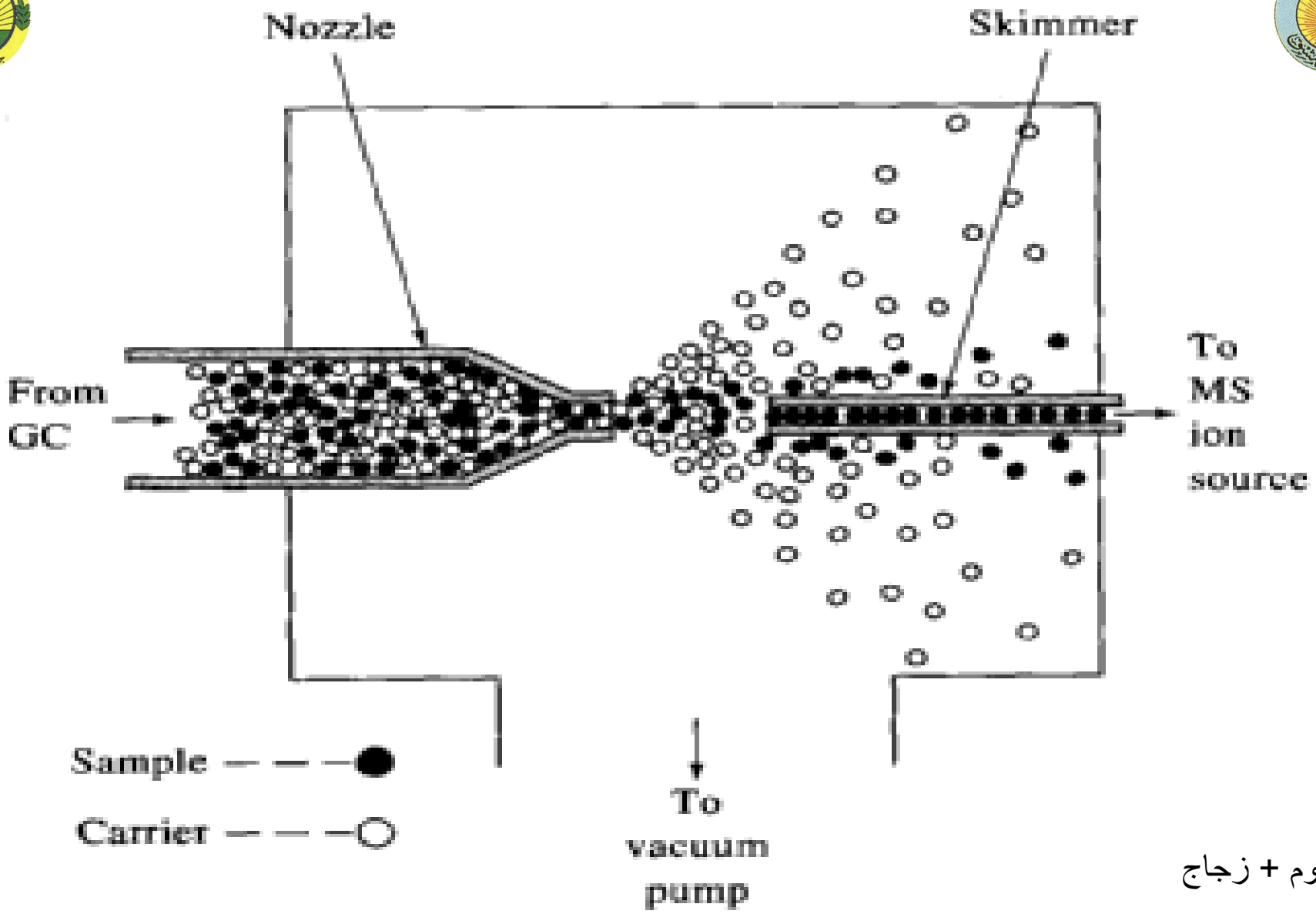
Liquid Chromatography

- Mass Spectrometry

For LC, the other two methods are not practical due to the solvent.



Schematic of a typical capillary gas chromatography/mass spectrometer.



Schematic of a jet separator.

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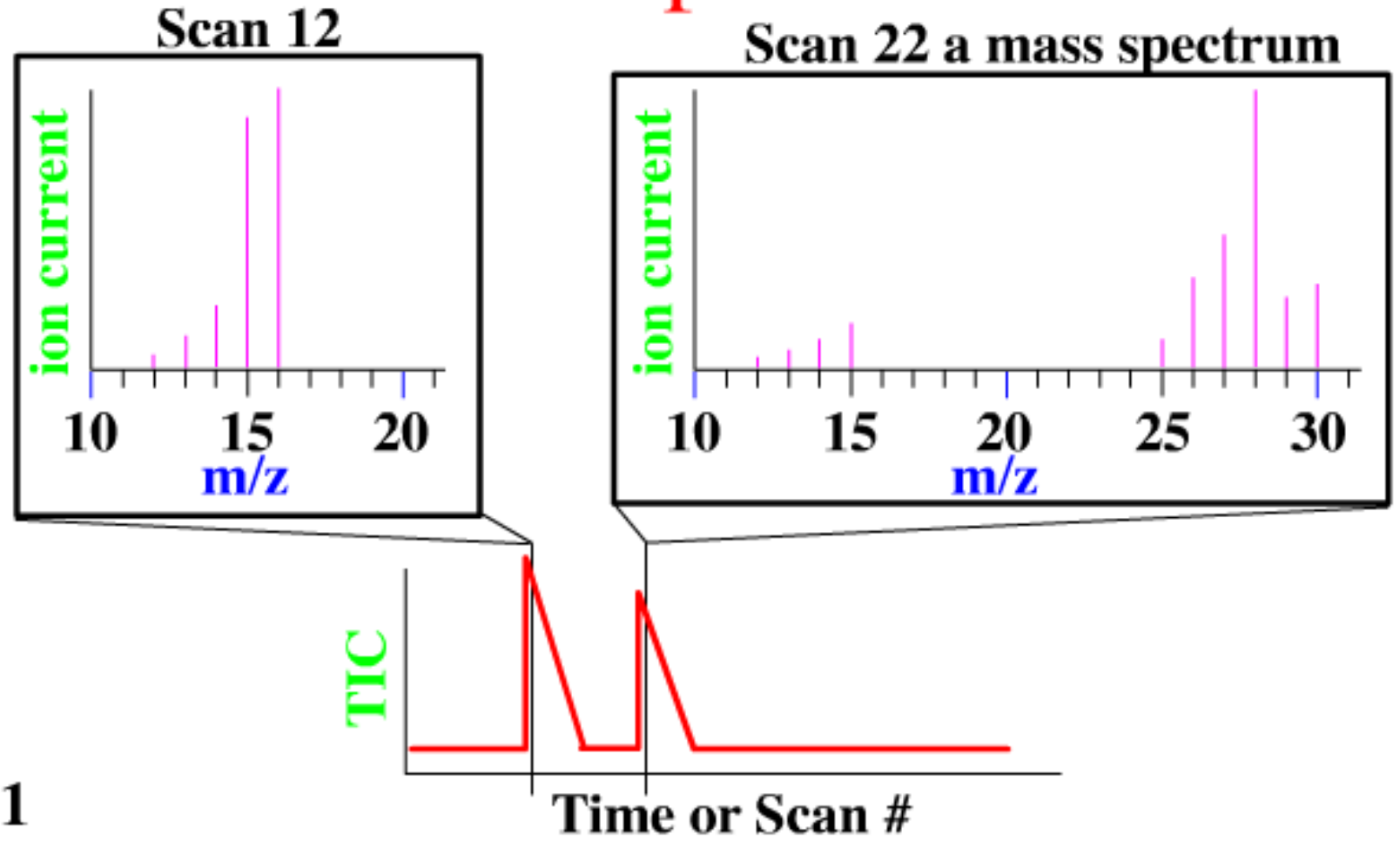
Total Ion Chromatograms and Mass Spectra

Each GC/MS run generates a chromatogram (TIC versus time or scan number) like other GC chromatography but also a mass spectrum for *each* scan--often literally hundreds of mass spectra in a normal run. The chromatographic signal via the MS scanning start when the ionization source is turned on.

Total Ion Chromatograms and Mass Spectra



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Total Ion Chromatograms and Mass Spectra

To reiterate: GC/MS chromatography yields a familar chromatogram and the success of separation—the first job of a chromatographer—can be confirmed. But there is another dimension of data: each MS scan can be examined individually using the MS software.

Total Ion Chromatogram

So exactly how *is* the chromatogram generated in GC/MS?

Total Ion Chromatogram

The answer involves a method of generating a signal that varies, like an FID or ECD detector, with the amount of analyte eluting from the GC column.

Total Ion Chromatogram

Each MS scan involves a tabulated list of the mass detector's signal for each m/z fragment. All of these intensities of each scan are added together and they yield...

Total Ion Chromatogram

the total ion current (TIC), a detector signal that varies over time, the typical components of a chromatographic plot: signal versus time. (Note that the total ion chromatogram is also often abbreviates as TIC.)

Total Ion Chromatogram

Resume

m/z	Intensity
30	3005
29	2385
28	10050
27	4856
26	2993
25	1534
15	1723
14	1543
13	1195
12	0805
	30089

Data from one MS scan

The ratios of these are constant for a given ionization process (EI, CI, etc.) and, most important, for a given compound.

Total ion current (TIC) for a particular scan (a single datum)

tal Ion Chromatogram

Data from one MS scan

m/z	Intensity	Normalized Abundance
30	3005	29.9%
29	2385	23.7%
28	10050	100.0%
27	4856	48.3%
26	2993	29.8%
25	1534	15.3%
15	1723	17.1%
14	1543	15.4%
13	1195	11.9%
12	0805	8.0%
	30089	

Most mass spectra are normalized. All peaks are ratioed to the base peak, the most intense peak, and expressed as a % of the base peak.

Total ion current (TIC) for a particular scan (a single datum)

Resume

Pl

Int

Ion

Mass A

Mass D

Chromato

Mass S

Prof .J .Al-Zehouri

Q&A