

## Main Components of Chromatograph

- Carrier gas (mobile phase)

– N<sub>2</sub>, He, H<sub>2</sub>

- Injector
- Column
- Detector

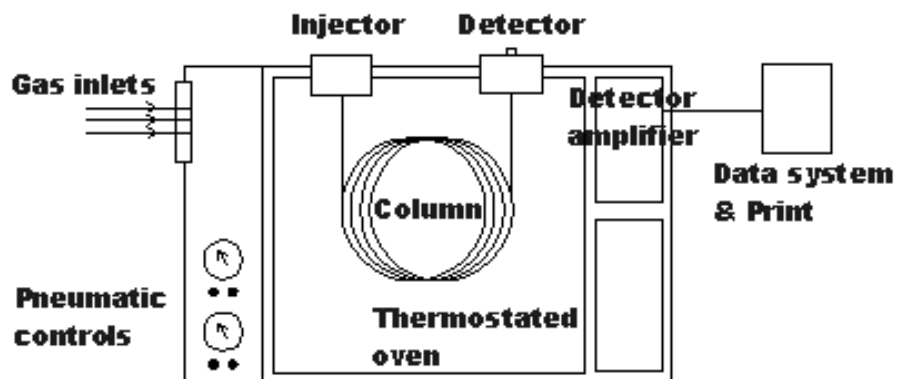
oven

- Integrator or Computer

Materials based on the

D.C.Harris, Exploring Chemical Analysis, 3<sup>rd</sup> ed, W.H. Freeman & Co. 2004

## Gas Chromatograph

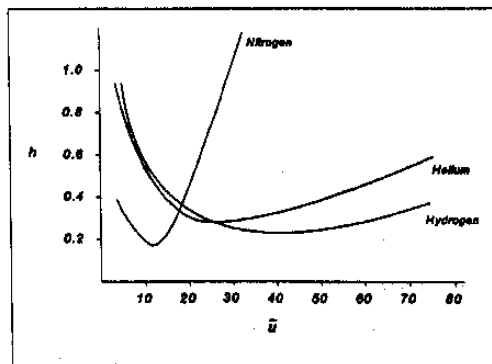


## Gas Chromatography

employs gas as a mobile phase (carrier) – He, H<sub>2</sub>, N<sub>2</sub>

Which gas is the best ?

Based on the van Deemter curve



The best for most operation conditions is hydrogen, but it is explosive.

Helium is employed especially with MS.

The conditions for the use of nitrogen has to be carefully selected (e.g. flow rate)

## GC Components

- Injector – transfers the analyte onto the column
- Column – separates the analytes
- Detector – recognizes separated analytes and translates them into graphical form (chromatogram)

# Injectors

Injectors are usually heated to **ensure analyte's transfer to a gas phase**. Volatile liquid or gaseous sample is injected through a septum. Vapor is swept through column

- **Split/splitless injector** usually consists of heated liner (a glass sleeve, prior to the column. (200 - 300 °C)
  - Split (dilution) only part of sample is introduced on the column  
1:25 - 1:600
  - Splitless - all the sample is introduced (but only for limited time period)
- **On-column injector** for the analytes which are thermally unstable
  - Analytes are injected directly on the column,  
This techniques is suitable for thermally unstable compounds

# Injection

- **Volume** Injected is Typically 0.1-3 $\mu$ L (liquid)
- **The injected volume is limited by the volume of solvent as a vapor phase.**

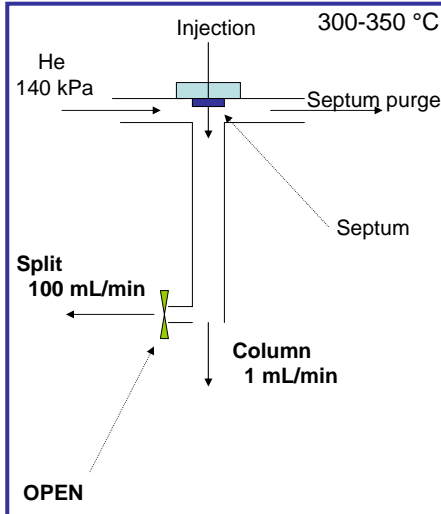
At 200°C and pressure on column 100 kPa

- 1  $\mu$ L of hexane (l) forms 222  $\mu$ L (g)
- 1  $\mu$ L of methylene chloride (l) forms 310  $\mu$ L
- 1  $\mu$ L of water (l) form 1111  $\mu$ L

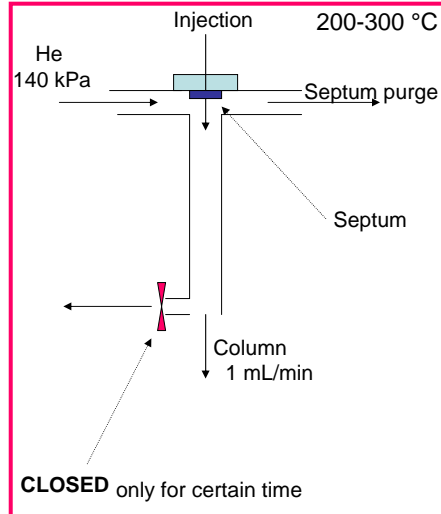
**volume of vapor > then volume of injector = backflash (system contamination)**

- **Concentration**
  - **Is defined by column retaining capacity**
    - Columns with a thicker film thickness (a stationary phase) retain more of the analyte

# Split versus Splitless



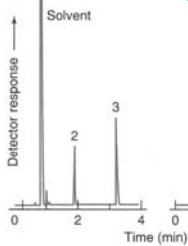
1% of sample introduced on the column.  
Higher precision.  
Eliminates interferences from matrix.



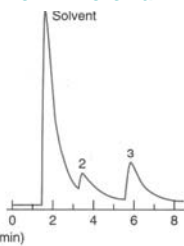
Most of the sample introduced on column.  
To obtain narrow peaks a cold trapping is used.

## Injection and Temperature program

### SPLIT



### SPLITLESS for whole run

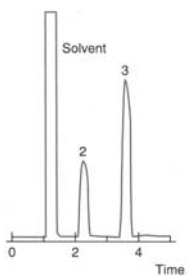


**Split injection** - a fraction of a solute (solvent) is injected, therefore peaks are sharp.

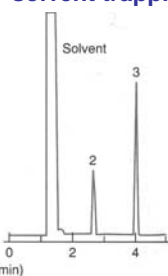
### Splitless injection – for trace analysis

The split valve is closed and most of the sample is introduced on the column.  
The volume of the gas going through the injector is only ca. 1 ml/min. Thus, sample components are transferred to the column for long time. Thus peak tailing.

### SPLITLESS 30 s



### SPLITLESS 30 s solvent trapping



**Splitless time** - If the split valve is opened after certain time 20 - 120 s, the transfer of sample is stopped. Still the transfer can be long, causing an increased peak width.

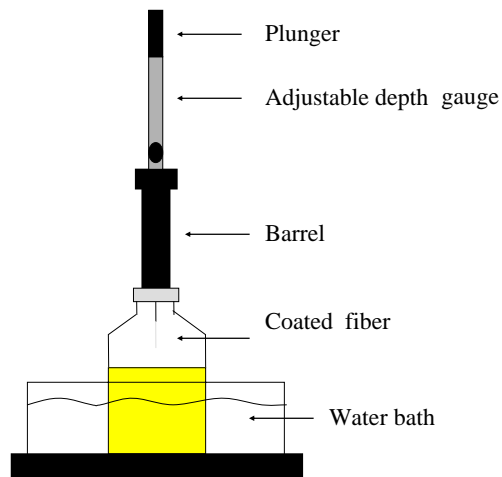
**Solvent trapping** - Injecting the sample to the column at temperature below boiling point of a solvent <20°C, after 30s (splitless time) a fast increase in the temperature to 20°C above solvent's boiling point.

Fast transfer from gas to liquid and again to the gas phase sharpens the elution band.

## On column injection

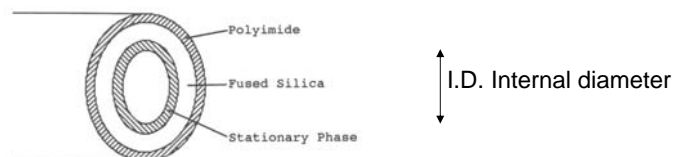
- On column injection for samples which would decompose at higher temperatures
  - Injects the sample **directly on the column** or the guard column.
  - All the sample is introduced on the column.
  - Also all interfering components are injected.
  - In past, the column has to be ca. 0.53 mm I.D. so the syringe needle can fit in.

## Volatile Compounds Isolation by SPME



# Columns

- **Packed**
  - solid particles either porous or non-porous coated with thin (1 μm) film of liquid
  - 3 - 6 mm ID; 1 - 5 m length
- **Capillary** (open tubular) silica columns
  - 0.1 - 0.5 mm I.D. (internal diameter); 15 - 100 m length
  - Inner wall modified with thin (0.1-5 μm) film of liquid (stationary phase)
  - easy to install
  - well defined stationary phase

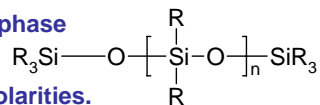


Optimal flow rate depends on carrier gas, I.D., film thickness  
As the linear velocity, I.D. and increases also van Deemter curve is steeper.

## Stationary Phases

- Must have:
- (1) low volatility
  - (2) thermal stability
  - (3) chemical inertness
  - (4) solvation properties giving suitable values for  $k'$ ,  $\alpha$ .

Most common are polysiloxanes stationary phase

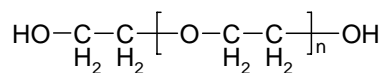


Nature of R varied to give different polarities.

For non-polar columns R = CH<sub>3</sub> or phenyl  
Best for non-polar analytes (hydrocarbons, PAHs etc.)

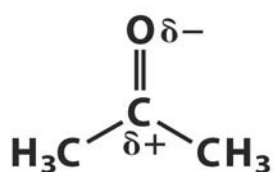
R = 50% CH<sub>3</sub> and 50% cyanopropyl have increased polarity.  
Best for alcohols, acids etc.

Greater polarity using polyethylene glycols:

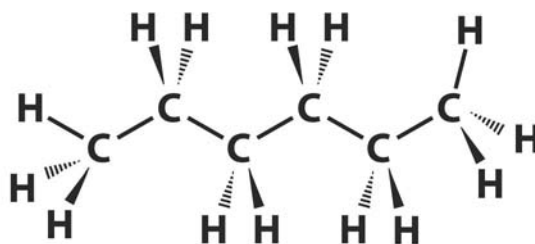




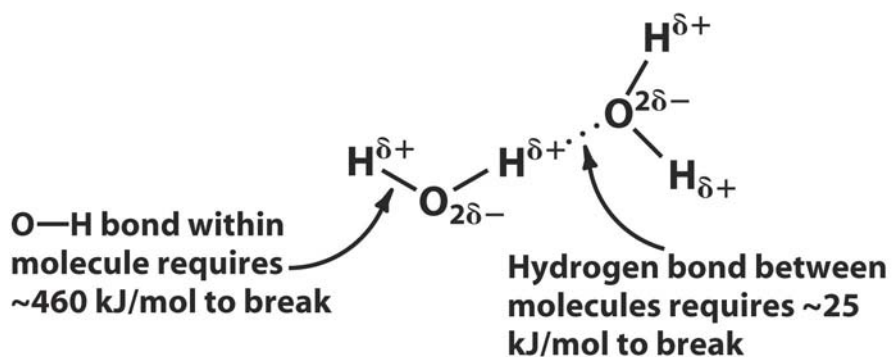
**Polar molecules attract each other by electrostatic forces. Positive attracts negative.**



**Acetone**



**Hexane**




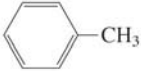

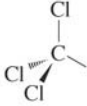
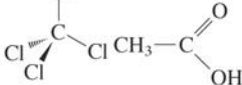
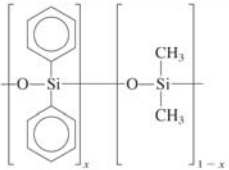
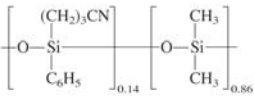
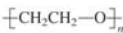
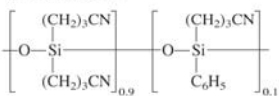
Typical nonpolar and weakly polar compounds		Typical polar compounds	
	octane (C <sub>8</sub> H <sub>18</sub> )	CH <sub>3</sub> OH	methanol
	toluene	CH <sub>3</sub> CH <sub>2</sub> OH	ethanol
	diethyl ether (C <sub>4</sub> H <sub>10</sub> O)	CHCl <sub>3</sub>	chloroform
			carbon tetrachloride
			acetic acid
		CH <sub>3</sub> C≡N	acetonitrile

TABLE 22-1 Common stationary phases in capillary gas chromatography

Structure	Polarity	Temperature range
 (Diphenyl) <sub>1</sub> (dimethyl) <sub>1-x</sub> polysiloxane	$x = 0$ Nonpolar $x = 0.05$ Nonpolar $x = 0.35$ Intermediate polarity $x = 0.65$ Intermediate polarity	$-60^{\circ}$ to $360^{\circ}\text{C}$ $-60^{\circ}$ to $360^{\circ}\text{C}$ $0^{\circ}$ to $300^{\circ}\text{C}$ $50^{\circ}$ to $370^{\circ}\text{C}$
 (Cyanopropylphenyl) <sub>0.14</sub> (dimethyl) <sub>0.86</sub> polysiloxane	Intermediate polarity	$-20^{\circ}$ to $280^{\circ}\text{C}$
 Carbowax (polyethylene glycol)	Strongly polar	$40^{\circ}$ to $250^{\circ}\text{C}$
 (Biscyanopropyl) <sub>0.9</sub> (cyanopropylphenyl) <sub>0.1</sub> polysiloxane	Strongly polar	$0^{\circ}$ to $275^{\circ}\text{C}$

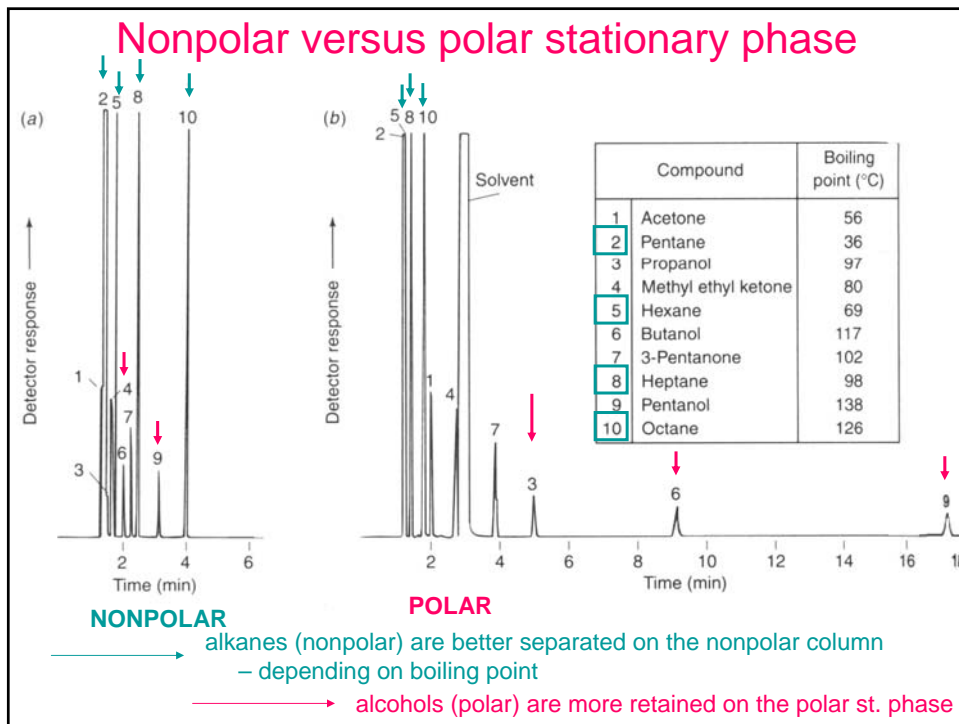
## Polarity of analytes

<b>Nonpolar</b>	<b>Weak and intermediate polarity</b>
Saturated hydrocarbons	Ethers
Olefinic hydrocarbons	Ketones
Aromatic hydrocarbons	Aldehydes
Halocarbons	Esters
Mercaptans	Tertiary amines
Sulfides	Nitro compounds without $\alpha$ -H atoms
CS <sub>2</sub>	Nitriles compounds without $\alpha$ -H atoms
<b>Strong and intermediate polarity</b>	<b>Strong polar</b>
Alcohols	Polyhydroxyalcohols
Carboxylic acids	Amino alcohols
Phenols	Hydroxy acids
Primary and secondary amines	Polyprotic acids
Oximes	Polyphenols
Nitro compounds with $\alpha$ -H atoms	
Nitriles compounds with $\alpha$ -H atoms	

## Derivatization

- Nonpolar stationary phase gives more reproducible data
- To be able to analyze polar analytes on nonpolar columns a derivatization can be used. Derivatization involves a chemical modification of the analytes.
- **Methylating agents:** use of diazomethane Carboxylic acids R-COOH can be analyzed as methyl esters  

$$\text{R-COOCH}_3$$
- **Silylating agents**  $\text{Si}(\text{CH}_3)_3$  can be also used

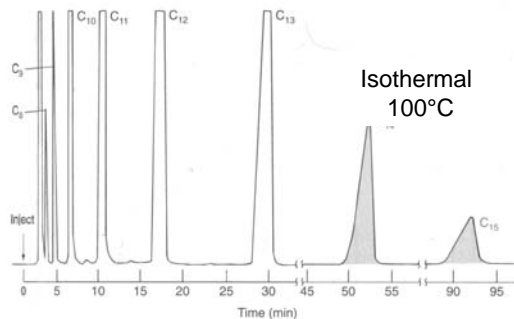


## GC - Modes of Separation

- Isothermal GC
- Programmed temperature GC
  - Raising column temperature (GC oven)
    - Decreases retention time
    - Sharpens peaks
    - only thermally stable compounds
- Programmed pressure GC

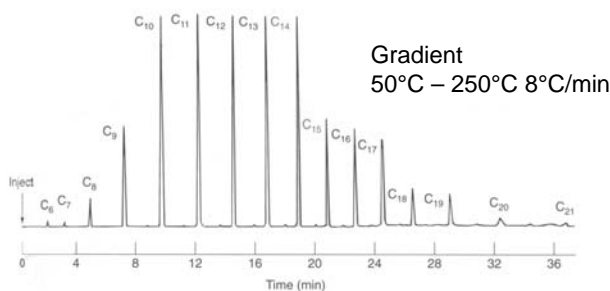
Always check for the **column temperature limit**.  
Nonpolar columns are more heat resistant.

## Temperature Effect



### Increase in temperature

- Decreases retention time
- Sharpens peak



## Selecting temperature conditions

- Temperature of injector  
ensures evaporation of sample,  
but do not decompose it (200 – 300 °C)
- Temperature of the column (GC oven)  
Effect of injection  
For the split injection– no specific requirements  
For the splitless and on column injection – solvent trapping technique  
Oven temperature - optimized to improve the separation
- Temperature of the detector  
has to be high enough to prevent condensation of  
analytes on the detector

## Detectors for GC

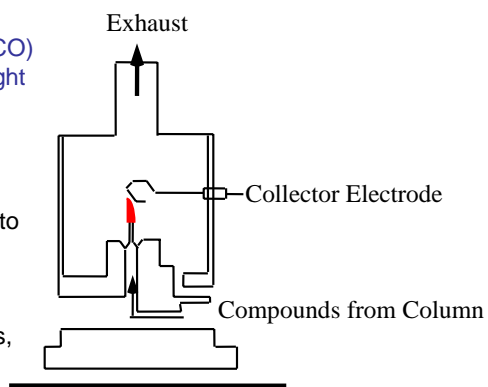
- **Flame ionization detector (FID)**
  - destruction of combustible sample in flame produces measurable current
- **Electron capture detector (ECD)**
  - radioactive
  - good for  $X^-$ ,  $NO_2^-$
- **Thermal conductivity detector (TCD)**
  - change in resistance of heated wire
- **Mass spectrometr (MS)**

## Flame Ionization Detector (FID)

Eluate is burned in the mixture of hydrogen and air  
Carbon atoms (except -COOH and -CO) produce CH radicals, which are thought to produce  $CHO^+$  ions

Cations are collected on the cathode producing current (signal)  
The response is directly proportional to the solute mass over 7 orders of magnitude.

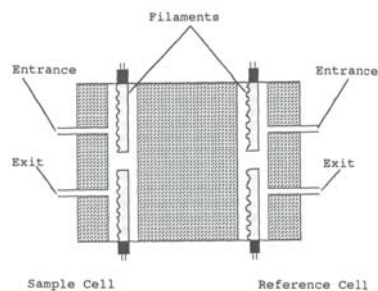
FID is insensitive to nonhydrocarbons,  $CO$ ,  $CO_2$ ,  $NH_3$ ,  $H_2S$  etc.



## Thermal conductivity detector (TCD)

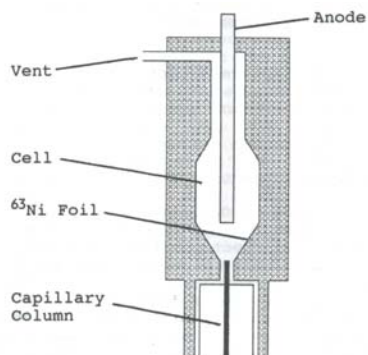
- **Universal detector**
- Measures the ability of substance to **transport heat** from a hot region to a cold region.
- The carrier from column passes through the sample cell, carrier without the sample goes through a reference cell. **Gas flowing over the filament will cool it, absorbing some heat.** The difference between amount of heat loss from carrier alone and from carrier with a sample is the measured signal.
- The carrier gas selection depends on its thermal conductivity
 

H <sub>2</sub>	0.170 J/(Kms)
He	0.141 J/(Kms)
N <sub>2</sub>	28 J/(Kms)
- The sensitivity is inversely proportional to a flow rate, TCD is more sensitive at lower flow rate.



## Electron Capture Detector (ECD)

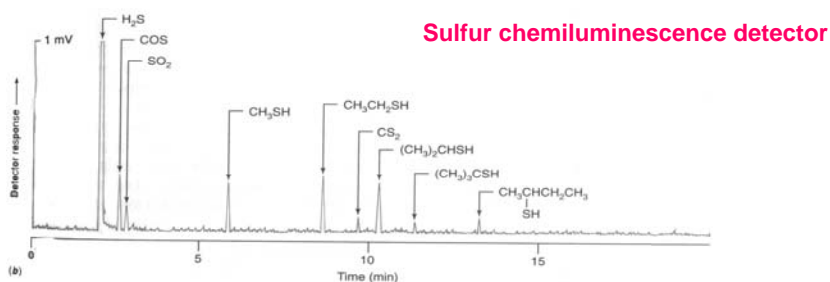
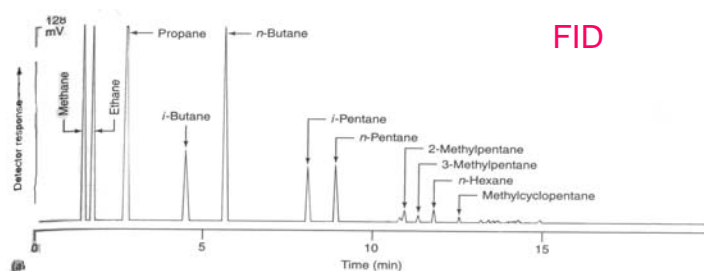
- **Selective detector for electron capturing compounds** – halogens, carbonyls, nitriles, nitro, organometallic compounds
- Radioactive <sup>63</sup>Ni emits high energy electrons β-rays which ionize a “makeup gas” nitrogen or 5% CH<sub>4</sub> in Ar – forming plasma containing the “thermal electrons”. Thermal electrons are captured on the electrode – signal. If electron capturing compound is present the number of thermal electrons on the electrode is decreased.
- The amount of the loss of detector background current is translated into a detector signal



## Other detectors - SELECTIVE

- **Photoionization detector (PID)** – Uses vacuum UV source to ionize aromatic and unsaturated compounds. Electrons produced by ionization are collected and measured.
- **Nitrogen phosphorus detector** (alkali flame) Sensitive to compounds containing N and P. Ions produced by those compounds in contact with  $\text{Rb}_2\text{SO}_4$  (or cesium) glass beads form great current.
- **Sulfur chemiluminescence detector** - Exhaust from FID where S is oxidized to SO which reacts with  $\text{O}_3$ , and forms excited  $\text{SO}_2^*$ , which emits blue light and UV radiation.
- **Atomic emission detector** – eluate goes through a Helium plasma in microwave cavity, the atoms are excited and emission is monitored. This technique can detect most of the elements.

## Detector Selectivity



## Gas chromatography/Mass Spectrometry

- The Mass spectrometer is the only detector which **does not** require higher temperature than is the temperature of GC column.
- The outlet of capillary column of GC is placed directly to ionization source  
The MS employed consists of EI or CI ionization and RF analyzer such as quadrupole or ion trap.

## MS

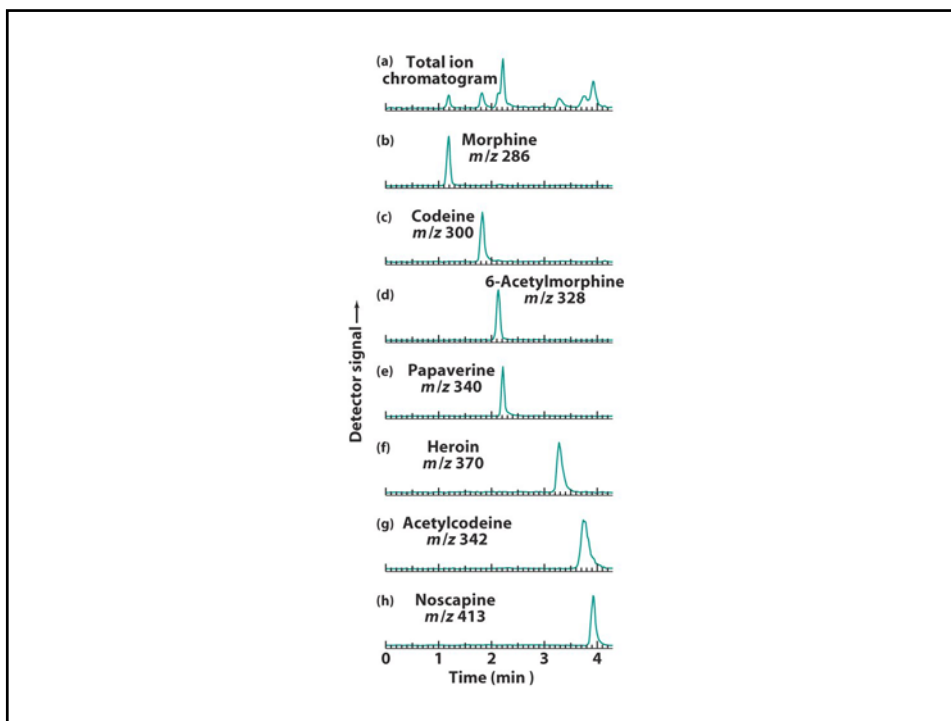
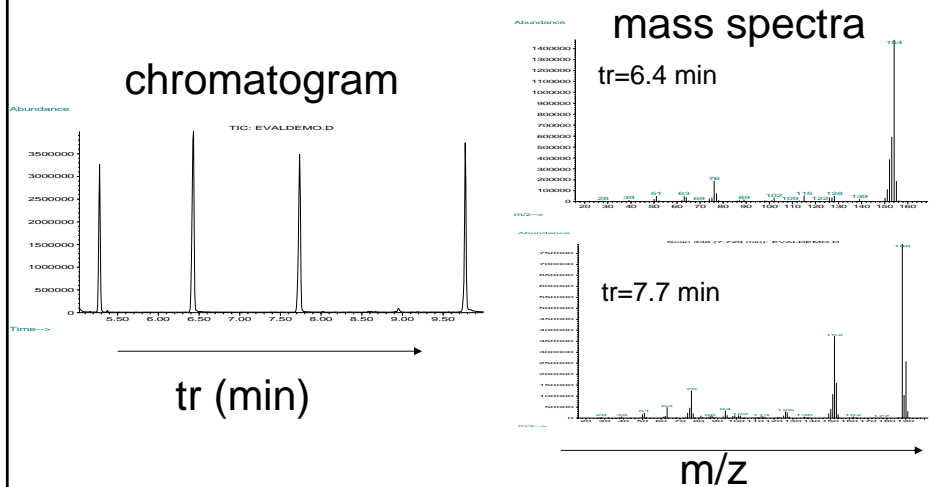
**Mass spectrometer** is the detector which fragments the molecules of eluting compounds. The individual fragments are then detected.

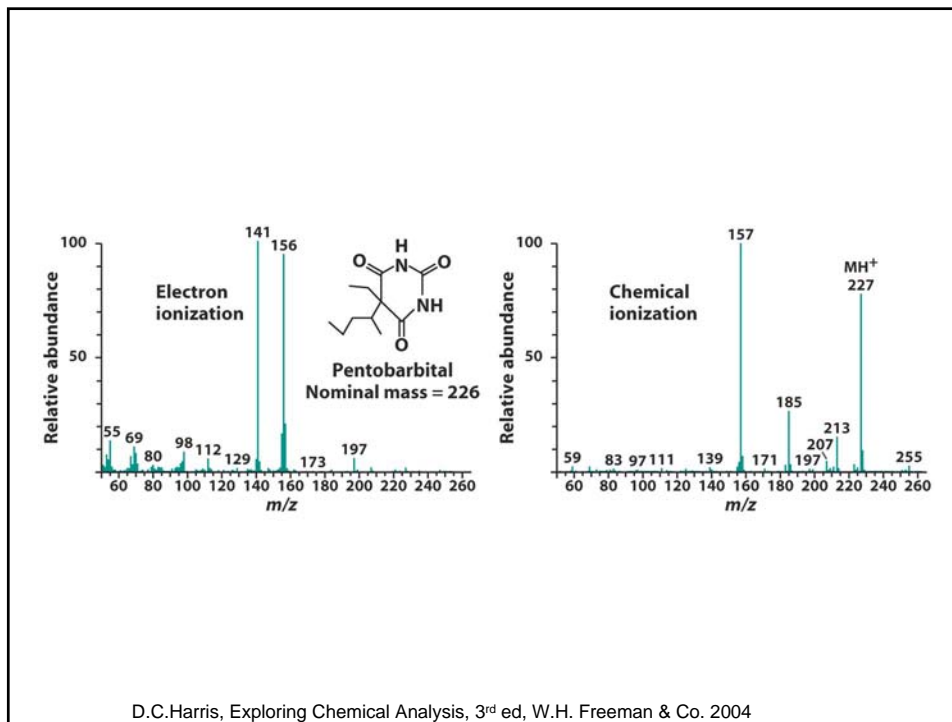
### **using GC/MS**

qualitative information : retention times  
masses of fragments of the compound

## Chromatogram versus Mass spectrum

The analytes can be monitored as **total ion current (TIC)** each data point in the chromatogram is represented by spectra of ions from MS  
Therefore for each peak on the chromatogram we can get the spectrum and attempt to identify the compound.





## Detectors

- Universal (TCD, MS)
- Selective (FID, ECD, PID, N and P detector, Sulfur chemiluminescence)
- Destructive
- Non destructive (TCD)

## Quantification in chromatography

- Area or height of the peak is proportional to the concentration of the analyte.
- The area is a more precise measure. Nevertheless when peaks coelute (are not separated on the baseline), the height may be used.
- Both the external and internal method of calibrations are employed.