An Introduction to Gas Chromatography Mass Spectrometry

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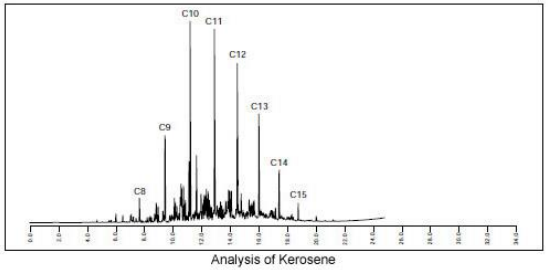
Recommended Textbooks:-

"*Analytical Chemistry*", G. D. Christian, P. K. Dasgupta, K.A. Schug, Wiley, 7th Edition

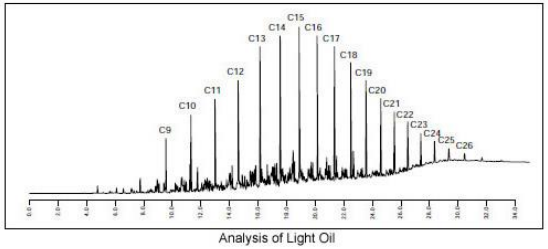
"*Trace Quantitative Analysis by Mass Spectrometry*", R.K. Boyd, C.Basic, R.A. Bethem, Wiley

"*Mass Spectrometry Principles and Applications*", E. de Hoffmann, V. Stroobant, Wiley

Mass Spectrometry is an analytical technique that forms ions from atoms or molecules and measures their *mass-to-range (m/z) ratios in* gas phase.

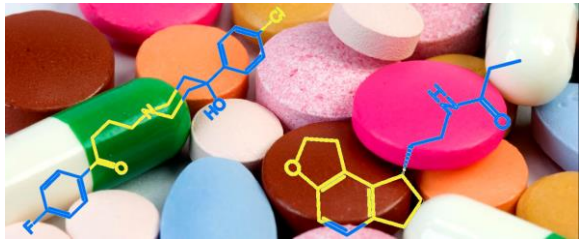


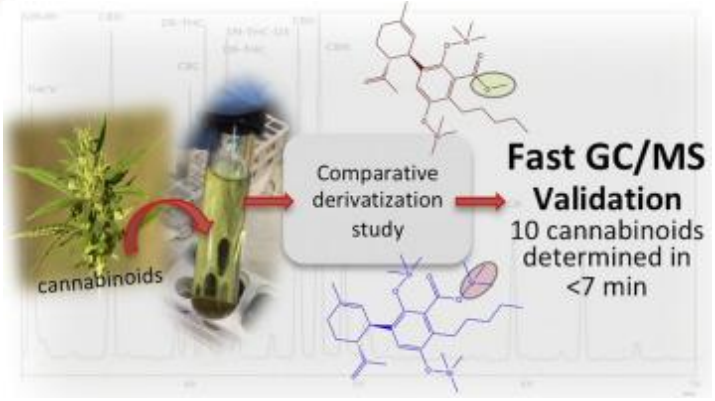
Analysis of Kerosene



Analysis of Light Oil

Mass Spectrometry is a primary tool used for almost every discipline. MS can provide information about molecular and elemental composition and also quantify the abundance of individual chemical components. It is highly selective techniques, meaning that it can differentiate between multiple compounds within a complex chemical or biological sample.



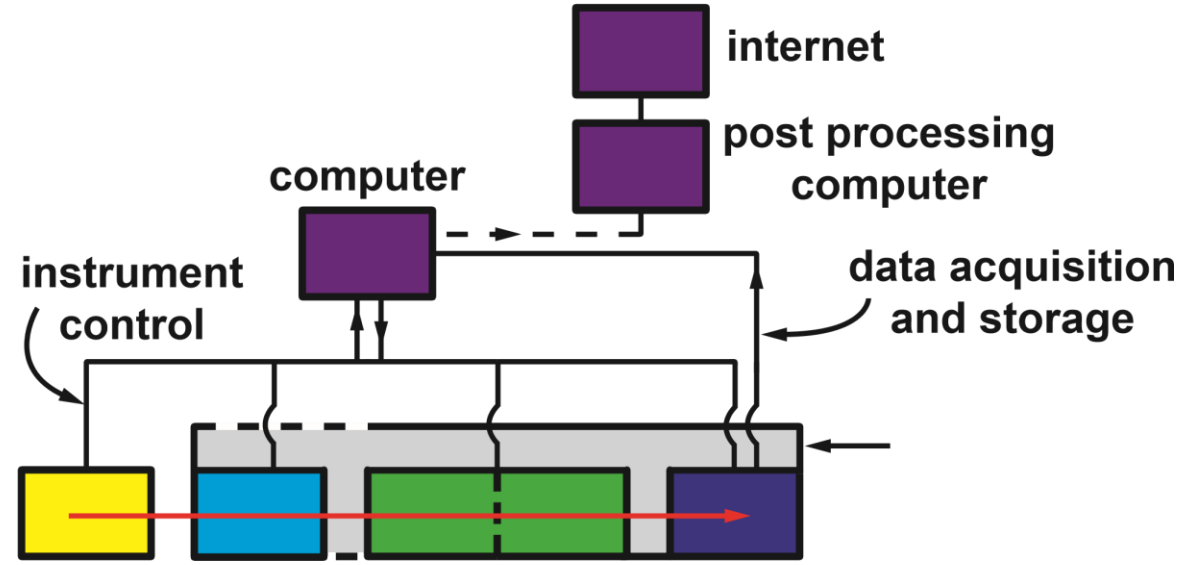


Lecture Overview

- Mass spectrometry instrument and definition
- Gas Chromatography mass spectrometry instrument overview
- Chromatography: Principles and Theory
  - Principles of chromatographic separations
  - Classification of chromatographic techniques
  - Gas chromatography (GC)
  - Theory of column efficiency in chromatography
  - Rate theory of chromatography
- Gas chromatography mass spectrometry (GC-MS)
- GC mobile phase
- Gas chromatography columns
- Ionisation methods
  - Electron Impact Ionisation (EI) /Chemical Ionisation (CI)
- Quadrupole (Q) mass analyser

A mass spectrometer is an analytical instrument that produces a beam of gas ions from samples (analytes), sorts the resulting mixture of ions according to their *mass-to-charge* (*m/z*) ratios using electrical or magnetic fields, and provides analog or digital output signal (peaks) from which the mass-to-charge ratio and the intensity (abundance) of each detected ionic species may be determined.

Block diagram of a mass spectrometer



sample inlet	ion source	analyser	detector
GC	EI	Quadrupole	electron multiplier:
LC	CI	ion trap	discrete dynode
CE	APCI	TOF	continuous dynode
gas	APPI	magnetic	MCP
probe	ESI	orbitrap	
	MALDI	ICR	
	DESI		
	DART		

Multiple forms exist for each instrument component, and they can usually be mixed and matched. Analysers can be used in single, e.g., Q or TOF, or in multi-analyser formats, e.g., QTOF and TOF/TOF, with a collision cell incorporated between the two analysers. The computer controls the instrument, acquires data and enables routine data processing, e.g. producing and quantifying spectra.

Key equations

Plate height

$$H = \frac{L}{N}$$

Plate number

$$N = 5.545 \left(\frac{t_R}{w_{½}}\right)^2$$

Adjustment retention time

$$t'_R = t_R - t_M$$

Retention factor

$$k = \frac{t'_R}{t_M}$$

van Deemter equation
Packed GC Column

$$H = A + \frac{B}{\bar{u}} + C\bar{u}$$

Golay equation
Capillary GC column

$$H = \frac{B}{\bar{u}} + C\bar{u}$$

Resolution

$$R_s = \frac{t_{R2} - t_{R1}}{(w_{b1} + w_{b2})^2}$$

Separation factor

$$\alpha = \frac{t'_{R2}}{t'_{R1}} = \frac{k_2}{k_1}$$

Resolution

$$R_s = \frac{1}{4}\sqrt{N}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k_2}{k_{ave}+1}\right)$$

When chromatography was invented?

In 1901 Mikhail Tswett invented adsorption chromatography during his research on plant pigment. He separated different coloured chlorophyll and carotenoid pigments of leaves by passing an extract of the leaves through a column of calcium carbonate, alumina and sucrose eluting them with petroleum ether/ethanol mixtures. Mikhail coined the term chromatography in a 1906 publication, from the Greek words *chroma* meaning “colour” and *graphos* meaning “to write”.

The International Union of Pure and Applied Chemistry (IUPAC) has drafted a recommended definition of chromatography:-

“**Chromatography** is a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary (stationary phase), while the other (the mobile phase) moves in a definite direction”. [L.S. Ettre, “Nomenclature for Chromatography”, *Pure & Appl. Chem*., 65 (1993), 819-872].

There are two types:- (a) Gas Chromatography (**GC**) and (b) Liquid Chromatography (**LC**).

Gas chromatography separates gaseous substances based on partitioning in a stationary phase from a gas phase. Liquid chromatography includes techniques such as size exclusion (separation based on molecular size), ion exchange (separation based on charge) and high-performance liquid chromatography (HPLC separation based on partitioning from a liquid phase)

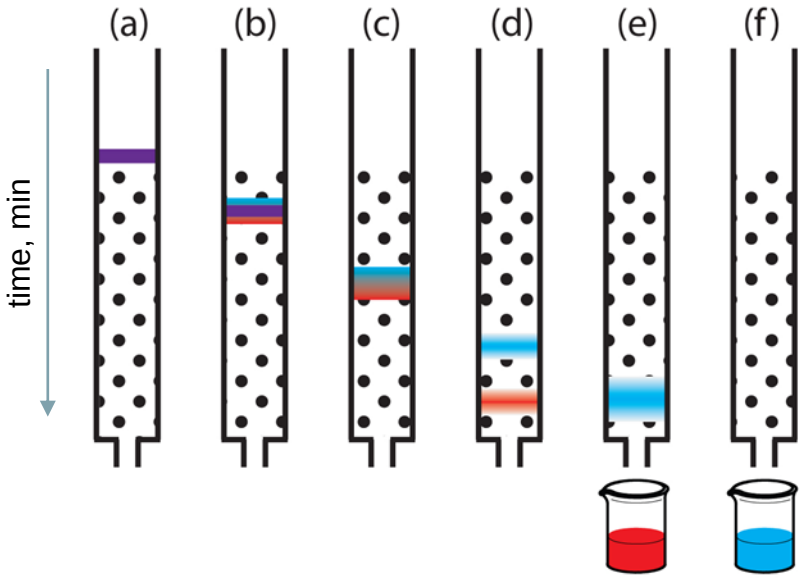
Principles of chromatographic separations

While the mechanisms of retention for various types of chromatography differ, they are all based on the dynamic distribution of an analyte between a fixed stationary phase and a flowing mobile phase. Each analyte will have a certain affinity for each phase.

$$K = \frac{c_s}{c_m}$$

K- *partition constant*

where c_s and c_m are the stationary and the mobile phases concentrations.



The distribution of the analyte between two phases is influenced by:-

(a) temperature, (b)the physico-chemical properties of compound, (c) the stationary and mobile phases.

Analytes with a large K value will be retained more strongly by the stationary phase than those with a small K value.
The result is that the latter will move along the column (be ELUTED) more rapidly.

Classification of chromatographic techniques

Chromatographic processes can be classified according to the type of equilibration chemistry involved, which is determined by the type of the stationary and mobile phases.

There are various bases of equilibration:-

1. Adsorption
2. Partition
3. Ion exchange
4. Size dependent pore penetration

More often that not, analyte stationary-phase-mobile-phase interactions are governed by a combination of such processes.

Adsorption Chromatography

The stationary phase is a solid on which the sample components are adsorbed. The mobile phase may be a liquid (*liquid-solid chromatography*) or gas (*gas-solid chromatography*); the components distribute between two phases through a combination of sorption and desorption processes.

An example is thin-layer chromatography (TLC)

- the stationary phase is planar, in the form of a solid supported on an inert plate, and the mobile phase is a liquid.

Partition chromatography

The stationary phase is usually a liquid supported on a solid or a network of molecules, which functions as a liquid, bonded on the solid support. The mobile phase may be a liquid (*liquid-liquid partition chromatography*) or a gas (*gas-liquid chromatography, GLC*).

Normal phase chromatography has a polar stationary phase (*e.g.* cyano groups bonded on silica gel) and the mobile phase is non-polar (*e.g.* hexane). When analytes dissolved in the mobile phase are introduced into the system, retention increases with increasing polarity.

Reversed phase chromatography has a non-polar stationary phase and a polar mobile phase, the retention of analytes decreases with increasing polarity.

Ion Exchange Chromatography

Ion exchange chromatography uses support with ion exchange functionalities as the stationary phase. The mechanism of separation is based on ion exchange equilibria. Hydrophobic interactions play a strong role in most ion exchange separations , nevertheless, particularly in anion exchange chromatography.

Size exclusion chromatography

Analytes are separated according to their size by their ability to penetrate into porous pockets and passages in the stationary phase

In every case, successive equilibria determine to what extent the analyte stays behind in the stationary phase or moves along with the eluent (mobile phase).

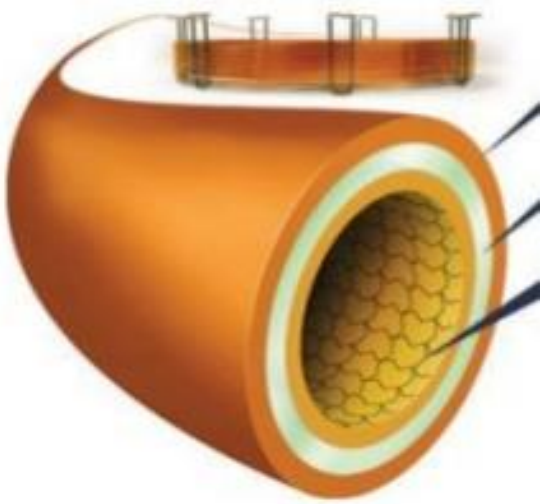
Gas chromatography (GC)

Two types of GC:-

(a) Gas-solid (adsorption) chromatography

(b) Gas-liquid (partition) chromatography

Gas-liquid chromatography used in the form of a capillary column, in which a virtual liquid phase, often polymer, is coated or bonded on the wall of the capillary tube.



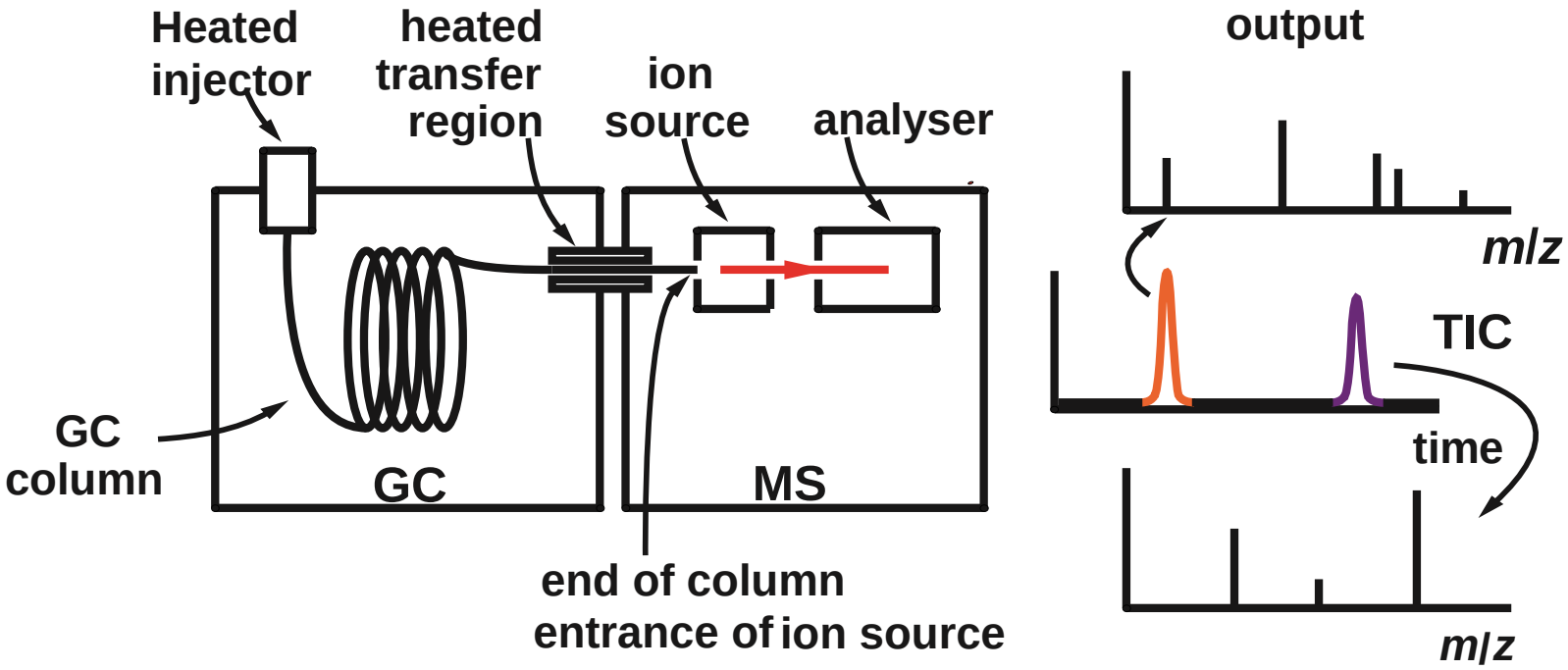
Special high temperature polyimide coating

Fused silica

Stationary phase with Engineered Self Cross-linking (ESC) technology

The determination of organic compound for example separation of benzene and cyclohexane (bp 80.1 and 80.8ºC) is extremely simply by GC-MS analysis.

Gas chromatography-mass spectrometer (GC-MS)



The sample is converted to the vapor state (if not already in a gas) by the injection into a heated port, and the eluent is a gas (the carrier gas). The stationary phase is non-volatile liquid or a liquid-like phase bonded on the capillary wall, which determines interactions of analytes and stationary phase by partition/adsorbability/polarities/any other chemical interactions. The sample injection port, column and detector (transfer line) are heated to temperatures usually about 50ºC above the boiling point of the highest analyte. The injection port and transfer line are usually kept warmer than the GC column to prevent sample condensation and promote the sample vaporisation. Separation occurs as the vapor components equilibrate between carries gas and the stationary phase. The carrier gas is a chemically inert gas (argon, helium, nitrogen). The components of the sample emerge from the GC column at a constant flow rate and enter the MS source via a heated transfer region. The analytical data consists of total ion chromatograms (TICs) and the mass spectra of the separated components.

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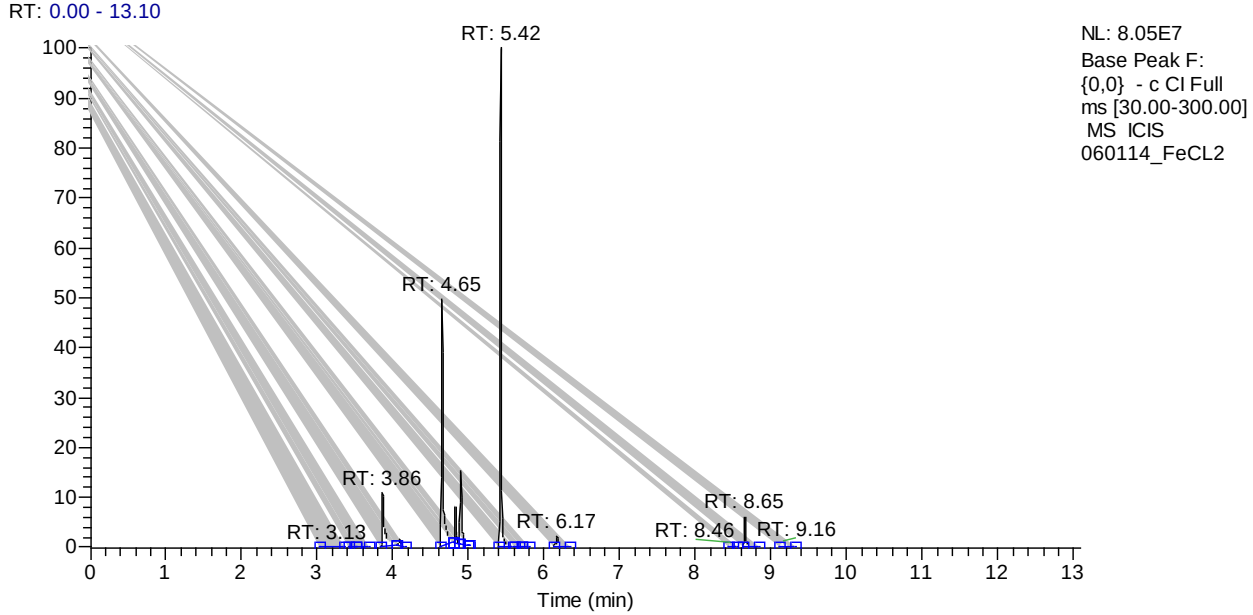

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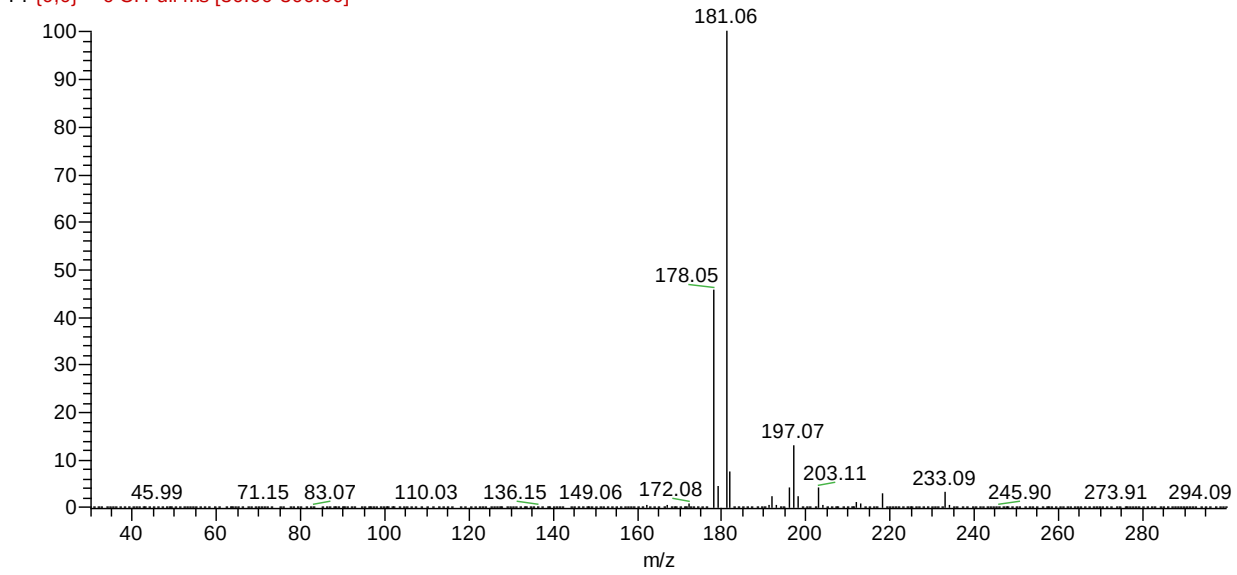


Theory of column efficiency in chromatography

Theoretical Plates theory

Band broadening in chromatography is the result of several factors, which influence the efficiency of separations. The separation efficiency of a column can be expressed in terms of the number of theoretical plates in the column.

$$H = \frac{L}{N}$$

H - the plate height (has dimensions of length, μm)

L - the column length

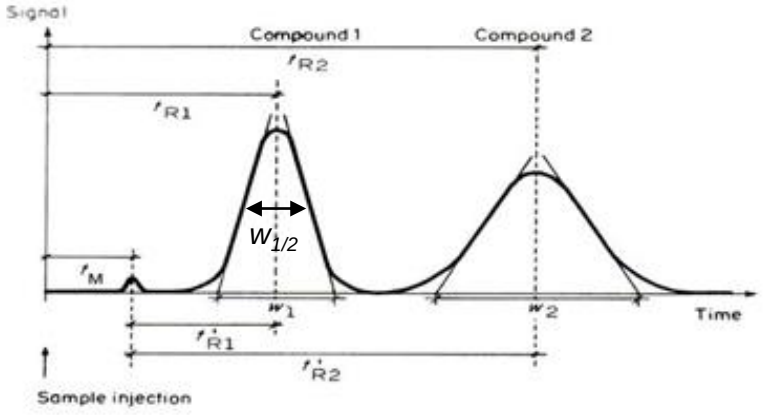
N - the number of theoretical plates

The more the number of plates, the more efficient is the column.

Experimentally, the plate height is a function of the variance, σ^2, of the chromatographic band and the distance, x, it has travelled through the column, and is σ^2/x; σ is the standard deviation of the Gaussian chromatographic peak.

The width at half-height, $w_{1/2}$, corresponds to 2.355σ, and the base width w_1 corresponds to 4σ. The number of plates, N, for an analyte eluting from a column:-

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



Theoretical Plates

Putting in $w_{1/2}=2.355\sigma$ then $N = 5.545\left(\frac{t_R}{w_{1/2}}\right)^2$

(N, the number of plates of a column, is strictly applicable for that specific analyte, t_R is the retention time, $w_{1/2}$ is the peak width at half-height in the same units as t_R)

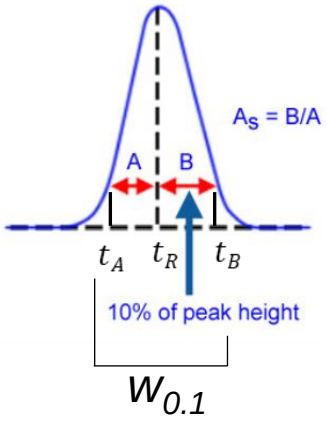
$$N = 16\left(\frac{t_R}{w_b}\right)^2$$

The *effective plate number* corrects theoretical plates for dead volume and hence is a measure of the true number of useful plates in a column:

$$N_{eff} = 5.545\left(\frac{t'_R}{w_{1/2}}\right)^2$$

t'_R is the adjusted retention time $t'_R = t_R - t_M$

t_M is the time required for the mobile phase to traverse the column and is the time it would take for an unretained analyte to appear.



For asymmetric peaks, the efficiency is determined by the Foley-Dorsey equation.

$$N_{sys} = \frac{41.7\left(\frac{t_R}{w_{0.1}}\right)^2}{\frac{B}{A}+1.25}$$

A+B = $w_{0.1}$ are the widths from t_R to the left and right sides

Once N is known, H can be obtained or $H_{eff} = L/N_{eff}$ and normally determined for the last eluting compound.

Rate theory of chromatography - the van Deemter equation

The retention factor, *k* is the ratio of the time the analyte spends in the stationary phase to the time it spends in the mobile phase.

$$k = \frac{t'_R}{t_M}$$

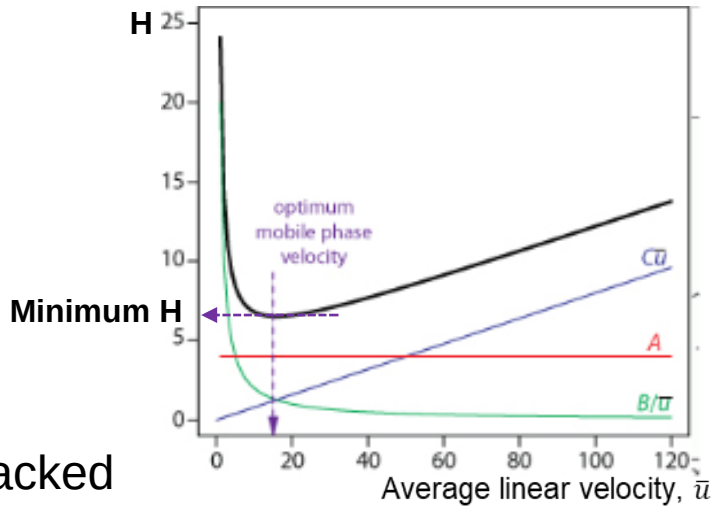
$$H = A + \frac{B}{\bar{u}} + C\,\bar{u}$$ *For a packed GC column the van Deemter equation*

A, B and *C* are constants for a given system and related to the three major factors affecting H, and $\bar{u}$ is the average linear velocity of the carrier gas in cm/s.

$\bar{u} = L/t_M$

t_M is the time for an unretained substance to elute

The general flow term for chromatography is the mobile-phase velocity, *u*. However, in GC, the linear velocity will be different at different positions along the column due to the compressibility of gases. The average linear velocity $\bar{u}$ is used.



The significance of the three terms A, B and C in packed column GC is shown as a plot of H as a function of carrier gas velocity.

Rate theory of chromatography - the van Deemter equation

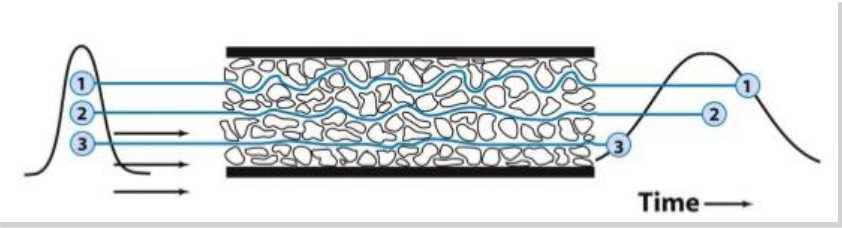
A- Eddy diffusion and is due to the variety of variable length pathways available between the particles in the column and is independent of the gas- and mobile-phase velocity and relates to the particle size and geometry of packing.

$A = 2\lambda d_p$

λ- an empirical constant

(depend how well the column is packed)

d_p -the average particle diameter



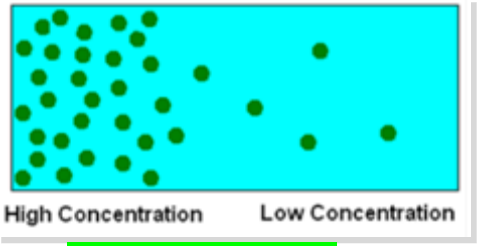
GC is used at modest pressures, and very fine tightly packed support are not used.

B - Longitudinal (axial) or molecular diffusion of the sample components in the carrier gas, due to concentration gradients within the column.

$B = 2\gamma D_m$

γ- an obstruction factor, typically equal to 0.6 to 0.8 in a packed GC column

D_m -the diffusion coefficient



Molecular diffusion

Molecular diffusion is a function of both the sample and the carrier gas. In a given analysis, the sample components are fixed, and the only way to change B or $B/\bar{u}$ is by varying the flow rate of the carrier gas. High flow rates reduce the contribution of molecular diffusion and the total analysis time.

Rate theory of chromatography - the van Deemter equation

C – the interphase mass transfer term and is due to the finite time required for analyte distribution equilibrium to be established between the two phases as it moves between the mobile and stationary phases. The *C*-term has two separate components, C_m and C_s, respectively, representing mass transfer limitations in the mobile and the stationary phases.

The C_m term originates from non-uniform velocities across the column cross section.

$C_m = \frac{C_1 \omega d_p^{\,2}}{D_m} u$ *for uniformly packed columns*

C_1 – a constant; ω – related to the total volume of mobile phase in the column

The stationary phase mass transfer term, C_s, is proportional to the amount of stationary phase, and increases with the retention factor for the analyte, and the thickness of the stationary phase film d_f through which the anlyte must diffuse;

$\frac{d_f^{\,2}}{D_s}$ represents the characteristic time for the analyte to diffuse in and out of the stationary phase.

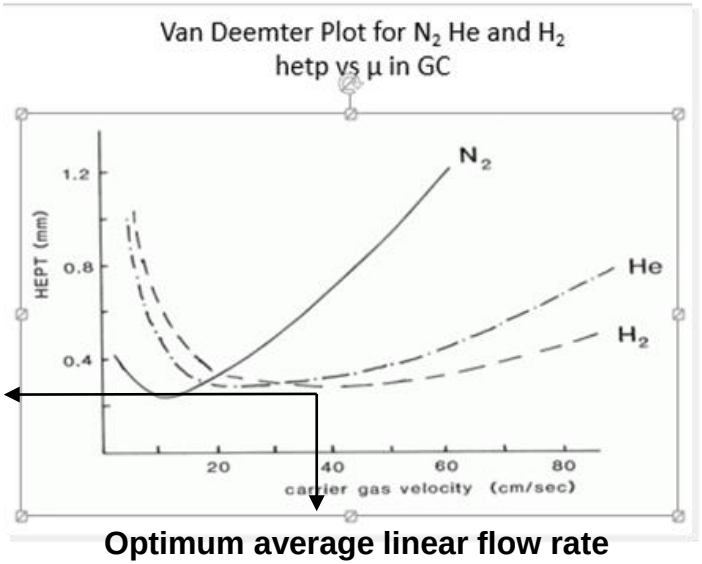
Most used are Open tubular (capillary) columns have no packing, A-term in van Deemter equation disappears.

$H = \frac{B}{\bar{u}} + C\,\bar{u}$ *Golay equation*

An efficient packed GC column will have several thousand theoretical plates, and capillary columns have plate counts depending on the column internal diameter 3,800 plates/m for 0.32 mm i.d. column a film thickness of 0.32 µm to 6,700 plates/m for a 0.18 mm i.d column with 0.18 µm film thickness (for an analyte of k = 5). The GC columns are typically 20-30 m long and total plate counts can be well in excess of 100,000.

GC mobile phase

The mobile phase (carrier gas) is almost always helium, nitrogen or hydrogen, with helium most popular. Gases should be pure and chemically inert. Impurities level should be less 10 ppm.



Flow rate is one of the parameters that determine the choice of carrier gas via the van Deemter plot, the minima in these plots, defined as the optimum values of *u.*

Hydrogen provides the highest value of u_{opt} of three common carrier gases, resulting in the shortest analysis time. The van Deemter curve is very flat, which provides a wide range over which high efficiency is obtained.

Retention factor efficiency and resolution

The retention factor k

$k = \frac{t'_R}{t_M}$ is a direct measure of how strongly an analyte is retained by the column under the given conditions.

If a pair of analytes are poorly separated, separation (resolution) improves if chromatographic conditions (temperature in GC) are altered to increase k.

While a large retention factor favours good separation, large retention factors mean increased elution time, so there is a compromise between separation efficiency and separation time. The retention factor could be increased by increasing the stationary-phase volume.

Resolution in chromatography

The resolution of two chromatographic peaks:-

$R_s = (t_{R2} - t_{R1})/[(w_{b1} + w_{b2})/2]$

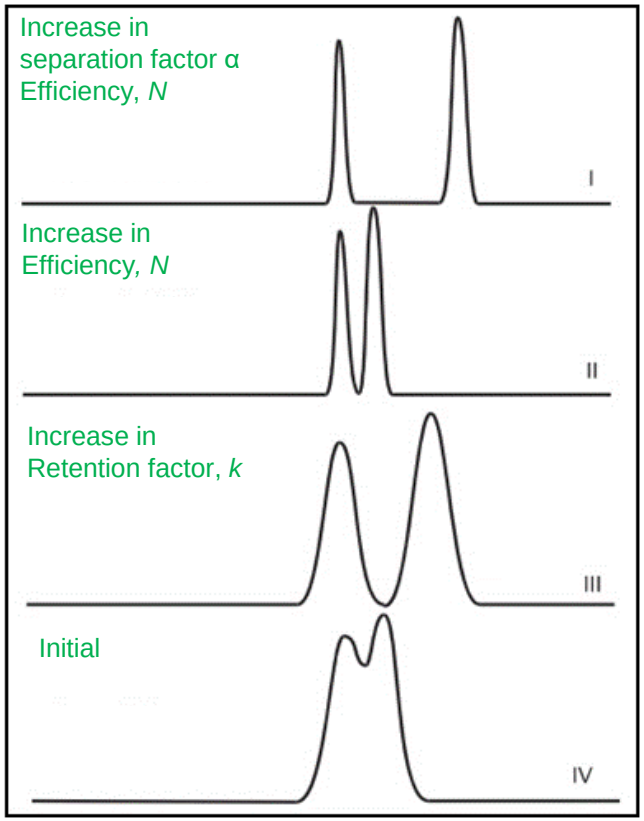
t_{R1} and t_{R2} are the retention times of the two peaks (peak 1 elutes first)

w_{b1} is the baseline width of the peaks.

The separation factor, α, also the selectivity and is a thermodynamic quantity that is a measure of the relative retention of analytes.

$$\alpha = \frac{t'_{R2}}{t_{R1}} = \frac{k_2}{k_1}$$

k_2 and k_1 are the retention factors of the adjusted retention times. This describes how well the chromatographic conditions discriminate between the two analytes.



$$R_s = \frac{1}{4}\sqrt{N}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k_2}{k_{ave}+1}\right)$$

k_{ave} is the mean of the two capacity factors.

N is proportional to L, the R_s is proportional to $\sqrt{L}$. So doubling the column increases the R_s by $\sqrt{2}$ or 1.4. The retention times would be increased in direct proportion to the length of the column.

Gas chromatography columns

The two types of columns are:-

- Packed columns
- Capillary columns

Packed columns can be in any shape, 1 to 10 m long and 0.2 to 0.6 cm in diameter. They made of stainless steel, nicker or Teflon. Long columns require high pressure and longer analysis time. The column is packed with small particles that may themselves serve as the stationary phase (adsorption chromatography) or more commonly are coated with a non-volatile liquid phase or varying polarity (partition chromatography).

Gas solid chromatography (GSC) is for separation of small gaseous species such as H_2, N_2, CO_2, CO, O_2, NH_3 and CH_4 and volatile hydrocarbons, using high surface area inorganic packings such as alumina or porous polymer. The gases are separated by their size due to retention by adsorption on the particles.

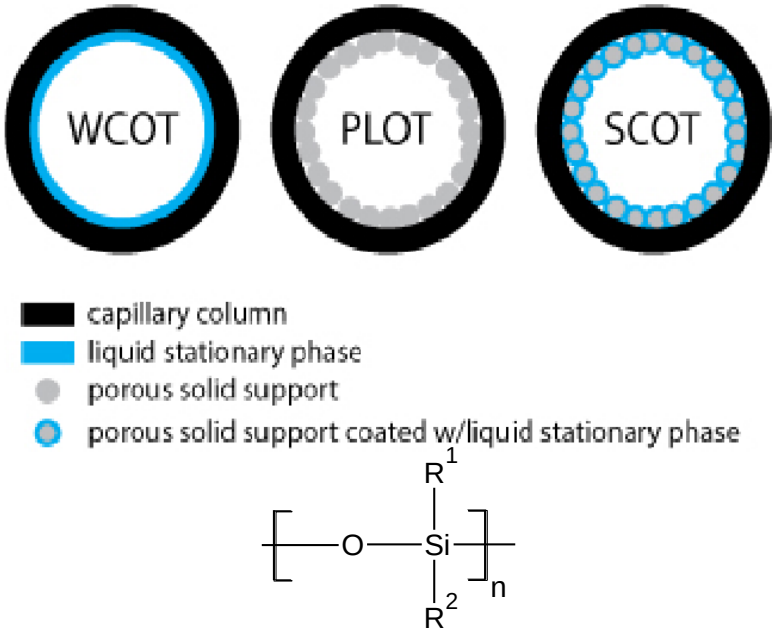
The solid support for a liquid phase have a high specific surface area, chemically inert, thermally stable and have uniform sizes. The most common used supports are prepared from diatomaceous earth, a spongy siliceous material. Particles have diameters in the range of 60 to 80 mesh (0.18 to 0.25 mm), 80 to 100 mesh (0.15 to 0.18 mm) or 100 to 120 mesh (0.12 to 0.15 mm)

Capillary columns most popular used

Capillary columns are made of thin silica (SiO_2) coated on the outside with a polyimide polymer for support and protection of the fragile silica capillary, allowing then to be coiled. The inner surface of the capillary is chemically treated by reacting the Si-OH group with a silane-type reagent.

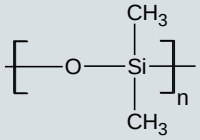
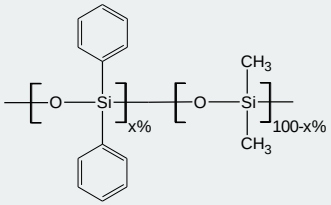
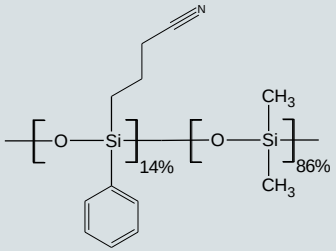
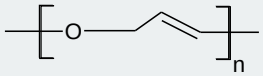
The capillaries are 0.10 to 0.53 mm internal diameter, with lengths of 15 to 100 m can have several hundred thousand plates.

There are three types of open-tubular columns:-
Wall coated open tubular (WCOT) have a thin liquid film coated on and supported by the walls of the capillary. The stationary phase is 0.1 to 0.5 µm thick.
In support coated open-tubular (SCOT) columns, solid microparticles coated with the stationary phase (much like in packed column) and attached to the walls of the capillary.



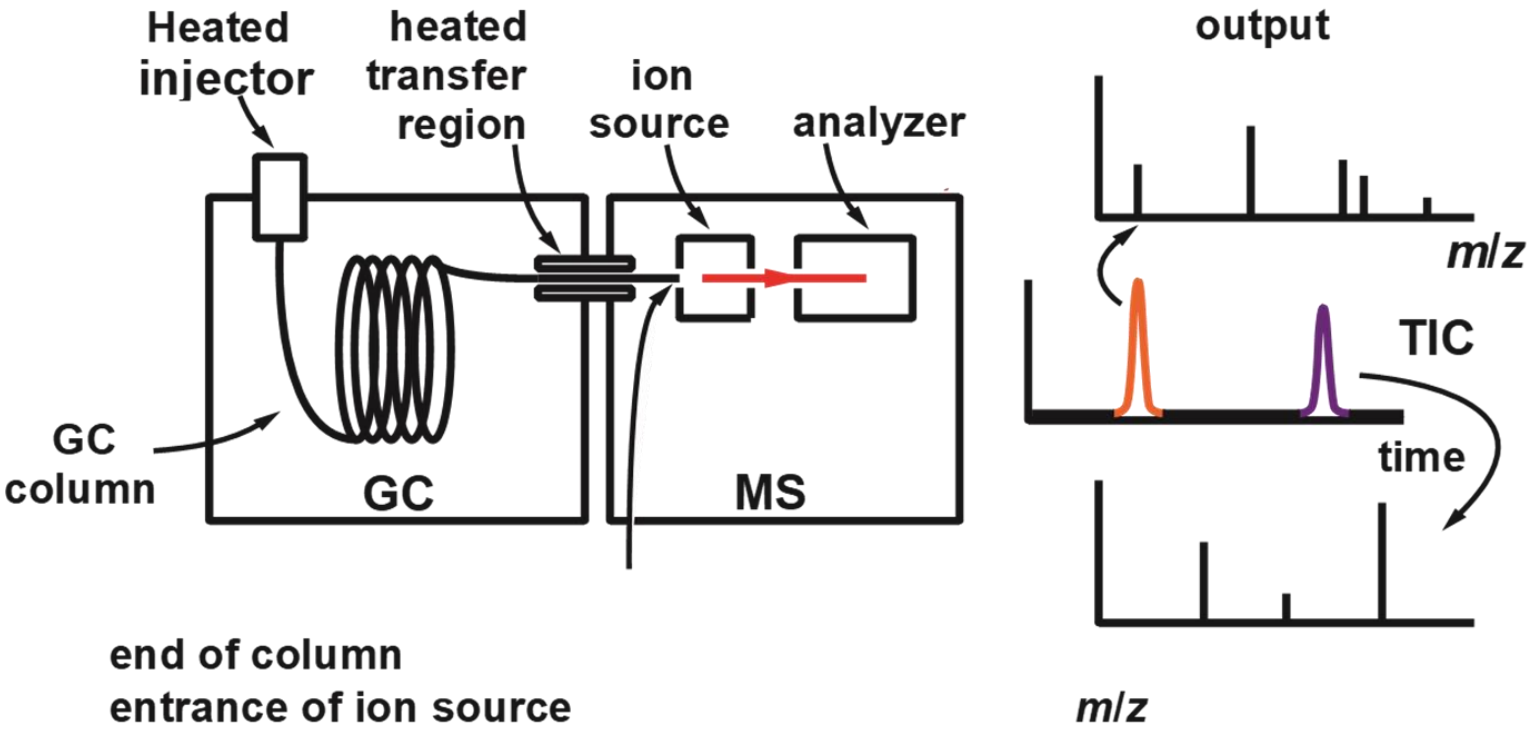
Porous layer open tubular (PLOT) columns, have solid-phase particles attached to the column wall, for adsorption chromatography. Particles alumina or porous polymers are used.

Capillary fused silica stationary phases

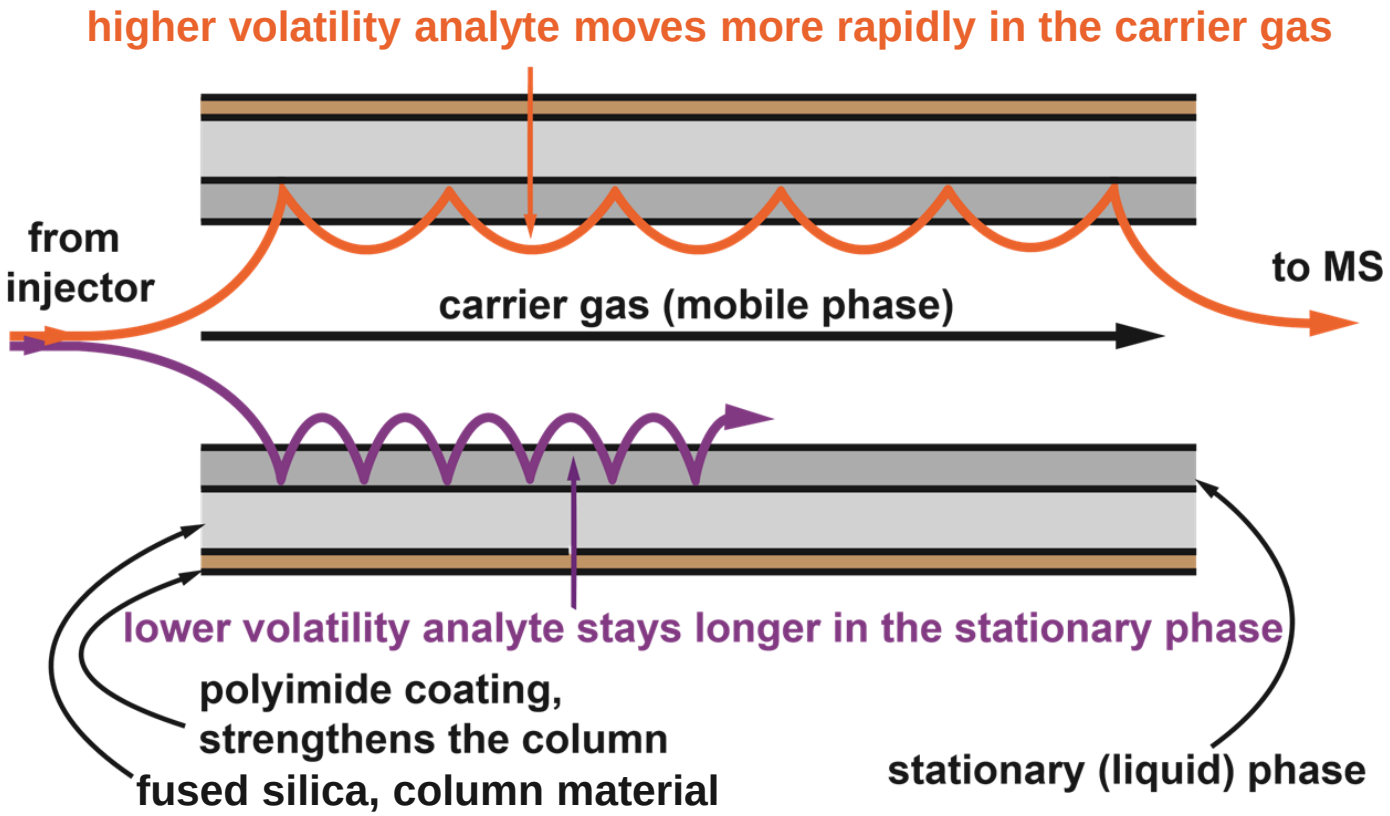
Phase	Polarity	Use	Max Temp. (°C)
100% dimethyl polysiloxane 	Nonpolar	Basic general purpose phase for routine use. Hydrocarbons, polynuclear aromatics, PCBs	320
Diphenyl, dimethyl polysiloxane 	Low (x=5%) Intermediate (x=35%) Intermediate (x=65%)	General purpose, good high temperature characteristics. Pesticides.	320 300 370
14% cyanopropylphenyl-86%dimethylsiloxane 	Intermediate	Separation of organochlorine pesticides listed in EPA 608	280
Poly(ethyleneglycol) Carbowax 	Very polar	Alcohols, aldehydes, ketones and separation of aromatic isomers	250

Phases are selected based on their polarity, keeping in mind that “like dissolve like”. A polar stationary phase will interact more with polar compounds and vice versa. Non-polar liquid phase are nonselective so separations tend to follow the order of the boiling points of analytes. Polar liquid phases exhibit several interactions with analytes such as dipole interactions, hydrogen bonding, and induction forces, there is often no correlation between the retention factor or volatility.

Gas chromatography mass spectrometry (GC-MS)



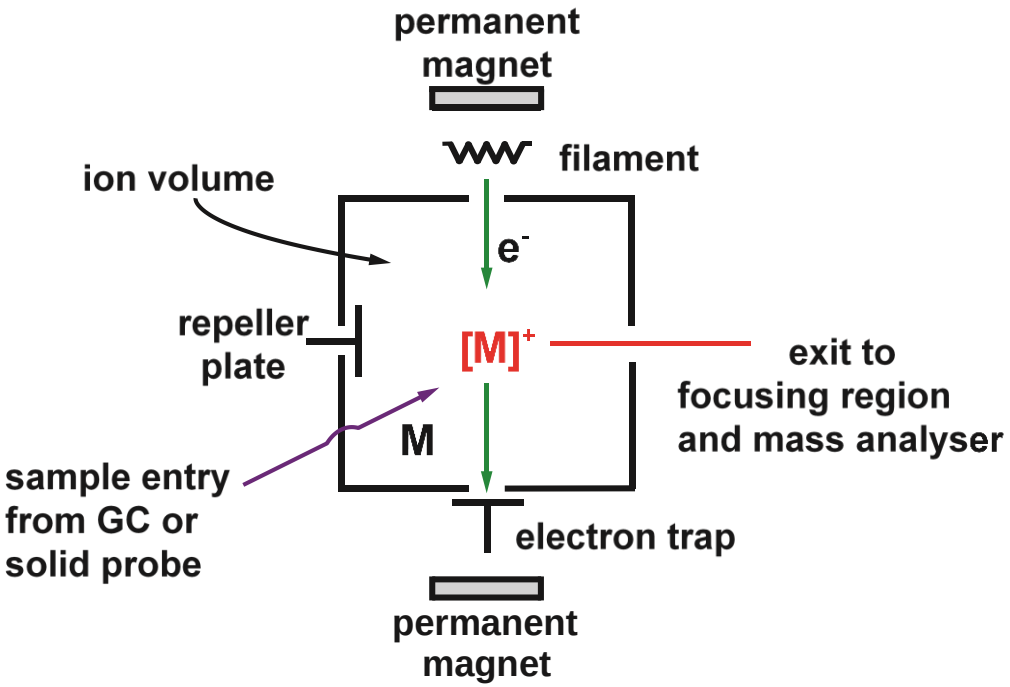
Samples are introduced into the GC using a heated injector. Components are separated on a column, according to a combination of molecular mass and polarity, and sequentially enter the MS source via a heated transfer region. The analytical data consists of total ion chromatograms (TIC) and the mass spectra of the separated components.



Analytes condense at the entrance of the column and are subsequently separated based on their molecular mass and polarity. These properties determine analyte volatility and as a result the retention times in the stationary liquid phase and the gaseous mobile phase. More volatile components elute first as they are carried through the column by the carrier gas at lower temperatures. Increasing the oven temperature enables the transfer of compounds with higher boiling points from the stationary phase into the vapour phase and their elution from the column.

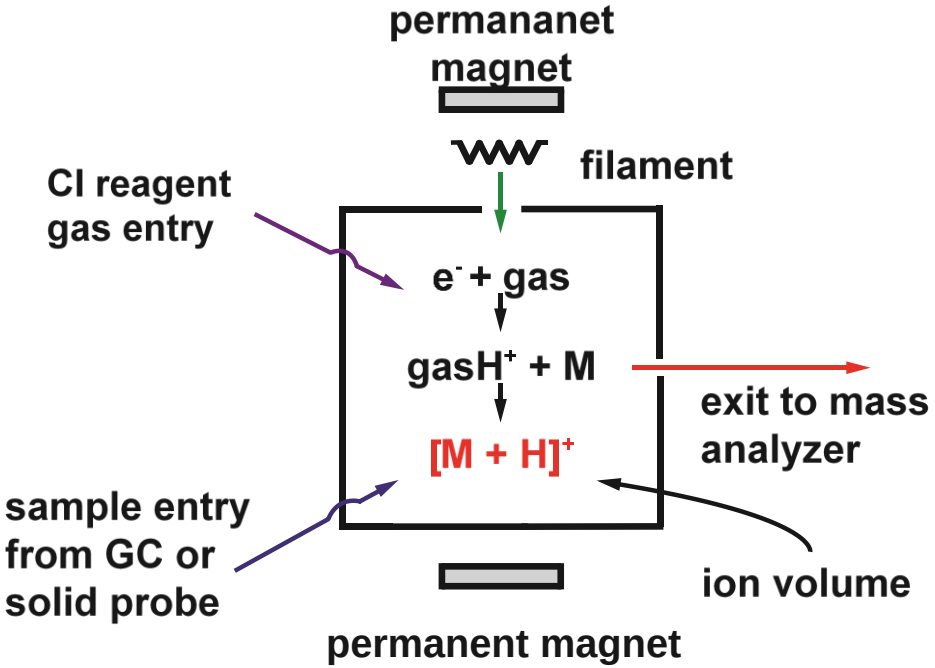
Ionisation methods – Electron Impact Ionisation (EI)

- Sample molecules are vaporised and introduced into the EI source (the analysis of gases or small volatile molecules)
- Derivatisation is required for the analysis of non-volatile thermally-labile compounds
- Electrons emitted from filament
- The electrons are accelerated into the region containing gaseous sample called the “source block” by potential of 70 eV (commonly used in EI)



- Energetic electrons can ionise molecules
  $e^- + M \rightarrow M^{+\bullet} + 2e^-$
- Weak magnetic field (B) collimates beam, forcing it into narrow helical path, thus increasing ionising path length
- Gaseous sample bombarded by electron beam – if electron energy (EE) > ionisation energy (IE), an electron is displaced ($M \rightarrow M^{+\bullet}$)
- +ve potential applied to repeller plate to push ions formed in the volume through exit to focusing region and mass analyser
- At 70 eV, the molecular ion ($M^{+\bullet}$) formed may fragment.
- Ions are accelerated out of the ion source and transmitted the mass analyser to the detector.

Chemical ionisation (CI) source



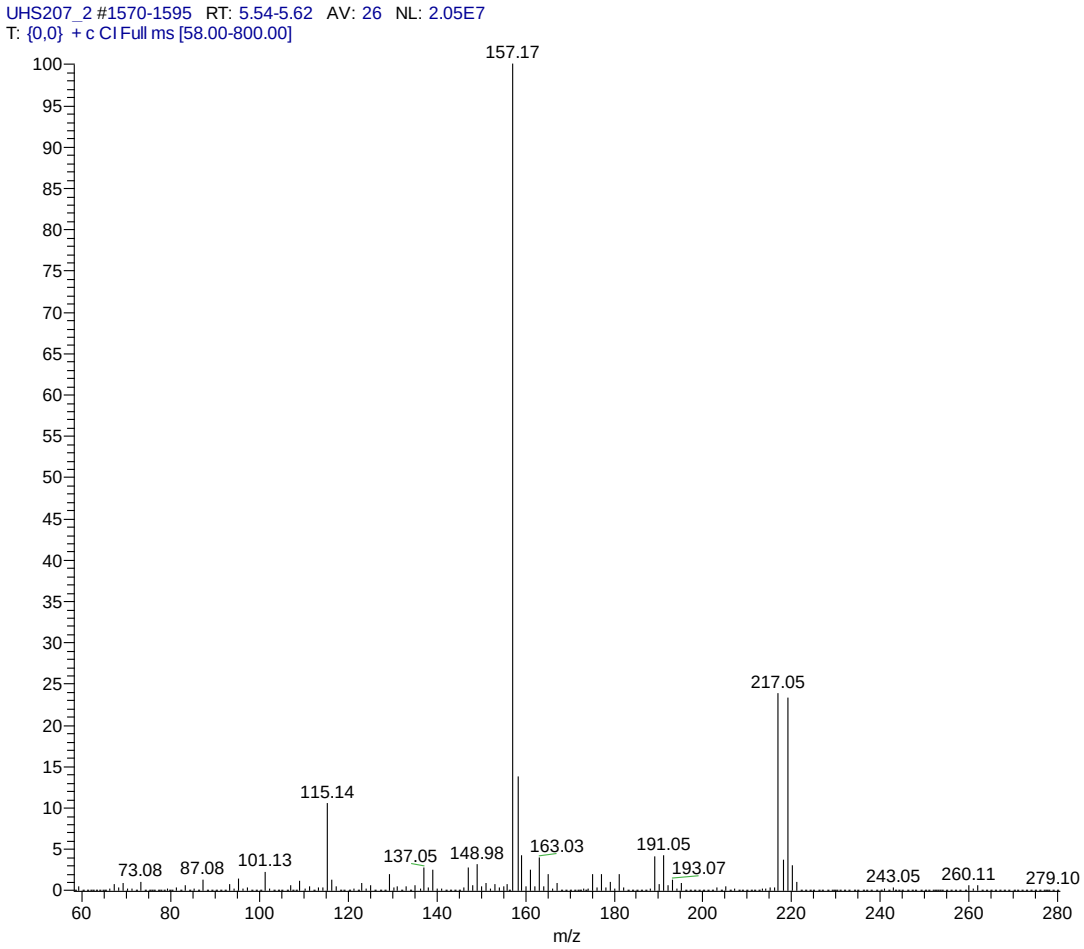
- CI volume similar to EI, but made more gastight by narrowing entry and exit slits
- CI does not require magnet or trap since beam does not penetrate thorough gas chamber
- Effective, high-speed pumping system required to maintain source pressure of ~10^{-4} mbar, analyser requires differential pumping
- Electrons from the filament react with a reagent gas (methane, isobutane or ammonia) generating protonated reagent species that transfer a proton onto, or form an adduct with the analyte.
- Reagent ions produced by EI at high pressure ($1x10^{-4}$ mbar)

  $NH_3 - e^- \rightarrow NH_3^{+\cdot} + 2e^-$

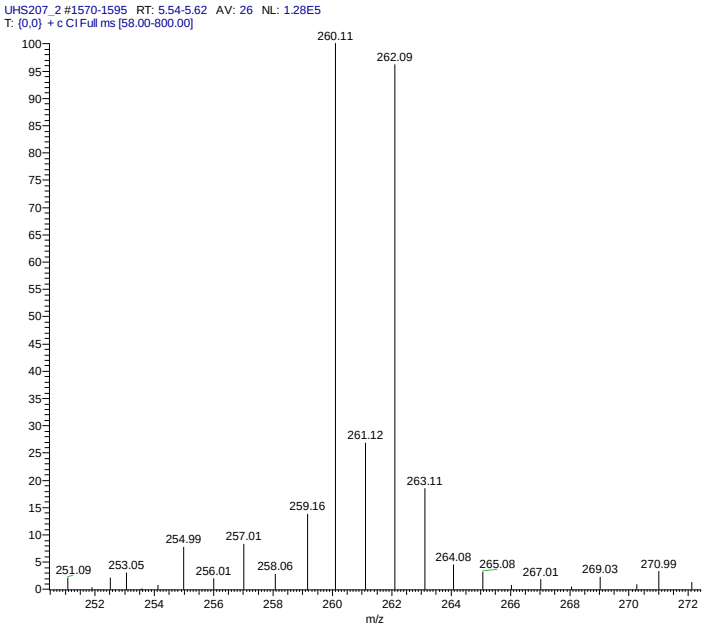
  $NH_3^{+\cdot} + NH_3 \rightarrow NH_4^{+\cdot} + NH_2$
- These ions are only slightly reactive with reagent gas itself, but readily react to ionise the sample via ion-molecule reactions in which the reagent ions act as Brönsted acids (proton donor)

e.g., $M + [NH_4]^+ \rightarrow [M + H]^{+\cdot}$ and/or $[M + NH_4]^{+\cdot}$

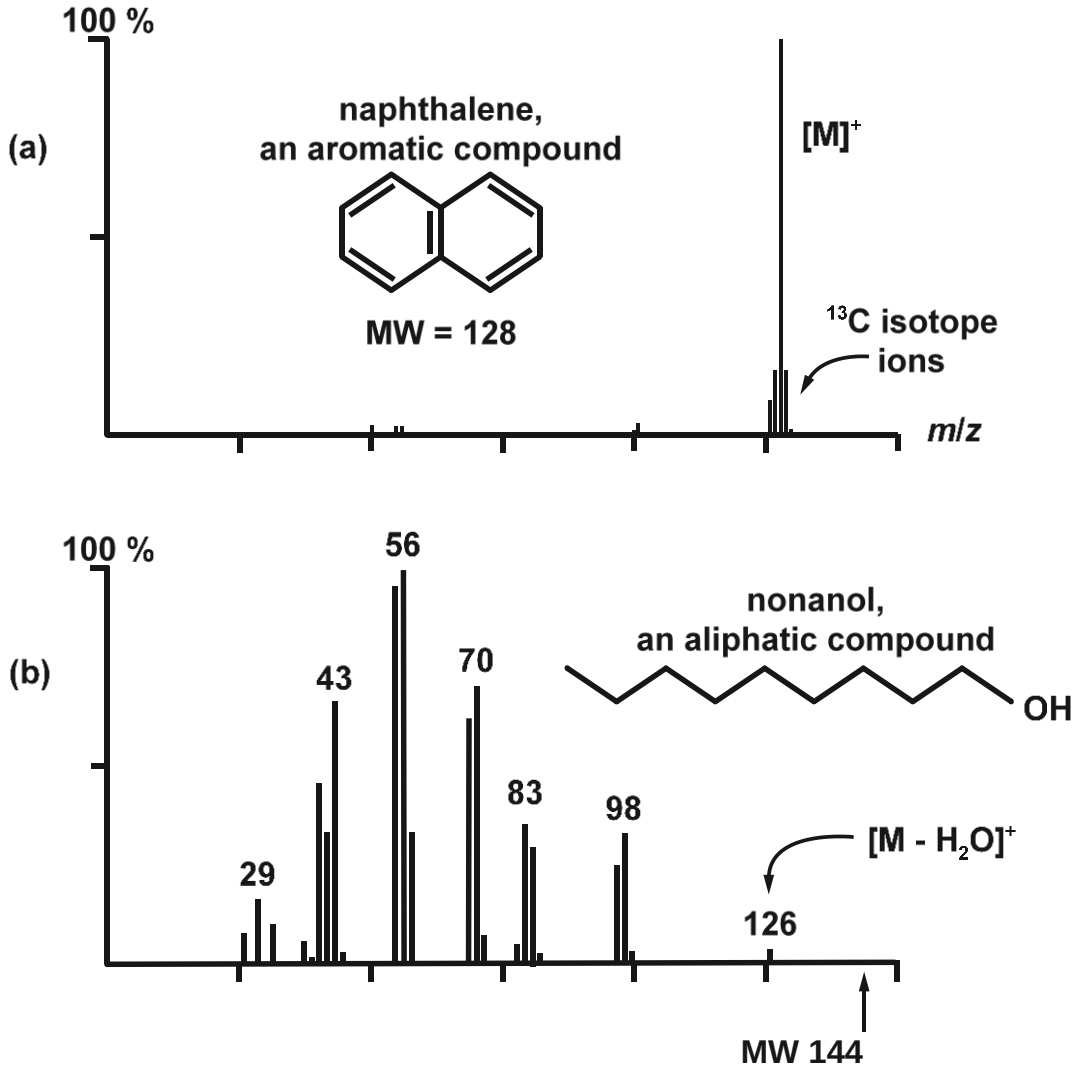
EI spectrum showing molecular ion $M^{+\cdot}$



$M^{+\cdot}$ at *m/z* 260/262 Br isotope



Comparison of the EI spectra for (a) an aromatic and (b) an aliphatic compound

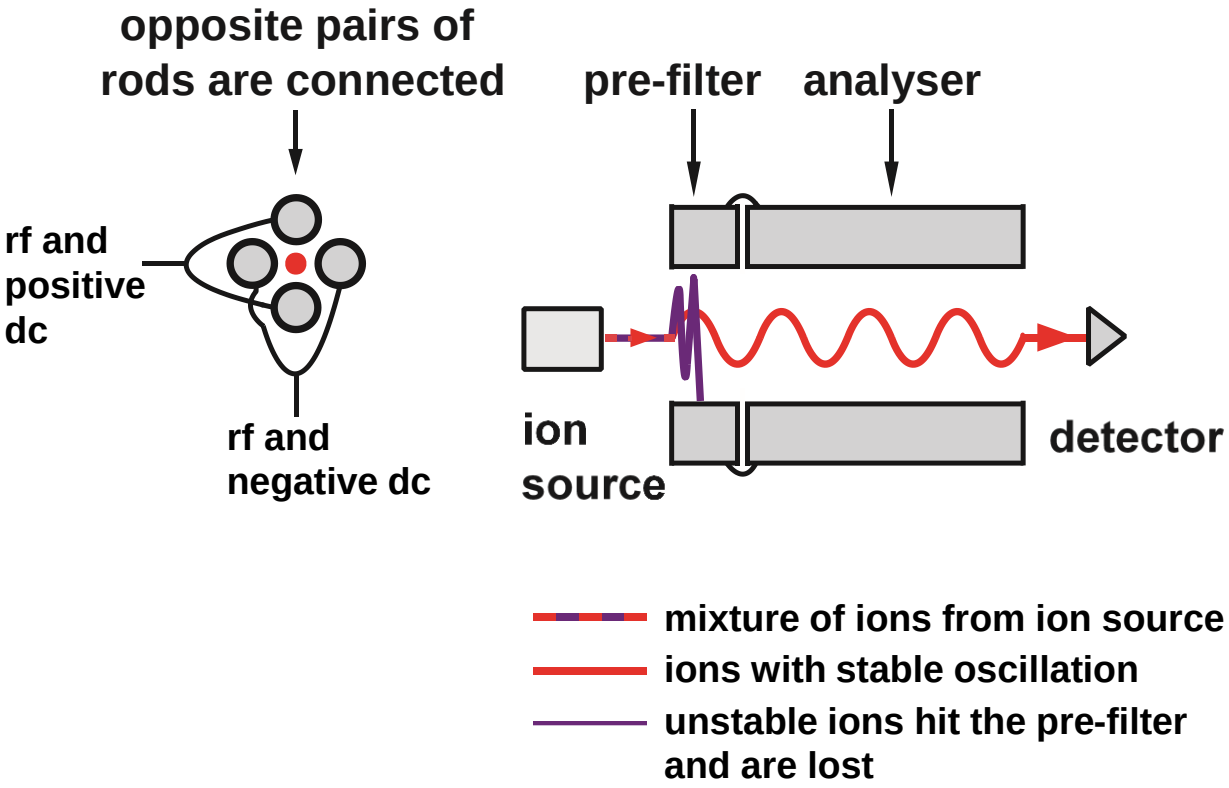


Libraries (EI).

i. Over the past forty-fifty years, since mass spectrometry has become a standard tool, libraries of mass spectra have been generated.

ii. The newest libraries contain hundreds of thousands of EI mass spectra from which an unknown compound can very often be identified.

Quadrupole (Q) analyser



- Mass filter –separation accomplished using combination of DC and RF electric fields. For any given set of rf and dc voltages, on the opposing pairs of rods,
- Only ions of with stable trajectory transmitted and enabling them to reach the detector.
- Unstable ions hit the initial part of the analyser, often a pre-filter, are discharged and lost.
- Scan DC/RF $\rightarrow m/z$
- *Low resolution instrument*

Mass of the elements.

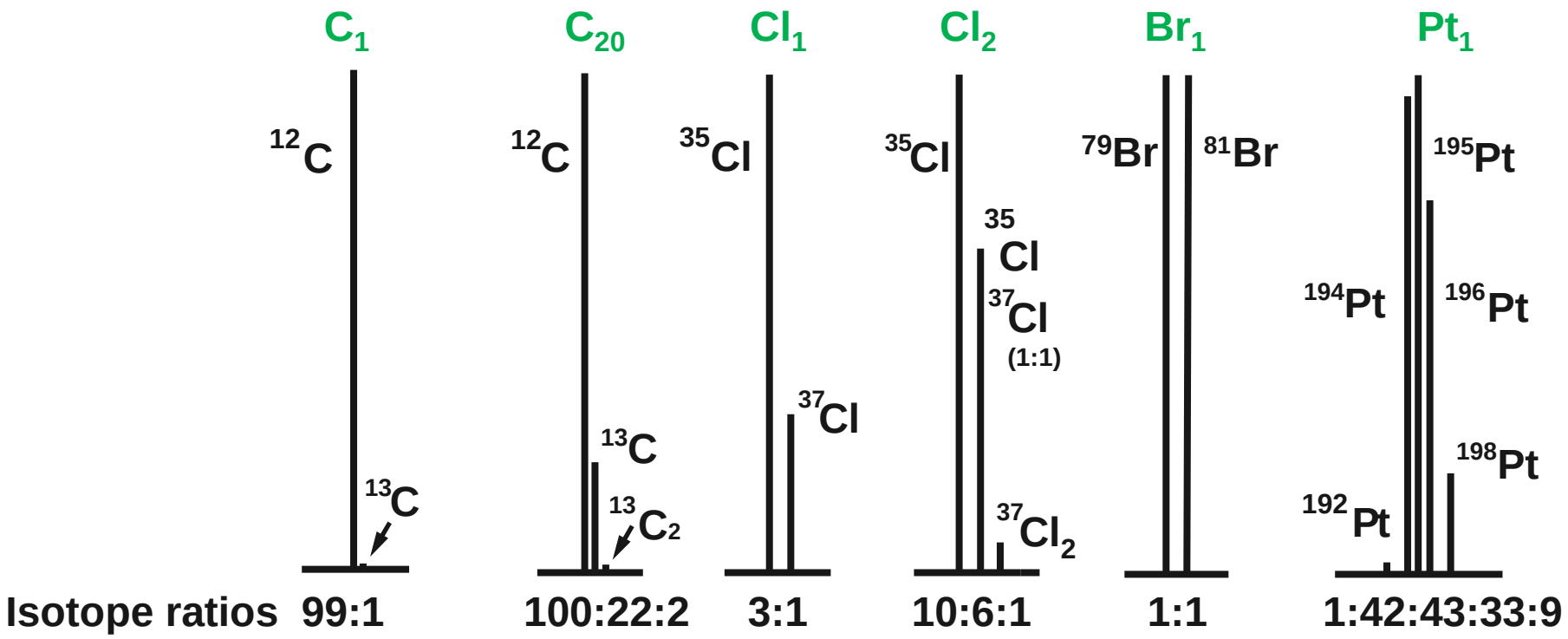
1. Today carbon **^{12}C** is taken to have an atomic mass of **12.000000000 Da.**
2. The atomics masses of the other elements and their isotopes are measured relative to this.
3. The relative atomic masses of some elements are listed below:-
   ^{12}C =12.00000000
   ^{1}H = 1.007825035
   ^{14}N =14.003074002
   ^{16}O =15.99491463
4. The molecular mass of ammonia (NH_3) =14.003074002+(3x1.007825035) =17.026549
   The molecular mass of OH = 15.99491463+1.007825035 =17.00274
5. By **accurately measuring** the molecular mass of a sample its **elemental composition** can be determined.

Monoisotopic mass- the mass of an ion which is made up of the lightest stable isotopes of each element (includes the mass defect, where ^{1}H=1.0078, ^{12}C=12.0000, ^{16}O=15.9949 etc).

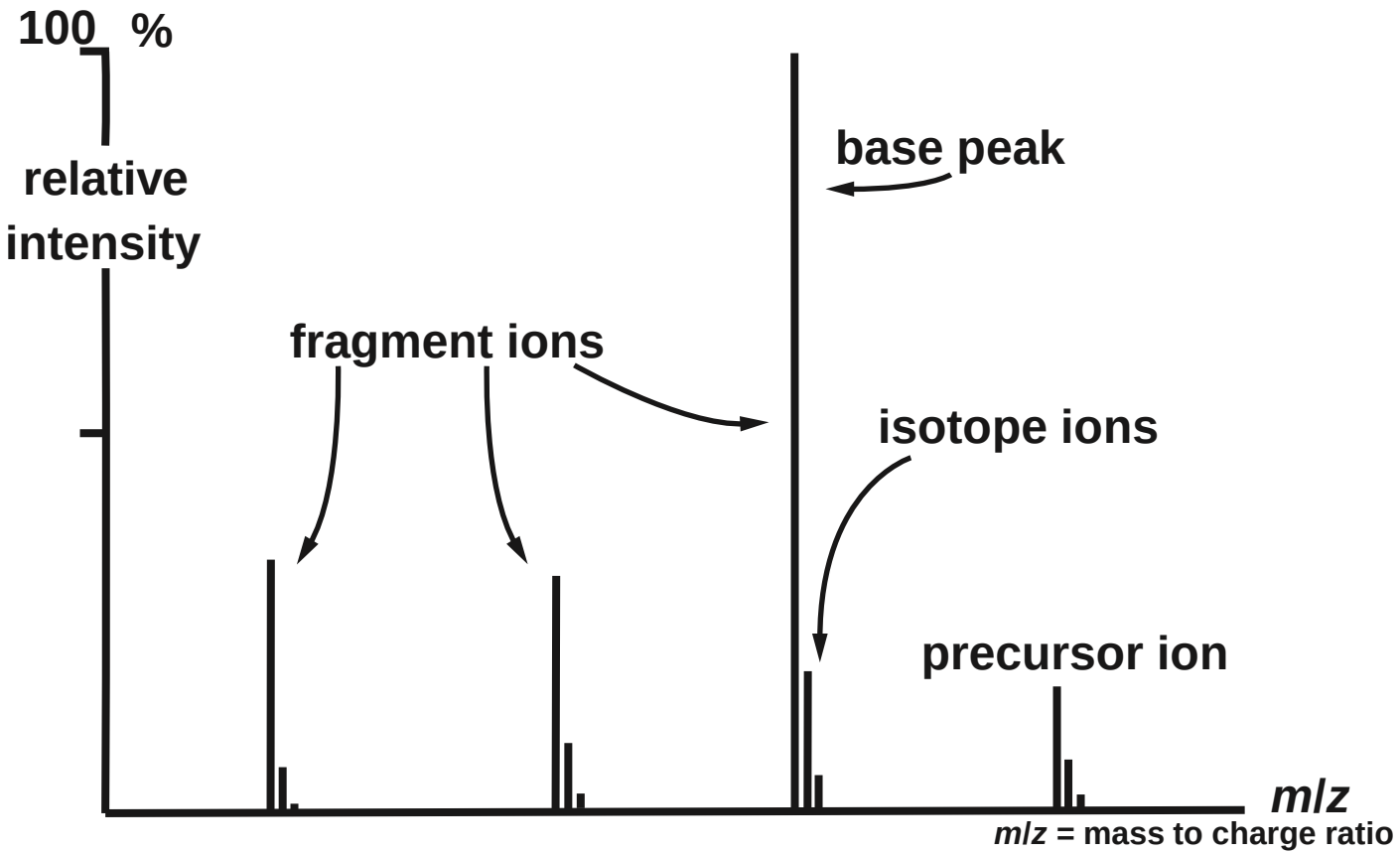
Average mass- the mass of an ion calculated using the relative average isotopic mass of each element (where, C=12.0111, H=1.00797, O=15.9994 etc).

Isotopic Abundance- the naturally occurring distribution of the same element with different atomic mass e.g. ^{12}C=12.0000=98.9%,^{13}C=13.0034=1.1%

Example of isotope patterns

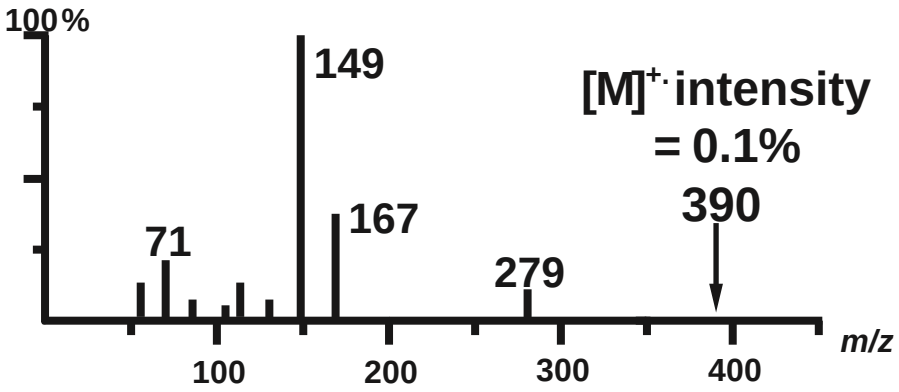


Basic features of mass spectra

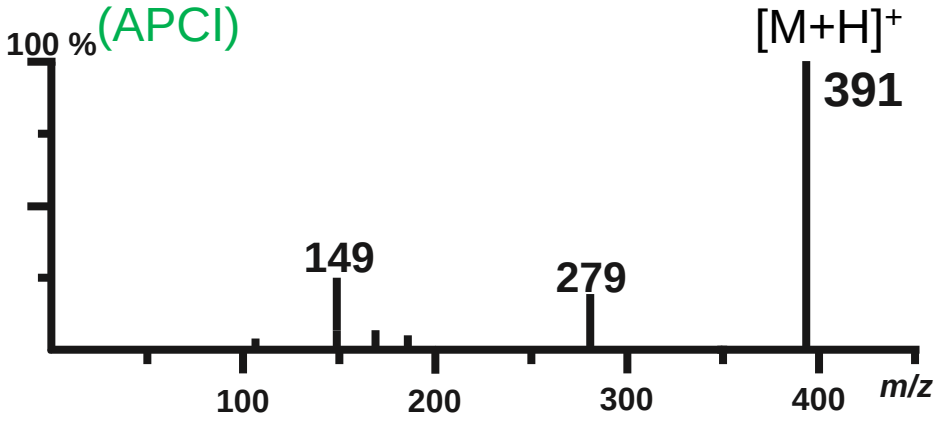


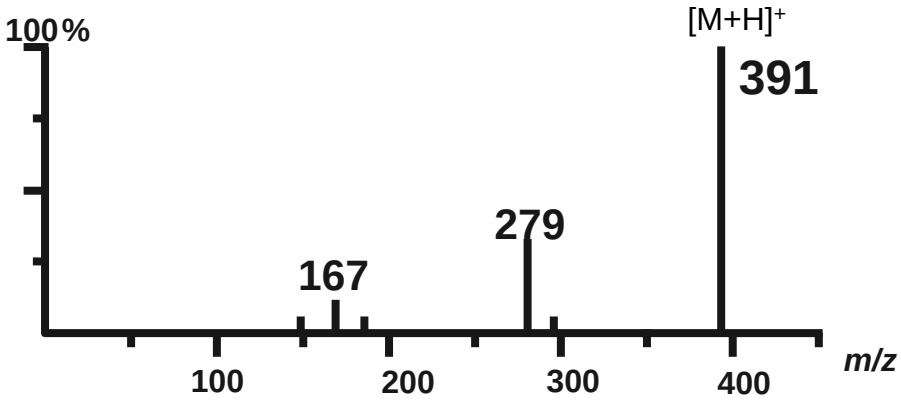
- Energy is added to molecules during ionisation. The distribution of the energy may result in the breaking of chemical bonds and, consequently, in fragment ion formation. The fragmentation may be so extensive that no precursor ion is observed.
- The form of the molecular/precursor ion depends on the mode of ionisation and can include for EI $[M]^{+\cdot}$ and CI $[M+H]^{+\cdot}$ $[M+NH_4]^{+\cdot}$, for ESI $[M]^+$, $[M + H]^+$ and other adduct ions, e.g., $[M + Na]^+$. The base peak represents the most stable ion resulting from the ionisation process and is, therefore, the most intense (abundant) peak in the spectrum. The intensities of all other ions are usually normalised with respect to the base peak.
- Ions, normally of lesser intensity and to the right of each precursor/fragment ion, generally represent isotopic species. Typically, but not always, isotope ions reflect the presence of carbon-13 (^{13}C).

Electron ionisation (EI)

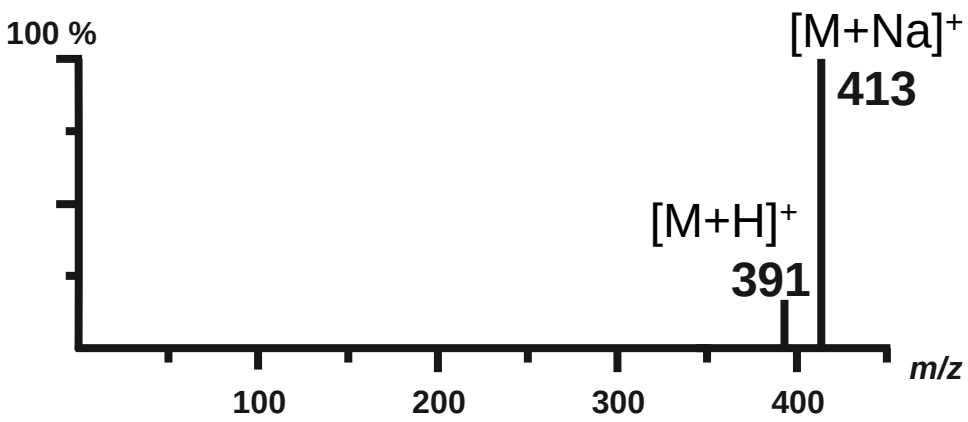


atmospheric pressure chemical ionisation (APCI)





Chemical ionisation (CI) with ammonia gas

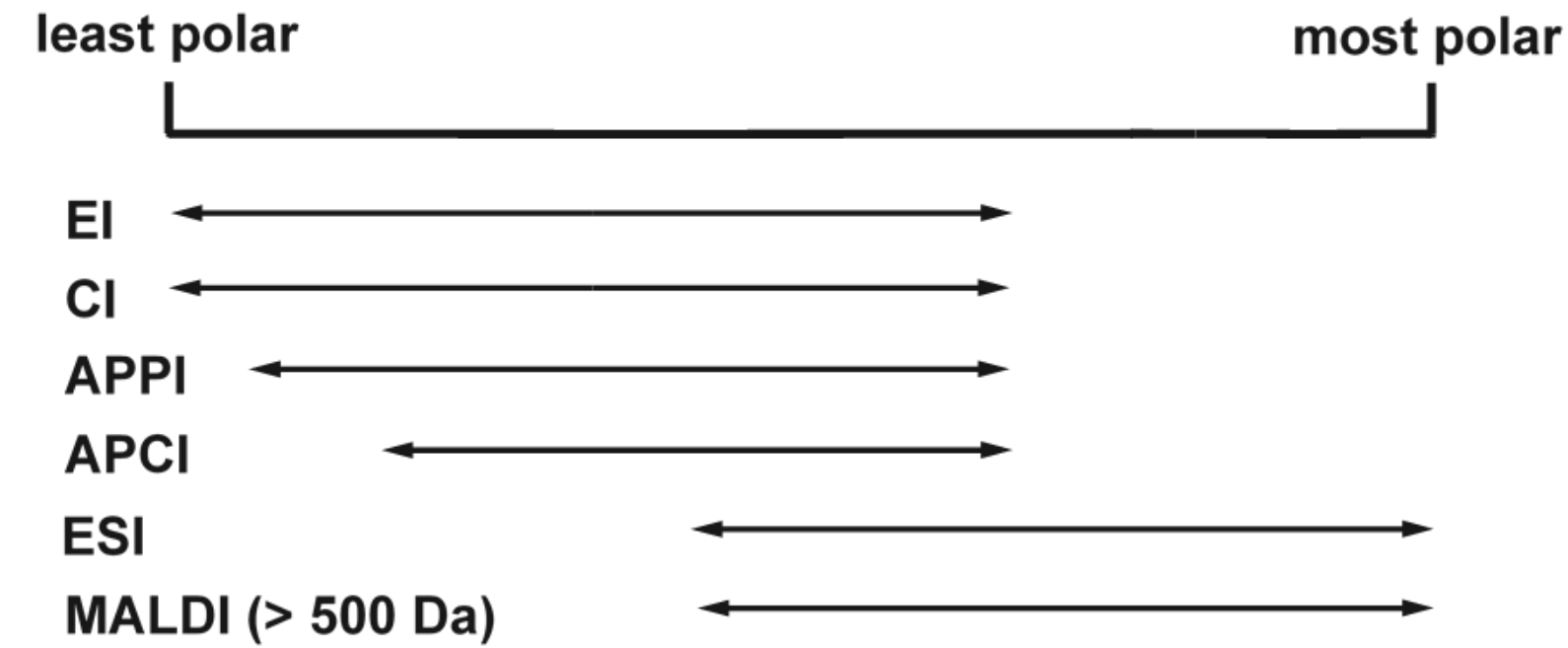


Electrospray ionisation (ESI)

Spectra (simplified by removing the isotope peaks) illustrating how data varies, depending on the ionisation method.

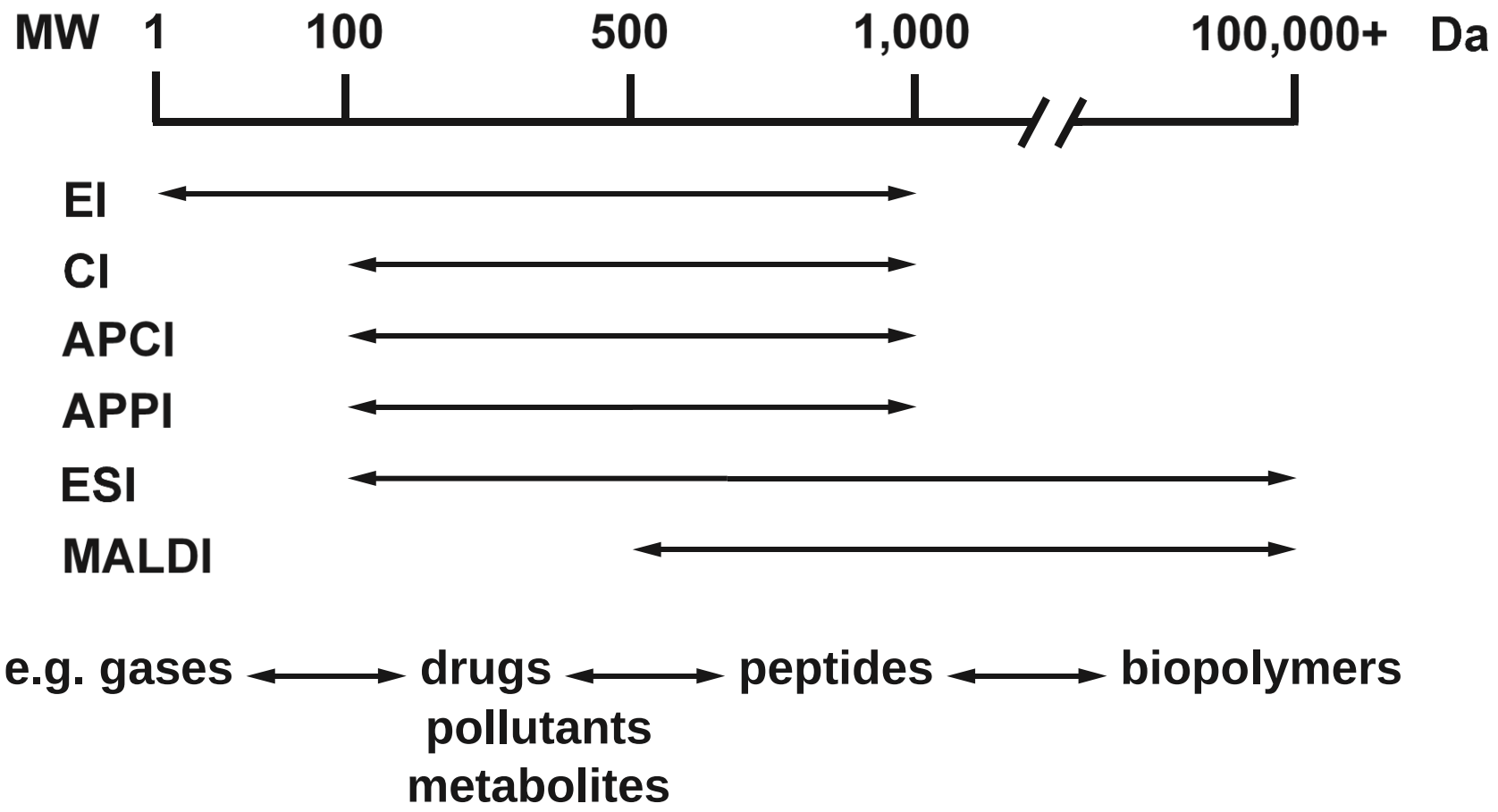
Mass spectra of dioctylphthalate under different ionisation conditions

The choice of ionisation method is often determined by the polarity of the analyte



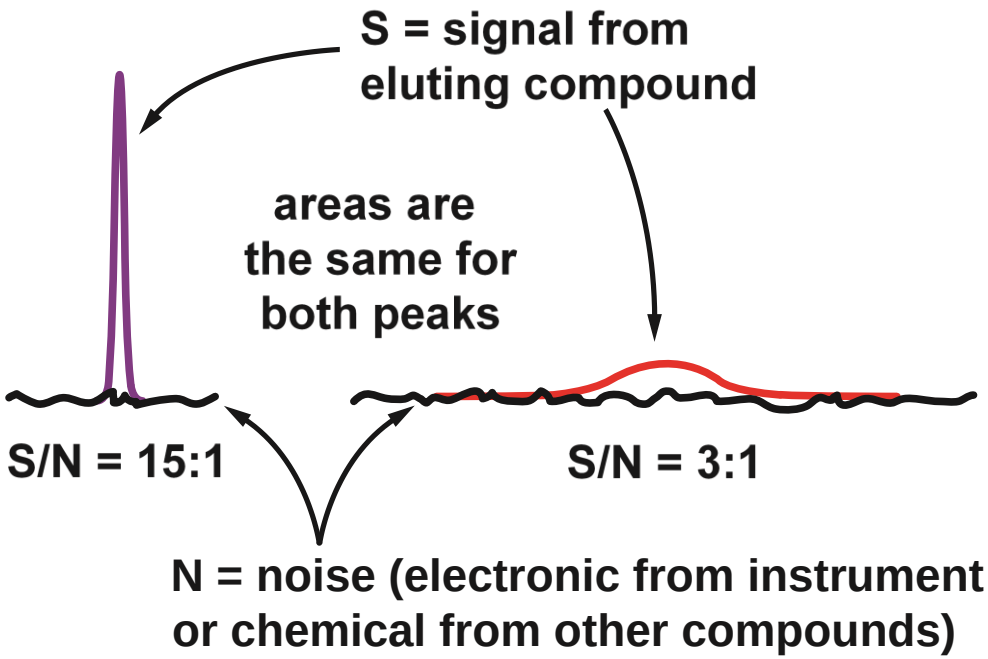
e.g. alkanes → drugs → metabolites → biopolymers

Mass ranges for different ionisation methods



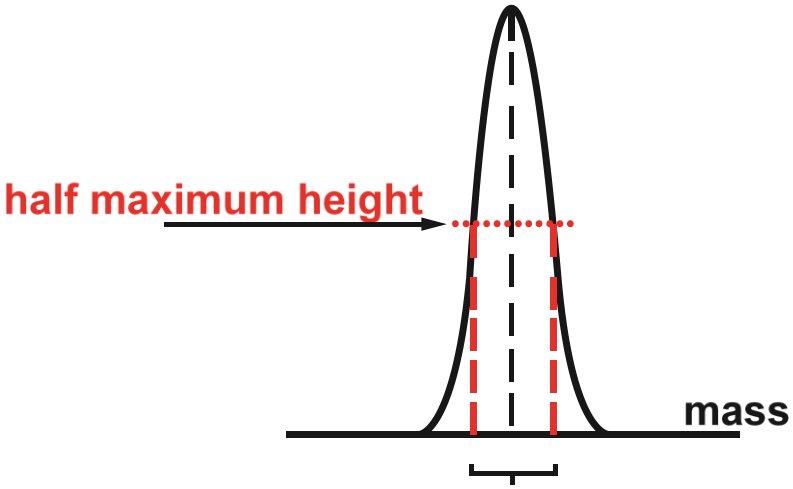
Several ionisation methods are applicable for compounds in the 100 to 1,000 Da range. The method chosen is often determined by the nature of the objective, e.g., EI for structural information and CI for quantification. Above 1,000 Da, ESI or MALDI is selected.

Signal-to-noise ratio vs. peak width



The signal-to-noise (S/N) ratio improves when the width of the chromatographic peak is reduced. The amount of material injected is the same in both cases shown. However, the number of ions arriving per unit time at the detector, i.e., the concentration, increases as the peak narrows. The higher concentration improves the S/N ratio. In the illustration the detection limit is increased by a factor of five.

The resolution of one mass from another and the sensitivity of ion detection are arguably the most important performance parameters of a mass spectrometer.



$$\text{Resolution} = \frac{\text{mass}}{\text{width at half maximum height}}$$

Resolution is a measure of the ability of a mass analyser to separate ions with different *m/z* values.

Resolution determined experimentally from the measured width of a single peak at a defined percentage height of that peak and then calculated as $m/\Delta m$, where m equals mass and Δm is the width of the peak.

The full width of the peak at half its maximum height (FWHM) is the definition of resolution used most commonly.