An Introduction to Gas Chromatography Mass Spectrometry

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Textbooks:-

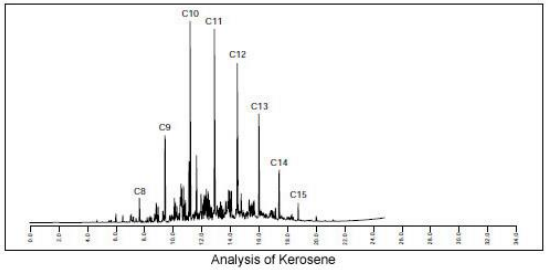
"*Analytical Chemistry*", G. D. Christian, P. K. Dasgupta, K.A. Schug, Wiley, 7th Edition

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

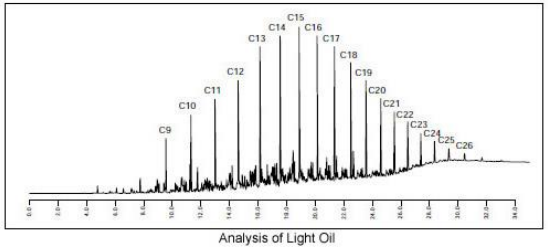
"*Mass Spectrometry for the Novice*", J. Greaves and J. Roboz

"*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.

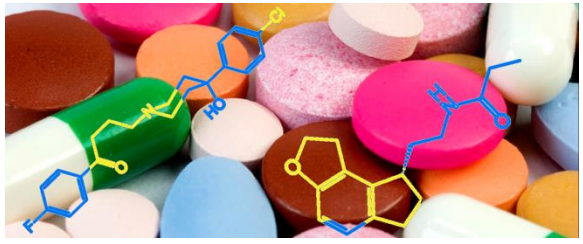


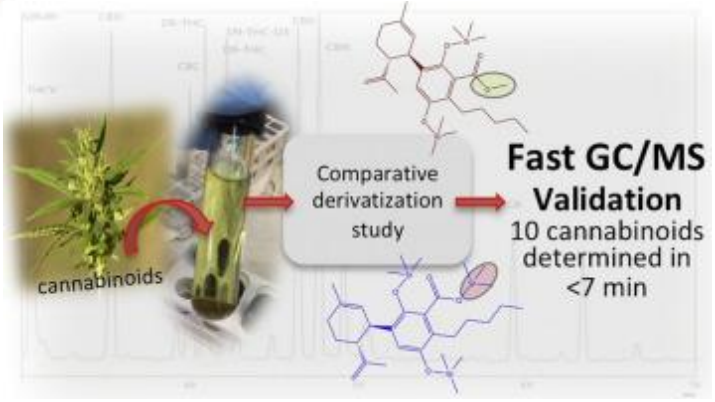
Analysis of Kerosene



Analysis of Light Oil

Mass Spectrometry 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.

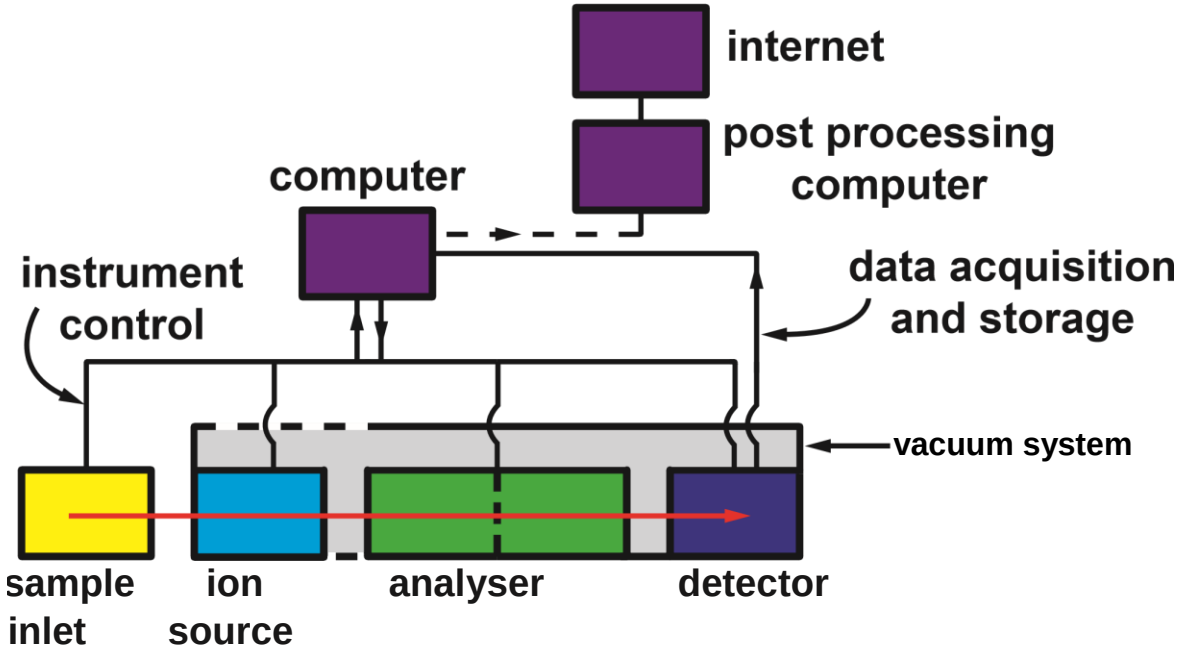




Lecture Overview

- Mass spectrometry definition
- Overview of mass spectrometry instruments
- 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 –the Van Deemter equation
- GC mobile phase
- Gas chromatography columns
- Gas chromatography mass spectrometry (GC-MS)
- Ionisation methods
  - Electron Impact Ionisation (EI) /Chemical Ionisation (CI)
- Quadrupole (Q) mass analyser
- An operation of the Q Exactive Orbitrap mass spectrometer

What are the major components 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.

Definitions concerning instruments, mass and *m/z* and ions

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.

What are ions? Ions are atoms, molecules or fragments of molecules that carry one or more positive or negative electrical charges.

What is mass to charge (*m/z*) ratio? of an ion is the number obtained by dividing the mass of the ion (*m*) by the number of electrical charges (*z*) acquired by the sample during the ionisation process. The *m/z* of an ion is dimensionless number:- *m* and *z* are always written in italics.

What is *m* in *m/z*? The scales of atomic masses are based upon an agreed standard by IUPAC. Today carbon **^{12}C** is taken to have an atomic mass of **12.000000000 Da.** The atomics masses of the other elements and their isotopes are measured relative to this.

^{12}C =12.00000000
^{1}H = 1.007825035
^{14}N =14.003074002
^{16}O =15.99491463

What is *z* in *m/z*? The electrical charge (positive of negative) present on an ion is represented by *z*. In most cases there is only one charge on an ion; thus, the measured *m/z* value is equivalent to the mass of the ion (*z*=1)

What is ionic mass? The ionic mass of an ion takes into account the mass of an electron (0.000548Da =0.548 mDa) that is removed or added during the formation of the ion.

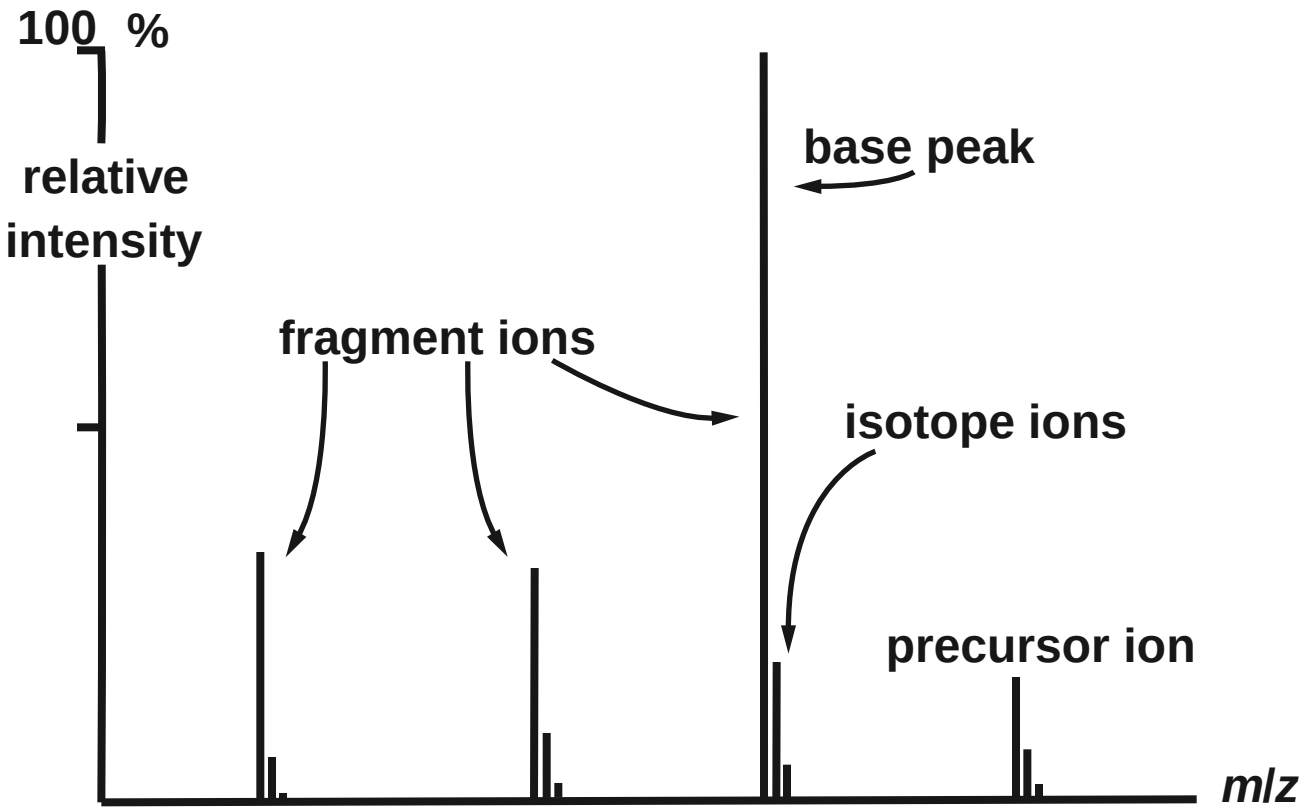
Monoisotopic mass- the mass of an ion which is made up of the lightest stable isotopes of each element (includes the mass defect, where ^{1}H=1.0078, ^{12}C=12.0000, ^{16}O=15.9949 etc).

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%

Isobaric mass empirical formulae that have the same nominal mass but different exact masses
The molecular mass of ammonia (NH_3) =14.003074002+(3x1.007825035) =17.026549
The molecular mass of OH = 15.99491463+1.007825035 =17.00274

What is a mass spectrum?

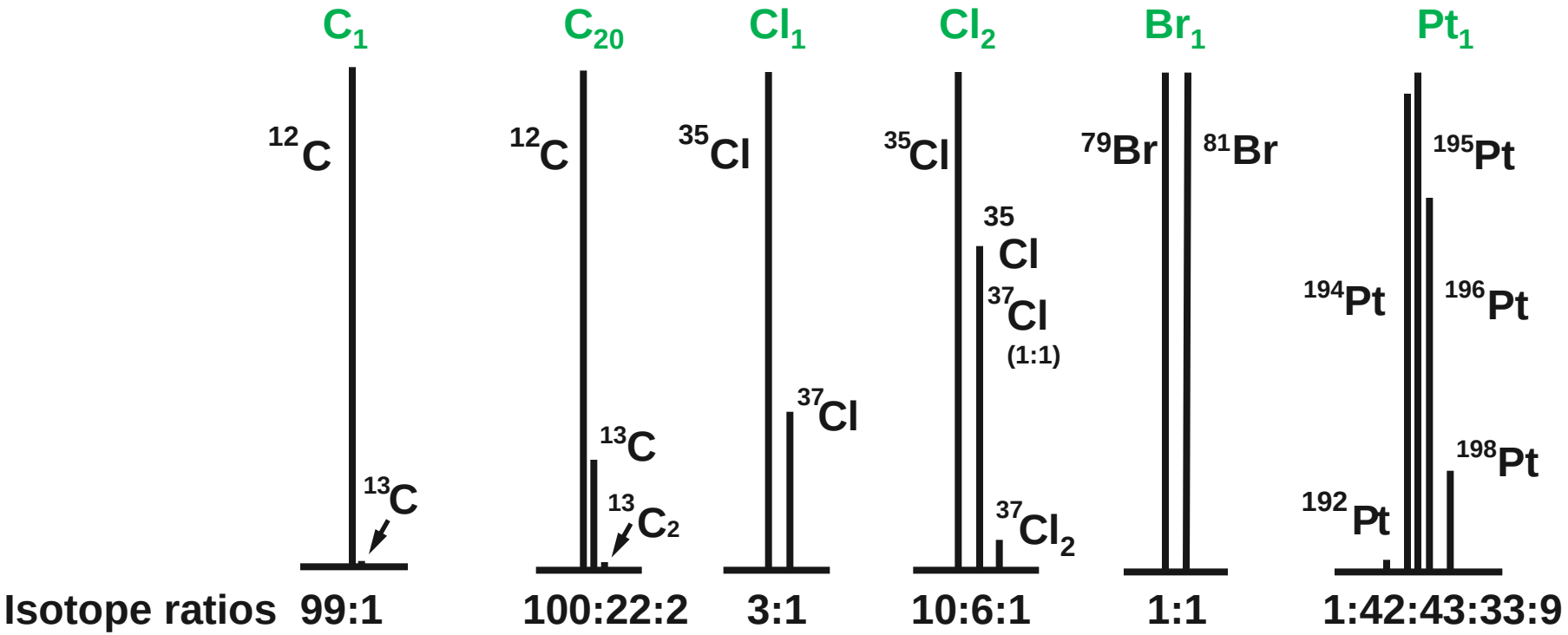


***m/z* = mass to charge ratio**

- 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).

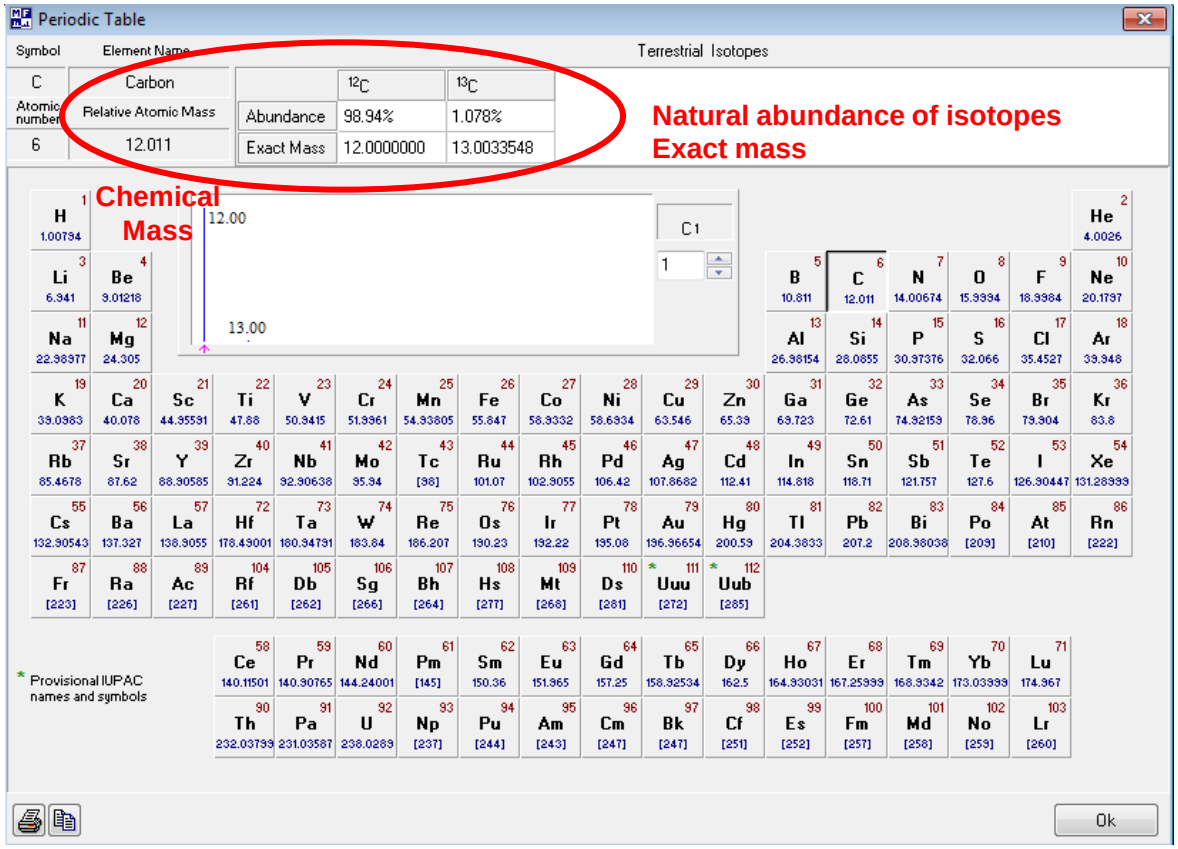
What are isotope peaks?

The atoms of elements are composed of protons, electrons and neutrons. An element is defined by the number of protons present in the nucleus. The number of neutrons may vary over a small range, yielding products – **isotopes**



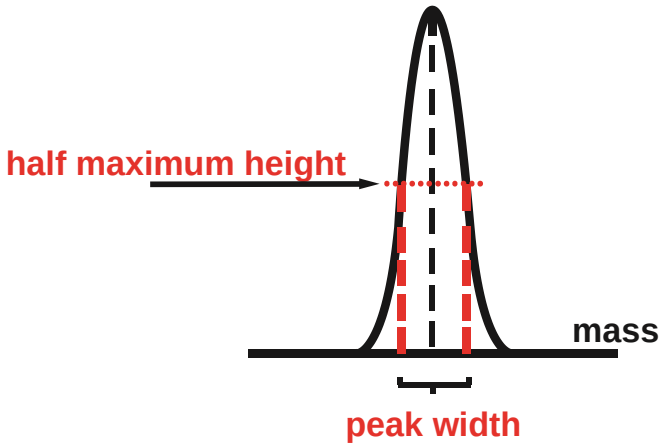
The presence of the ^{13}C isotope, which occurs with a natural abundance of 1.1% per carbon atom, results in the isotope peaks that are commonly found on the right-hand side of all ions in mass spectra.

What Is An Isotope?



Each element can have different natural isotopes with different abundances

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



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

Calculation of resolution (FWHM, full width at half maximum height definition)

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 most used.

What is MS/MS?

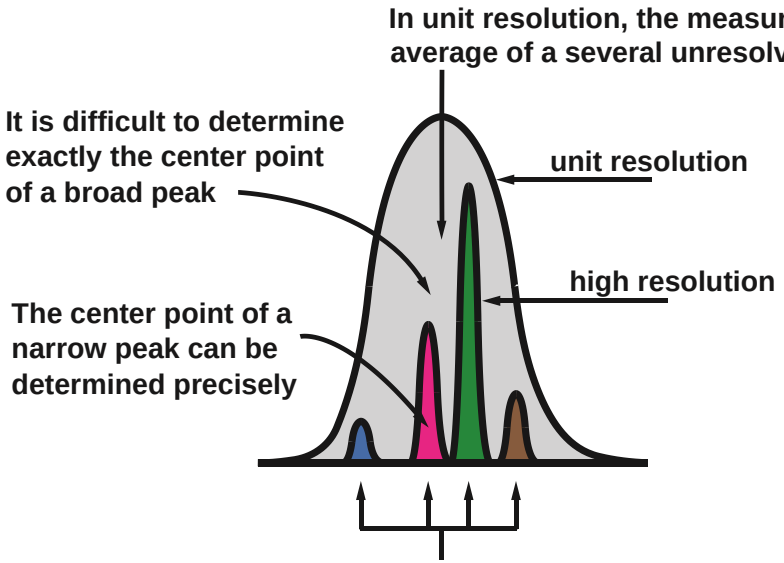
Tandem mass spectrometry conducting two mass separations consecutively within the same instrument. MS/MS is based on analysing the products of *controlled collisions* between selected ions (*precursors*) and neutral gas molecules in pressurised collision cells that are placed in specific regions of the instrument.

Tandem-in-space the first analyser to select *specific ions* from the total beam arriving from the ion source. Next, the selected ions undergo collision-induced dissociation (CID) in a pressurised cell followed by analysis of the *product ions* in the second analyser.

In *tandem-in-time* the same analyser is used for both scans, but in different time (an ion trap analyser).
Selected ion monitoring (SRM) mode of MS/MS operation

What is accuracy of mass measurement?

The difference between the calculated and experimentally determined masses of an ion provides a numerical measure of accuracy of the experimental data.



Isobaric ions have the same nominal mass but different empirical formulae, and thus different accurate masses. Isobaric peaks can be resolved by high resolution.

High resolution improves the process of accurate mass measurement

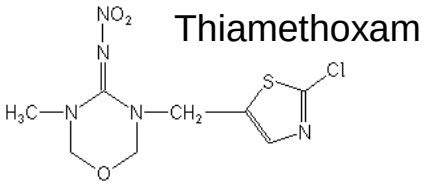
Theoretical *m/z* 500.0025
Measured *m/z* 500.0000

ppm Error = $(m_{theoretical} - m_{measured}) / m_{theoretical} \times 10^6$

ppm Error = $(500.0025 - 500.0000)/500.0000 \times 10^6 = 5$ ppm

Scan speed is the rate at which mass spectra are acquired, and measured in masses/unit time.

Resolution significance

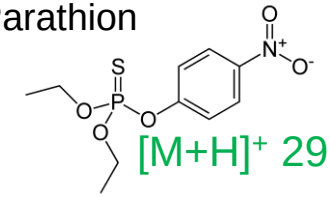


Thiamethoxam

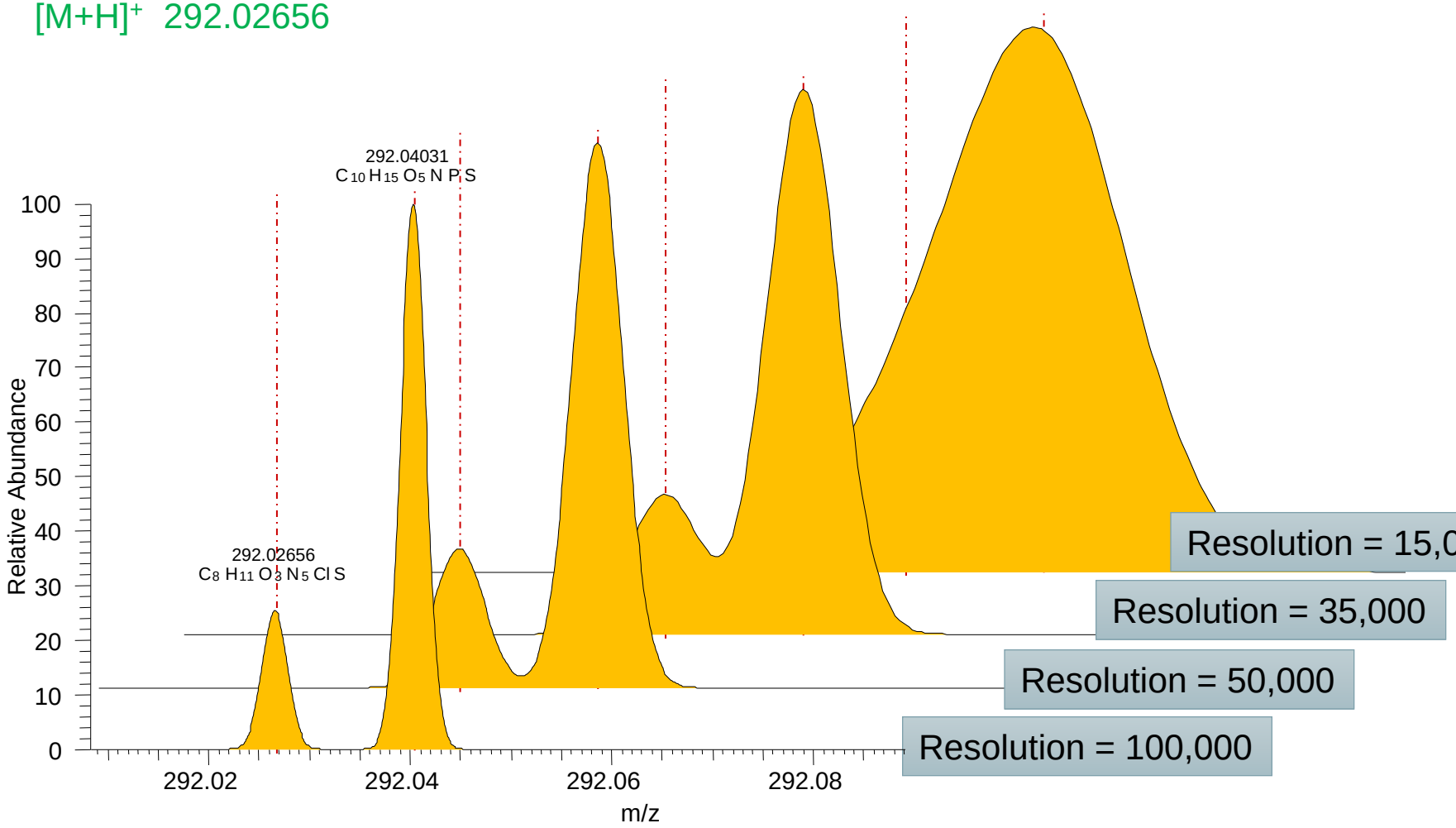
$[M+H]^+$ 292.02656

Δm
0.0138 Da

Parathion



$[M+H]^+$ 292.04031



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**)
(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)

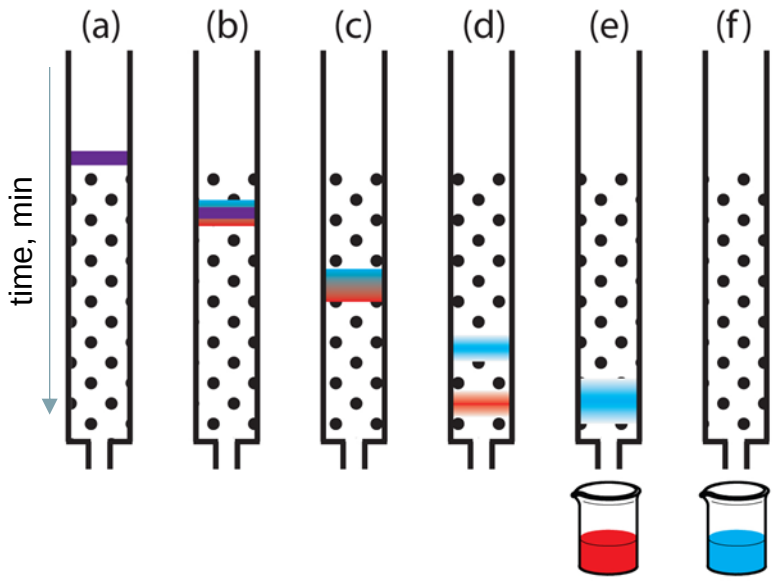
Principles of chromatographic separations

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 partition ratio is simply the ratio of the time a solute spends in the stationary phase to that it spends in the mobile phase

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.

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.

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*).

Key equations for chromatography

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 Capillary (open tubular) GC Column	$H = A + \frac{B}{\bar{u}} + C\bar{u}$
Golay equation Packed GC column	$H = A + \frac{B}{\bar{u}} + C_s\bar{u} + C_m\bar{u}$
Resolution	$R = \frac{t_{R2} - tR_1}{(W_{b1_+} W_{b2})^2}$
Separation factor	$\alpha = \frac{t'_{R2}}{t_{R1}} = \frac{k_2}{k_1}$
Resolution	$R_s = \frac{1}{4}\sqrt{N}\left(\frac{a-1}{a}\right)\left(\frac{k_2}{k_{ave}+1}\right)$

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

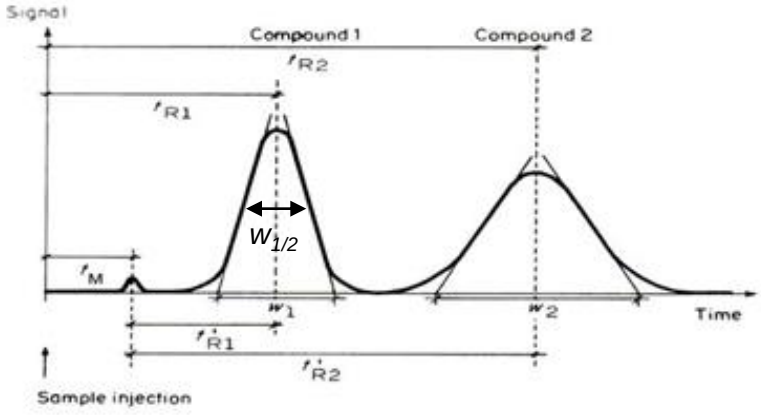
N - the number of theoretical plates

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 $N= 5.545(\frac{t_R}{w_{1/2}})^2$

(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)

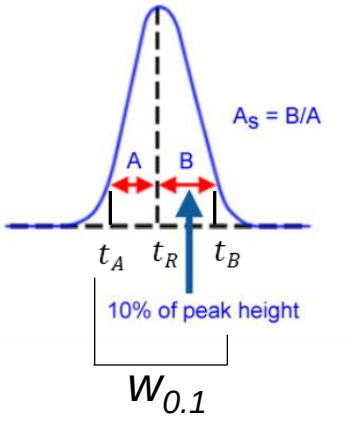
$N = 16(\frac{t_R}{w_b})^2$

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:

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

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.

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

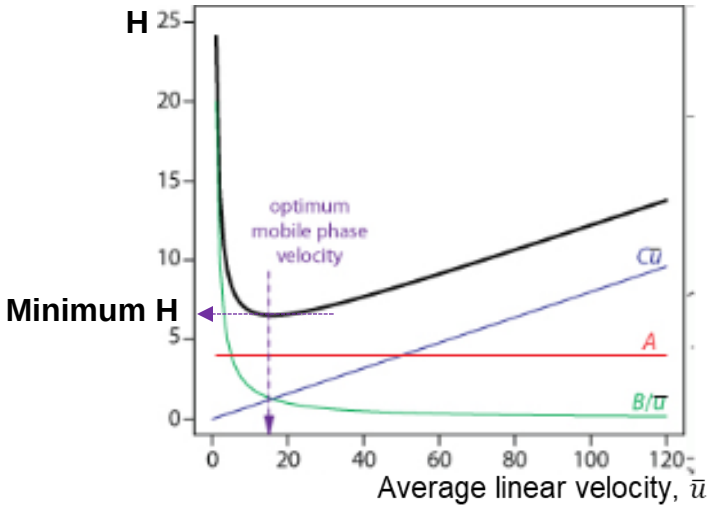
$$H = A + \frac{B}{\bar{u}} + C\,\bar{u}$$ *For a packed GC column the van Deemter equation*

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 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

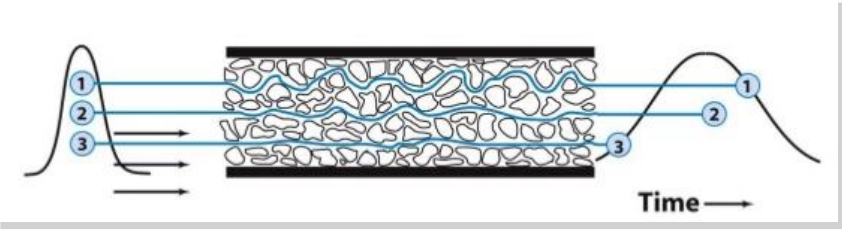
A- Eddy diffusion and is due to the variety of variable length pathways available between the particles in the column and is independent of the gas- and mobile-phase velocity and relates to the particle size and geometry of packing.

$A = 2\lambda d_p$

λ- an empirical constant

(depend how well the column is packed)

d_p -the average particle diameter



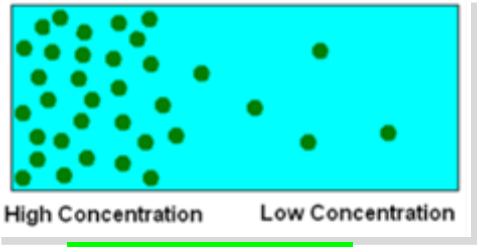
GC is used at modest pressures, and very fine tightly packed support are not used.

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

$B = 2\gamma D_m$

ɣ- 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;

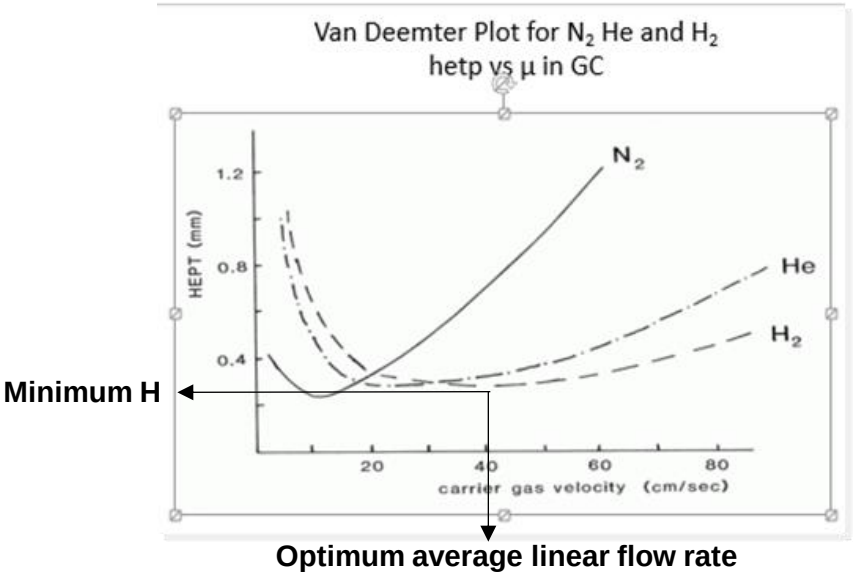
$\frac{d_f^{\,2}}{D_s}$ represents the characteristic time for the analyte to diffuse in and out of the stationary phase.

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*

GC mobile phases and the Van Deemter plot

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.



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:-

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

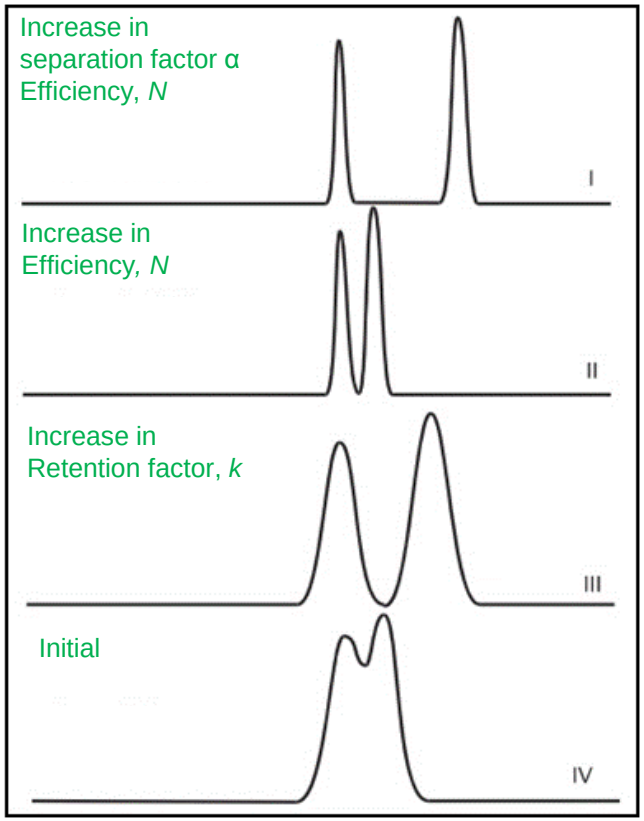
t_{R1} and t_{R2} are the retention times of the two peaks (peak 1 elutes first)

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.

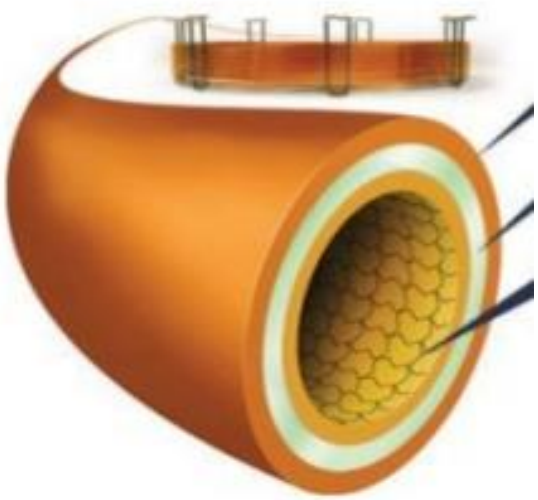
Gas chromatography (GC)

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

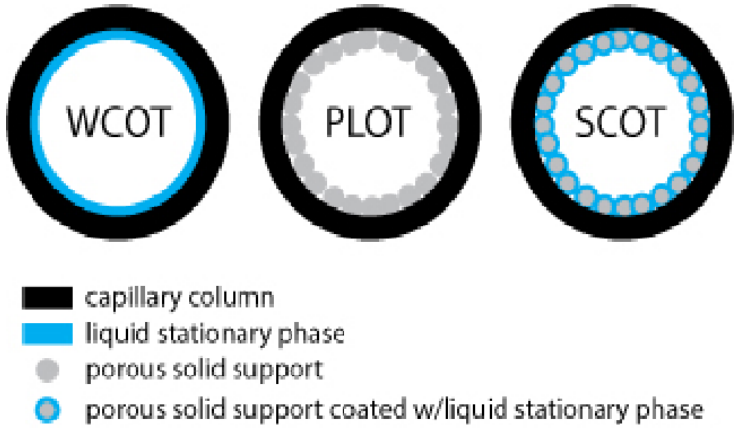
The inner surface of the capillary is chemically treated to minimise interaction of the sample with the silanol groups (Si-OH) on the tubing surface, by reacting the Si-OH group with a silane-type reagent.

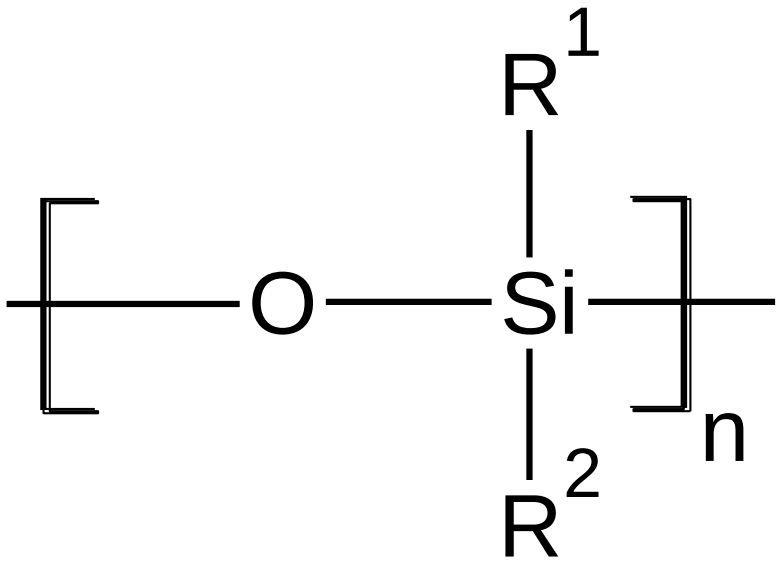
The capillaries are 0.10 to 0.53 mm internal diameter, with lengths of 15 to 100 m.

Capillary columns most popular used

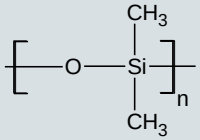
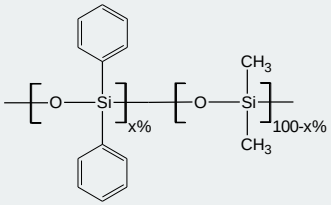
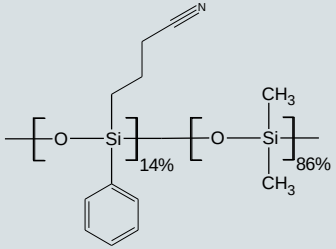
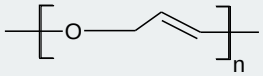
There are three types of open-tubular columns:-

- 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.

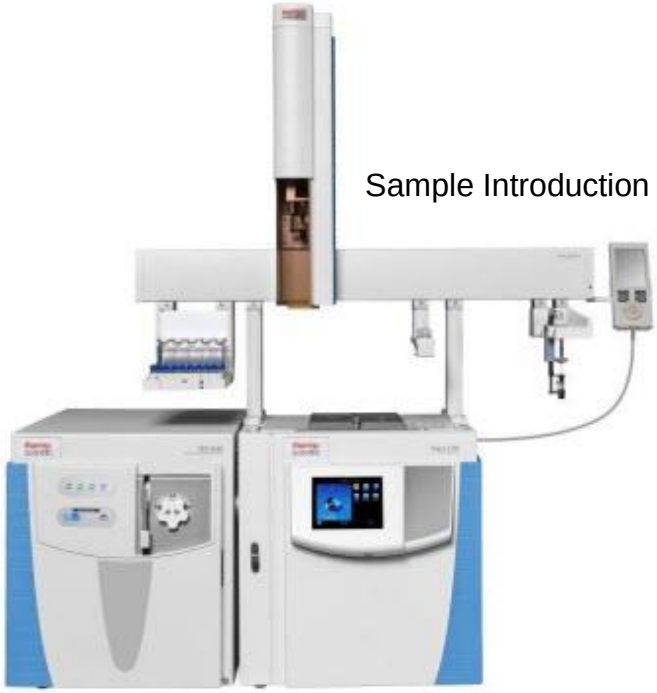




Capillary fused silica stationary phases

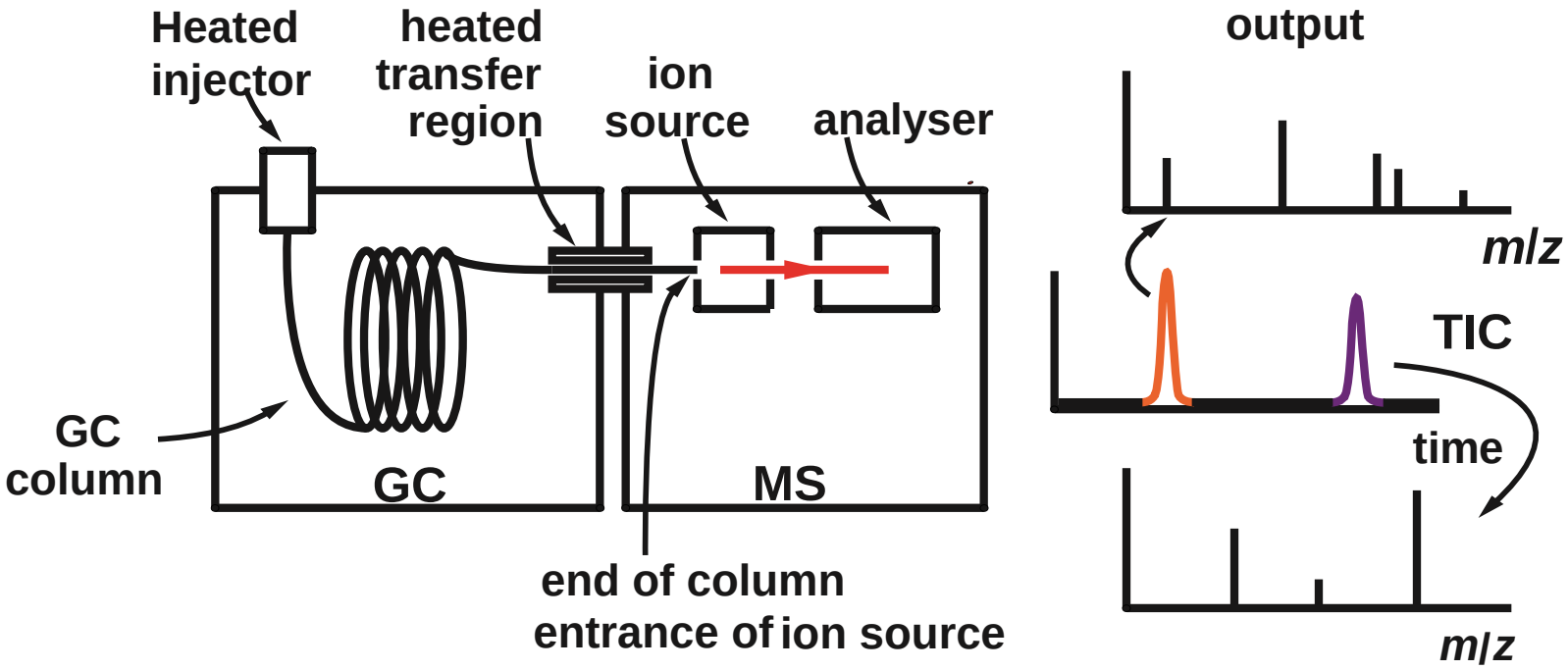
Phase	Polarity	Use	Max Temp. (°C)
100% dimethyl polysiloxane 	Nonpolar	Basic general purpose phase for routine use. Hydrocarbons, polynuclear aromatics, PCBs	320
Diphenyl, dimethyl polysiloxane 	Low (x=5%) Intermediate (x=35%) Intermediate (x=65%)	General purpose, good high temperature characteristics. 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. A phase should be selected in which the solute has some solubility. 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.

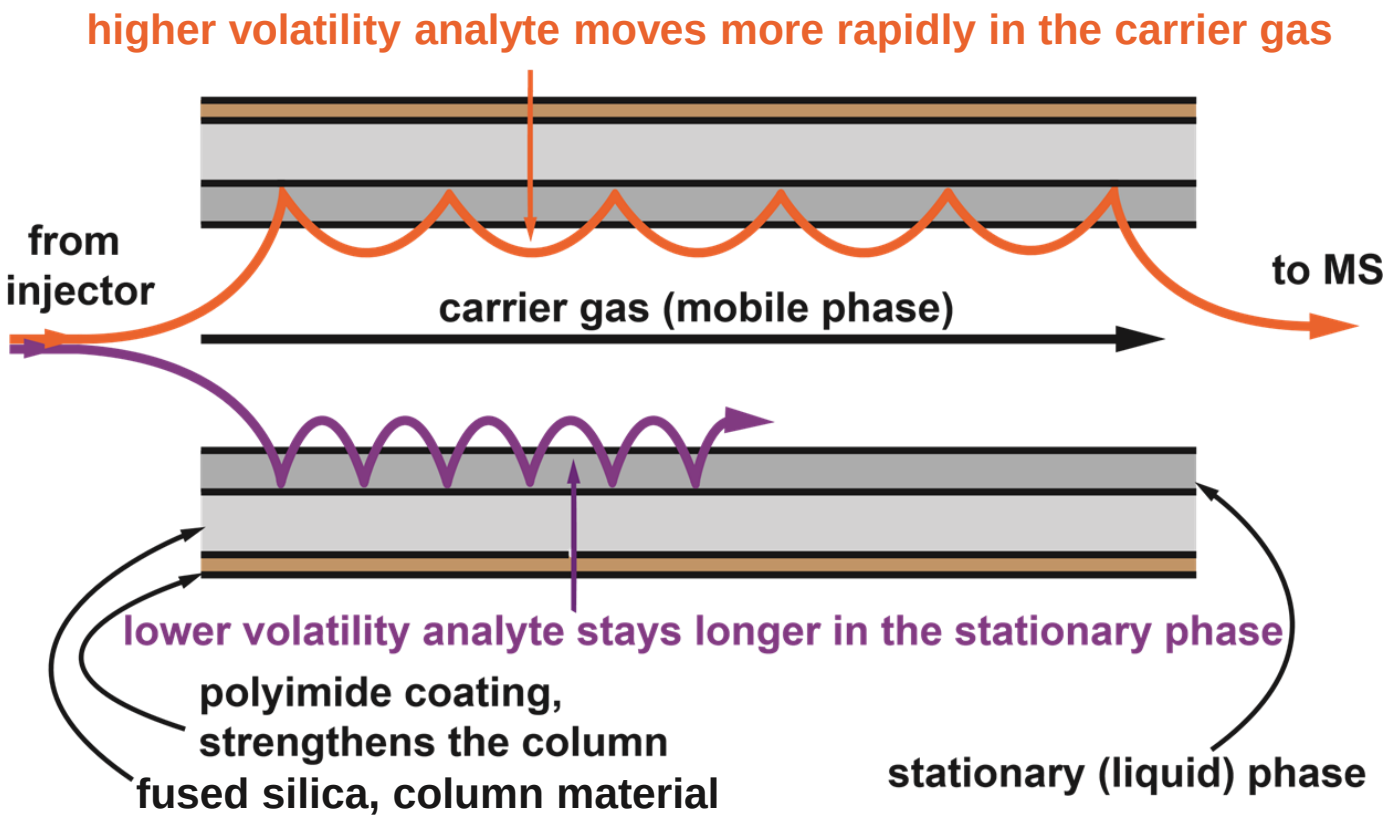

Sample Introduction
Ion source and mass detection
Gas Chromatograph


Sample Introduction
Q Exactive mass spectrometer
Ion source
Gas Chromatograph

Gas chromatography-mass spectrometer (GC-MS)



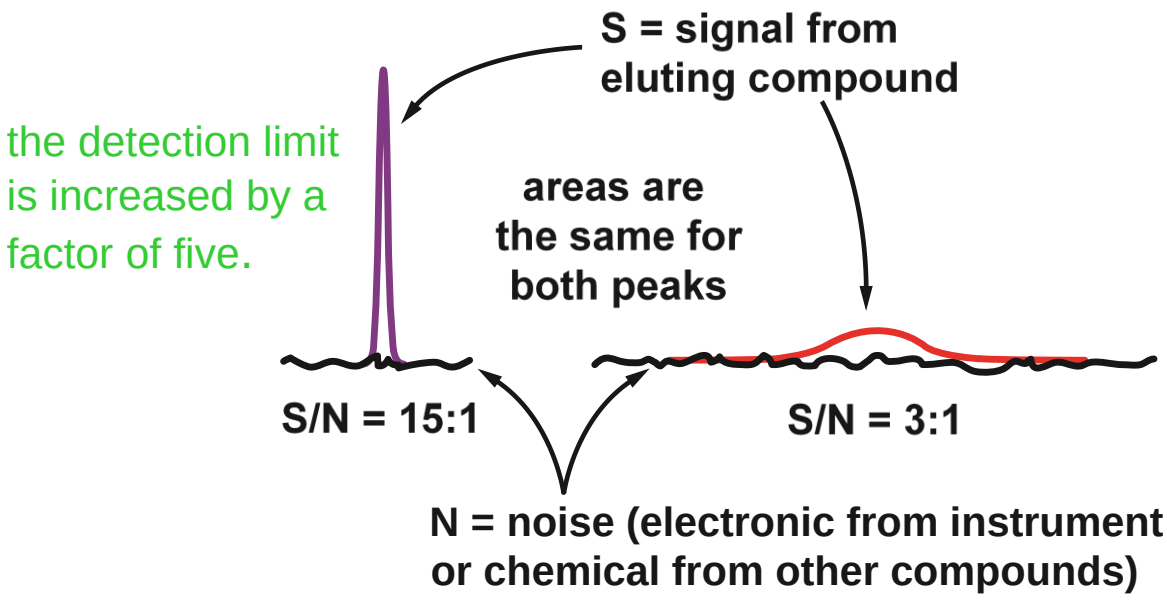
The sample is converted to the vapor state (if not already in a gas) by the injection into a heated port, and the eluent is a gas (the carrier gas). The stationary phase is non-volatile liquid or a liquid-like phase bonded on the capillary wall, which determines interactions of analytes and stationary 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 carrier 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 data consists of total ion chromatograms (TICs) 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.

Signal-to-noise ratio vs. peak width

The sample capacity of capillary columns is limited (pico- to nanograms per sample component). The column produce peaks that well separated and only a few seconds wide.



- 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.

Methods of sample introduction into the GC-MS instrument

- **Liquid injection**
- **Headspace analysis** for species with low molecular masses and high volatility
- **Thermal desorption** for analytes trapped by passage through a cartridge packed with a retentive material such as Tenax
- **Solid phase microextraction fiber (SPME)** elution for compounds that have been adsorbed onto the fiber from liquid or gaseous matrices

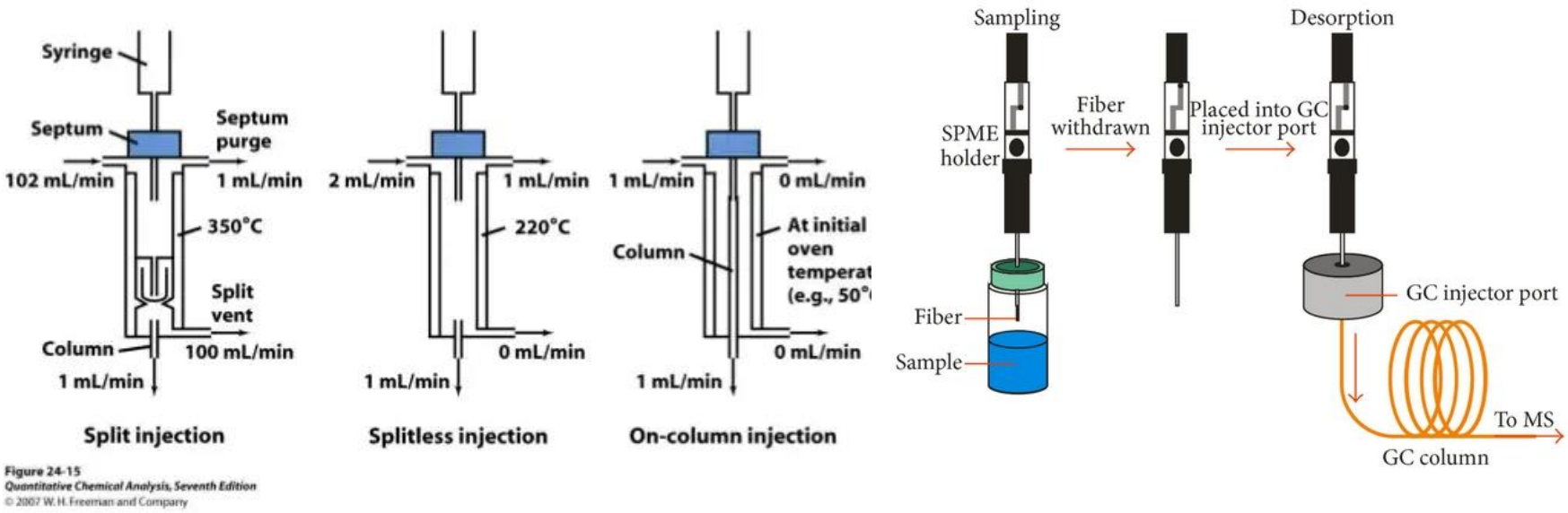
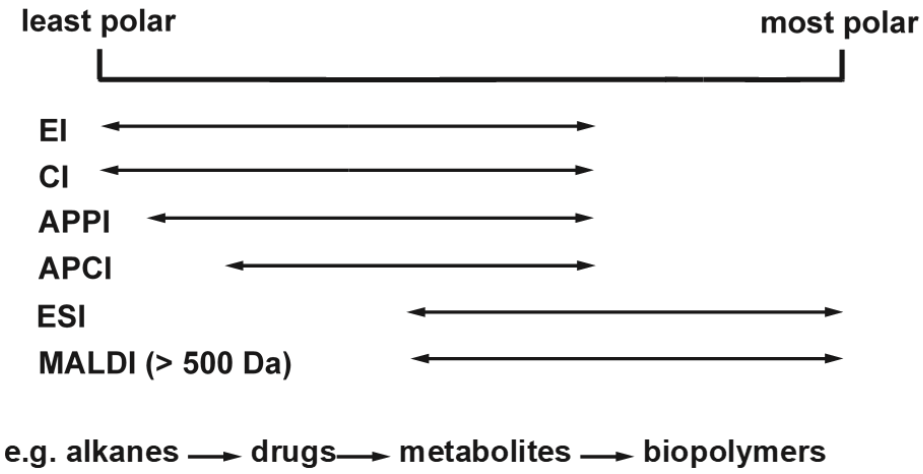


Figure 24-15
Quantitative Chemical Analysis, Seventh Edition

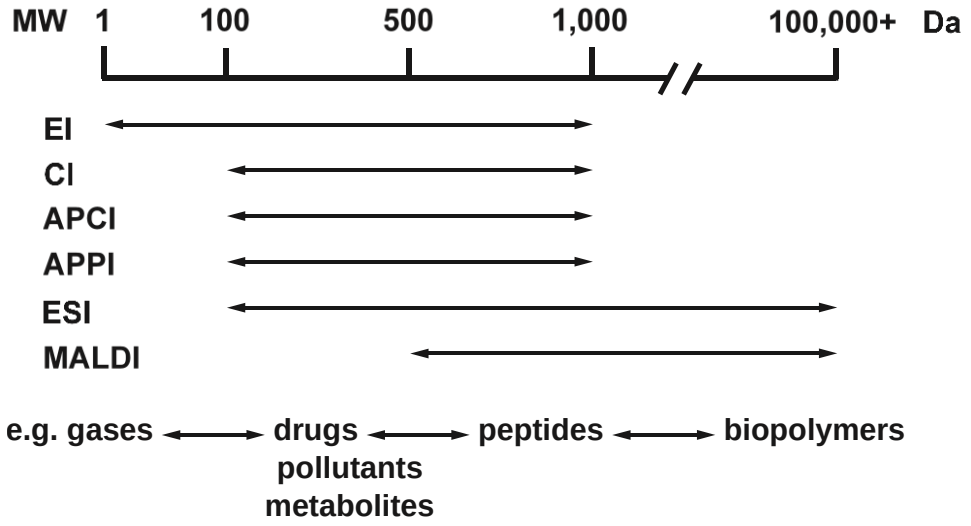
The need to volatilize samples has always been a limiting factor in GC-MS. As a rule of thumb, “one polar group good, two polar groups bad and do not run acids” is a useful guidelines. Primary amines (NH_2-), hydroxyls (-OH), and carboxylic acids (-COOH) are the most relevant polar groups.

Ion sources and methods of ionisation

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



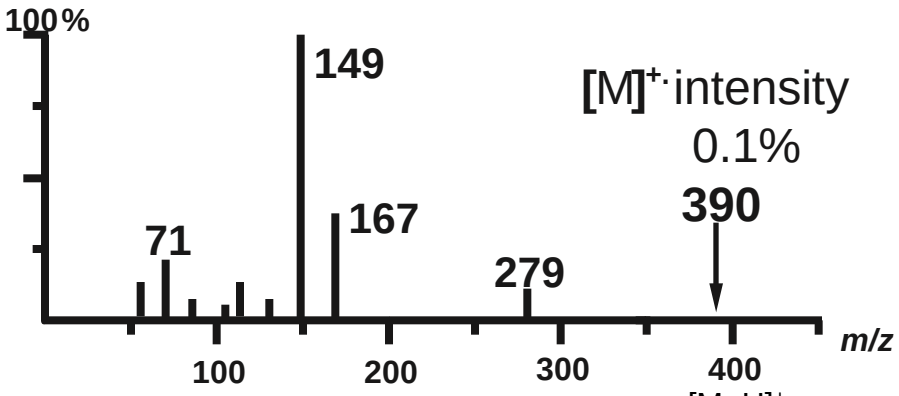
Several ionisation methods are applicable for compounds in the 100 to 1,000 Da range. The method chosen is often determined by the aim, e.g., EI for structural information and CI for quantification. Above 1,000 Da, ESI or MALDI is selected.



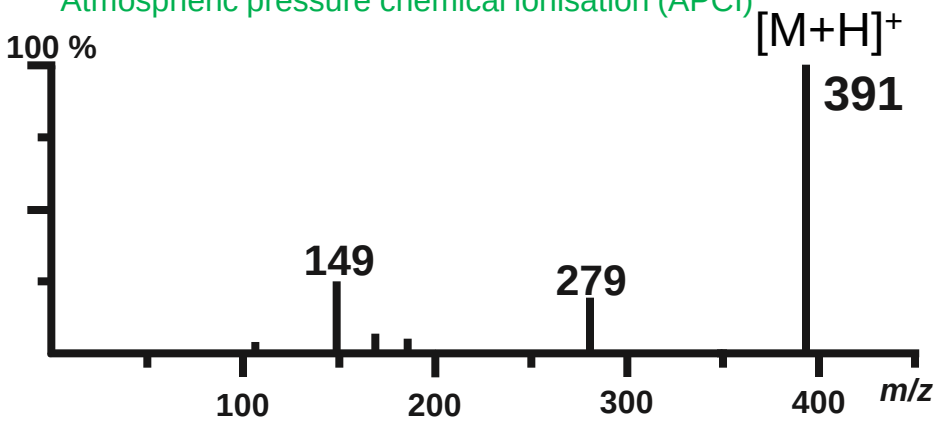
Mass ranges for different ionisation methods

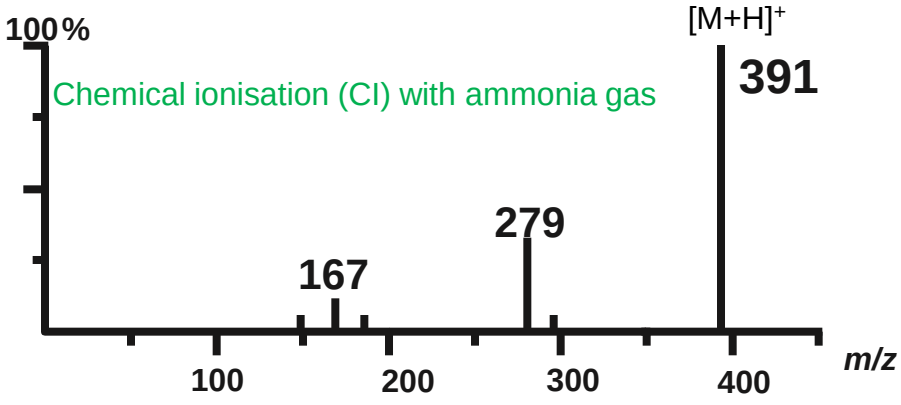
Mass spectra of dioctylphthalate under different ionisation conditions

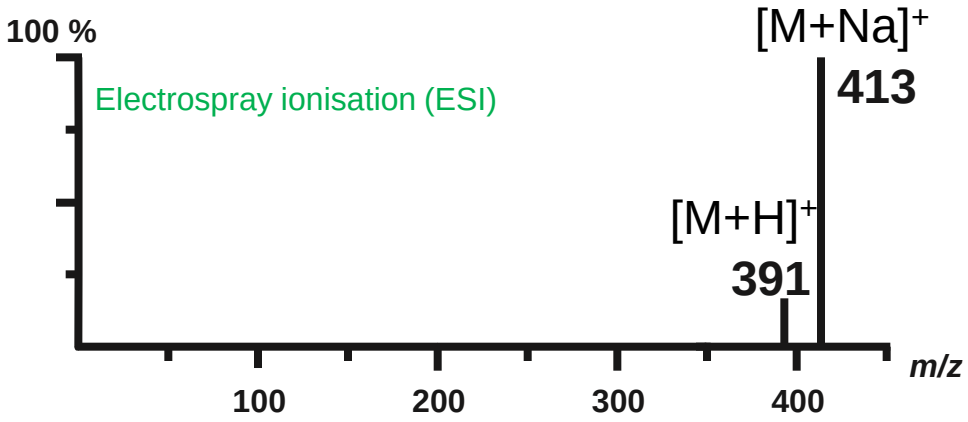
Electron ionisation (EI)

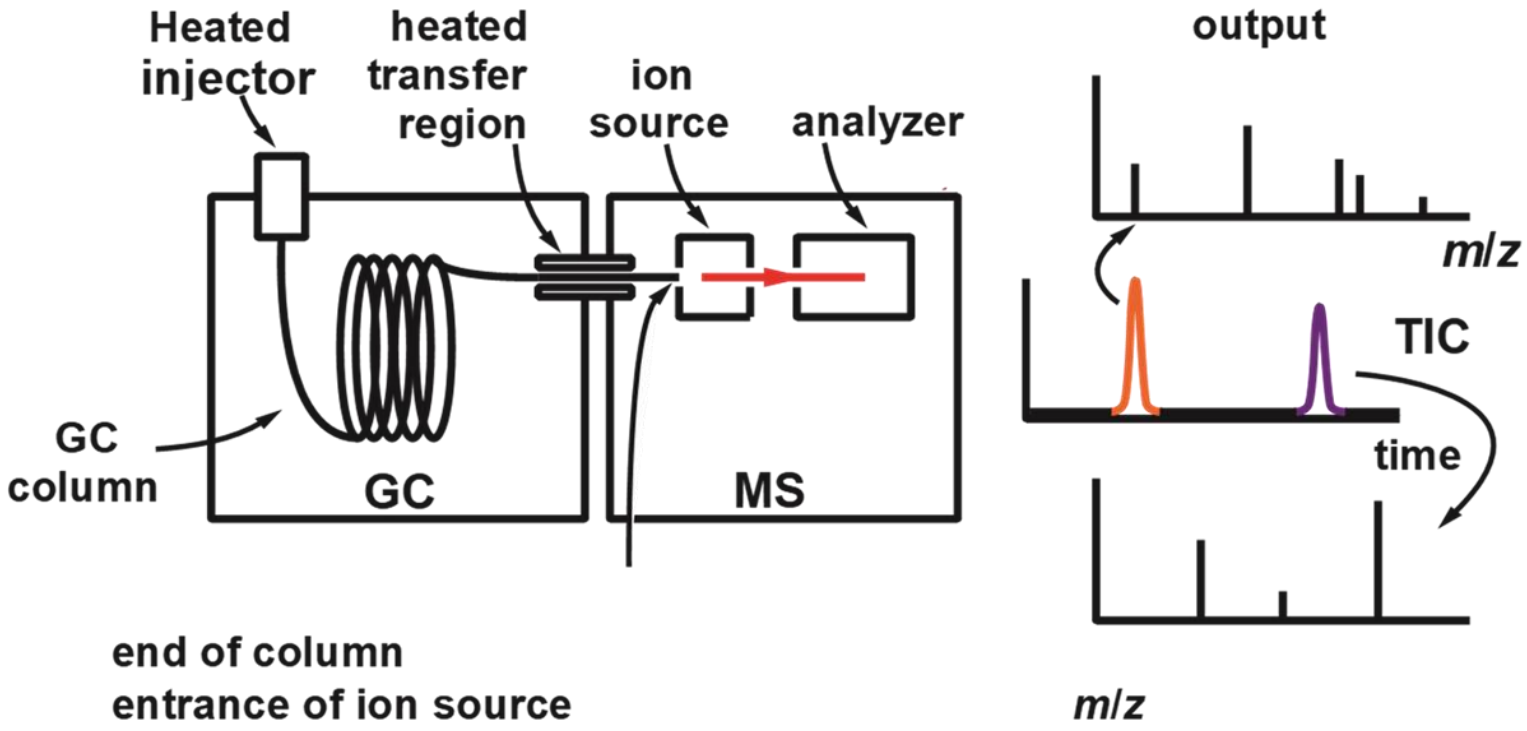


Atmospheric pressure chemical ionisation (APCI)





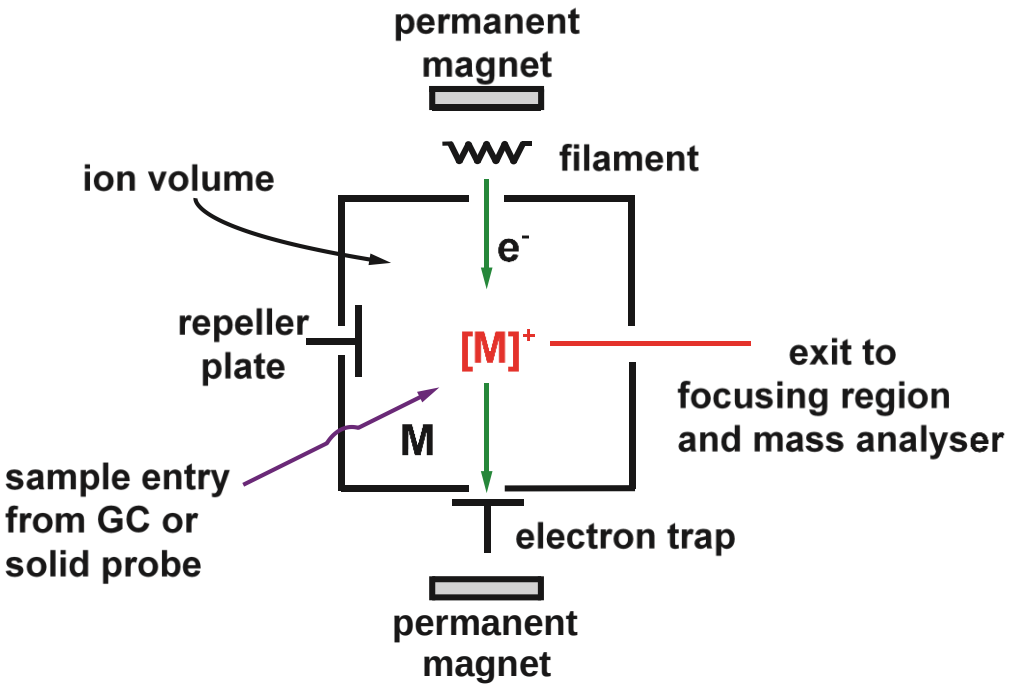




Samples are introduced into the GC using a heated injector. Components are separated on a column, according to a combination of molecular mass and polarity, and sequentially enter the MS source via a heated transfer region. The analytical data consists of total ion chromatograms (TIC) and the mass spectra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

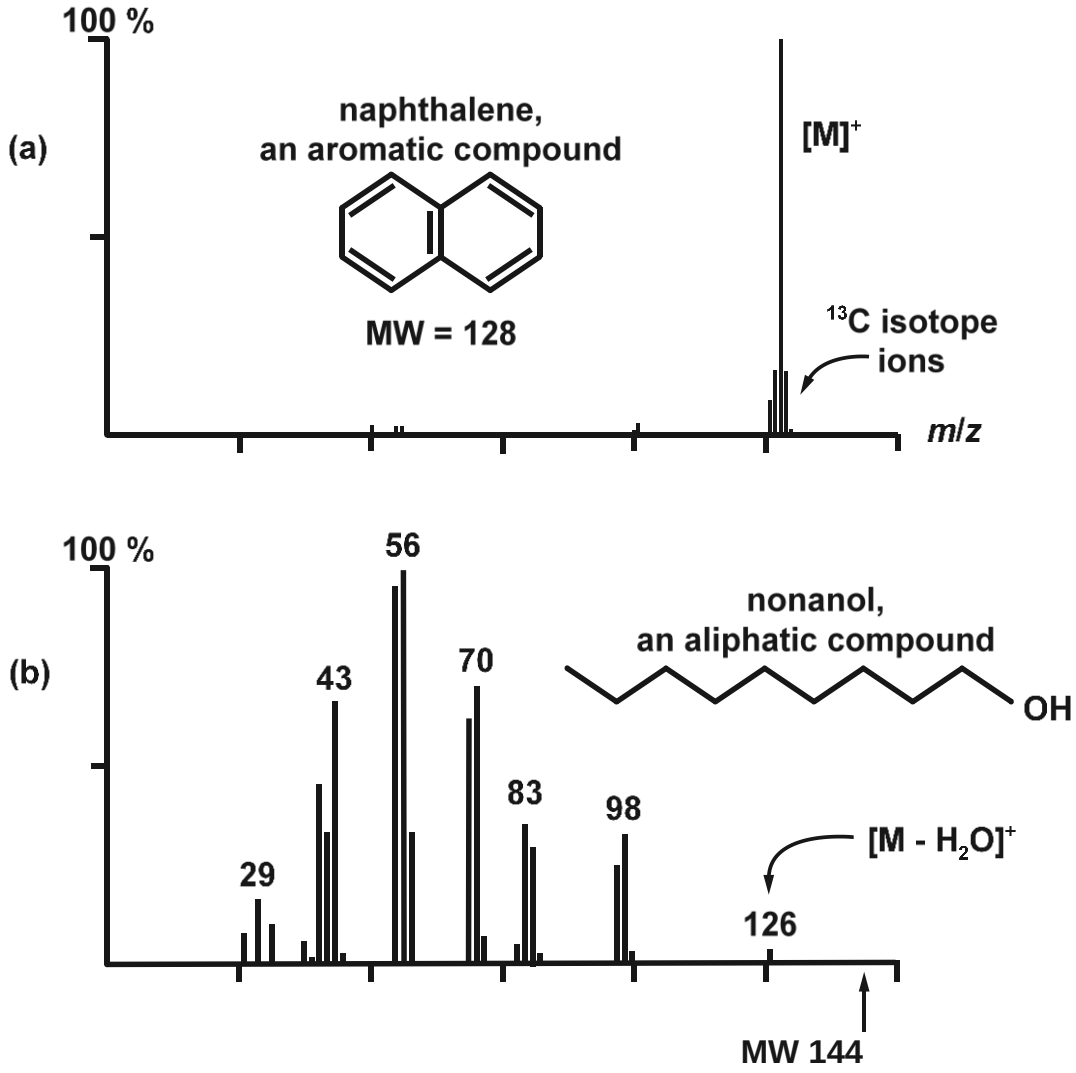
Ionisation methods – Electron Impact Ionisation (EI)

- The ion source are held at 250℃ and under vacuum 10^{-7}-10^{-2} Torr range.
- 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)
- A potential difference between the filament and a trap on the opposite side of the source, directs the electrons across the ion volume.



- 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.

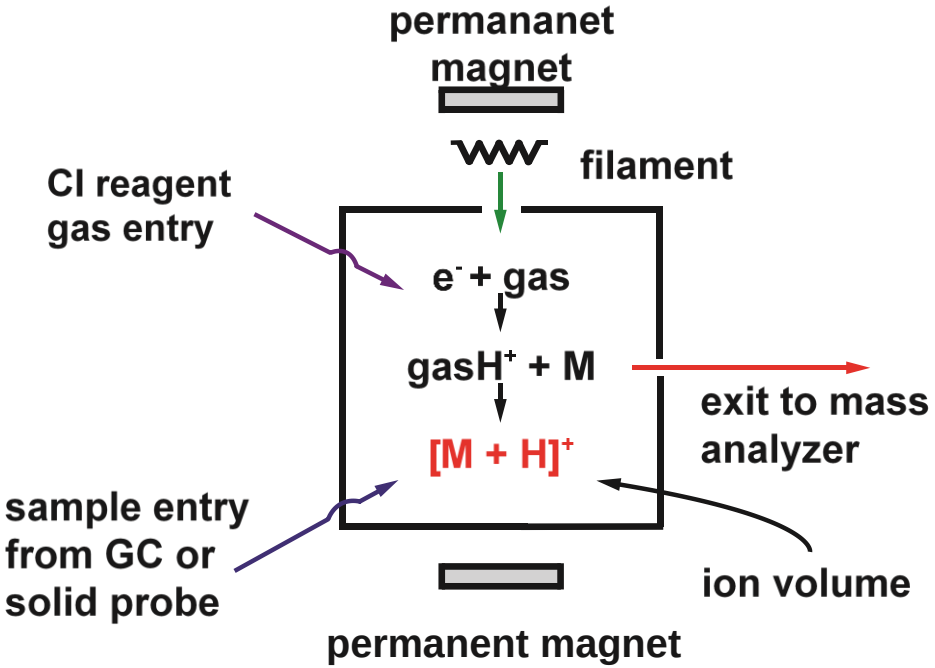
Comparison of the EI spectra for (a) an aromatic and (b) an aliphatic compound



The reproducibility of EI spectra, both in terms of fragmentation and between instruments, has led to the creation of large libraries of spectra.

The current NIST/EPA/NIH library contains close to 250,000 EI spectra.

Chemical ionisation (CI) source



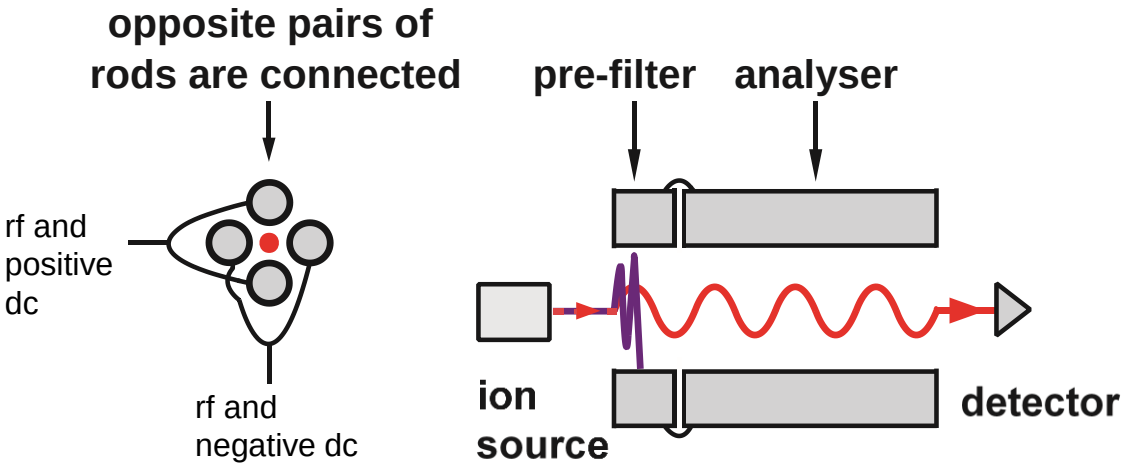
- CI volume similar to EI, but made more gastight by narrowing entry and exit slits
- CI does not require magnet or trap since beam does not penetrate thorough gas chamber
- Effective, high-speed pumping system required to maintain source pressure of ~10^{-4} mbar, analyser requires differential pumping
- 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)

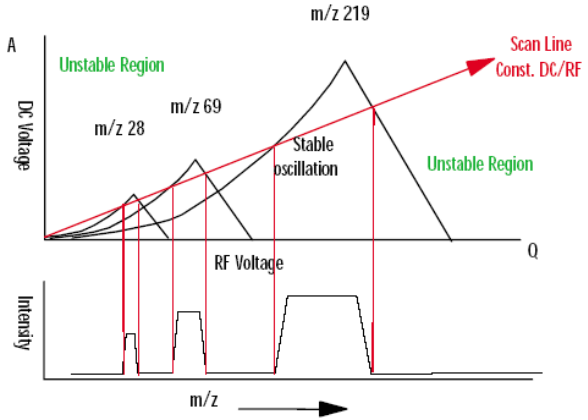
  $NH_3 - e^- \rightarrow NH_3^{+\cdot} + 2e^-$

  $NH_3^{+\cdot} + NH_3 \rightarrow NH_4^{+\cdot} + NH_2$
- 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 $[M + NH_4]^{+\cdot}$

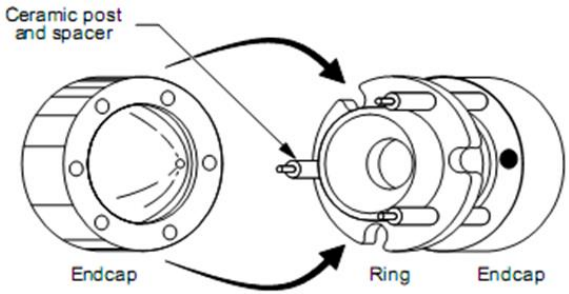
Quadrupole (Q) analyser

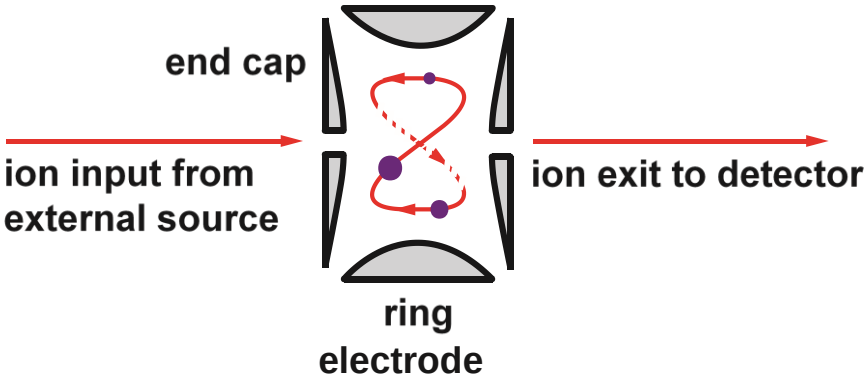


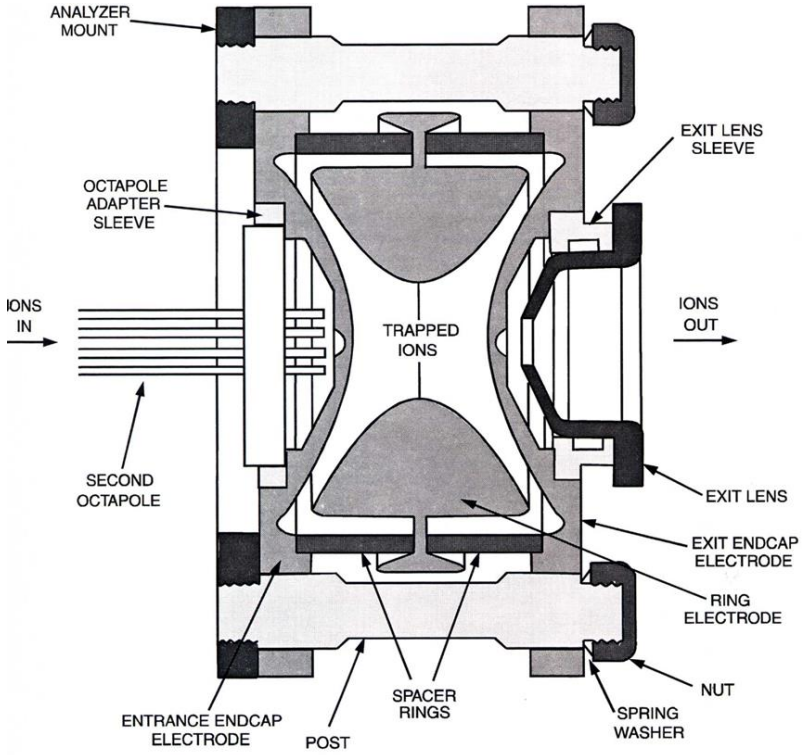


- The voltage placed on one pair of rods is comprised of a positive direct current (dc) combined with a superimposed radio frequency (rf) voltage. The other pair of rods carries a negative dc voltage with an rf component that 180° of phase with that on the first pair. Mass separation is based on the fact that ions begin to oscillate upon entering the field produced by the superimposed rf and dc voltages. For any field, derived from the combination of the voltages, only ions with specific *m/z* values have a stable oscillatory trajectory along the axis of the quadrupole to the detector.
- Unstable ions hit the initial part of the analyser, often a pre-filter, are discharged and lost.
- Scan DC/RF →*m/z*
- *Quadrupoles are usually operated to achieve unit mass resolution to enable optimum sensitivity*

Quadrupole ion trap (QIT) analyser



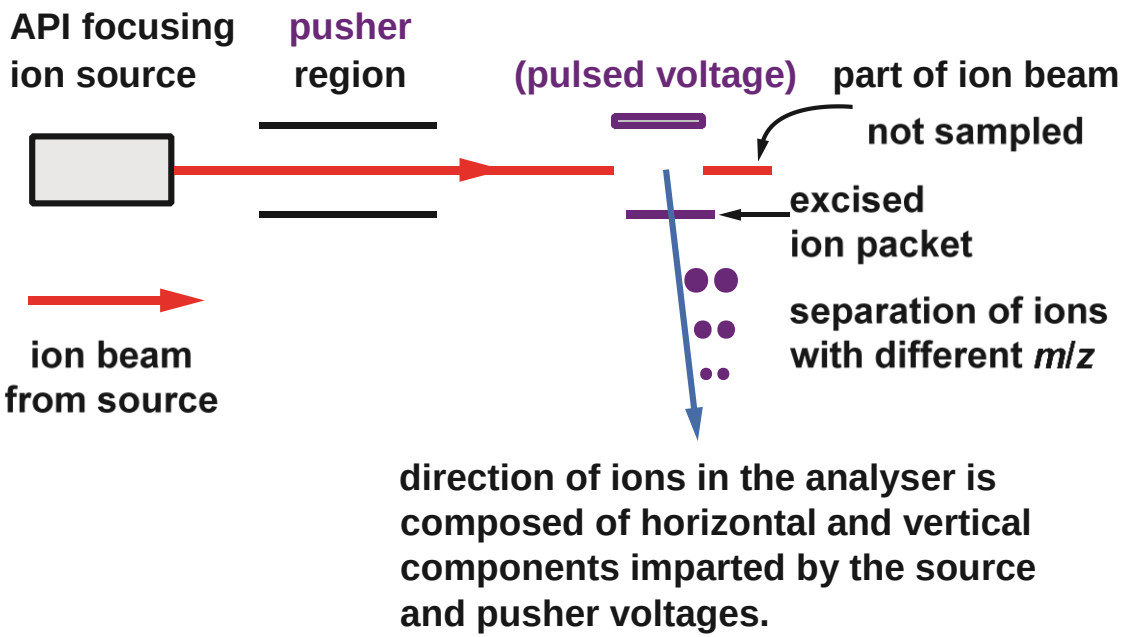




- Ions of all *m/z* values enter the ion trap at the same time. A three-dimensional quadrupole field, created by a combined rf/dc field on the ring electrode and grounded end caps, restrains all injected ions in a complex sinusoidal path within the cell. Helium gas is supply into the cell the gas causes the ions to cluster and remain close to the centre of the cell, where electrical field most symmetrical, resulting ions staying in their most stable orbits.
- By changing the voltages progressively ions with different *m*/*z* values reach their resonance and then are ejected sequentially onto the detector, and mass spectra recorded.
- MS^n fragmentation (*tandem in time*) takes place when a specific *m*/*z* is isolated in the trap and undergoes excited in the presence of a neutral gas, providing MS/MS data. The isolation/fragmentation cycle can be repeated to obtain MS^n data.

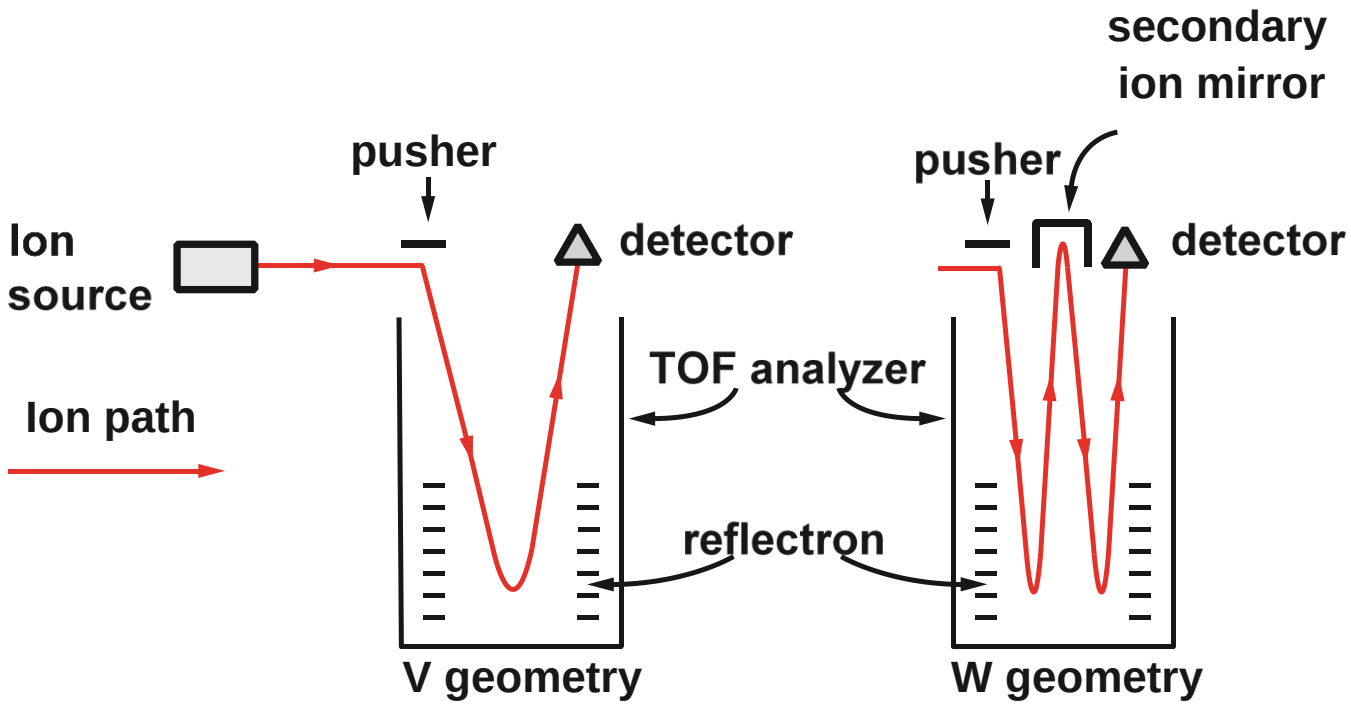
Injection of ions into an orthogonal TOF analyser

The basic principle of TOF analyser:- pulses of ions are accelerated from the ion source into the analyser tube, and the time for an ion to travel through a field-free region to the detector is measured. The time-of-flight for the passage of an ion in a TOF analyser is a function of its momentum, and therefore its *m/z.*
The acceleration voltage and consequently the kinetic energy (momentum), is the same for all ions thus, those ions with the lowest *m/z* will travel fastest and arrive at the detector first, followed by the sequential arrival of ions with successively higher *m/z.*



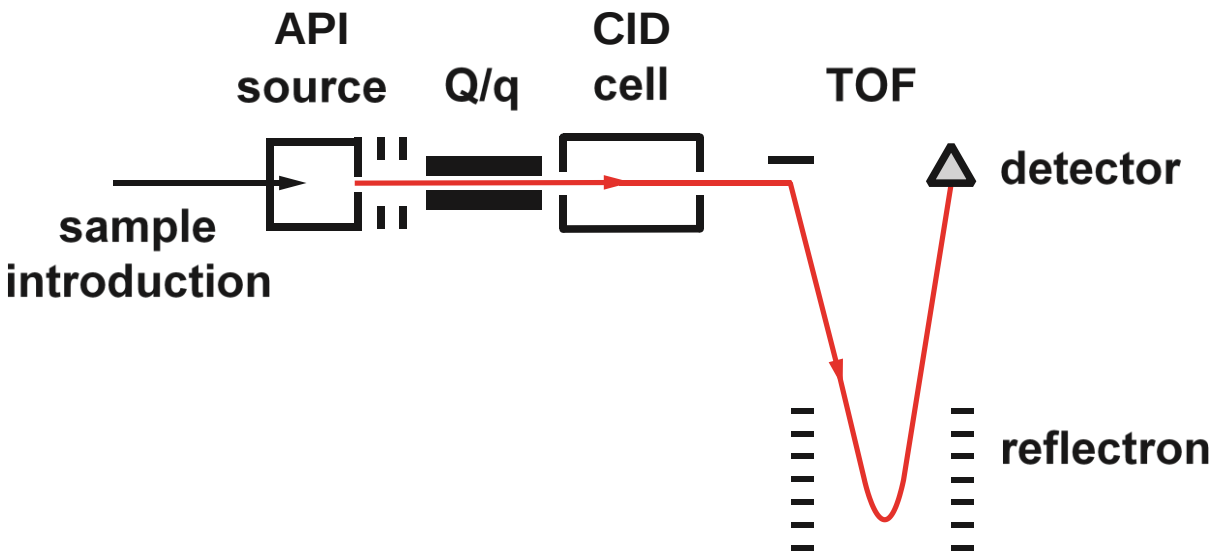
The individual packets of ions required in TOF analysis must be excised from the ion beam, and accelerated orthogonally into the analyser using a pulsed pusher voltage. Only part of the continuous ion beam can be sampled as the excised ion packet must traverses the analyser before another set of ions can be introduced.

Principle of orthogonal TOF analysers with V and W geometries



- Ions arriving from the source are accelerated orthogonally into the analyser by a pulsed voltage from the pusher.
- Ions separate based on their momenta. As they travel through the analyser the lightest, fastest, ions (lowest *m*/*z*) will arrive at the detector first.
- Some horizontal momentum imparted in the source remains so that ions travel at an angle into and out of the reflectron thus attaining a characteristic ‘V’ trajectory.
- Increasing the distance that the ions travel improves mass resolution, e.g., by using ‘W’ geometry.

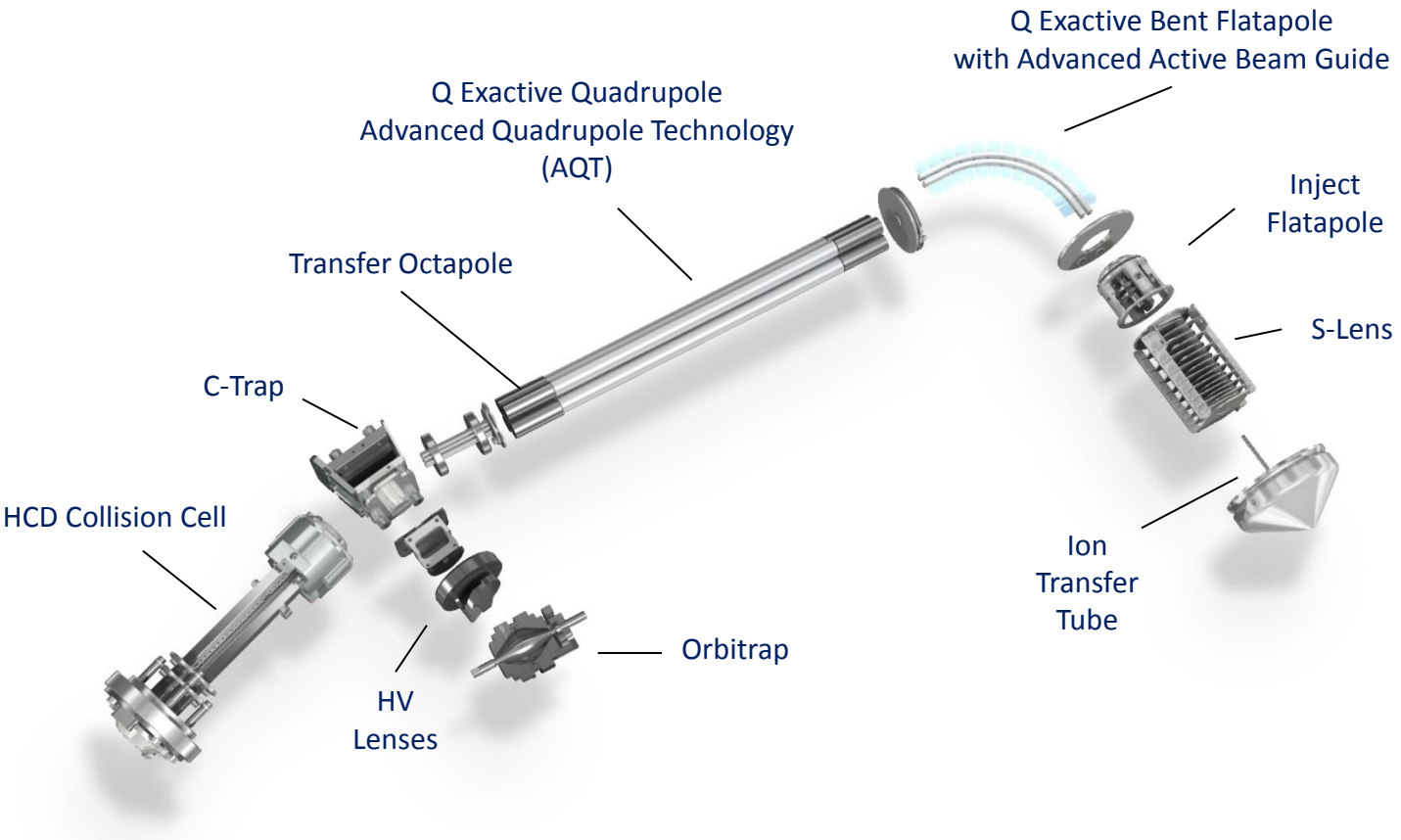
Principle of a QTOF MS/MS instrument - *tandem in space*



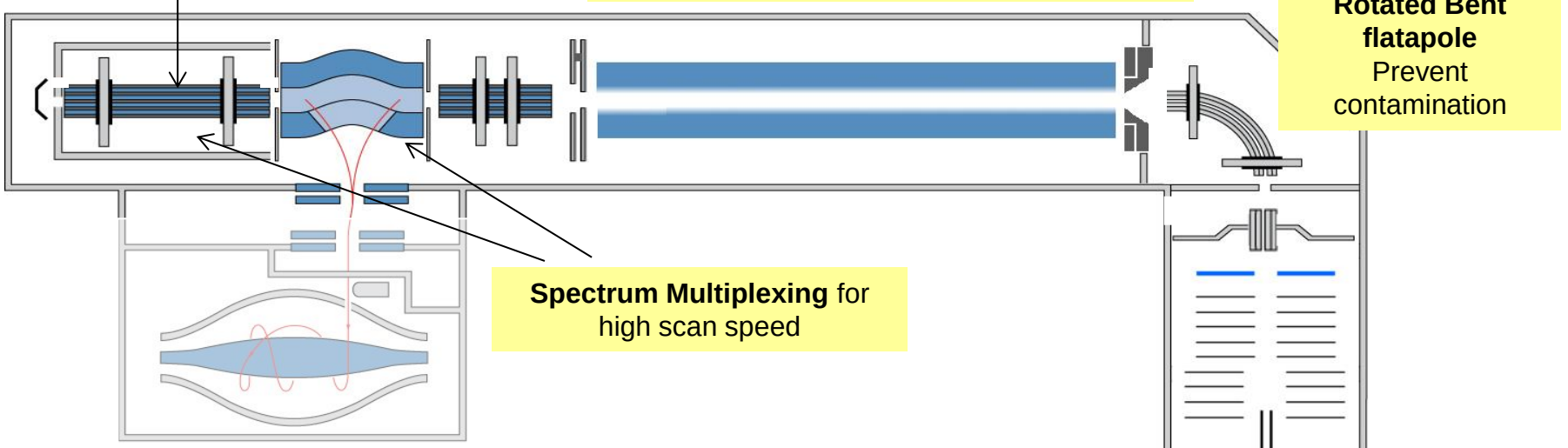
Quadrupoles can set as *broad band pass* (q) the rf voltages can be applied to the quadrupole, but without being combined with a dc field. Under these conditions all ions, *e.g.* those emerging from the ion source that enter the q are transmitted, regardless of their *m/z* values.

The quadrupole can be used in broad band pass (q) mode, to pass all ions to the time-of-flight (TOF) analyser, or in narrow band pass (Q) mode to select an ion with a specific *m/z* for collision induced dissociation (CID). The CID cell also has a q function by which the fragments formed are constrained and transferred to the TOF analyser. CID fragment ions are separated and collected in the TOF analyser.

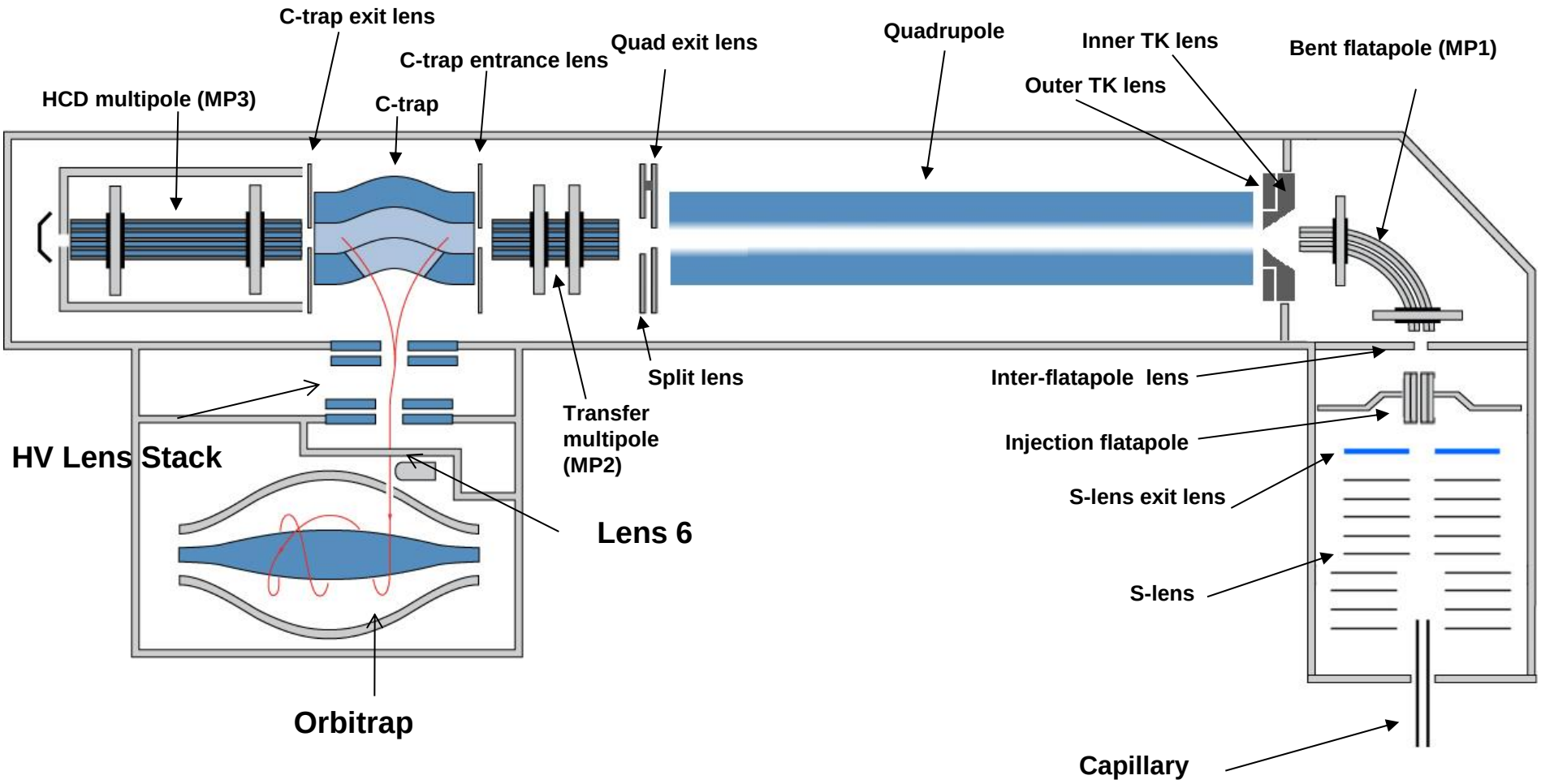
Q Exactive Plus mass spectrometer



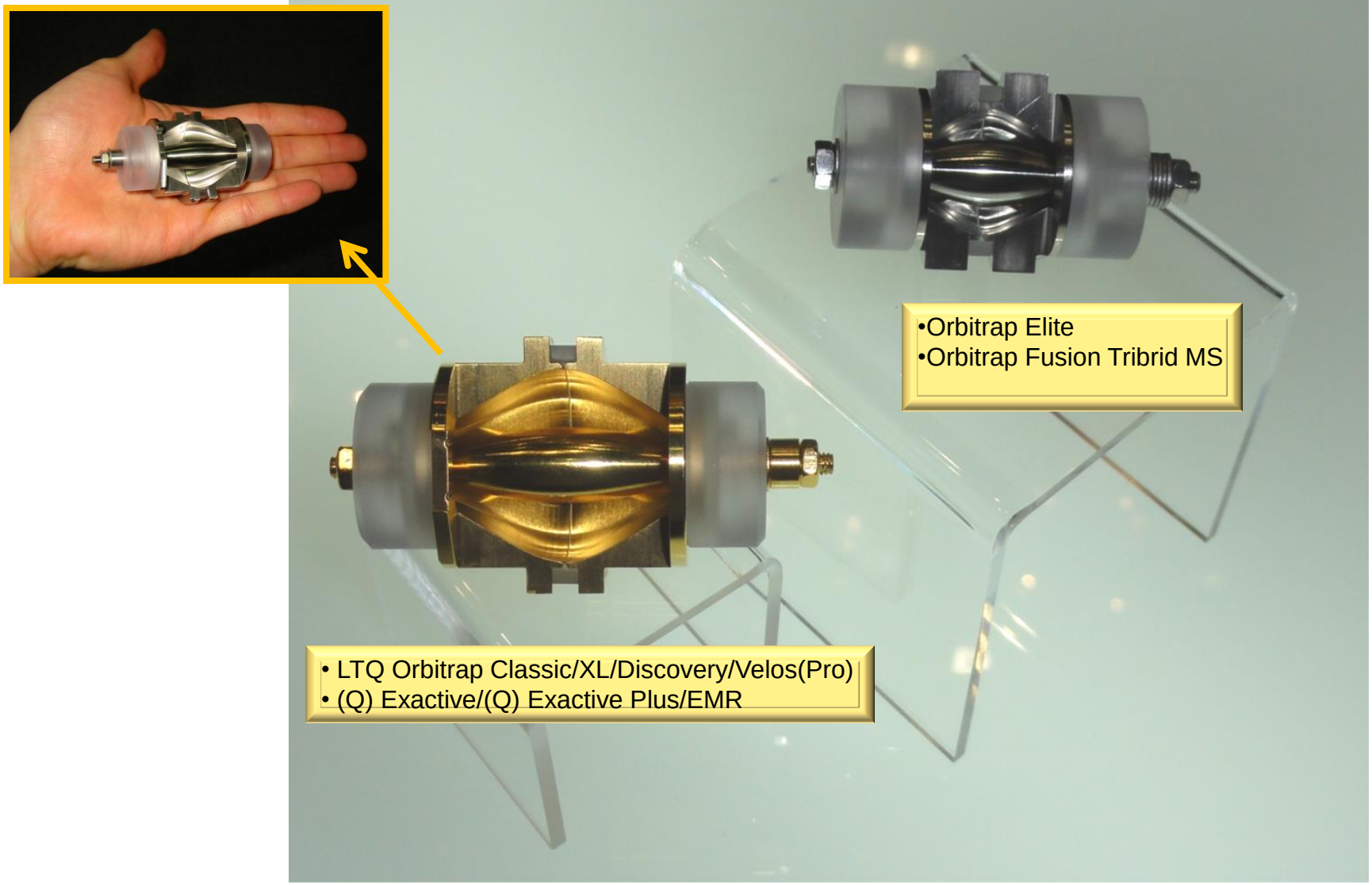
Q Exactive Plus



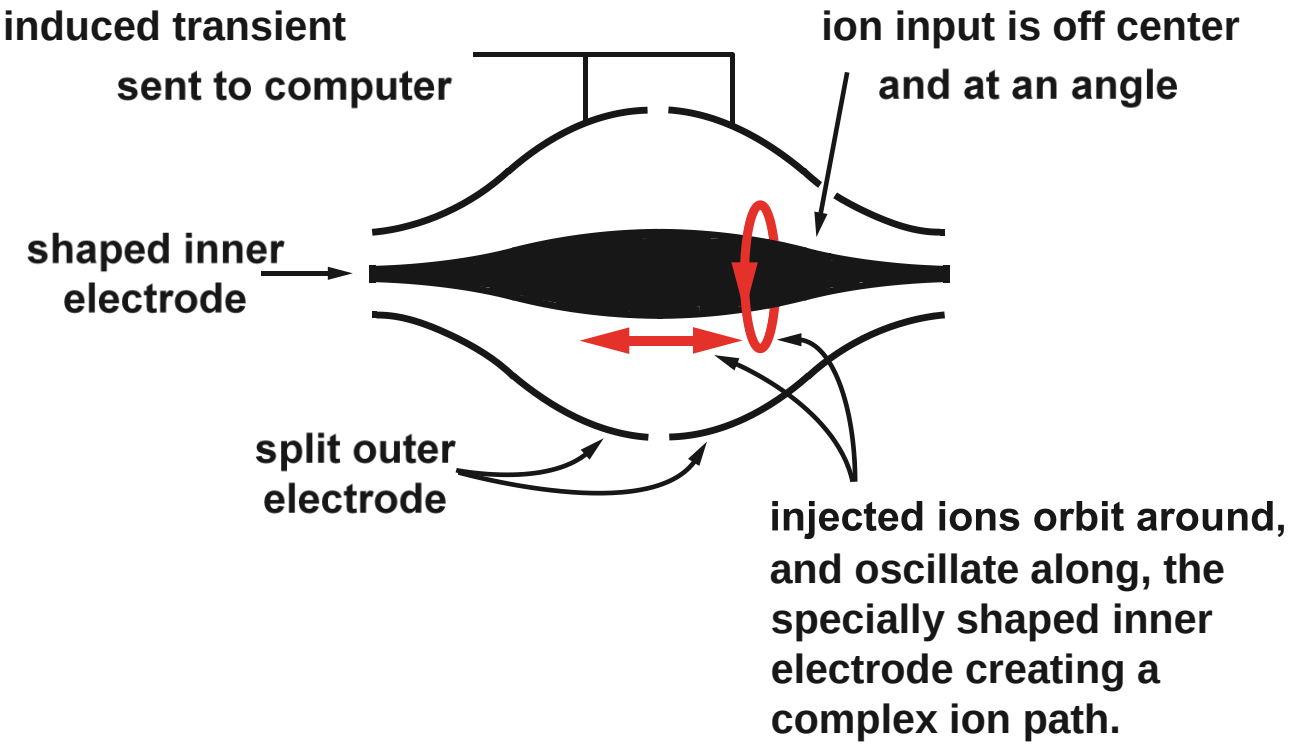
Q Exactive – Bent and rotated flatapole



Original Orbitrap and High Field Orbitrap Real Size Cutaways



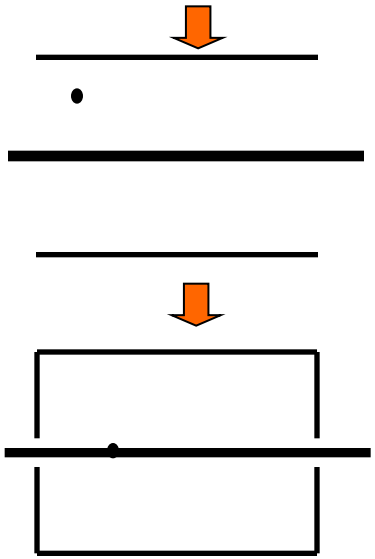
Brief operational principle of Orbitrap analyser



Ions are collected in a specialised component called the C-trap and then injected into the orbitrap as high-speed pulses. Ions are injected at an angle and offset from the centre of the trap. The momentum of the ions causes them to orbit around, and oscillate along, the central spindle-like electrode. The lateral oscillation of the ions along the inner electrode induces a transient (image) current in the split outer electrode. The recorded image current is interpreted using Fourier transform analysis to provide *m*/*z* values and intensities.

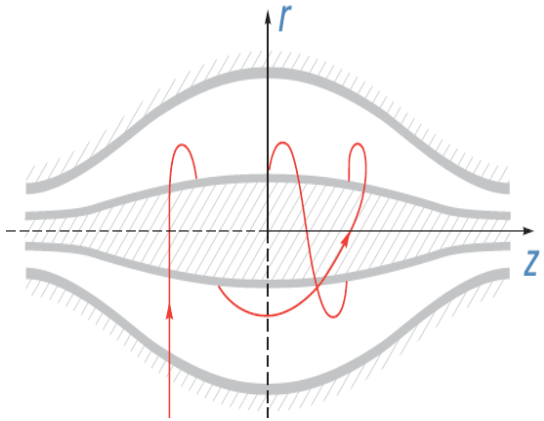
Principle of Trapping in the Orbitrap

- The Orbitrap is an ion trap – but there are no RF or magnet fields!
- Moving ions are trapped around an electrode
  - *Electrostatic attraction is compensated by centrifugal force arising from the initial tangential velocity*
- Potential barriers created by end-electrodes confine the ions axially
- One can control the frequencies of oscillations (especially the axial ones) by shaping the electrodes appropriately
- Thus we arrive at …



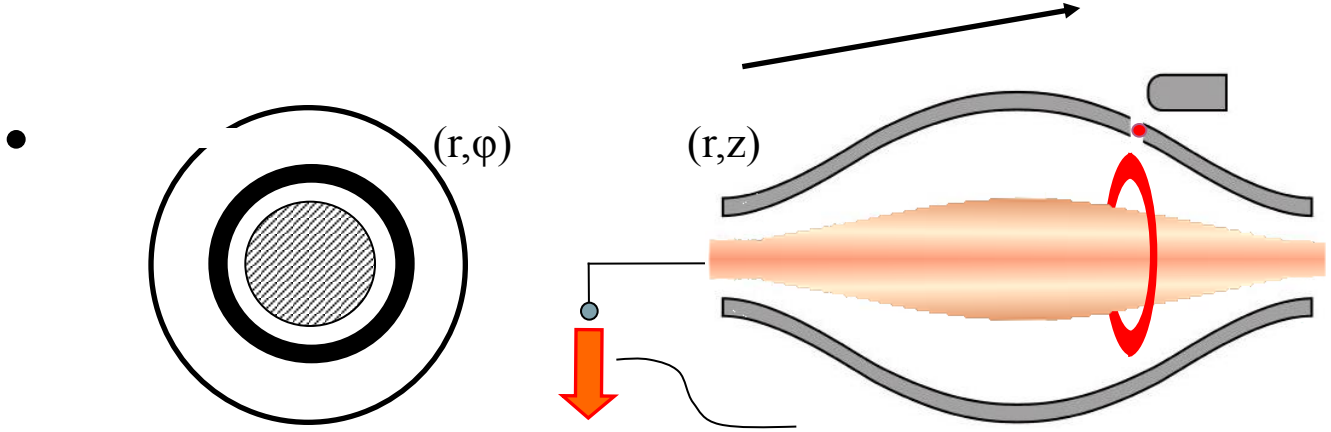
Orbital traps

Kingdon (1923)

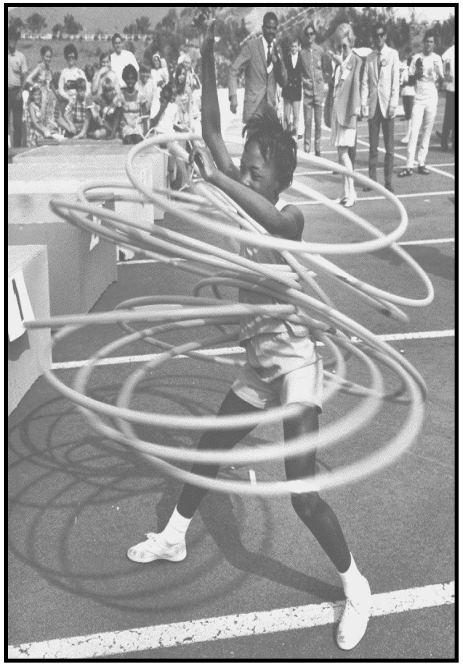


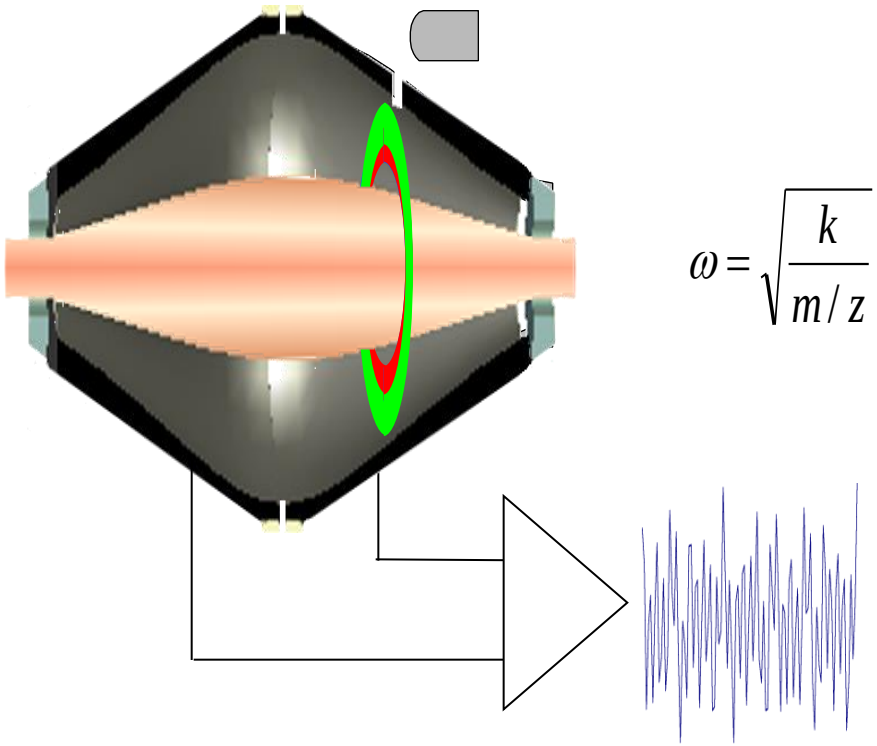
Ion Rotations in the Orbitrap

- ✓ A short ion packet of one *m/z* enters the field tangentially, off-equator
- ✓ Ions are squeezed towards the central electrode by increasing voltage applied on this electrode
- ✓ In the axial direction, ions are forced to move away from the narrow gap towards the wider gap near the equator
- ✓ This initiates axial oscillations without the need for any further excitation ("excitation by injection")
- ✓ After the voltage increase stops, ion trajectories become a stable spiral
- ✓ Angular spreading forms ROTATING RINGs bouncing back and forth



Retaining the Ions in the Orbitrap



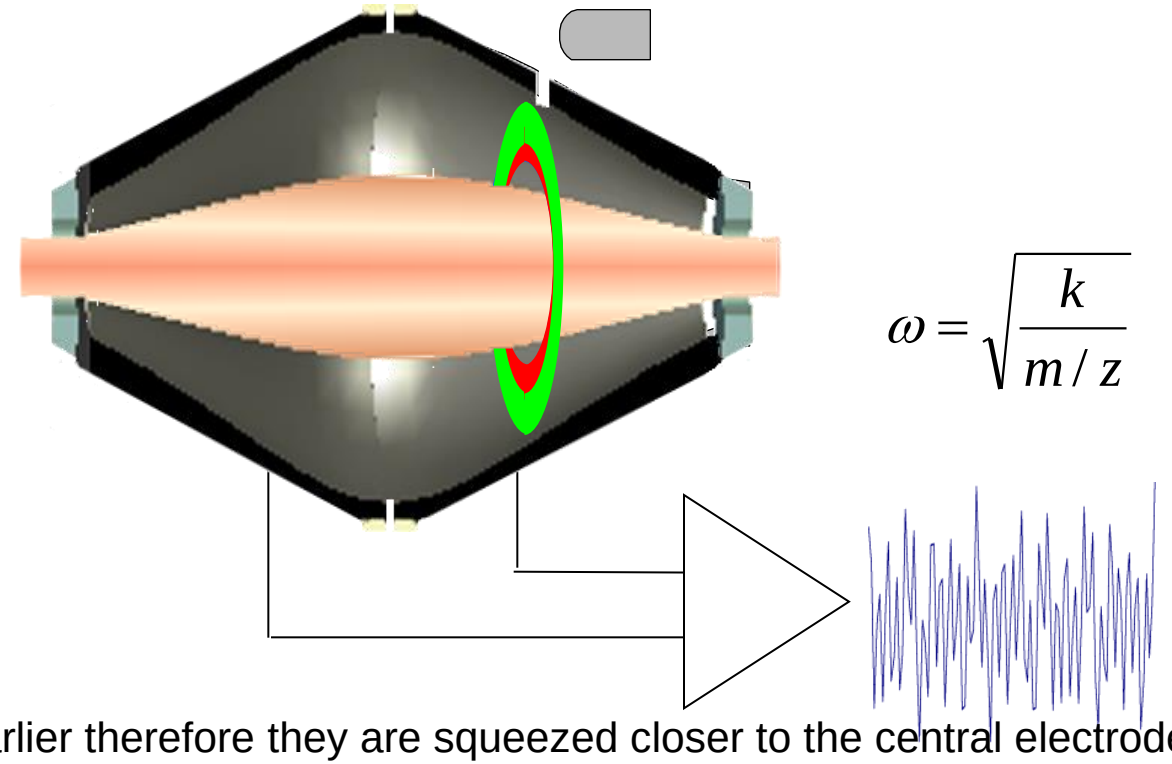


$$\omega = \sqrt{\frac{k}{m/z}}$$

- Many ions in the Orbitrap generate a complex signal whose frequencies are determined using a Fourier Transformation
- Lighter ions enter Orbitrap earlier, therefore they are squeezed closer to the central electrode than heavier ions

Detection of ions in the Orbitrap

- Frequency of axial oscillations of each ring induces an image current on split outer electrodes
- Multiple ions in the Orbitrap generate a complex signal whose frequencies are determined using a Fourier Transformation

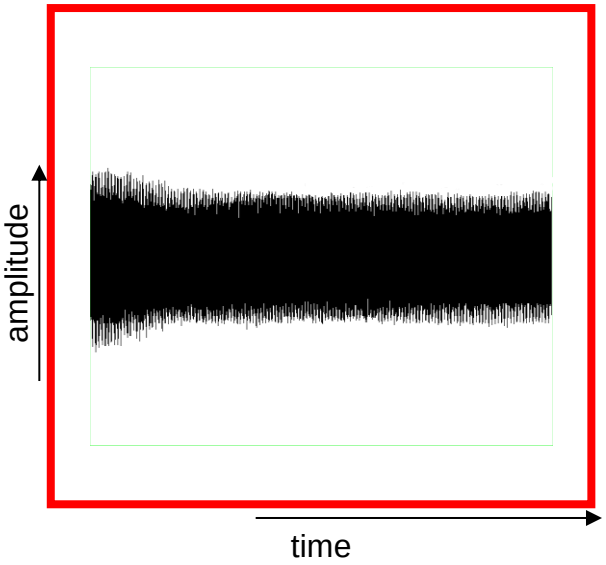


$$\omega = \sqrt{\frac{k}{m/z}}$$

- Lighter ions enter Orbitrap earlier therefore they are squeezed closer to the central electrode than heavier ions
- **An image current transient of these oscillations is converted to a frequency spectrum using a Fourier transform similar to the approach used in FTICR**
- **eFT: enhanced FT transformation algorithm for high resolution at the same transient time**

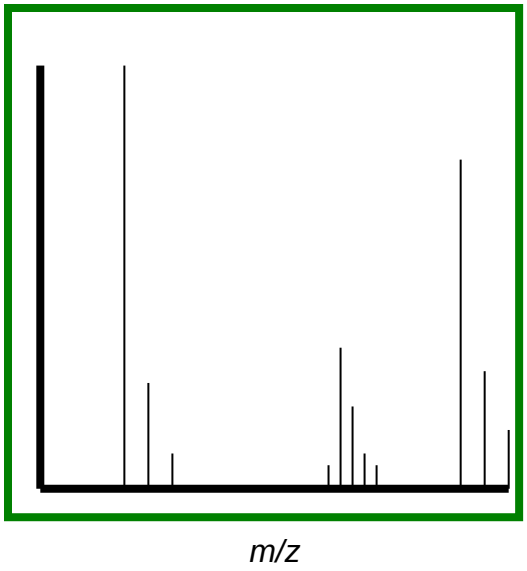
  & Higher Resolution in shorter detection time

From “time domain” to mass spectrum



“time domain” signal

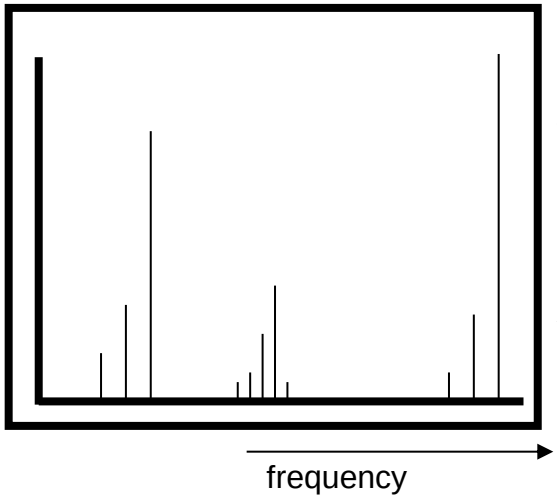
(what the trap delivers)



Frequencies are converted into masses.

Result:
A mass spectrum

$$\omega_z = \sqrt{\frac{k}{m/z}}$$

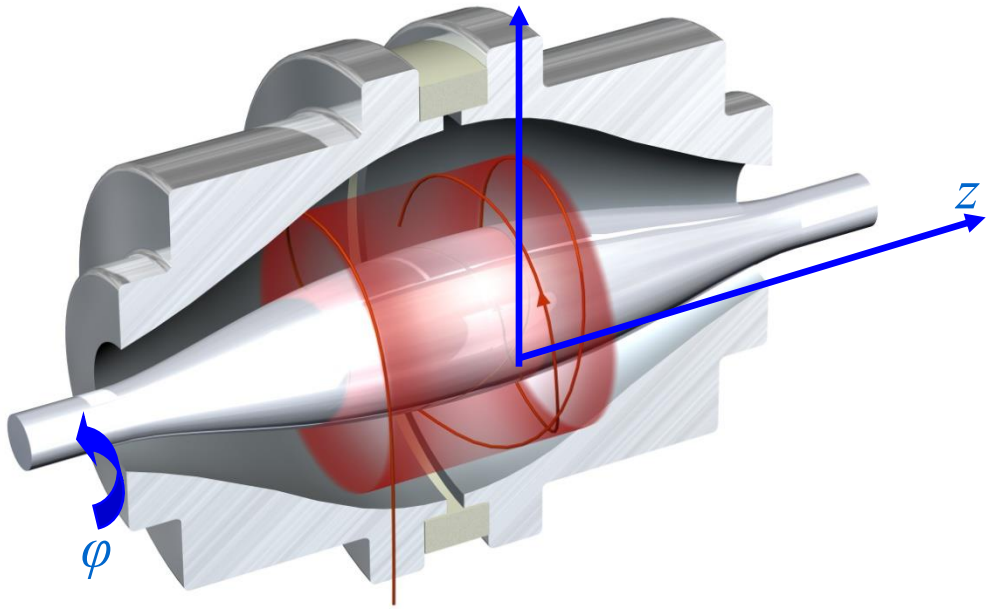


Fourier Transformation extracts the frequencies and intensities.

Result:
A frequency/intensity spectrum

- Characteristic frequencies:
  - Frequency of rotation ω_{φ}.
  - Frequency of radial oscillations ω_{r}.
  - Frequency of axial oscillations ω_{z}.

$$\omega_{\varphi} = \frac{\omega_z}{\sqrt{2}} \sqrt{\left(\frac{R_m}{R}\right)^2 - 1}$$



$$\omega_r = \omega_z \sqrt{\left(\frac{R_m}{R}\right)^2 - 2}$$

$$\omega_z = \sqrt{\frac{k}{m/q}}$$

For reference: ideal potential distribution in the Orbitrap

$$U(r,z) = \frac{k}{2} \cdot \left\{ z^2 - r^2/2 + R_m^2 \cdot \ln(r/R_m) \right\}$$

Only this frequency does not depend on energy, angle, etc. and could be used for mass analysis !