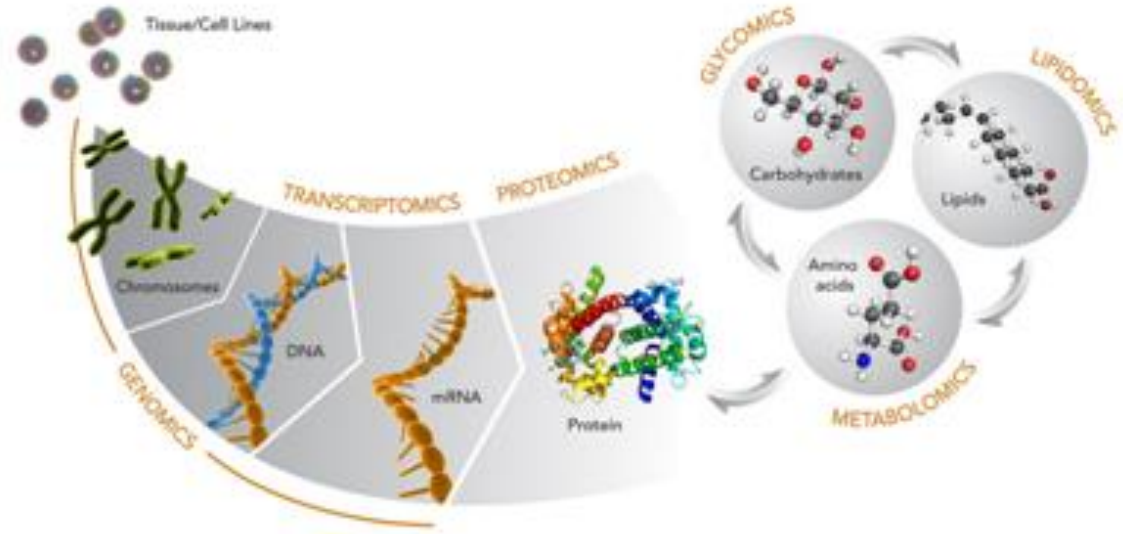
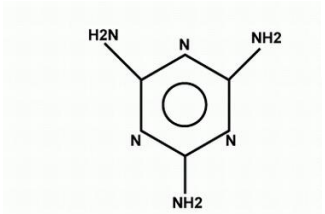
Mass spectrometry techniques measure the mass/ charge ratio of analytes. They enable both IDENTIFICATION and QUANTIFICATION of analytes by measuring molecular mass (m/z) or their fragmentation products.

Mass spectrometry (MS), especially combined with chromatography and computer technologies, has been widely used in organic chemistry, biochemistry, drug metabolism, clinical, toxicology, determination of pesticides, environmental protection, petroleum chemistry, geochemistry, food chemistry, chemical plants, cosmic chemistry and chemical defense and other fields.



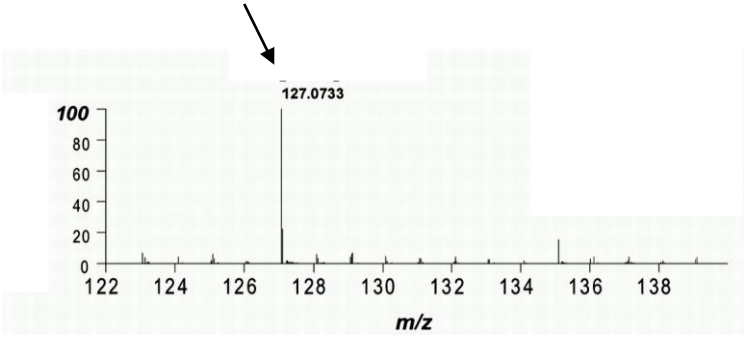
MASS/CHARGE RATIO

A mass spectrometer is based on electromagnetic fields, acting only on ionic species. In fact electrically charged particles are affected by a magnetic field while electrically neutral ones aren't.
A mass spectrometer measures the m/z value and a species able to produce ions with different charge will thus produce more signals at different m/z values of the mass spectrum (this is not an issue for small molecules, forming only single charged ions)

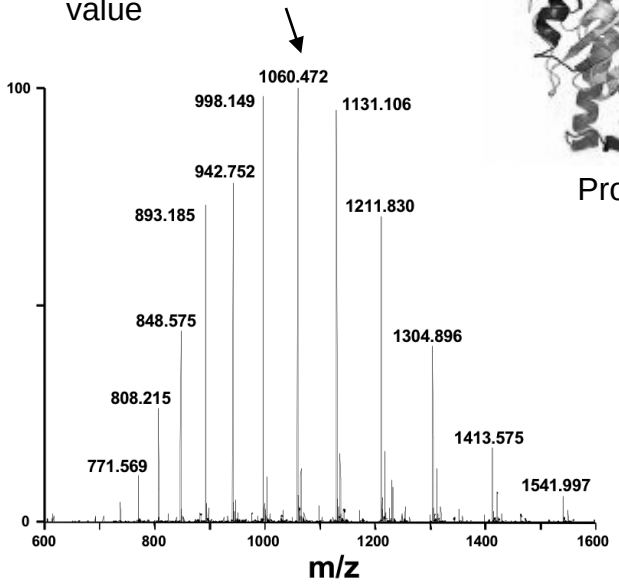


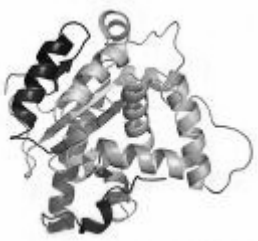
Small molecule

Single charge ion (z = +1)



Different ions with same mass m and different charge z, so different m/z value

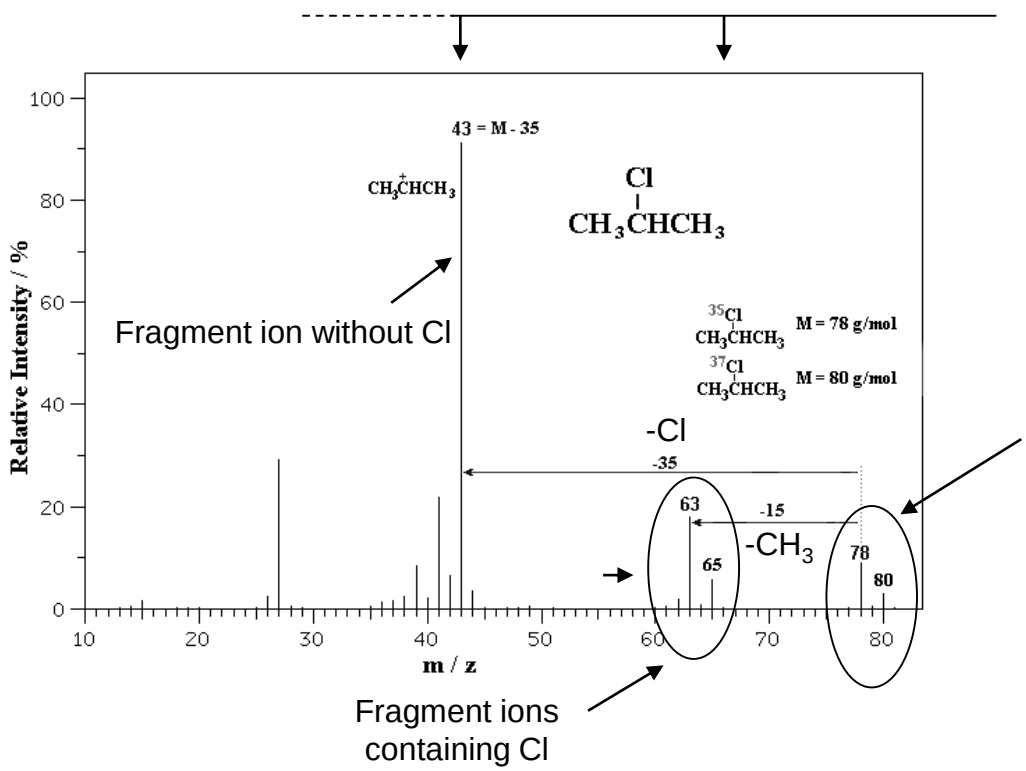




Protein

MASS SPECTRA

The mass spectrum of a compound graphically represents masses (or better m/z values) of all ions that are obtained analyzing the compound with a mass spectrometer in given experimental conditions.
It represents the ID of a compound and contains all information necessary for its identification and for understanding its molecular structure.



FRAGMENT IONS: Molecular ions formed in the initial ionisation dissociate to fragment ions (with minor mass) because of excess internal energy remaining after ionisation.

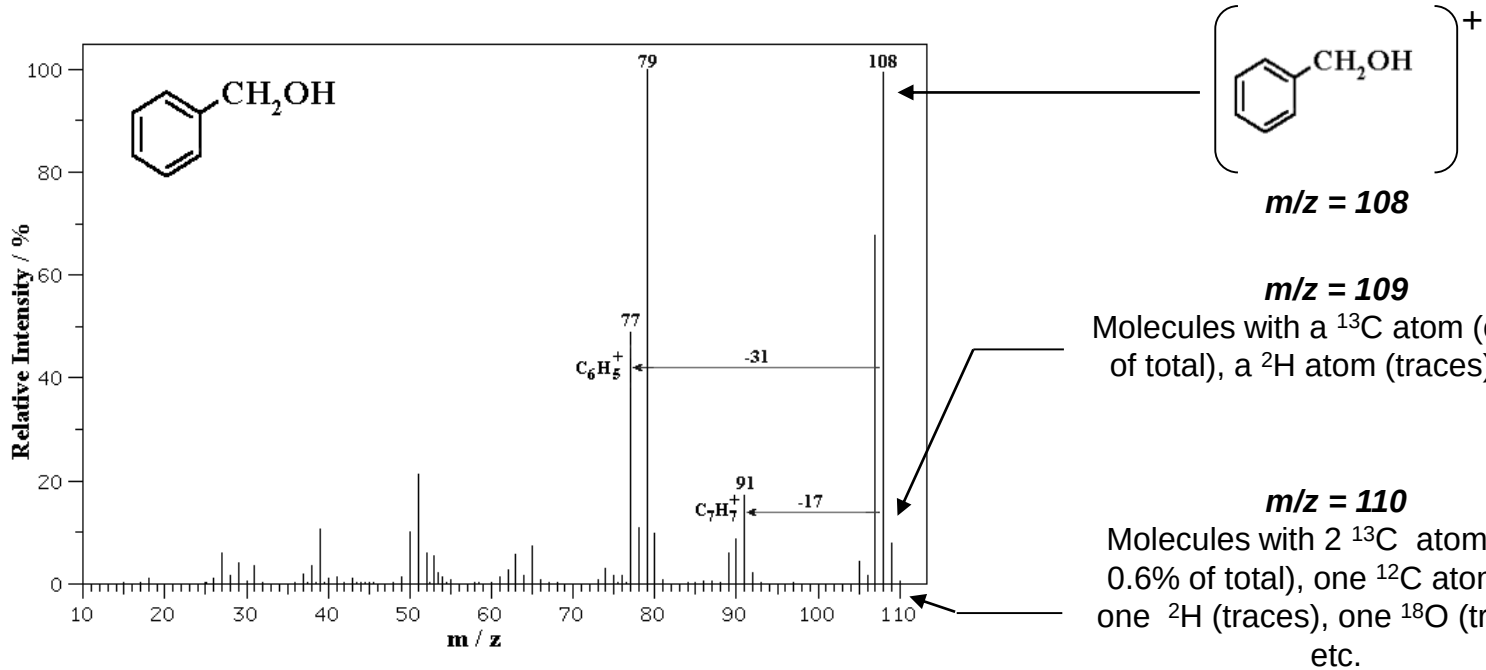
MOLECULAR ION: is the ion deriving form the original species in its intact form, but it does not necessarily represents the most intense signal of the mass spectrum.

Mass may differ from nominal one (e.g., H addition or removal produce masses of m + 1 or m - 1).
In this case two molecular ions are present with masses of m and m + 2, corrisponding to two molecules with Cl isotopes ^{35}Cl e ^{37}Cl.

ANALYTE IDENTIFICATION

The easiest way to identify an analyte from a mass spectrum is using the m/z value.

M/z values are dependent on the exact molecular mass, therefore mass spectrometry enables to identify different isotopes of the analyte.
One molecule will produce signals corresponding to all possible isotopic forms (e.g., presence of ^{13}C is responsible for isotopes of most organic molecules).



m/z = 108

m/z = 109
Molecules with a ^{13}C atom (ca. 8% of total), a ^{2}H atom (traces), etc.

m/z = 110
Molecules with 2 ^{13}C atoms (ca. 0.6% of total), one ^{12}C atom and one ^{2}H (traces), one ^{18}O (traces), etc.

ANALYTE IDENTIFICATION

Nevetheless, knowledge about m/z value of an ion is not a sufficient information for a definitive and nonambiguous identification of the ion, especially if the value m/z has been calculated with low accuracy.

The capability of a mass spectrometer to distinguish two different species having different m/z values is linked to its resolution R:

$$R = \frac{m/z}{\Delta m/z}$$

m/z represents nominal value.

Δm/z is the minimum difference of m/z value that can be distinguished at the given m/z value

Instruments with a resolution of about 1000 can distinguish molecules of the same species containing different isotopes (e.g., $^{12}CO_2$ e $^{13}CO_2$, with m = 44 e m = 45) but not species having same nominal mass (e.g., N_2 e CO, having both m = 28).

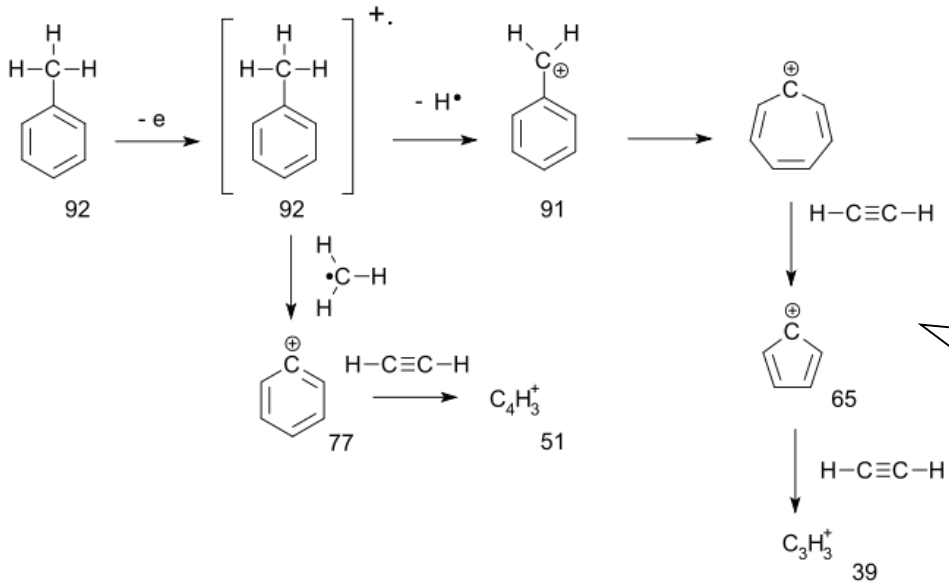
N_2 and CO have slightly different masses (m_{CO} = 27.994915 e m_{N_2} = 28.006148) and a high resolution instrument (R = 10^5 - 10^6) can distinguish them : high resolution instruments enable to identify the **molecular formula** of a substance by measuring the molecular mass.

https://iupac.org/

ANALYTE IDENTIFICATION

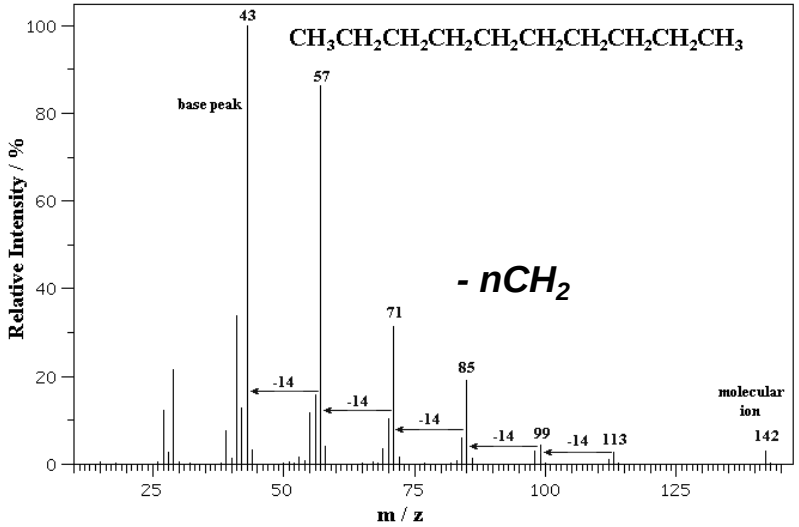
To achieve absolute identification of a target analyte with mass spectrometry measurements performed at low resolution **fragmentation pattern** is used.

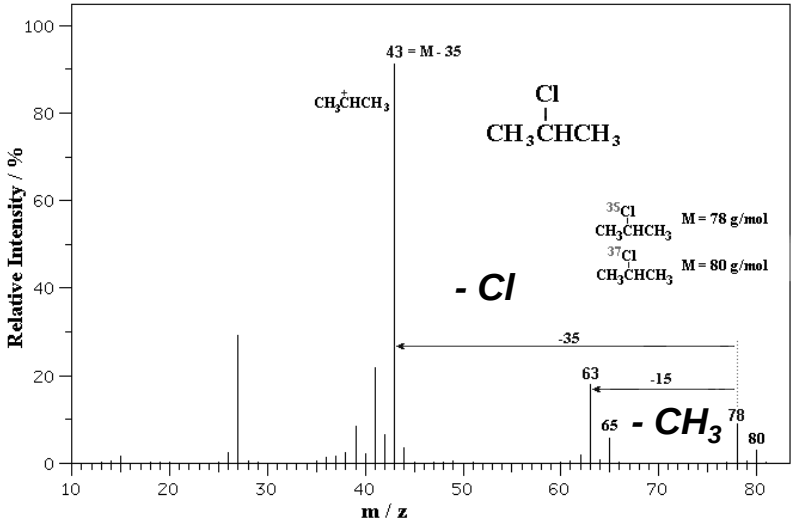
By providing high energy, ions of minor size are produced depending on the original structure of the molecule. These ions are specific for each species and enable its identification.

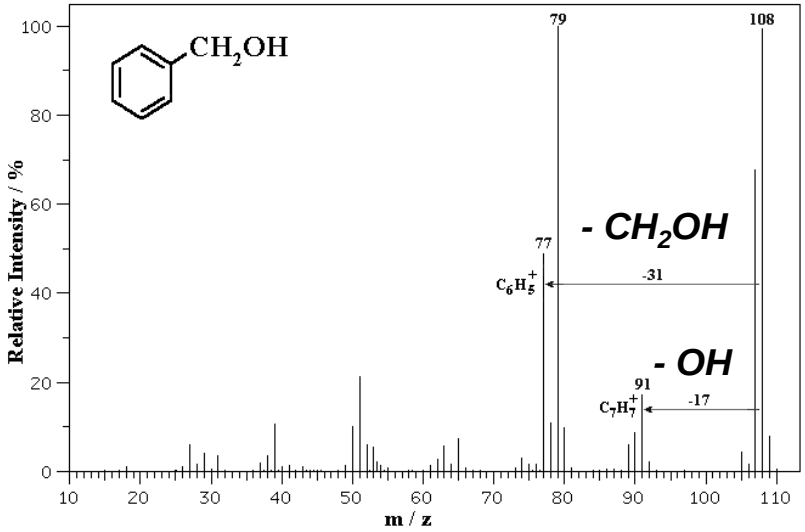


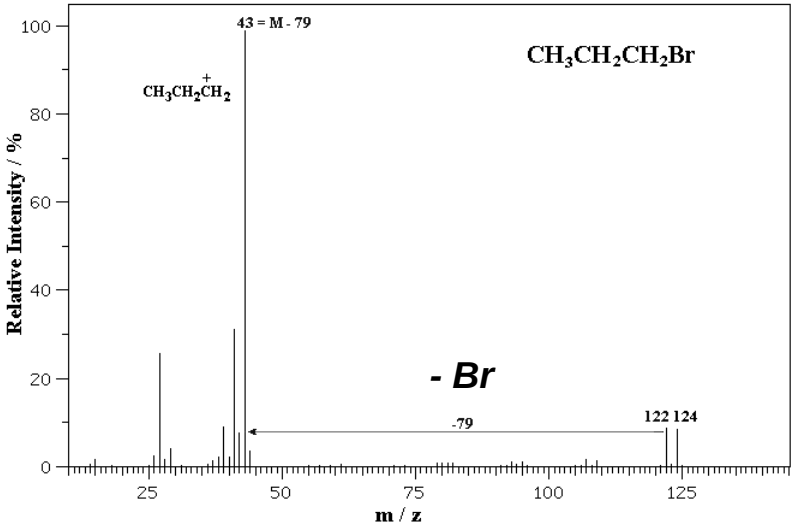
Toluene fragmentation: many species with m=92 could be present in sample, but none of them will provide the same fragments as toluene**.**

ANALYTE IDENTIFICATION







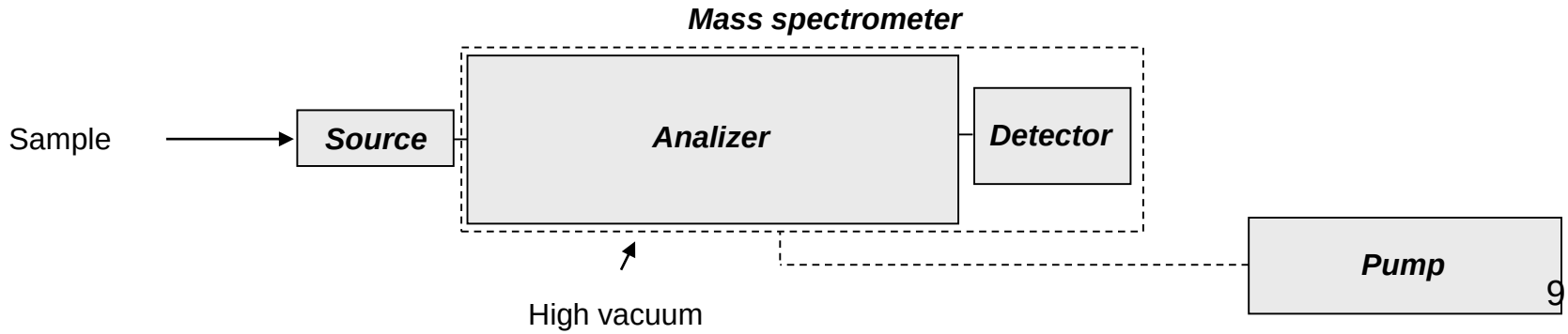


INSTRUMENTS

Tre main components:

- ***Ionization source***: converts the analyte into a ionized form in the gas phase, thus suitable to be analyzed with the mass spectrometer.
- ***Analizer:*** selects ions according to their m/z and send them to the detector.
- ***Detector:*** produces a signal proportional the ions that arrive.

Movement of the ions requires **high vacuum** (10^{-5} - 10^{-6} mBar): coupling with ionization surces working at atmospheric pressures represents a technical challenge.



IONIZATION SOURCES – ELECTRON IMPACT (EI)

The sample for analysis is introduced into the ion source, either through a solids inlet or through a gas chromatography column. A beam of electrons produced by a heated filament of either Tungsten or Rhenium collides with the sample gas molecules, removes an electron and produces a positively charged ion corresponding to the relative molecular mass of the sample being analysed.
Electron impact is an energetic ionisation technique and also produces fragment ions which are smaller parts of the original molecule.

$$M + e^- (70\ eV) \rightarrow \underbrace{M^{+\bullet}}_{\text{Molecular ion}} + e^- + e^- \rightarrow \text{Fragments}$$

Electron impact sources generally produce positive ions. In case target molecule contains alogens (electronegative elements such as Cl, Br, F) negative ions can be produced but the process is less efficient.

$$M + e^- (70\ eV) \rightarrow \underbrace{M^{-\bullet}}_{\text{Molecular ion}} \rightarrow \text{Fragments}$$

Ionization sources-chemical ionization (CI)

Chemical ionization is a **low energy ionization** process that avoids excessive fragmentation. CI uses a reagent gas to gently transfer protons to the sample, usually producing (M+H)+ quasimolecular ions. Such ions have very little tendency to fragment because little excess energy is imparted to them. The **reagent gas (methane,** isobutane, or ammonia) is present in the ion source at a pressure of 1 torr. It is ionized by an electron beam and the resulting ions undergo a complex series of ion-molecule reactions to produce species such as $CH5^+$ in methane.

$$CH_4 + e^- \, (70\ eV) \rightarrow CH_4^{+\bullet} + 2e^-$$

$$CH_4^{+\bullet} + CH_4 \rightarrow CH_5^+ + CH_3^\bullet$$

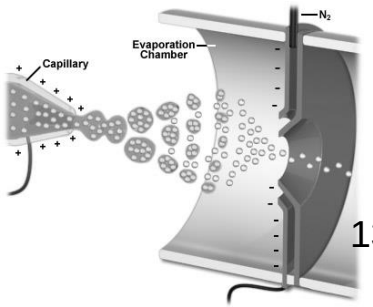
Cation CH_5^+ is very acidic and can transfer a proton to every organic molecule. Produced ions have a low energy content and generally do not fragment.

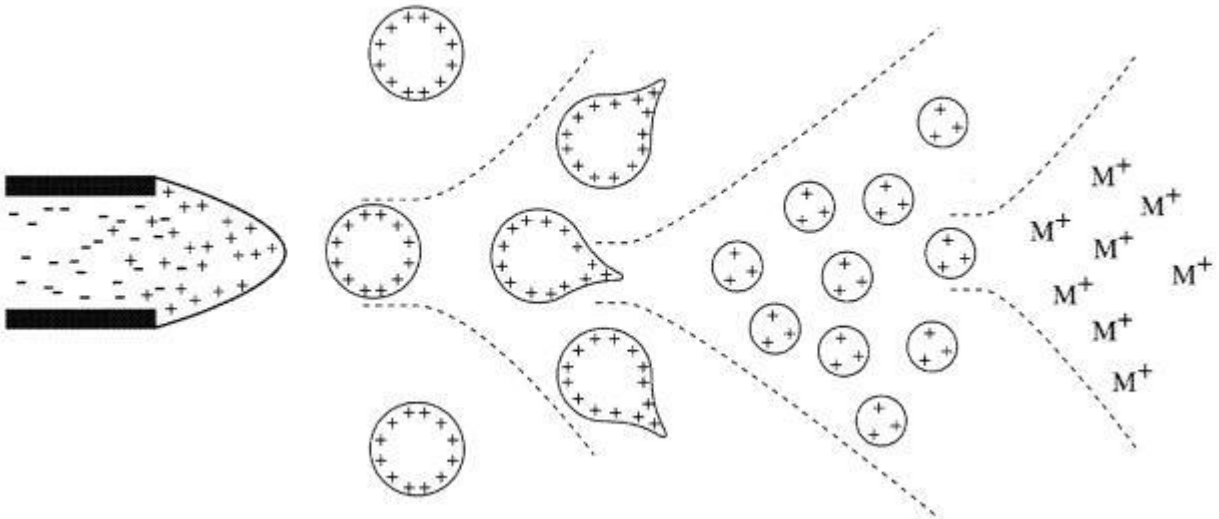
$$CH_5^+ + M \rightarrow MH^+ + CH_4$$

IONIZATION SOURCES - ELETTROSPRAY

ESI uses electrical energy to assist the transfer of ions from solution into the gaseous phase before they are subjected to mass spectrometric analysis.
The transfer of ionic species from solution into the gas phase by ESI involves three steps: (1) dispersal of a fine spray of charge droplets, followed by (2) solvent evaporation and (3) ion ejection from the highly charged droplets.

Sample is nebulized trough a needle maintained at a high voltage (e.g. 2.5 – 6.0 kV) negative or positive depending on the type of ions (positive for cations, negative for anions). The application of a nebulising gas (e.g. nitrogen) at high temperature, which shears around the eluted sample solution, enhances a higher sample flow rate.
The charged droplets are continuously reduced in size by evaporation of the solvent, leading to an increase of surface charge density and a decrease of the droplet radius. Finally, the electric field strength within the charged droplet reaches a critical point at which it is kinetically and energetically possible for ions at the surface of the droplets to be ejected into the gaseous phase.



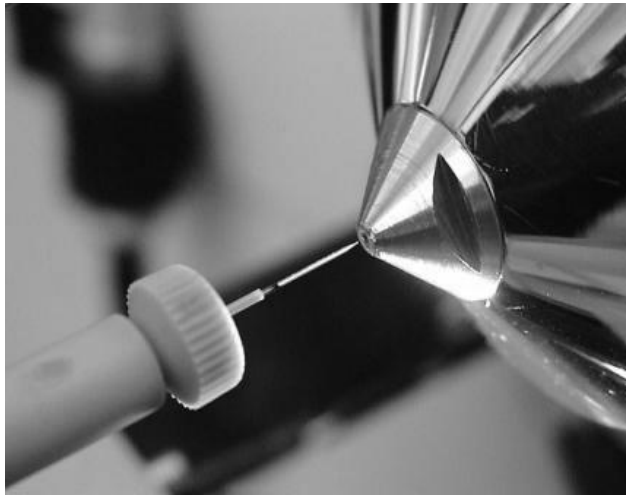


Mechanism of electrospray ionisation

IONIZATION SOURCES - ELETTROSPRAY

The emitted ions are sampled by a sampling skimmer cone and are then accelerated into the mass analyser for subsequent analysis of molecular mass and measurement of ion intensity.

ESI source



Sampling Cone

ANALIZERS

A mass analyzer is the component of the mass spectrometer that takes ionized masses and separates them based on charge to mass ratios and outputs them to the detector where they are detected and later converted to a digital output.

- Quadrupole Mass Analyzer
- Time of Flight Mass Analyzer
- Magnetic Sector Mass Analyzer
- Electrostatic Sector Mass Analyzer
- Quadrupole Ion Trap Mass Analyzers
- Ion Cyclotron Resonance

APPLICATIONS

In theory mass spectrometry allows to analyze complex samples (without pre-treatment) but resulting spectra would be too complex for correct interpretation.
Therefore separation techniques are required to simplify the sample and remove contaminants when complex samples are analyzed.

- **Coupling with gas-chromatography:** for the analysis of volatile substances with ionization obtained with electron impact or chemical ionization

- ***Coupling with HPLC:*** for the analysis of non volatile organic molecules with ESI

Exception: elemental analysis techniques based on the use of atomization sources such as inductively coupled plasma torch coupled to mass spectrometry (**ICP-MS techniques**). In this case, the relative simplicity of mass spectra (which only contain atomic species) allows to use this technique for direct sample analysis without any prior separation.

APPLICATIONS

Mass-spectrometry detection techniques coupled with separation techniques have significant advantages over other conventional detection techniques:

Mass spectrometry is universal: any substance capable of being ionized can be detected, even though with variable detection limits.

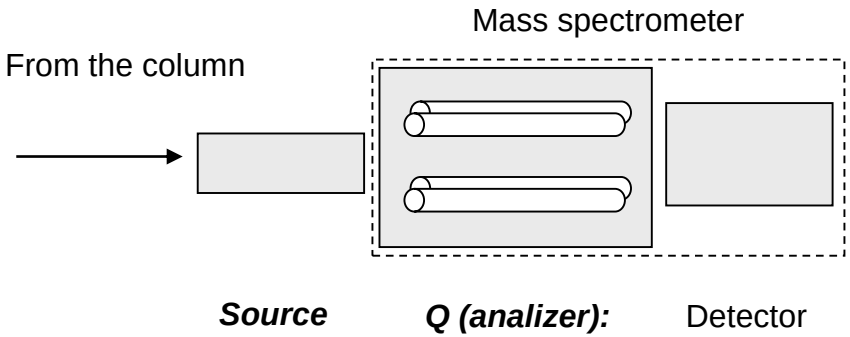
Mass spectrometry is sensitive, and its performance is often comparable or better than that of any other available detectors

It can be applied to all separation techniques: by modifying the ionization source, it can be used for elemental analysis (ICP-MS), gas chromatography (GC-MS) and liquid chromatography (HPLC-MS).

Allows both quantification and identification, based on the molecular mass or fragmentation spectrum. In fact, it is also able to distinguish and quantify co-eluted substances (ie not separated from the chromatographic column): the separating capacity of the chromatographic system is no longer a limit to the ability to identify the compounds present in the sample.

CONFIGURATIONS: SINGLE QUADRUPOLE

Single-analizer mass spectrometers (in this case single-quadrupole) are the simplest and cheapest tools.



Single-quadrupole mass spectrometer interfaced with an HPLC chromatograph

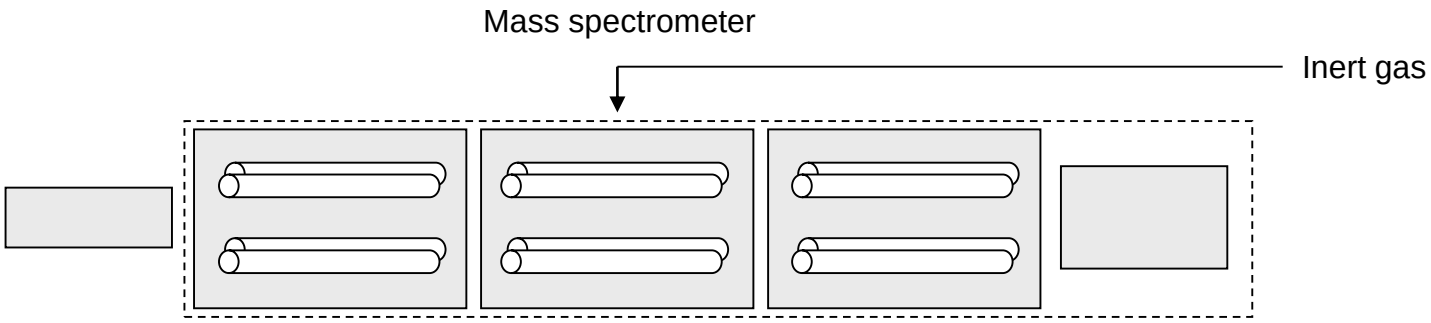
CONFIGURATIONS: SINGLE QUADRUPOLE

A single quadrupole mass spectrometer allows you to scan the ions produced in the source in order to identify ions at specific m / z values, but also to monitor the ions corresponding to the target analytes during a chromatographic separation. In this **Single Ion Monitoring (SIM) mode**, the quadrupole is set to pass only one ion with a given m / z ratio: every time this source is generated in the source, a signal is obtained in the chromatogram. This mode is suitable, for example, to obtain **a chromatogram relevant only to the presence of the species under consideration**. By rapidly changing different **Multiple Ion Monitoring (MIM**) values, different ions can be monitored simultaneously.

Ions produced by the source

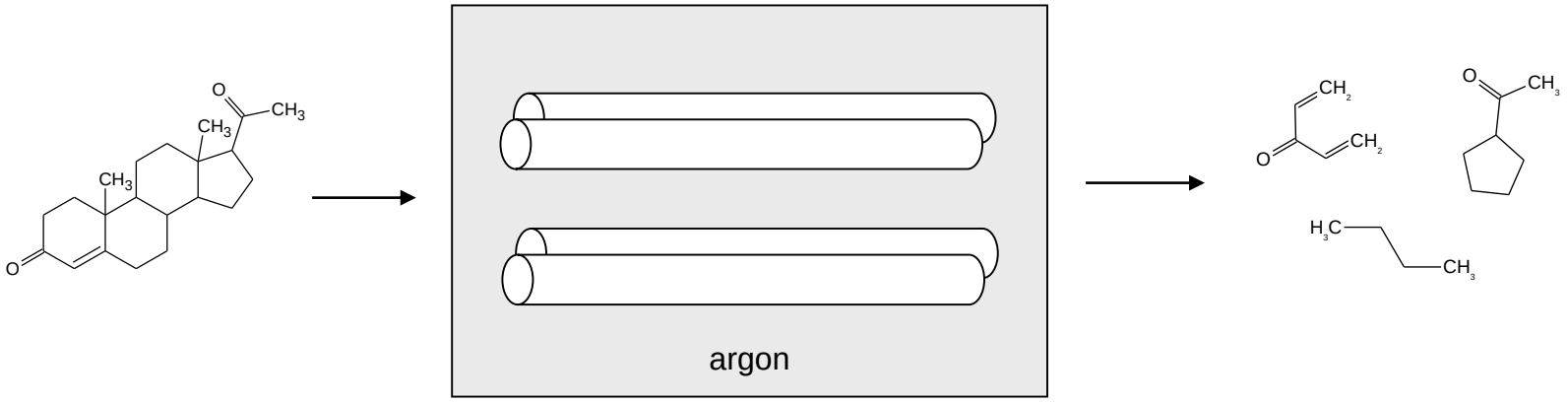
CONFIGURATIONS: TRIPLE QUADRUPOLE

In order to be able to use mass spectrometry techniques based on the fragmentation of the produced ions, however, instruments with several series analyzers are needed. The most common of these is the **triple quadrupole mass spectrometer**, which is equipped with three independent quadruples. Two of them (Q1 and Q3) act as analyzers, while the quadruple intermediate (Q2) acts as a collision cell, i.e. it allows to implement a controlled fragmentation of the ions of interest, colliding with accelerated by a predetermined potential with an inert gas (eg argon).



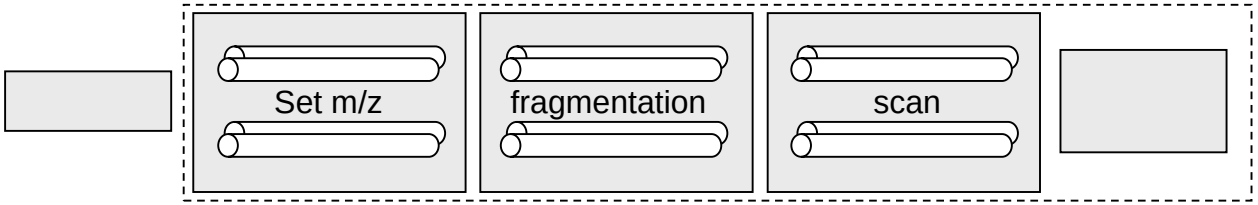
CONFIGURATIONS: TRIPLE QUADRUPOLE

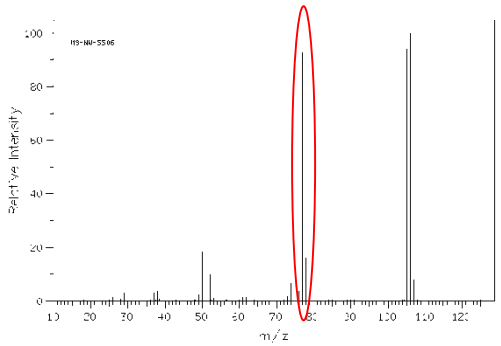
The **collision cell**, which is structurally similar to a quadrupole (but may also be an exapole or other geometry) has the function of fragmenting the ions by accelerating and colliding with an inert gas (usually argon). The electrodes within the cell have the function of accelerating the ions to a given energy (the magnitude of the fragmentation process depends on the energy of the ions themselves) and not allowing its dispersion during collision processes.



CONFIGURATIONS: TRIPLE QUADRUPOLE

A triple quadrupole tool allows to study and exploit fragmentation phenomena. For example, it is possible to study the fragmentation spectrum of a specific ion (**Product Ion Scan**): the first quadruple is set to pass only that ion, which is then fragmented into the collision cell. The third quadrupole scans the fragment ions by providing the fragmentation spectrum of the selected ion. This mode is suitable for **confirming the identity of an ion through the study of its fragmentation products.**

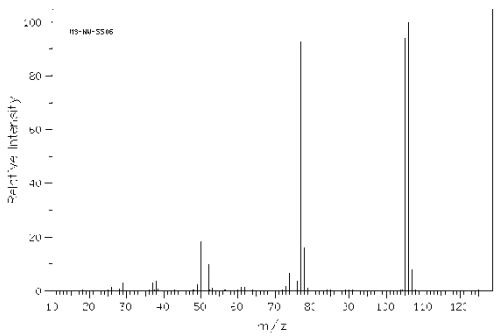




Ions produced by the source

m/z selected from Q1

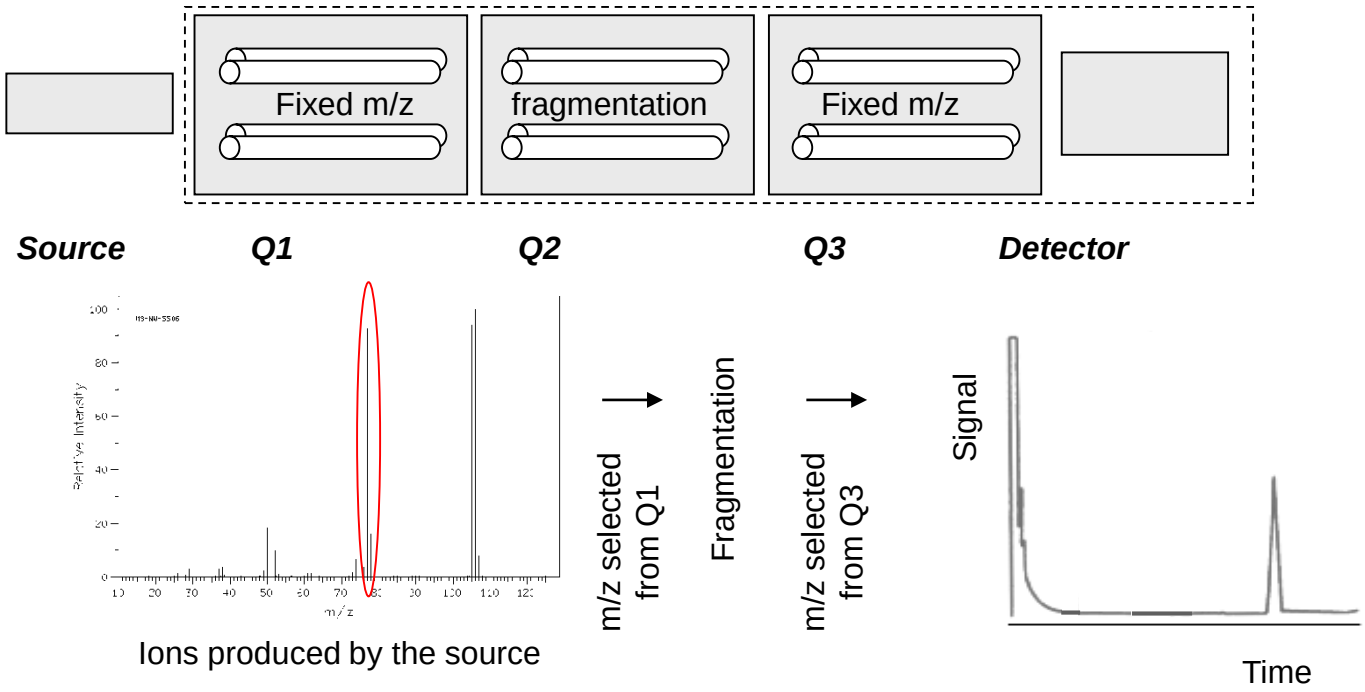
Fragmentation



Fragmentation spectrum of the ion selected from Q1 and obtained from Q3

CONFIGURATIONS: TRIPLE QUADRUPOLE

Maximum selectivity is achieved through **Single Reaction Monitoring (SRM**) and **Multiple Reaction Monitoring (MRM**) techniques, which are based on a dual selection of ions and are used for quali-quantitative analysis of the target species in low concentrations and in complex matrices. In these modes, the first quadrupole is set in order to allow passage of target ion, which is then fragmented into the collision cell. The second quadrupole then selects one (or more) fragment ions with specific m / z values.



CONFIGURATIONS: TRIPLE QUADRUPOLE

In general, techniques based on fragmentation are also called "**tandem" or MS / MS mass techniques.**

The **Multiple Reaction Monitoring (MRM) mode** in which a source ion product is selected from the first quadruple and multiple fragments are revealed with fixed m / z values is the one that ensures maximum selectivity to confirm analyte identity. High selectivity typically also has the lowest detection limits. In "official" analyzes, the detection of molecular ion and its fragments is considered the confirmatory and ultimate analysis.