Mass spectrometry:
a quick overview

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Mass Spectrometry

Analytical technique used to measure the mass to charge ratio m/z of ions

- **Technique that allows:**

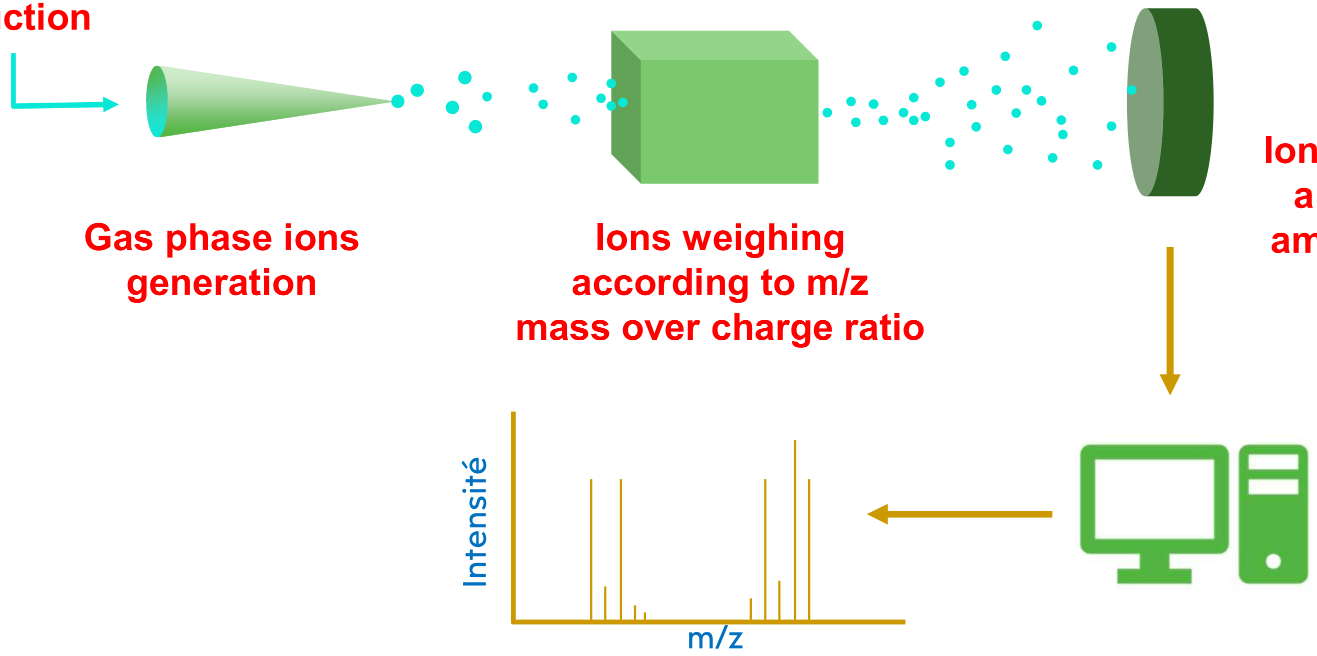
- Access to structural informations
- To establish the composition mixture
- Quantification

- **It is characterized by**:

- High sensitivity
- Specificity of informations
- Represented in many fields of applications

Mass Spectometry

Detection of ionic entities



Sample introduction

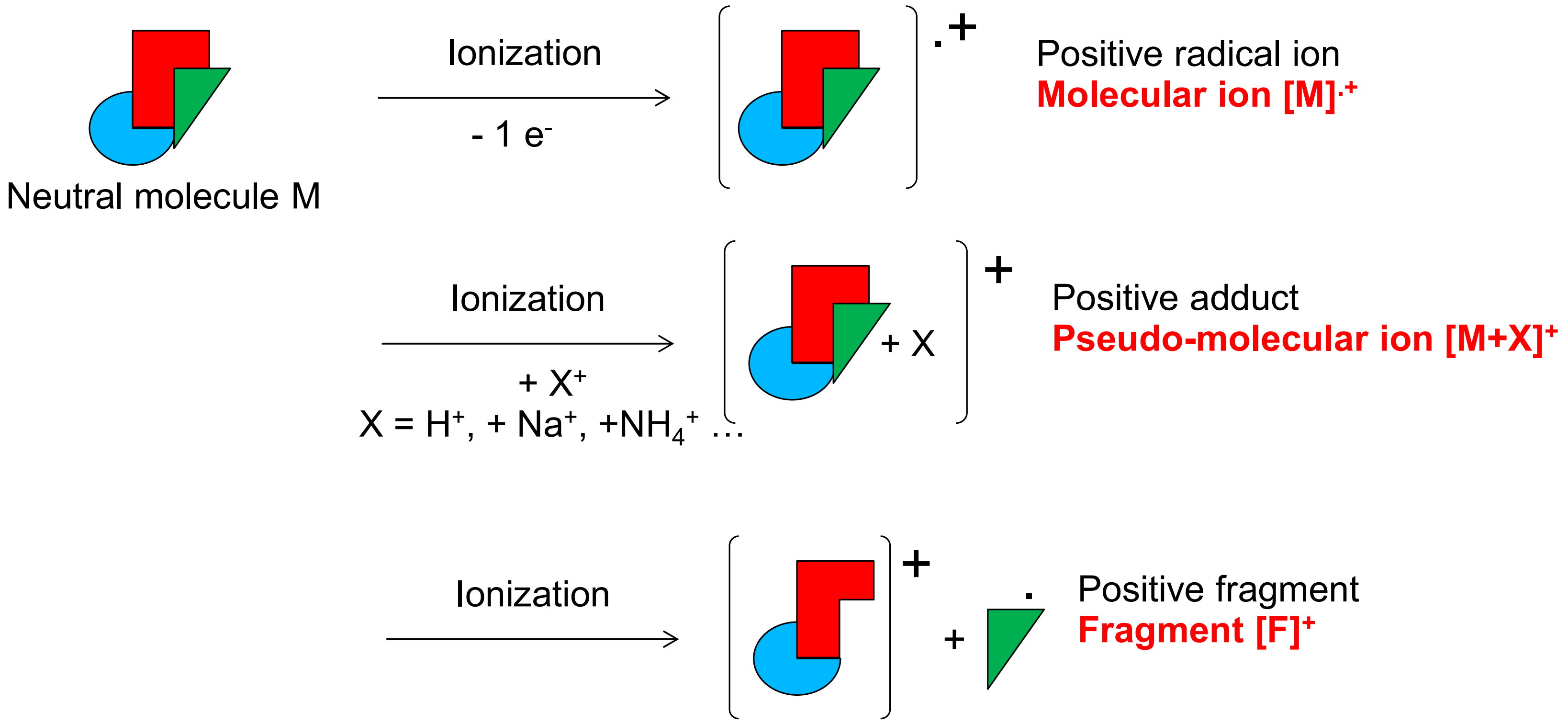
Gas phase ions generation

Ions weighing according to m/z mass over charge ratio

Ions counting and signal amplification

Signal processing and spectrum vizualisation

Mass Spectometry : different type of ionization



Neutral molecule M

Ionization

- 1 e^-

Positive radical ion

Molecular ion $[M]^{\cdot +}$

Ionization

+ X^+

X = H^+, + Na^+, +NH_4^+ …

Positive adduct

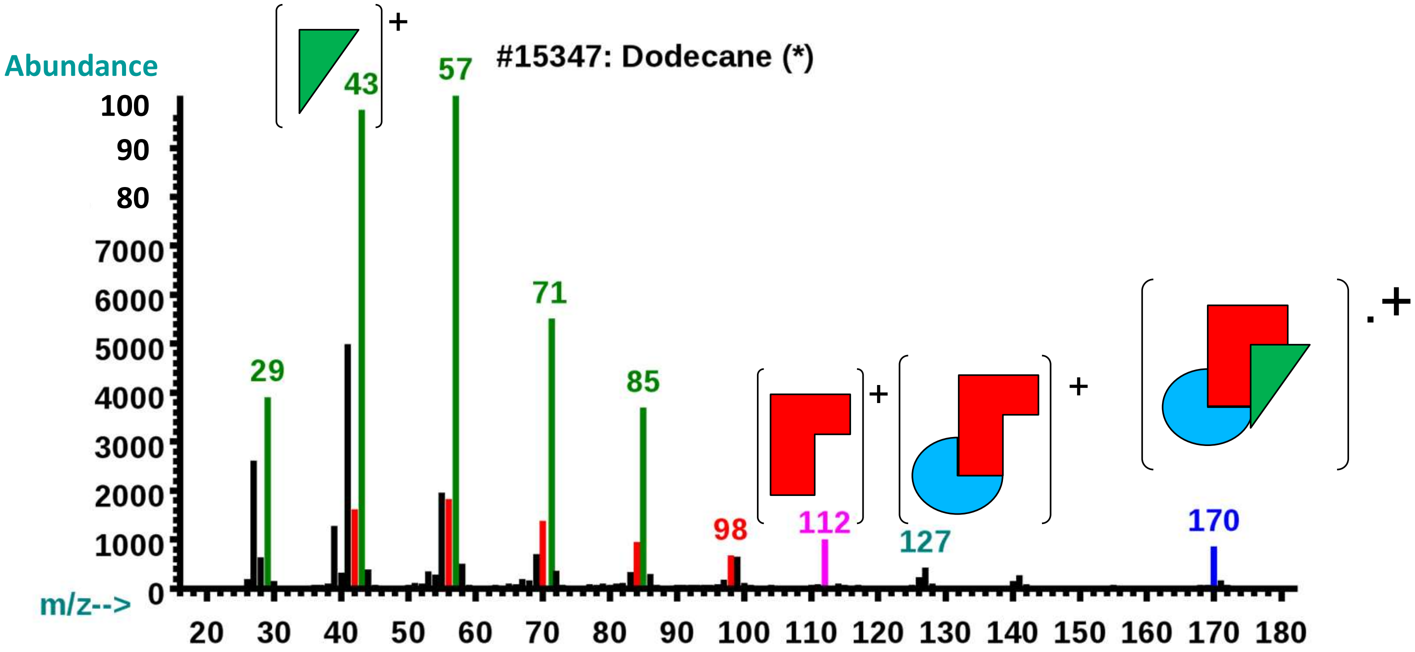
Pseudo-molecular ion $[M+X]^+$

Ionization

Positive fragment

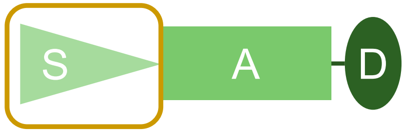
Fragment $[F]^+$

Mass Spectometry : a mass spectrum ?



x-axis : mass over charge ratio m/z expressed in Thomson
y-axis : ion intensity (absolute or relative abundance)

Ion sources

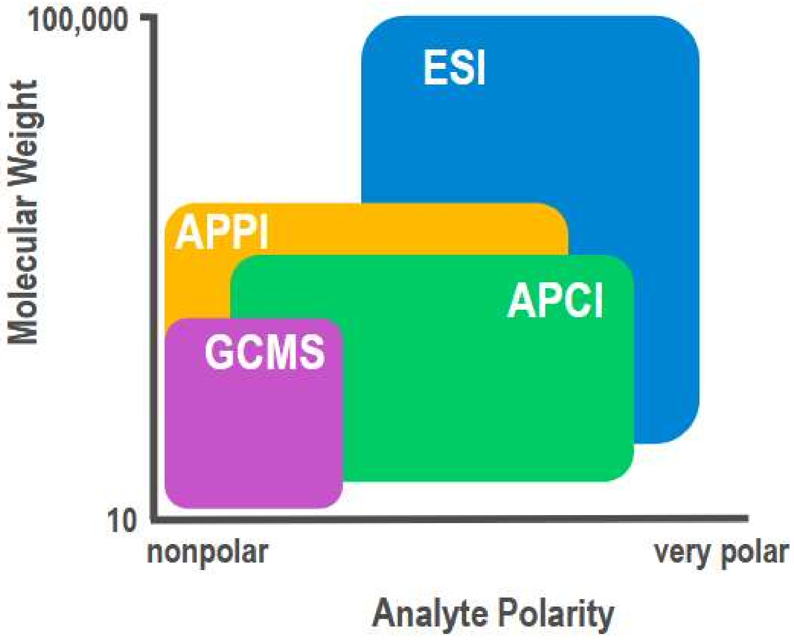


- Electron impact
- Chemical ionization
- Electropspray
- Atmospheric Chemical Pressure Ionization
- Matrix Assisted Laser Desorption

Ion sources

The choice of ionization sources depends on the molecules' physico-chemical properties

Polarity of analytes determines the ionization source.



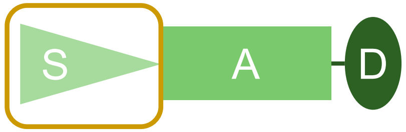
ESI	Electrospray ionization
APPI	Atmospheric pressure photo ionization
APCI	Atmospheric pressure chemical ionization

LC/MS sources

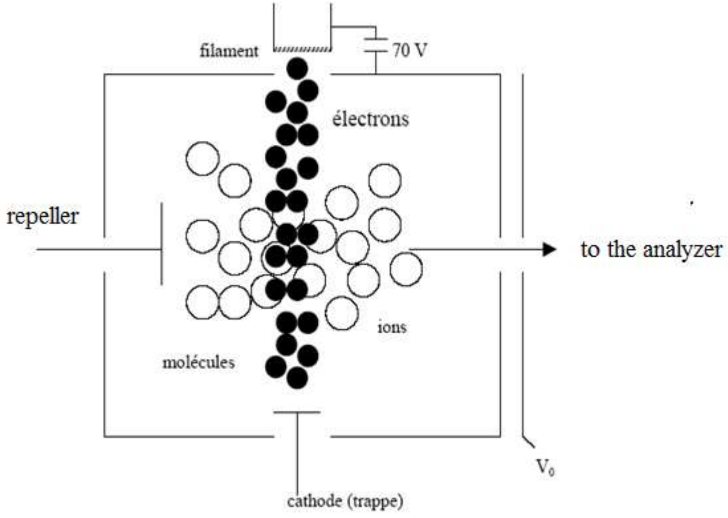
GC/MS	Gas chromatography / Mass spectrometry

Electron Impact (EI)
Chemical Ionization (CI)

Ion sources



Electron impact (EI)



1/ e^- produced by heating a metal filament **under vacuum**

2/ e^- accelerated by potentiel difference **=>** E_C= 70 eV

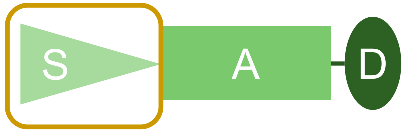
3/ transmission of part of the kinetic energy of the electron beam on the molecule

4/ ejection of an electron and formation of $[M]^{+\bullet}$ ion (molecular ion)

$$\boxed{M + e^{-} \xrightarrow{70\ \text{eV}} M^{\circ +} + 2e^{-}} \longrightarrow \textbf{Fragments}$$

=> Tearing an electron leads to an increase in the internal energy of the molecule and fragmentations: very energetic mode of ionization

Ion sources



Chemical ionization (CI)

Soft ionization in 3 steps

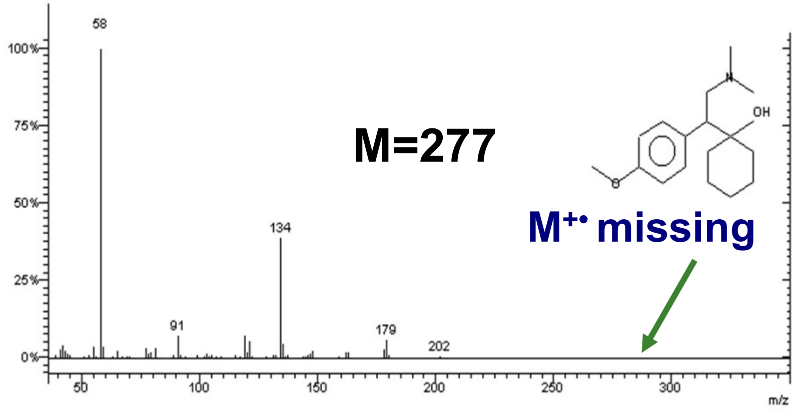
- Ionization of reactant gas by EI
- Molecule ion reaction
- Proton transfert

$GH^+ + M \rightarrow G + MH^+$ proton transfer

$G^{+\cdot} + A \rightarrow G^+ + A^{+\cdot}$ charge exchange

Venlafaxine mass spectra in EI and CI

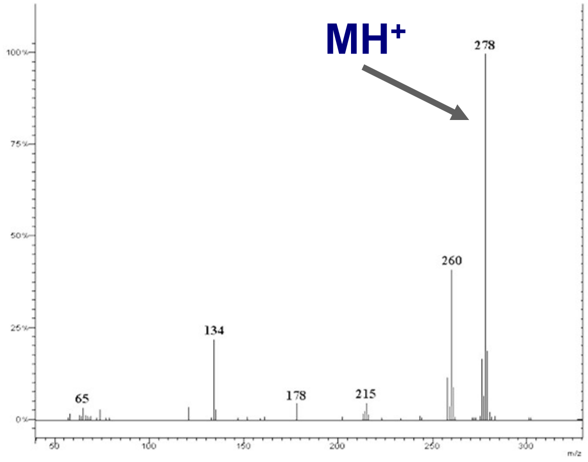
Electron impact ionization



M=277

$M^{+\bullet}$ missing

Chemical ionization (isobutan)

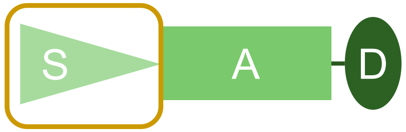


MH^{+}

Advantages	Disadvantages
Simple	Molecule must be volatile
Sensitive	molecule must be thermally stable
Fragmentation helps with identification of molecules	Extensive fragmentation- can't interpret data
Library-searchable fingerprint spectra	Useful mass range is low (<1000 Da)

Molecular weight information, selective, sensitive.

Ionization source:



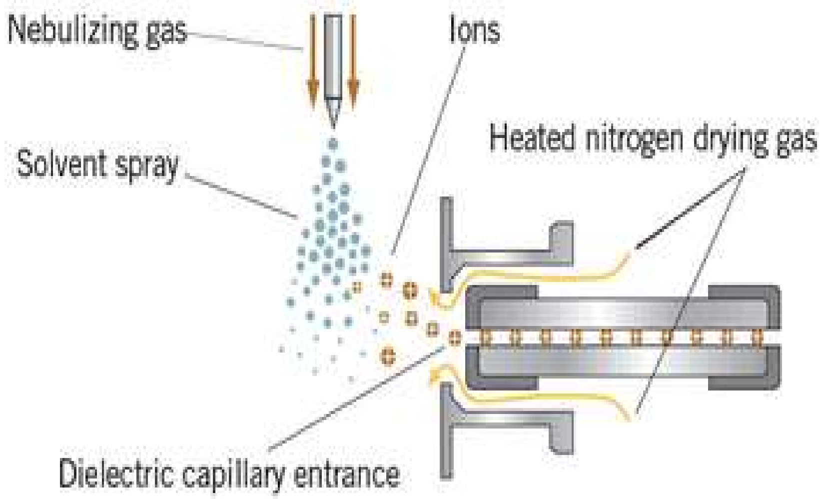
Electrospray (ESI)

Soft ionization technique **at atmospheric pressure**
Vaporization steps by ESI:

(1) dispersal of a fine spray of charged droplets
(2) solvent evaporation
(3) ion ejection from the highly charged droplets

Application to small polar molecules to large biomolecules (proteins, oligonucleotides…)

Ion production : single and multiple charged adducts



Ionization source:

Electrospray (ESI): process

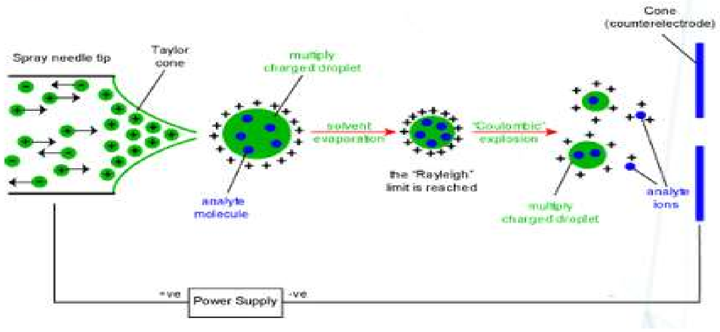
- The nebulizer produces a uniform droplet size.

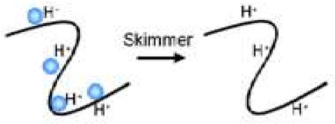
- Charged droplets are attracted toward the capillary.

- Heated nitrogen stream surrounding the capillary shrinks the droplets.

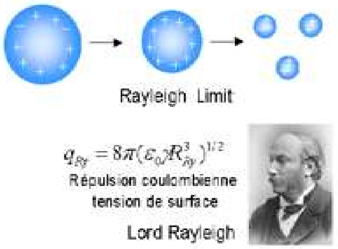
- Desolvation of droplets: shrink until the repulsive electrostatic (Coulombic) forces exceed the droplet cohesive forces, leading to droplet explosions.

- Repetition of the process until analyte ions are ultimately desorbed into the gas phase, driven by strong electric fields on the surface of the micro droplets.





Kebarle, *Anal. Chem.*, 1993, 65, 972A



$$q_{Ry} = 8\pi(\varepsilon_0 \gamma R_{Ry}^3)^{1/2}$$

Ionization source

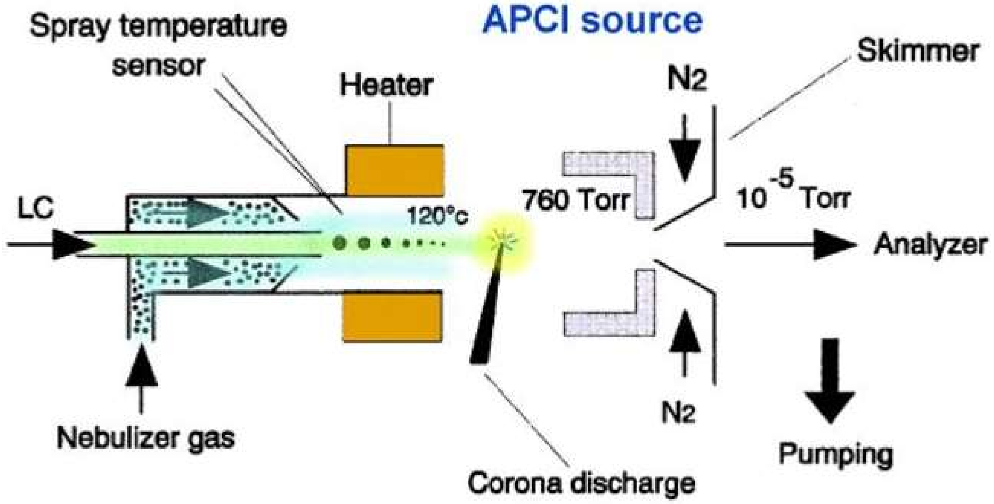
Atmospheric Pressure Chemical Ionization (APCI)

APCI is a gas-phase chemical ionization process.

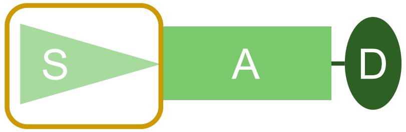
- Vaporization of the analyte before ionization.

- Droplets fully vaporized in a heated ceramic tube (~ 400 to 500°C).

Application to less polar and non-polar compounds



Ionization source



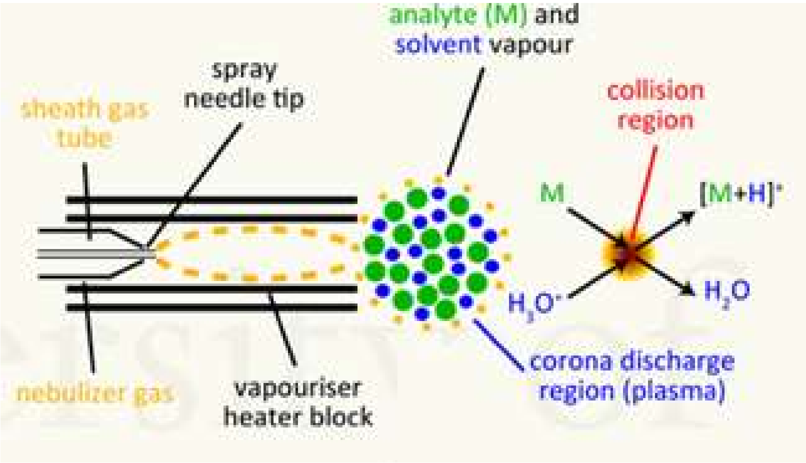
Atmospheric Pressure Chemical Ionization (APCI)

Principle similar to chemical ionization :

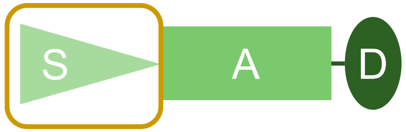
- After evaporation, reagent gas ionization.

- Charge transfer to the analyte.

Generation of single charged ions and adducts



Ionization source



Matrix Assisted Laser Desorption Ionization (MALDI)

Soft ionization technique

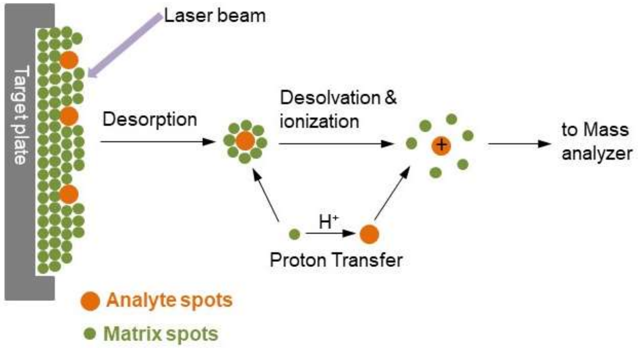
- Sample mixed with a suitable matrix material

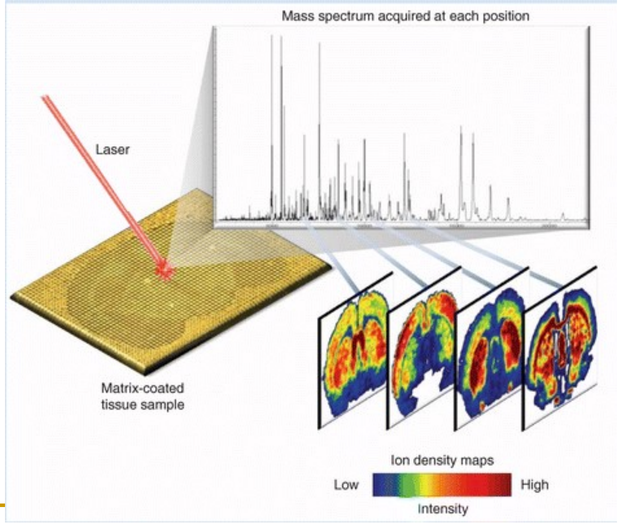
- Co-crystallization on a metal plate.

- Ionization by desorption of the sample and matrix material

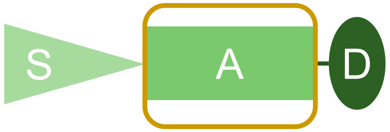
- The molecules are ionized via protonation or de-protonation.

Application of big molecules: proteins, peptides, DNA, and polysaccharides, polymers…..
Generation of single and multiple charged adducts.



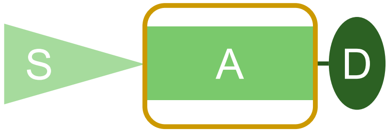


Mass Analyzer



- **Quadrupole**
- **Ion trap**
- **Time of Flight**
- **Orbitrap**

Analyzers characteristics



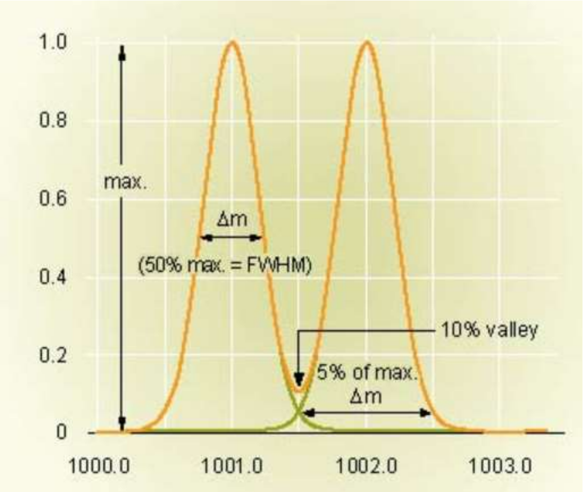
- **Limit in mass/charge ratio**: minimum and maximum values of the measurable m/z ratios expressed in Thomson (Th) or in mass unit (u or uma) when z = 1

- **Resolution**: ability to distinguish 2 close signals.

- **Mass accuracy**: accuracy of measured m/z ratios. Highly dependent on the stability and resolution of the analyzer and is expressed in % or parts per million (ppm).

- **Speed scanning**: measurable mass domain per unit of time expressed in amu per second or millisecond

Analyzers characteristics: resolution



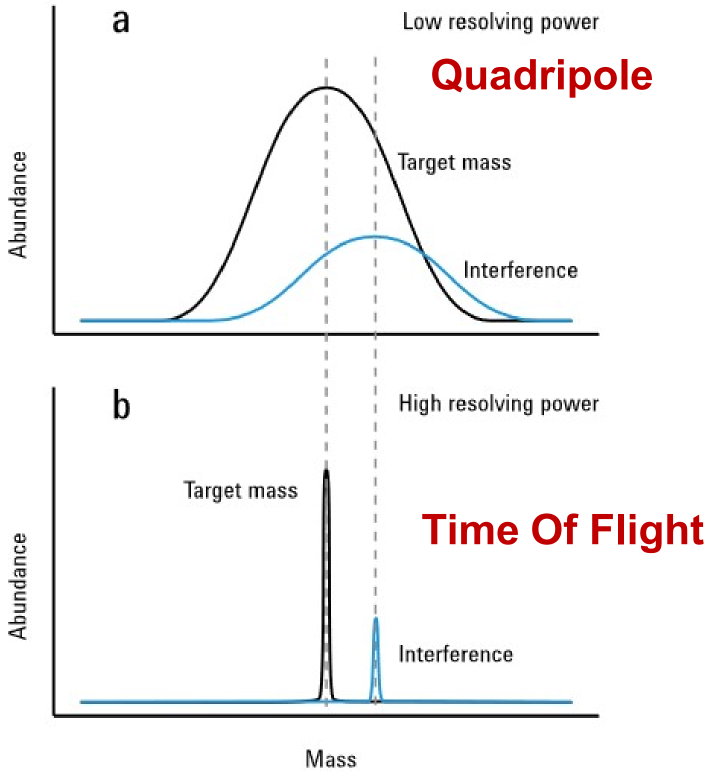
$$R = \frac{M}{\Delta M} = \text{resolution}$$

$$\Delta M = \text{resolving power}$$

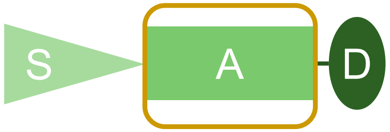
$$M = \text{mass of the (second) peak}$$

FWHM : full width at half maximum

Analyzer	Resolution	m/z range
Quadrupole	2 000	8 000
Ion trap	5 000	6 000
Time of flight	60 000	500 000
Orbitrap	480 000	4 000
FT -ICR	1 000 000	30 000



Mass analyzers



Quadrupole

Resolution: unitary (low resolution)

- Four parallel rods.

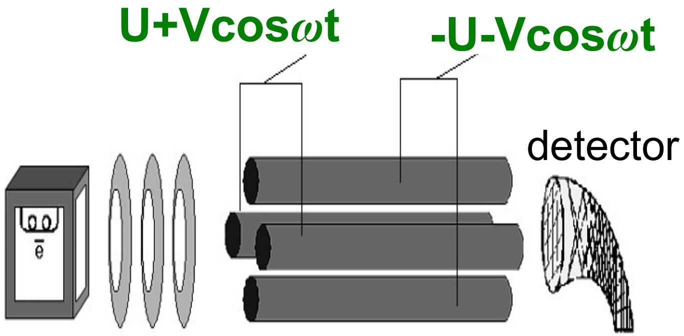
- Opposite rods have the same polarity whilst adjacent rods have opposite polarity.

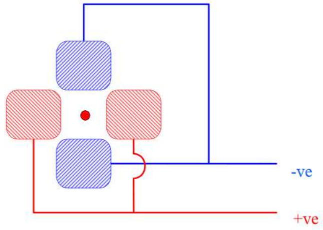
- Constant and variable tension applied : quadrupolar electrostatic field formed and traps the ions.

- Each rod is applied with a DC and an RF voltage. Ions are scanned by varying the DC/RF quadrupole voltages.

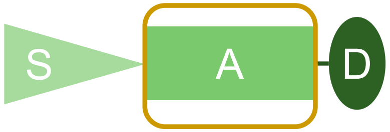
- A DC/RF value is specific to a m/z and stabilizes the trajectory of the ion

- Only ions with the selected mass to charge ratio will have the correct oscillatory pathway in the Rf field

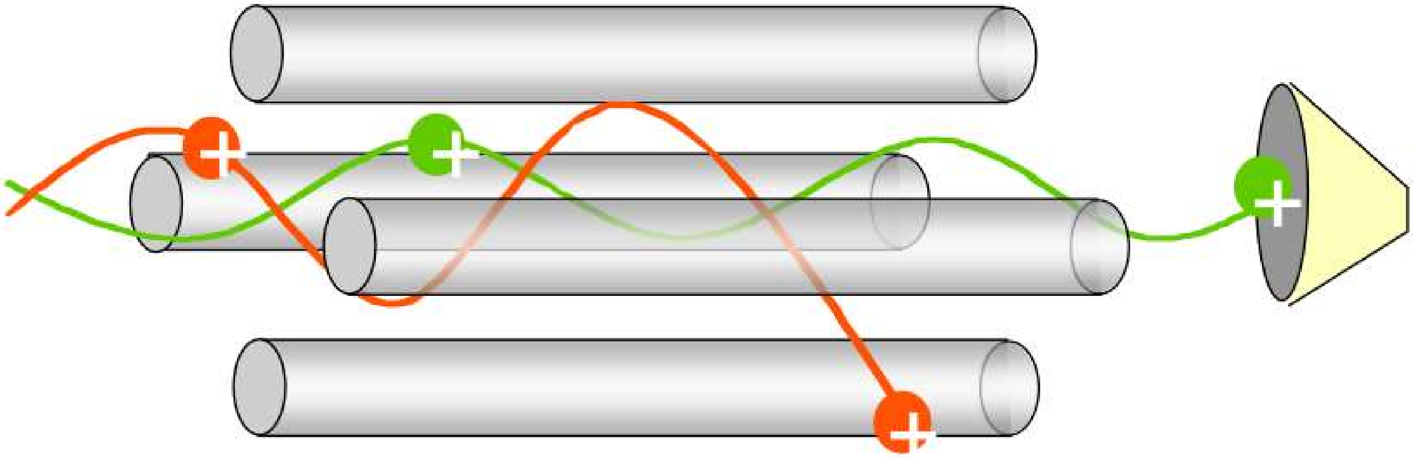




Mass analyzers

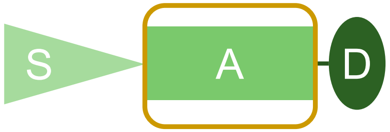


Quadrupole



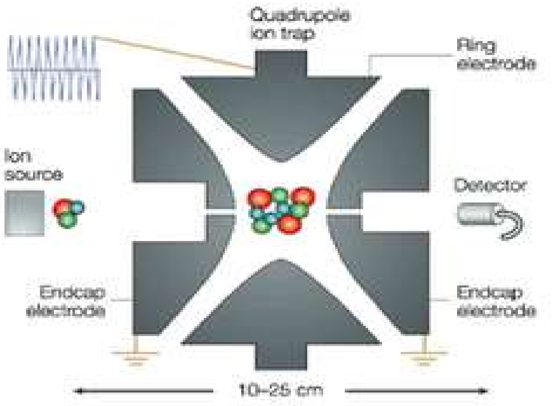
The 🟢 ion is transmitted along the quadrupole in a stable trajectory Rf field. The 🔴 ion does not have a stable trajectory and is ejected from the quadrupole.

Mass analyzers

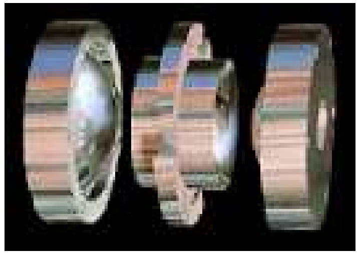


Ion trap

Resolution: unitary (low resolution)

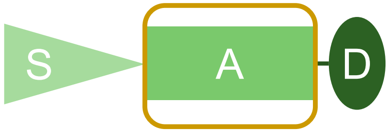


- 1 ring electrode and 2 end caps
- Principle very similar to quadrupole
- Ions stored by RF & DC fields
- Scanning field can eject ions of specific m/z



Advantages: **- successive fragmentations (MS^n)**
- High sensitivity full scan MS/MS

Mass analyzers



Time of flight (TOF)

Resolution: 3 or 4 digits (high resolution)

- Charged ions generated in the ion source enter the mass analyzer.

Analyzer components:
- Flight tube

- After ions are ionized, they arrive at the ion pulser.

- A high voltage pulse is applied which accelerates the ions into the flight tube.

- An ion mirror at the end of the tube reflects the ions and sends them to the detector that records their time of arrival.

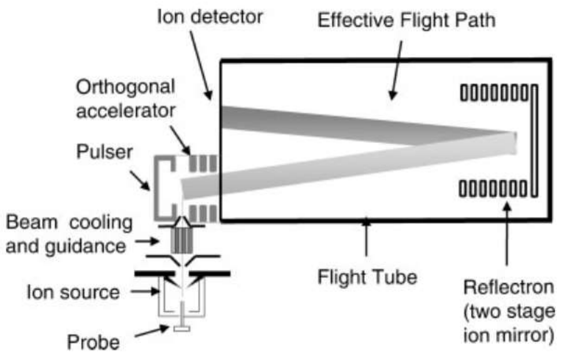
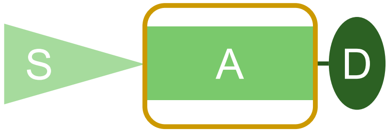


FIGURE 1. Schematic overview of the ToF mass spectrometer.

Mass Spectrometry Reviews DOI 10.1002/mas

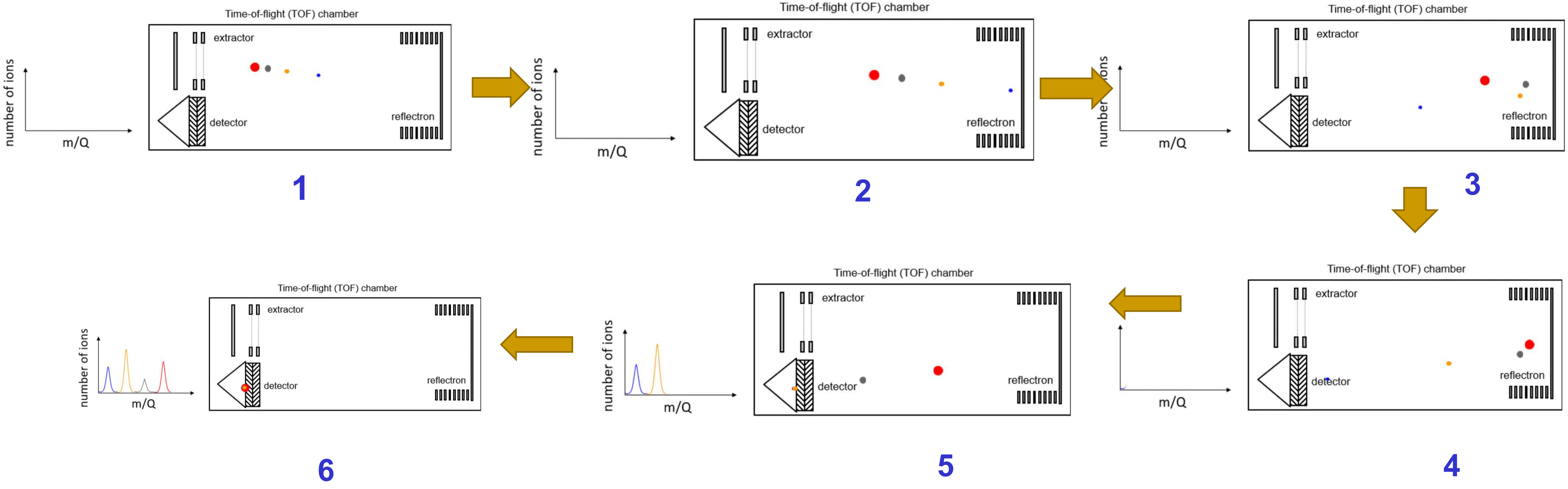
Mass analyzers



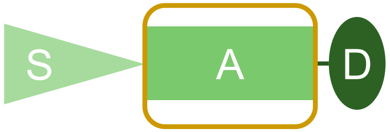
Time of flight (TOF)

Velocity depends on ion’s kinetic energy

Lighter ions travel faster and reach the detector in less time than the heavier particles that move slower and take longer to reach the detector

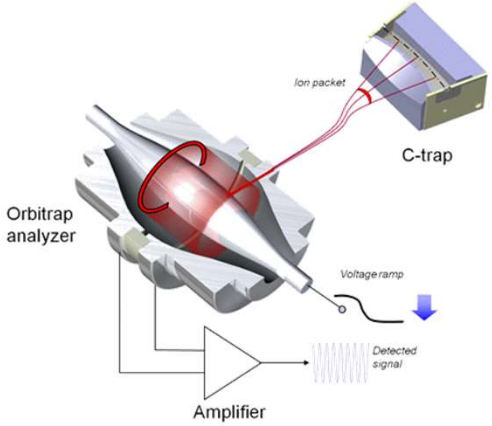


Mass analyzers



Orbital trap (Orbitrap®)

Resolution: 4 digits (high resolution)



- Recent concept : 1999, commercialization since 2005

- DC voltages applied to the electrodes

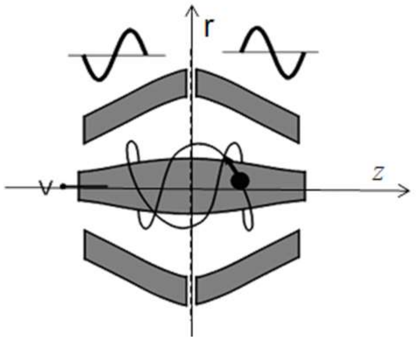
- Ions oscillation around the central electrode under the effect of DC voltages and the geometry of the trap.

- Current induced by the movement of the ions is recorded, amplified and converted by Fourier transform into frequencies:

deduction of m/z and corresponding intensities with a very high resolution.

Axial oscillation

$$\omega = \sqrt{\left(\frac{z}{m}\right)k}$$



-> only depends on m/z

Mass spectrometers: to sum up

- **Electron impact**
- **Chemical ionization**
- **Electropspray**
- **Atmospheric Chemical Pressure Ionization**
- **Matrix Assisted Laser Desorption**

Mass spectrometers: to sum up

Simple mass spectrometer MS

- **Electron impact**
- **Chemical ionization**
- **Electropspray**
- **Atmospheric Chemical Pressure Ionization**
- **Matrix Assisted Laser Desorption**

- **Quadrupole**
- **Ion trap**
- **Time of Flight**
- **Orbitrap**

Mass spectrometers: analyzers combination

Simple mass spectrometer MS

- Electron impact
- Chemical ionization
- Electropspray
- Atmospheric Chemical Pressure Ionization
- Matrix Assisted Laser Desorption

- Quadrupole
- Ion trap
- Time of Flight
- Orbitrap

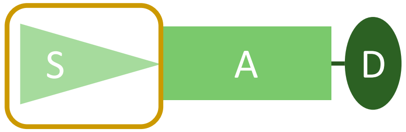
Tandem mass spectrometer MS/MS

Hybride mass spectrometer MS/MS

Acquisition modes

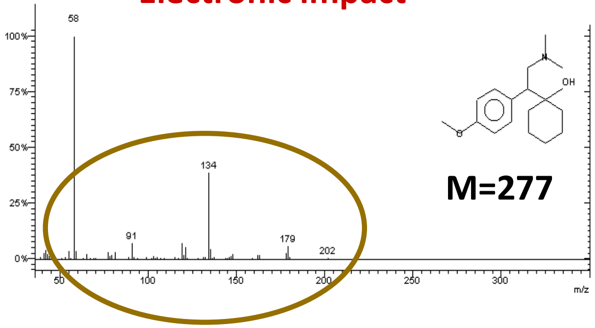
- Fullscan
- Single ion monitoring/recording
- Ion fragmentation

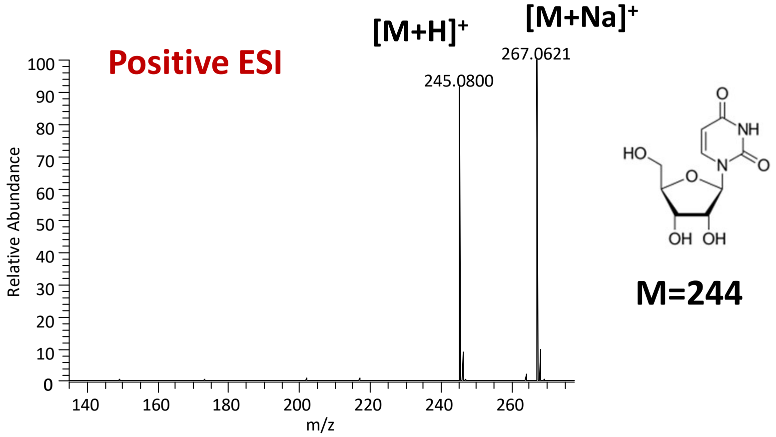
Acquisition modes



Scanning acquisition *"fullscan"*

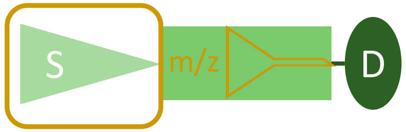
Electronic impact





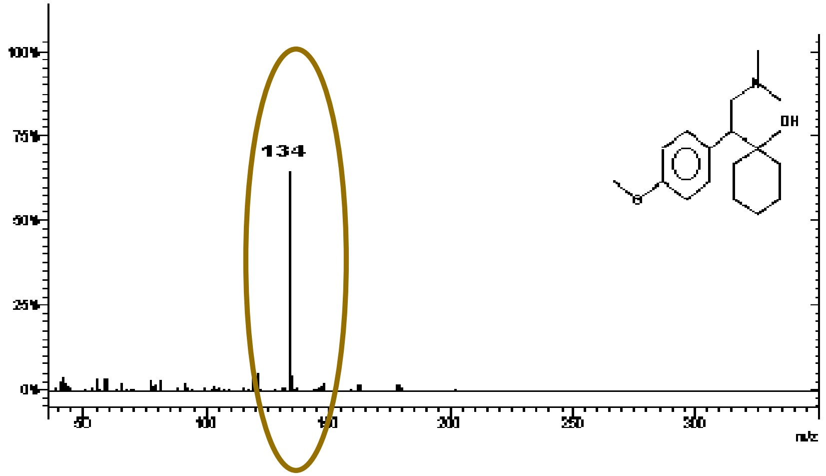
- Scanning of voltages DC and RF of the potential used
- Overview of all the ions produced in the source at a given time
- Source spectrum => "Inventory" of the sample
- Poor sensitivity, poor selectivity

Acquisition modes



"*Single Ion Monitoring*" (SIM), "*Single Ion Recording*" (SIR)

SIM on134 m/z ion



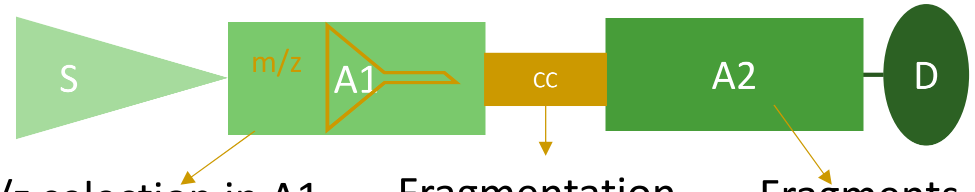
- Detection of one or more ions of the analyzed products
- The quadrupole field is fixed at values of DC and RF such that only a given ion of m/z reaches the detector
- Multiple values of DC and RF will be applied in turn to select multiple ions
- **Increasing sensitivity: ejection of unwanted ions**
- Poor selectivity

Acquisition modes

Ion fragmentation

- Structural elucidation: Interpretation of spectra unknown compounds spectra
- Selective and sensitive analyzes: Allows quantification while keeping structural information
- Detect and quantify trace molecules
- Remove interfering matrix

MS/MS analysis



m/z selection in A1
Parent ion

Fragmentation
in collision cell

Fragments
scanning in A2

- 1-Selection of an ion by SIM in A1 (often a quadrupole)

- 2- Activation by collisions between the ion and a collision gas in collision cell

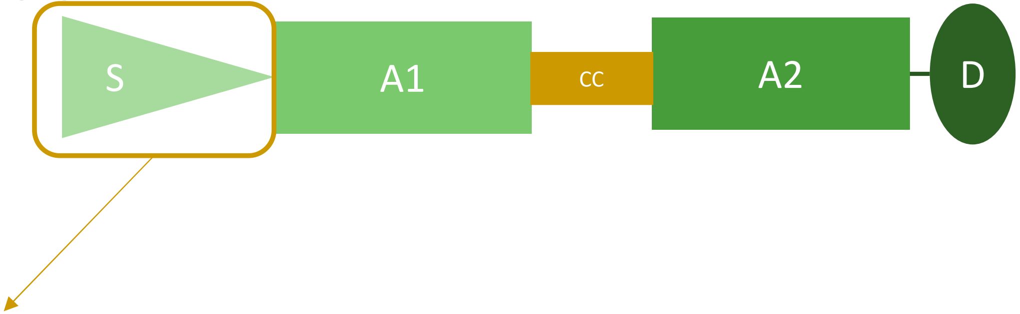
- 3- Fragmentations (daughter or fragment ions) in CC

- 4- Scanning fragment ions in A2 to obtain fragmentations spectrum

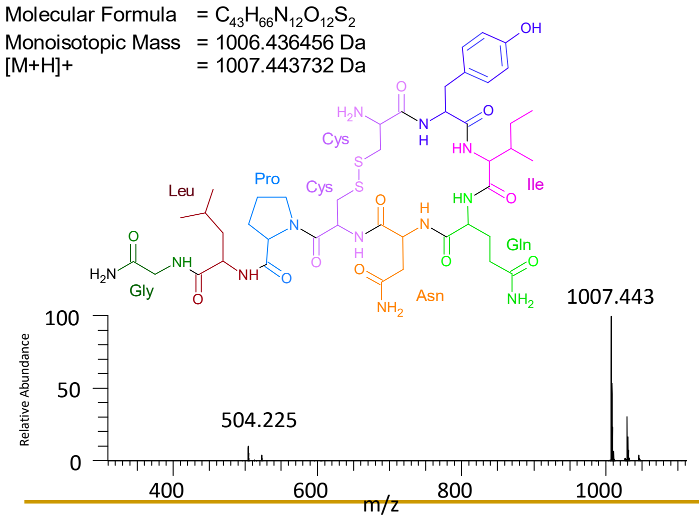
Sensitivity, selectivity, structural informations, rich spectrum, quantitative analysis

MS/MS analysis

Structural analysis



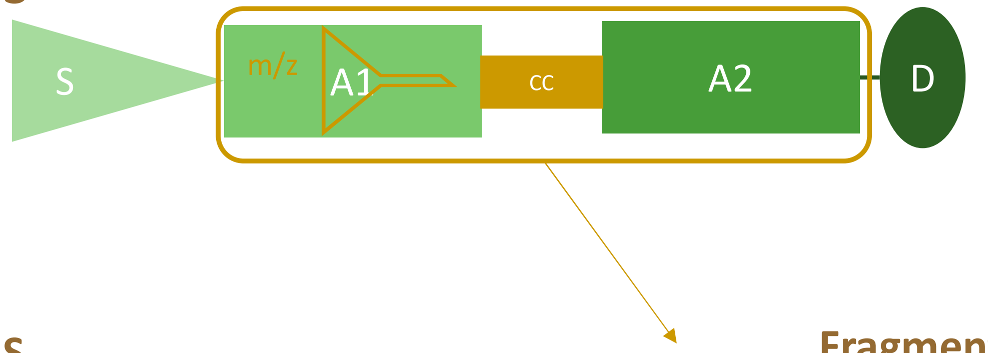
Fullscan MS



Molecular Formula = $C_{43}H_{66}N_{12}O_{12}S_2$
Monoisotopic Mass = 1006.436456 Da
[M+H]+ = 1007.443732 Da

MS/MS analysis

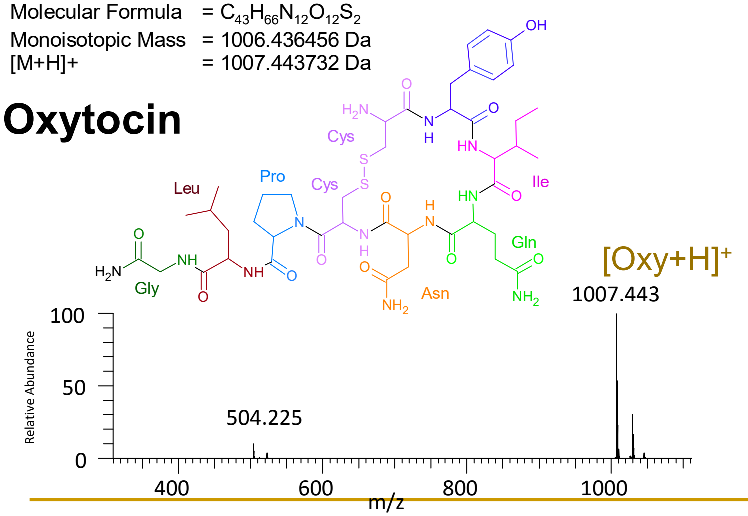
Structural analysis



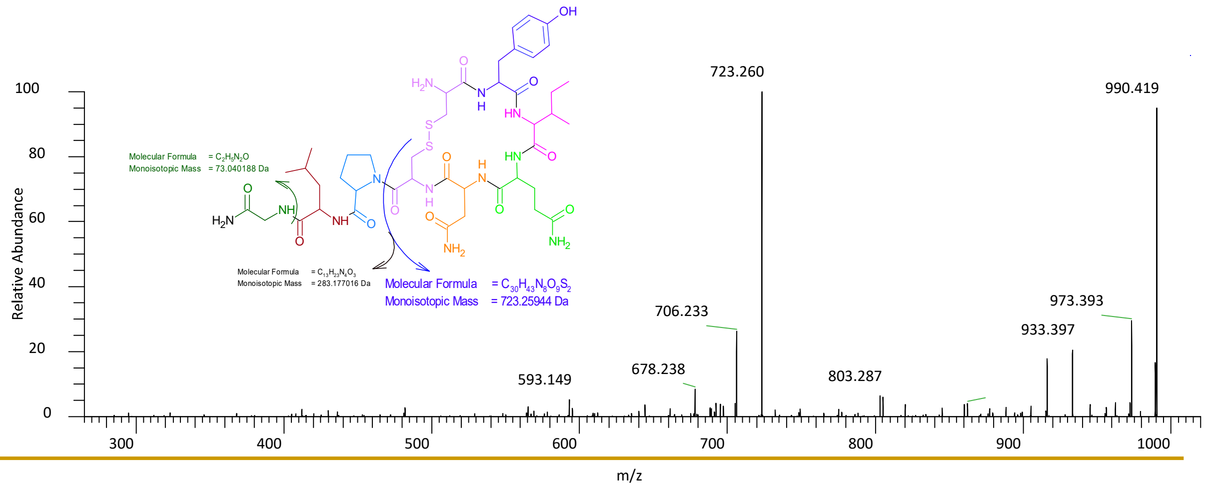
Fullscan MS

Molecular Formula = $C_{43}H_{66}N_{12}O_{12}S_2$
Monoisotopic Mass = 1006.436456 Da
[M+H]+ = 1007.443732 Da

Oxytocin

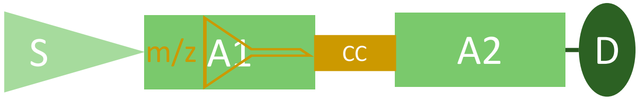


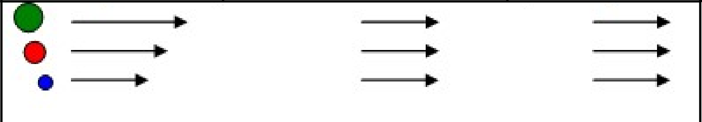
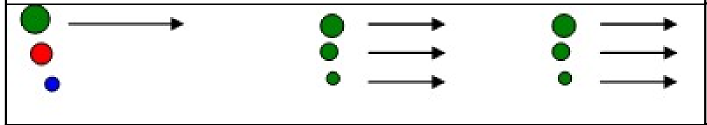
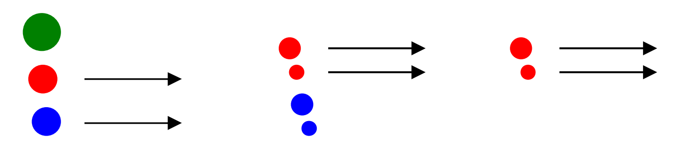
Fragmentation MS/MS



MS/MS analysis

Quantification in a tandem quadrupole



Q1	Collision cell	Q2	Scan type
			**Fullscan**
			**Fragmentation**
			**Multiple Reaction Monitoring (MRM)**

MS Spectrum

MS/MS Spectrum

MRM

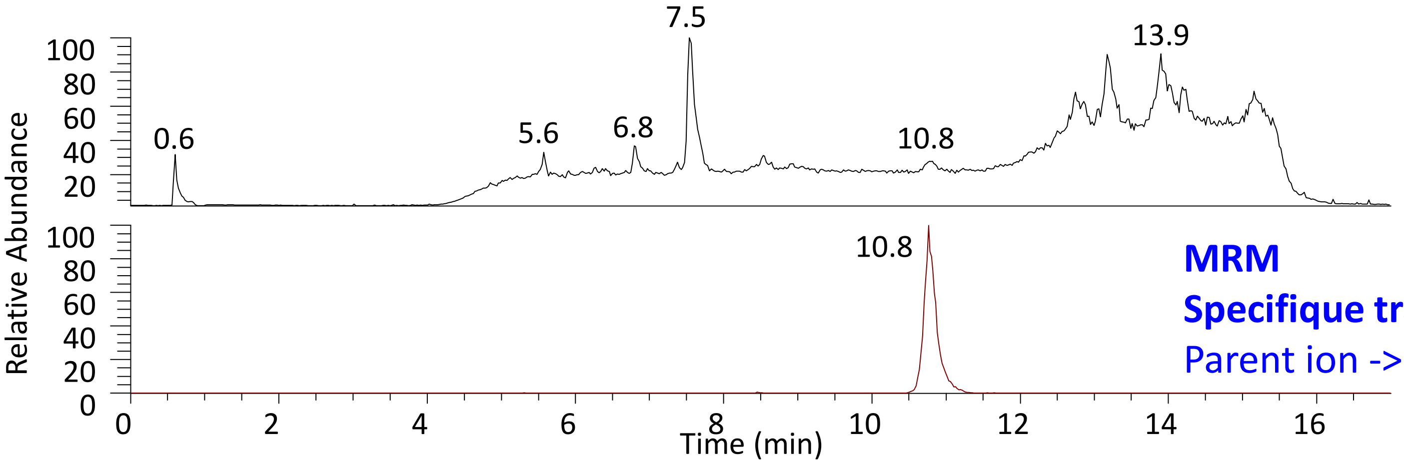
Acquisition modes

Ion fragmentation in a tandem quadrupole

Molecules of interest :

- active substance
- metabolites
- drugs

Example of a quantitative analysis :

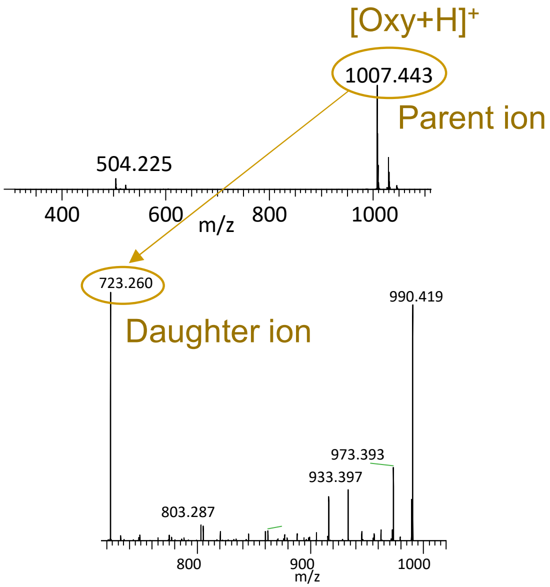


Fullscan

MRM

Specifique transition

Parent ion -> daughter ion



Selective quantification of molecule(s) in complexe biological sample.

Sensitivity gain

Mass spectrometry: things to remember

- Chromatography coupling (GC or LC)
- (vaporization)
- Ionization of molecules

- m/z separation
- (fragmentation)
- Low or high resolution MS

- m/z detection
- Intensities recording

- Structural informations

- Quantification