Université de Strasbourg

Licence 3

Analytical Chemistry

Mass Spectrometry

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Outline

1. General introduction of mass spectrometry (MS)

2. Fundamentals and Instrumentation of MS

   2.1. Ion source
   2.2. Analyzer
   2.3. Detector
   2.4. Principle of fragmentation

3. Applications

Did you know it?

Mass spectrometry is used for:

- Locate a deposit by analyzing hydrocarbons in rocks
- Detect and identify the use of steroids in athletes
- Study the composition of molecules found in space
- Detect the presence of dioxins in contaminated food
- Study genetic mutations
- Discover new pathological markers
- Analyze and date archaeological pieces

Introduction

History

- 1897: J. Thomson discovers the electron and determines its m / z ratio (Physic Nobel Prize)
- 1912: Construction of the first mass spectrometer

3 major periods:

-1912-1960: Elementary analysis and increase of resolution power

-1960-1980 Analysis of organic compounds, increase of the mass range, beginning of the exact mass evaluation for the determination of crude chemical formula

-1980- Analysis of biologics macromolecules

Brève historique:

Nowadays:

-Miniaturization of mass spectrometers (tanks, space stations, operating theaters, ...)

- Towards ever more resolute and therefore precise systems in molecular weight measurement

What is mass spectrometry?

➢ Analytical method to "weigh" the molecules with a very high precision.

➢ Its molecular weight is determined

Example of application:

* Search the signal of a given compound in a complex mixture (Carbon monoxide in Titan's atmosphere or a dopant in urine)

* Get a first data on an unknown molecule (molecule extracted from a medicinal plant)

Principle of mass spectrometry ?

➢ Analytical method for measuring the mass of molecules in relation to their number of charge

➢ Mass to charge ratio:

$$\frac{m}{z}$$

How to weigh a molecule?

- Work in the gas phase where the molecules are isolated
- Working with charged molecules (ionization)
- Use properties linking:

Energy / Path / Mass

Work in electric or magnetic fields

A mass spectrometer measures the mass of isolated molecules

Three steps:

1- Volatilize

- Separate the molecules from each other
- To move from the state of condensed matter to a gaseous state

2- Ionize

- Turning molecules into ions
- Use of an electric field

3- Analyze

- Calculate molecular mass from the ratio:

m / z = mass / number of charges

Ionization and fragmentation

Ionization:

1. By protonation: $A\text{-}BH^+$
2. By deprotonation: $A\text{-}B^-$
3. By loss of electron: $A\text{-}B^{+\bullet}$
4. By cationization: $A\text{-}B\text{-}Na^+$

Fragmentation :

- When ions have too much internal energyv

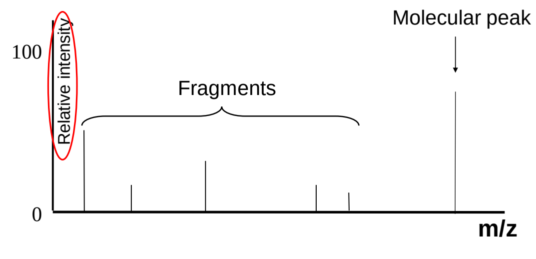
$$A\text{-}B^{+\bullet} \rightarrow \begin{cases} A^+ + B^\bullet \\ A^\bullet + B^+ \end{cases}$$

What information can mass spectrometry provide?

1- The molecular weight of a compound

2- The mass of some "pieces" of this compound called fragments

3- A measure of quantity

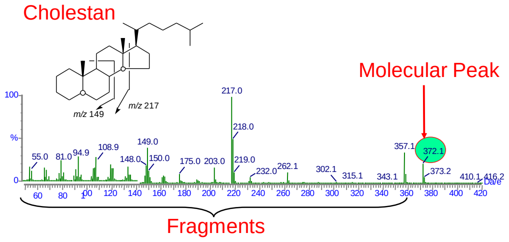


Example of cholestan

1- The m/z value of the molecular peak makes it possible to calculate the molecular mass

2- Fragmentation peaks make it possible to reconstitute part of the structure

3- Peak intensity allows for quantitative analysis



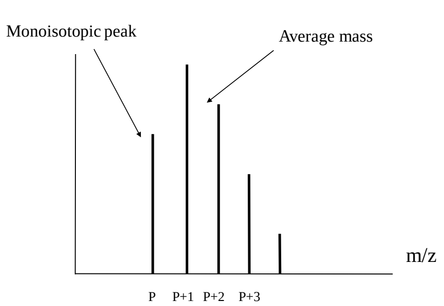
Example: MS spectra of cholestan obtained by electron impact MS

How to calculate the molecular mass?

Influence of isotopes

M	M+1	M+2	
^{12}C 98,9% ^{14}N 99,64% ^{16}O 99,8%	^{13}C 1,1% ^{15}N 0,36% ^{17}O ε	^{18}O 0,2%	1 major isotope
^{35}Cl 75,8% ^{79}Br 49,8%		^{37}Cl 24,2% ^{81}Br 50,2%	Extended distribution

Isotopic profile



What mass are we measuring?

Monoisotopic mass

It is the "exact" mass of the first peak of the isotopic profile, ie the mass which takes into account only the masses of the most stable isotopes (C^{12}, H^{1}, O^{16}, S^{32}, N^{14}, …).

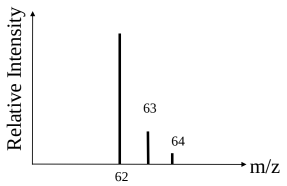
Chemical or average mass

It is the centroid of the masses constituting the isotopic profile ie the mass which takes into account the mass of the elements given by the periodic table (C=12,011).

The mass is expressed in Dalton (Da)

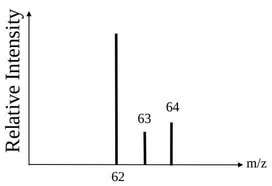
It depends on the resolution of the mass spectrometer

Why seek an isotopic profile?



HO—CH_2—CH_2—OH

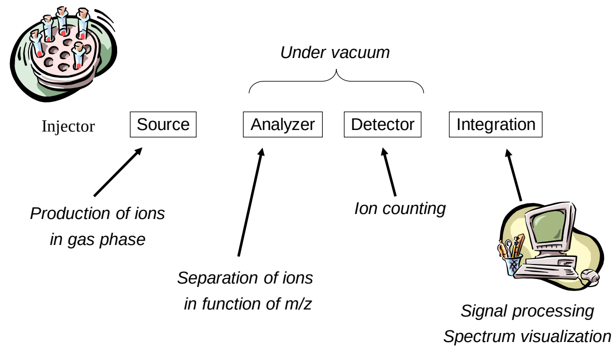
m/z = 64 : 2 C^{13} or 1 O^{18} (0.2%)



CH_3—CH_2—SH

m/z = 64 : 2 C^{13} or 1 S^{34} (4.2%)

Mass spectrometer



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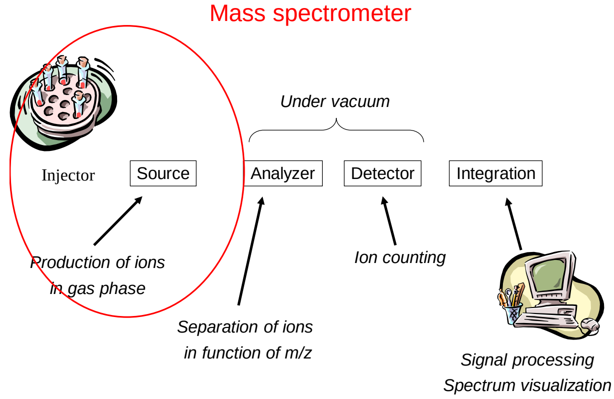
Mass spectrometer



The ion source: its role is to volatilize and ionize

There are many types of ion sources and each of these types of sources is based on a different physical principle.

The physical principle that makes it possible to volatilize and ionize a type of compound is chosen by the operator according to the characteristics of the analyzed molecule. The volatilization and ionization steps are successively or simultaneously depending on the type of source.

The main criteria for selection are:

- the volatility and thermal stability of the compound to be analyzed
- the chemical functions present and their ability to induce ionization
- The size of the molecules
- the quantities of product available

Different ionization methods

➢ Ionization of a neutral molecule by ejection or capture of an electron

$A\text{-}B^{+\bullet}$

➢ Ionization by protonation or deprotonation

$A\text{-}BH^{+}$ or $A\text{-}B^{-}$

➢ Ionization by adduct formation (ion-molecule reaction)

$A\text{-}B\text{-}Na^{+}$

Ion sources are classified in "hard" sources and "soft" sources

- Numerous ionization methods have been invented to ionize and volatilize increasingly fragile, large and polar molecules.
- "Hard ionizations" often generate molecular ions, with an odd number of electrons, which fragment a lot and sometimes even completely before having time to leave the source. Their fragments can be analyzed and give structural information.
- "Soft ionizations" generate ions with even-numbered electron, which are relatively stable and have sufficient lifetimes to cross the analyzer, reach the detector, and thus be measured.

Sources d'ionisation

EI Ionisation (Electron Impact)	Hard
CI Ionisation (Chemical Ionisation)	Soft
ESI Ionisation (electrospray)	Soft
MALDI(Matrix Assisted Laser Desorption Ionisation)	

Ionization Sources

EI Ionisation (Electron Impact)

CI Ionisation (Chemical Ionisation)

Small molecules

Volatile and non-heat-sensitive

ESI Ionisation (electrospray)

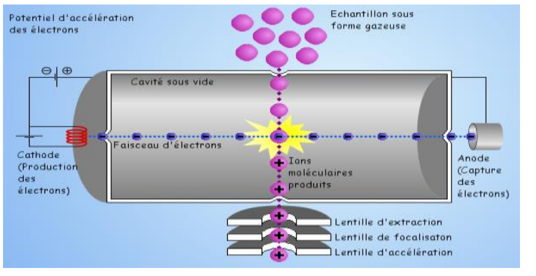
MALDI(Matrix Assisted Laser Desorption Ionisation)

LC-ESI-MS coupling

On small non-volatile molecules

Biomolecules (1 300 kDa) and non covalent complex

Electron Impact (EI)



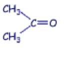
- a filament carried at high T ° C by passing a current emits electron e^- which can be accelerated by a certain ΔV.

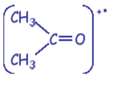
- E_{cin} of e- influences the ionization efficiency and the excitation energy of the formed ions

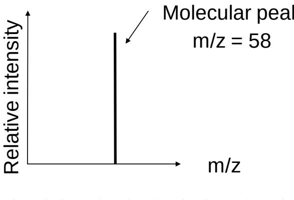
optimum efficiency: e^- accelerated beam at 70eV

Electron Impact (EI)

Example of acetone







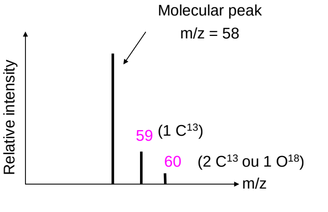
The notation $M^{+\bullet}$ means that it is the whole molecule after the loss of an electron. It is positively charged with an unpaired free electron.

Molecular ion

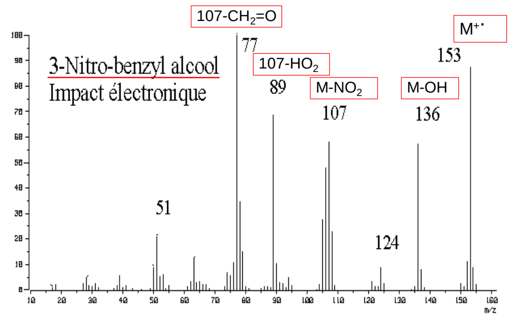
We can have on the spectrum of peaks having a mass greater than that of the molecular ion

related to the presence of isotopes

So for acetone :



Example of EI spectrum



Chemical Ionization Source (CI)

Complementary to the electron impact because produces ions with a small excess of energy

⟶ Little fragmentation

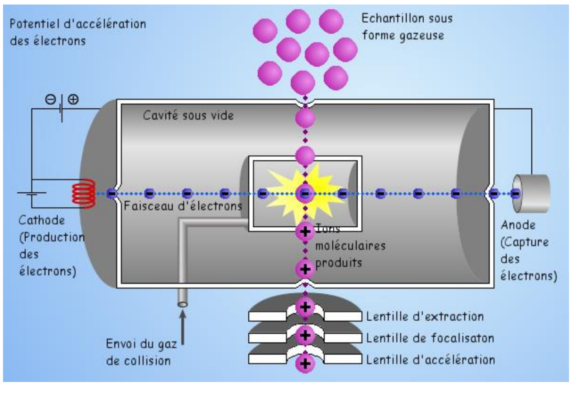
the molecular ion is easily recognizable!

Ionization is done by collision between the gaseous molecules of the sample and primary ions of a reactive gas present in the source:

the ionization is done by ion-molecule collisions

$GH \longrightarrow GH^{+\cdot}$	$GH^{+\cdot} + M \longrightarrow MH^{+} + G$
Ionization of the reactive gas by electron impact	Ionization of the molecule M by proton transfer

Chemical Ionization Source (CI)



Chemical Ionization Source (CI)

1. Reactive gas: example of methane

1. Formation of ions by EI

$$CH_4 + e^- \longrightarrow CH_4^{+\cdot} + 2e$$

$$CH_4^{+\cdot} \longrightarrow CH_3^+ + H^\cdot$$

$$CH_4^{+\cdot} + CH_4 \longrightarrow CH_5^+ + CH3^\cdot$$

$$CH_3^+ + CH_4 \longrightarrow C_2H_5^+ + H_2$$

2. Collision between ionizing species and the molecule to be analyzed

The major entity of the gaseous plasma is CH_5^+ which is a very strong acid (electrophilic), able to protonate most organic molecules

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

$$C_2H_5^+ + M \longrightarrow MC_2H_5^+$$

MH^+ is an (M+1) ion of low internal energy

Little fragmentation

The reactive gas must be chosen according to the molecule to be analyzed

The proton affinity of a B product is defined as the enthalpy of the reaction:

$BH^+ \longrightarrow B + H^+ \quad \Delta H^0 = AP(B)$

The chemical ionization of a M molecule can be considered as the sum:

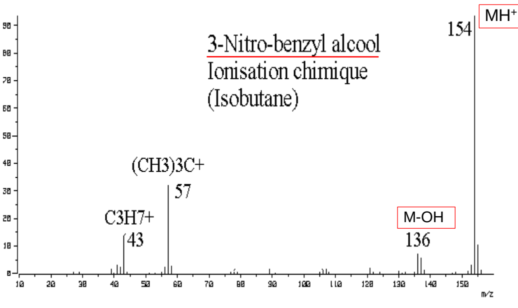
$BH^+ \longrightarrow B + H^+ \quad \Delta H^0 = AP(B)$

$M + H^+ \longrightarrow MH^+ \quad \Delta H^0 = -AP(M)$

The reaction takes place if it is exothermic ie if AP(M) > AP(B)

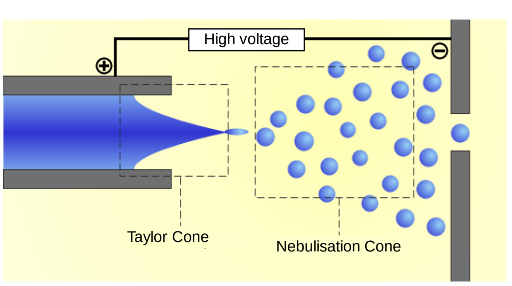
Reactive B	CH_4	H_20	NH_3	$n\text{-}C_4H_{10}$
Ion BH^+	CH_5^+	H_3O^+	NH_4^+	$C_4H_{11}^+$
AP(B) kJ/mol	540	742	858	723

Example of CI spectrum



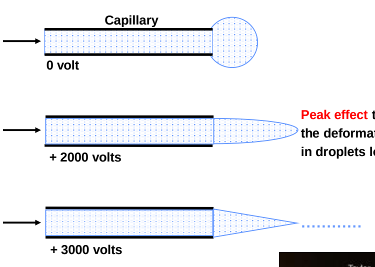
Electrospray ionisation (ESI)

based on the atmospheric pressure formation of charged molecules from a spray created in an electric field



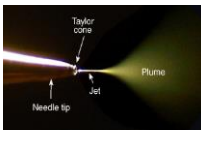
Electrospray ionization: principle of spray production

Required flow by a pump at p atm

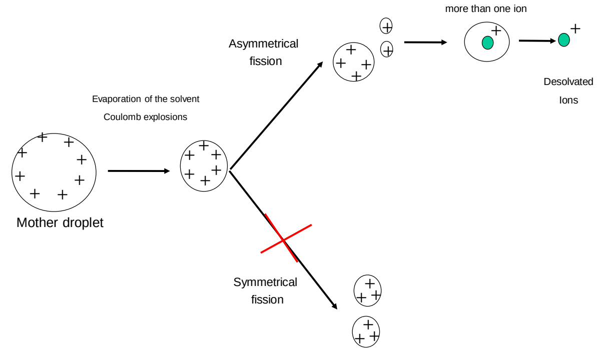


Peak effect that drives the deformation of the liquid in droplets loaded

Emission of a visible spray These droplets expelled dry, start fissions, and generate desolvated ions



Dole model



Advantage of electrospray

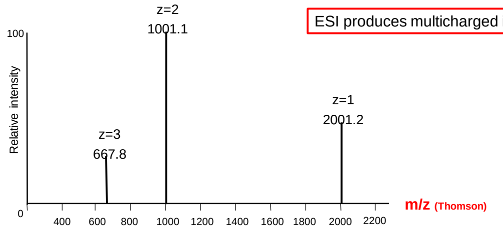
- ➢ Operates at low T ° C, at atmospheric pressure,
  - no degradation, no fragmentation
- ➢ Generates multicharged ions
- ➢ accurate measurement of the molecular weight (0.1%) or ± 1 Da M = 10000 Da
- ➢ Extraction des ions de large masse moléculaire (polymère, biomolécule)
- ➢Sensitive (C ~ µM)
- ➢ Extraction of polar molecules

Limitation of electrospray

- ➢ Little structural information except when performing MS / MS
- ➢ Very sensitive to the presence of salts or additives
  - suppression ion phenomena
  - imperative desalting

Interpretation of an electrospray spectrum

ESI produces multicharged ions



Charge state	m/z	Calculated masses
1	$2001.2 = (M + m_H) / 1$	2000.2 Da
2	$1001.1 = (M + 2m_H) / 2$	2000.2 Da
3	$667.8 = (M + 3m_H) / 3$	2000.4 Da

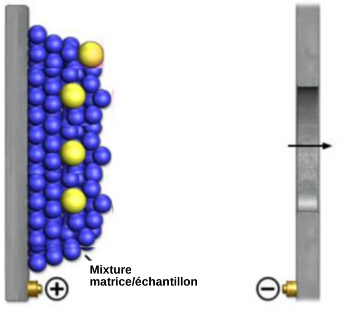
Mass of our compound: 2000,2 Da

Matrix Assisted Laser Desorption Ionisation (MALDI)

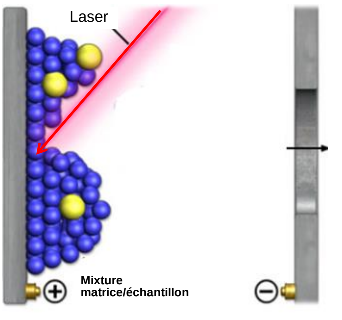
- MALDI is based on the use of a compound (the matrix) that absorbs at 337 nanometers
- The energy will be transferred to the sample by the matrix
- The ionized sample will be transferred to the analyzer

Generates ions at a single charge

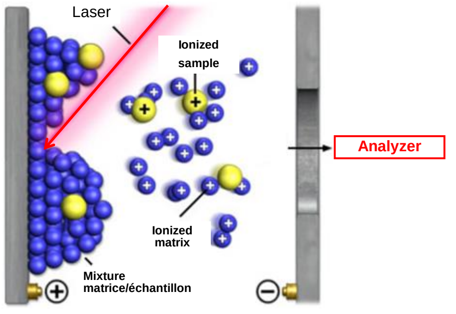
Principle of MALDI-MS



Principle of MALDI-MS



Principle of MALDI-MS

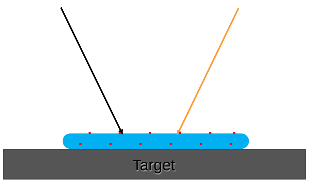


MALDI target

Sample preparation in MALDI-MS

Sample
Water 0,1 % TFA/acetonitrile (50/50)

matrix
α-cyano-4-hydroxycinnamique acid
In water 0,1 % TFA/acetonitrile (50/50)



- The analyte is diluted about 10,000 times in this matrix
- Slow and total evaporation of solvents
- Formation of large matrix crystals
- No coupling with possible chromatography

Characteristics of the matrix:

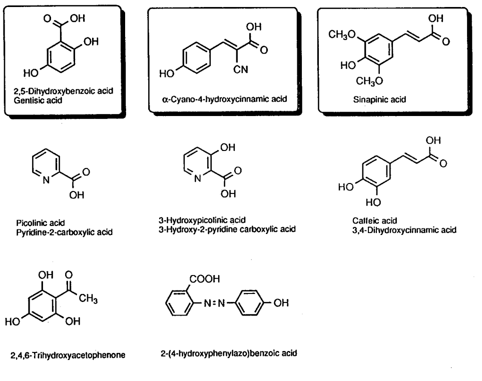
1. Low molecular weight (facilitate vaporization)
2. Acidic (acting as a source of protons)
3. Strong absorption in UV (absorbs laser irradiation)
4. Functionalized with polar groups (work in aqueous solution)

Role :

- Protect the analyte from destruction by a direct laser beam
- Facilitate its vaporization and ionization

Matrices commonly used in MALDI-MS

They absorb at 337 nm and crystallize easily



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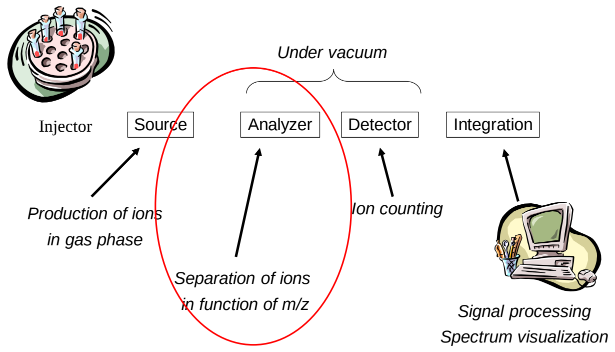
Mass spectrometer



The analyzer

There are different types of analyzers.

They are all based on different physical principles, but all analyzers measure m / z values.

This is a part of the vacuum apparatus($10^{-5} – 10^{-7}$ Torr)

BE:	Deflection by a magnetic field (the oldest)
Q:	Quadrupole
IT:	Ion Trap
TOF:	Time of Flight
FT-ICR:	Fourier Transform Ion Cyclotron Resonance

The ions generated in the source are sent (extraction and focalisation) to the analyzer by electrostatic fields that can be a few volts (Q, IT, FT-ICR) or several tens of kilovolts (TOF, B).

Notion of the “mean free path”

The mass spectrometer must be under a high vacuum because it is necessary to limit the collisions between the ions to be analyzed and the residual gas molecules:

- deviation of the ion from its trajectory
- unwanted reactions (fragmentation)

Mean free path: minimum distance between 2 shocks at a given pressure

According to the kinetic theory of gases:

$$L = kT / \sqrt{2}p\sigma$$

$$\boxed{L = 0{,}66 / p}$$

L : Mean free path (m)

k: Boltzmann constant ($1{,}38.10^{-21}$ J/K)

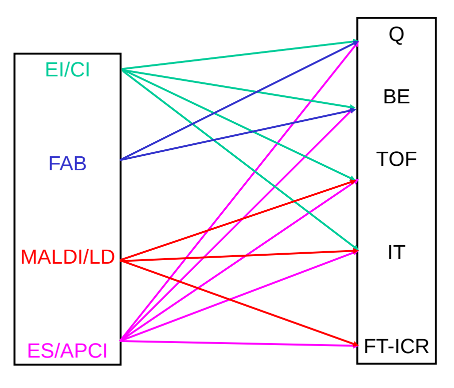
T: Temperature (300 K)

p: Pressure (Pa)

σ: collision section (45.10^{-20} m^2)

Commercial mass spectrometers

Many source/analyzer couplings are possible



The main features of an analyzer are:

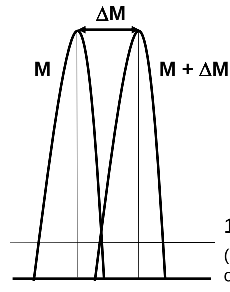
- Resolution R
- Mass range m/z
- Scanning speed in m/z
- Sensitivity

Often, with the same analyzer, one of these characteristics can be increased at the expense of others, but only within certain limits.

Each type of analyzer has its "strong point"

Resolution **R**

R measures the ability of an analyzer to distinguish ions separated by ΔM Dalton (M ion compared to M+ΔM ion)

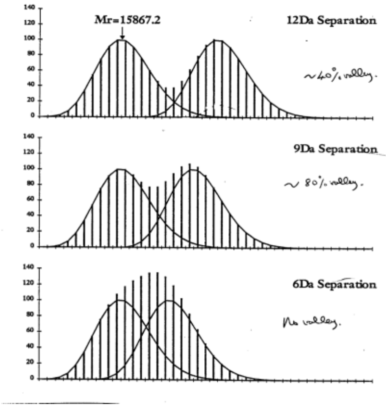


$$\boxed{R = M/\Delta M}$$

10 % Valley

(2 peaks overlap on 10% of their height)

The resolving power



Detection of 2 products
Good accuracy of
mass measurement

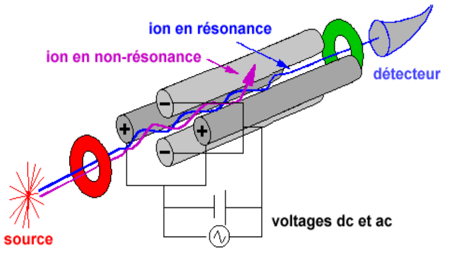
Loss of info: 2 products
Mass measurement
false

Characteristics of analyzers

Analyzer	Resolution	Mass range m/z
Quadripole (Q)	2 000	8 000
Magnetic (EB)	20 000	20 000
Time of Flight (TOF)	20 000	500 000
Ion Trap	5 000	6 000
FT-ICR	1 000 000	4 000

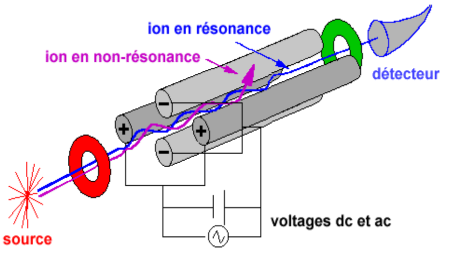
Quadrupole

Quadrupole has four parallel metal bars between which ions are injected with a kinetic energy of a few electron volts.



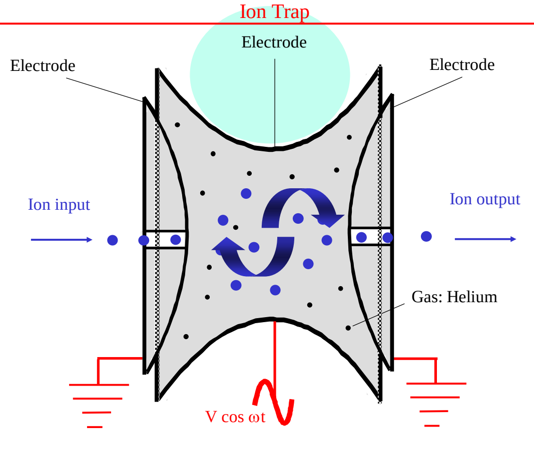
Quadrupole

The ions oscillate between the bars (slalom) thanks to oscillating electric voltages applied on the bars.



Ions of a single m/z value pass through the system without hitting the bars

Ion Trap



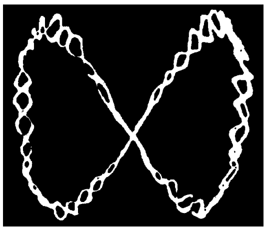
Ion Trap

Movement equation of ions identical to those for quadripole

The four parallel bars of the quadrupole filter are replaced by an "O-ring" whose interior is hyperbolic.

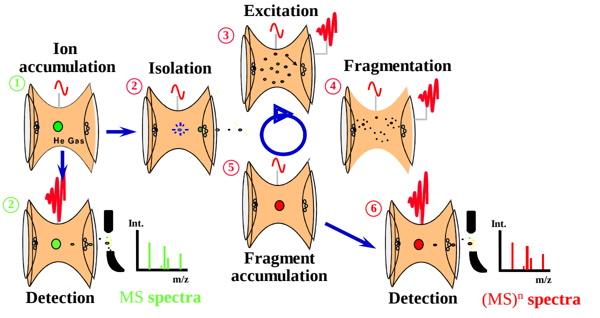
The functions that represent the voltages applied on the ring make it possible to calculate the equations of movement of the ions.

Ion trajectory



Curve of Lissajous

MS and (MS)^n analysis using an ion trap

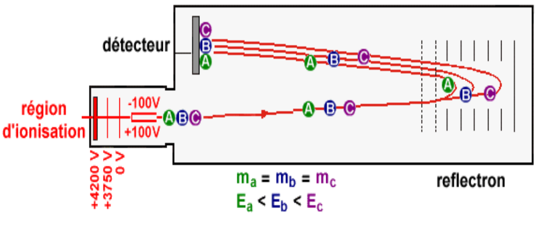


Time of Flight (TOF)

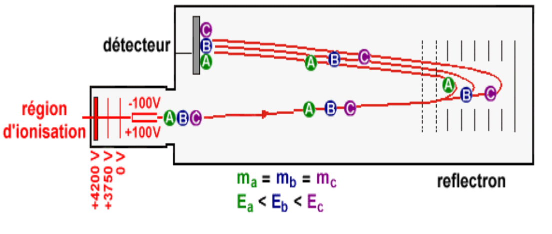
Separation of ions as a function of their speed when moving in a free zone of fields (flight tube)

Two types of use mode:

Linear mode and reflectron mode



Time of Flight (TOF)

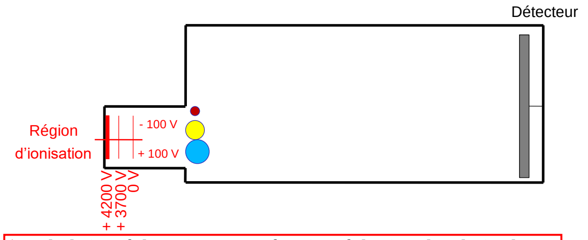


Ions arrive with their Ec ($mv^2/2$) in a free zone of fields

- Lighter are faster → 1st detected
- Heavier are slower → last detected

Time of Flight (TOF)

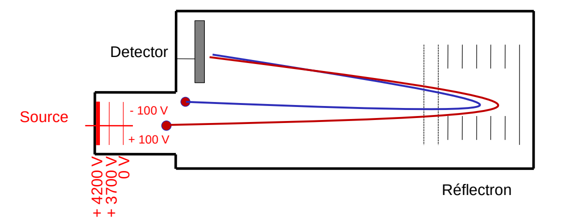
Linear mode



- **Calculation of the ratio m/z as a function of the time that the ion has to travel through the flight tube**
- **Extremely fast analysis speed**
- **Mass limit > 1 000 000, but 5000 resolution**
- **<u>Limitation</u> : Dispersive effect of Ec, lower resolution**

Time of Flight (TOF)

Reflectron mode

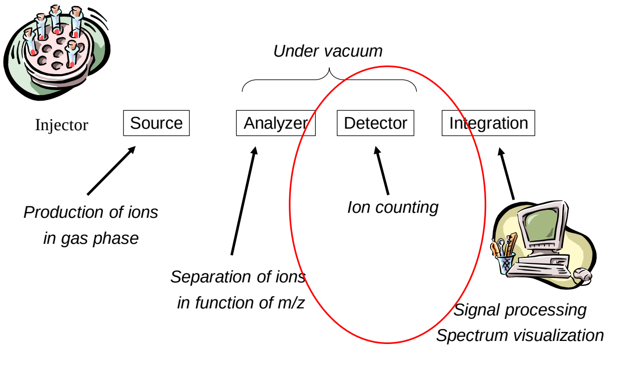


- **Correction of the dispersive effect of Ec (linear mode)**
  - **Time focusing at the detector**
- **Extremely fast analysis speed**
- **Mass limit = 30 000, but 5000 resolution**

Outline

1. General introduction of mass spectrometry (MS)
2. Fundamentals and Instrumentation of MS
   - 2.1. Ion source
   - 2.2. Analyzer
   - 2.3. Detector
   - 2.4. Principle of fragmentation
3. Applications

Mass spectrometer



Detector

Like analyzers and sources, there are different types of detectors. They are all based on different physical principles, but their role remains the same, counting ions.

This is a part of the vacuum apparatus(10^{-5} – 10^{-7} Torr)

- Photographic plates
- Faraday cylinder
- Electron multiplier
- Photon multiplier

Detector

Photographic plates: (historical detection) :

Principle : the blackening of the plate gives a relative value of the intensity of the flux (quantity of ion)

Limitation: very insensitive

Faraday cylinder:

Principle : charge transfer of the ion detected on a conductive surface, then amplification of the signal

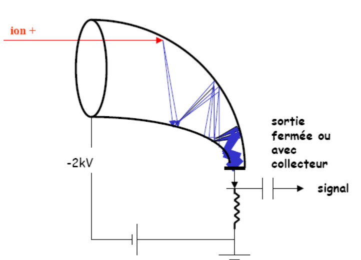
Advantage: specific

Limitation: not very sensitive, big background noise, slow in the measure

Detector

Electron multiplier (most common detector) :

Principle: signal doping by secondary electron formation using lead doped glass tubes (dynode)



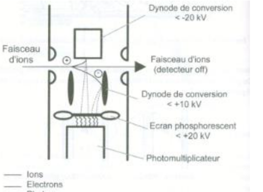
Advantage: good sensitivity and fast scanning

Limitation: less precise than the Faraday cylinder, limited life time

Detector

Photon multiplier:

Principle: doping of the signal by secondary electron formation (dynode). These are accelerated to the phosphorescent screen where they converted to photons. These photons are then detected by the photomultiplier.



Advantage : good sensitivity, very strong amplification gain

Limitation: scanning slower than electron multiplier, limited life time

Outline

1. General introduction of mass spectrometry (MS)

2. Fundamentals and Instrumentation of MS

   2.1. Ion source
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3. Applications

Fragmentation

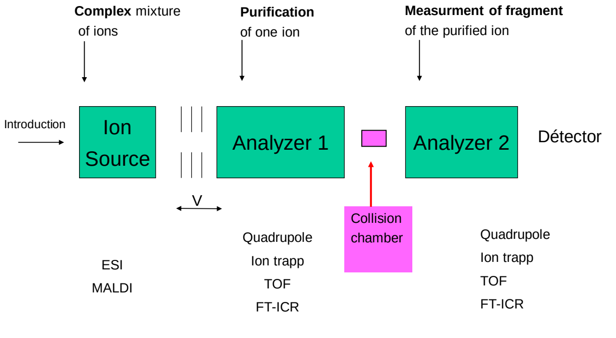
Principle: consists in "breaking" a molecule inside a mass spectrometer, in order to determine its structural properties

Moyens : coupling several analyzers and act sequentially

Multi-dimensional mass spectrometryMS^n

MS-MS is a powerful tool for determining structure

MS-MS multidimensional mass spectrometry



Fragmentation

Role of the first analyzer: select the ions with a designate m/z (parent ion)

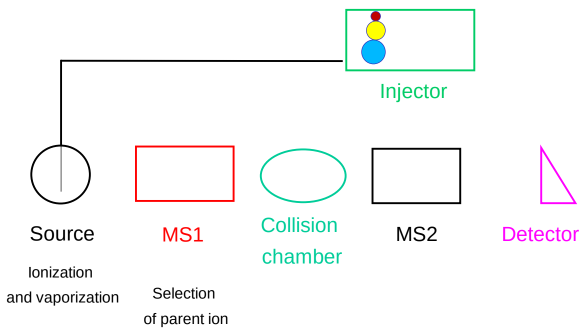
Purification of an ion present in a complex mixture

Role of the collision chamber: chamber in which the parent ion is going to be fragmented to give the son ions

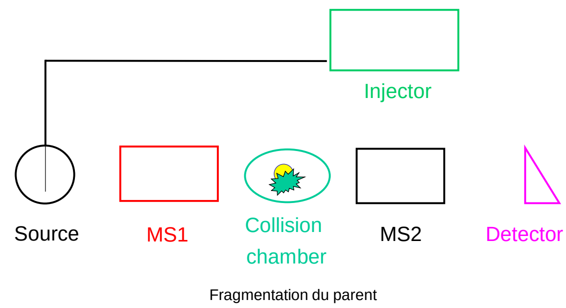
Example: Presence of a gas that will induce collisional fragmentation

Role of the second analyzer: measure fragment m/z

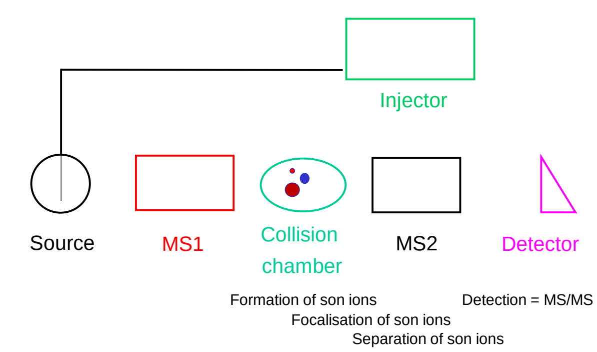
MS-MS multidimensional mass spectrometry



MS-MS multidimensional mass spectrometry

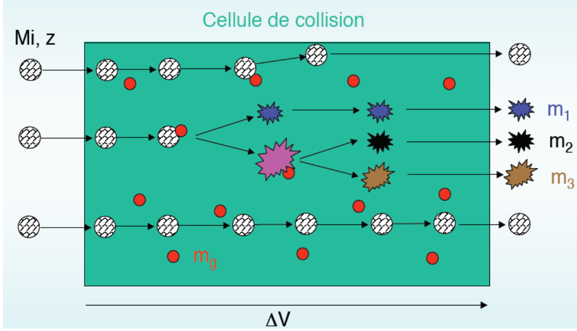


MS-MS multidimensional mass spectrometry



Fragmentation principle in the collision chamber

(Collision Induced Dissociation : CID)



Outline

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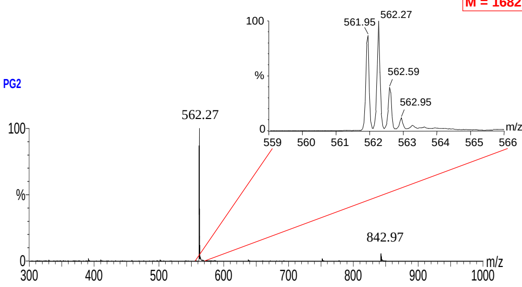
Application

Determination of the state of charge of a compound

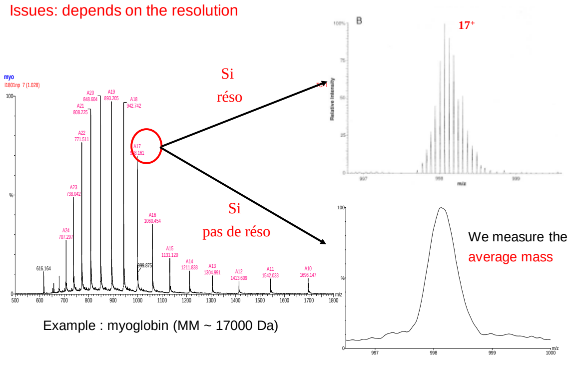
$\Delta m/z = 0.32$
↓
$z = 3$
$M = 1682.85$ Da



Application

Determination of the state of charge of a compound

Issues: depends on the resolution

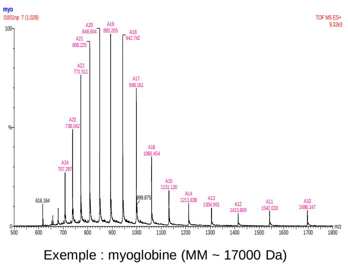


Example : myoglobin (MM ~ 17000 Da)

Application

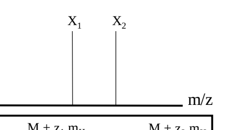
Determination of the state of charge of a compound

Cases of low resolution



Exemple : myoglobine (MM ~ 17000 Da)

Two consecutive peaks determine M and z_1



$$X_1 = \frac{M + z_1 m_H}{z_1} \qquad X_2 = \frac{M + z_2 m_H}{z_2}$$

$$z_2 = z_1 - 1$$

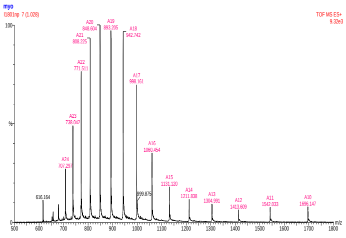
System of 2 equations with 2 unknowns

Calculation of z: $\Rightarrow$ $z_1 = \frac{X_2 - 1}{X_2 - X_1}$

Application

Determination of the state of charge of a compound

Cases of low resolution



Exemple : myoglobine (MM ~ 17000 Da)

Series of multicharged ions.

All these peaks correspond to the same molecule, but with a different number of protons.

The mass M and z are first calculated from two peaks.

Next, M is calculated from each of the peaks of the multicharged ion series.

In this example, 16 different charge states (10 to 25 charges) are observed.

The measured mass will therefore be the result of the average of these 16 measurements, hence the high accuracy obtained.

Application

Determination of the state of charge of a compound

Cases of low resolution

m/z	z	Mass
679,12	25	16953,00
707,31	24	16951,44
737,99	23	16950,77
721,5	22	15851,00
808,28	21	16952,88
848,53	20	16950,60
893,24	19	16952,56
942,67	18	16950,06
998,18	17	16952,06
1060,41	16	16950,56
1130,95	15	16949,25
1211,81	14	16951,34
Average :		**16951,65 +/- 0,17 Da**

The average of the values found for the molecular weight is calculated with a standard deviation.

The more multi-charged ions, the more precisely the mass can be measured

The calculated masses are **chemical masses** and **not monoisotopic masses** because the **resolution is not sufficient** to separate the isotopic peaks