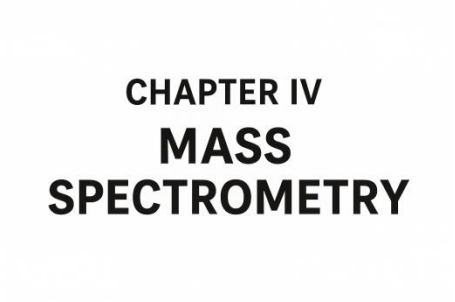
Chapter IV :

Mass Spectrometry :



IV.1. Introduction

Mass spectrometry (MS) is a powerful physicochemical analysis method used to **characterize matter** by precisely determining the **atomic or molecular masses** of the species present in a sample. The instrument used, called **a mass spectrometer,** measures the mass-to-charge ratio of the ions produced from the analyzed molecules.

This technique can be **combined with a separation method,** such as chromatography (GC-MS or LC-MS), to identify and quantify the constituents of a complex mixture.

Thanks to **technological advances,** the **miniaturization of instruments,** and the **emergence of new ionization methods,** mass spectrometry has established itself as **one of the most powerful and versatile analytical techniques.** It allows not only the **detection and identification of molecules** by measuring their mass, but also the **determination of their chemical structure** through the study of their ionic fragments.

Its **exceptional sensitivity** allows extremely low detection limits to be reached, often on the order of **nanograms (10^{-9} g)** or even **picograms (10^{-12} g)** , which makes it particularly useful for analyses requiring very small quantities of matter.

▪ II. Applications

Mass spectrometry is used in many **scientific and industrial fields,** including:

- **Organic chemistry:** identification and characterization of organic compounds, determination of molecular structures;
- **Inorganic chemistry:** analysis of elements, isotopes and metallic complexes;
- **Astrophysics:** study of the composition of meteorites and cosmic dust;
- **Biology and biochemistry:** identification of proteins, peptides and metabolites (proteomics, metabolomics);
- **Geochemistry:** isotopic dating and analysis of mineral samples;

- **Environment:** detection of pollutants at very low concentrations;
- **Medicine and pharmacology:** analysis of drugs, biomarkers and doping substances.

Its **high sensitivity** and **selectivity** make it a tool of choice for the **analysis of micro-samples,** such as those performed on **works of art** or **rare biological samples.** Today, mass spectrometry plays an essential role in **pollution monitoring, medical research,** and the **fight against doping, thanks to its ability to provide rapid, quantitative, and precise** analyses.

IV.2. Basic Principle

Mass spectrometry relies on **measuring the mass-to-charge ratio (m/z)** of ions from molecules or atoms present in a sample. This technique makes it possible to **determine the molecular mass** and **identify the chemical composition** of a compound.

The general principle comprises **three fundamental steps:**

(a) Ionization: A very small amount of the substance to be analyzed is converted into **ions** (positively or negatively charged) using an appropriate ionization method (such as electron impact, electrospray, or matrix-assisted laser desorption - MALDI). This step is essential, as only an ion can be manipulated and detected in a mass spectrometer.

- **By protonation ($A–BH^+$):** A **proton (H^+) is** added to the neutral molecule. → It becomes a **positive ion.** Example: basic molecules (amines, peptides).

$$\text{A-B} + H^+ \longrightarrow [\text{A-BH}]^+$$

- **By deprotonation ($A–B^-$):** A **proton is removed** from the molecule. → It becomes a **negative ion.** Example: acidic molecules (organic acids, phenols).

$$\text{A-B} - H^+ \longrightarrow [\text{A-B}]^-$$

- **By electron loss ($A–B^+ \cdot$)** : The molecule **loses an electron** during an electron impact (EI). → This results in a **positive radical molecular ion,** typical of EI spectra. Example : hydrocarbons, volatile organic compounds.

$$\text{A-B} + e^-_{(inc)} \longrightarrow \text{A-B}^{+\bullet} + 2e^-$$

- **By cationization ($A–B–Na^+$):** The molecule **binds to a metal cation** (often Na^+ or K^+). → This type of ionization is common in **electrospray**

ionization (ESI) or **MALDI.** Example : polar or non-basic compounds (sugars, polymers).

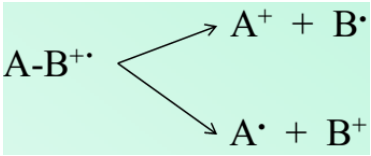
$$\text{A-B} + \text{Na}^+ \longrightarrow [\text{A-B} + \text{Na}]^+$$

(b) Fragmentation

Once ionized, molecules can contain an **excess of internal energy** . This energy causes the **breaking of certain chemical bonds** , forming **characteristic fragments** .

- These **fragment ions** produce **secondary peaks** in the spectrum.
- Analyzing these fragments allows us to **determine the structure** of the compound.

Example: The main peak corresponds to the **molecular ion (M^+),** while the smaller peaks correspond to **fragment ions (lower m/z).**



IV.3. Units of measurement in mass spectrometry

- **The Dalton (Da)**

The **Dalton (Da)** , also called **the unified atomic mass unit (u),** is the unit used to express the **mass of atoms, molecules, and ions.**

1 Dalton (1 Da) corresponds to **1/12 of the mass of a carbon-12 atom (^{12}C).**

$1\ \text{Da} = 1.660\ 539 \times 10^{-27}\ \text{kg}$

Example :

Molecule	Formula	Mass (in Da)
Hydrogen (H)	H	1.008 Da
Water (H_2O)	H_2O	18,015 Da
Glucose	$C_6H_{12}O_6$	180,156 Da
Medium protein	—	≈ 50,000 Da (50 kDa)

- **The Thomson (Th)**

The **Thomson (Th)** is the unit used to express the **mass/charge ratio (m/z)** of an ion.

1 Th=1 Da per unit of elementary charge (e)

1 Th = 1 Da / e

- If an ion has a **charge of +1** , its **m/z** is equal to its **mass in Da** .
- If the charge is **+2** , its **m/z** will be **half** of its mass.

Role :

- The Thomson expresses **what the spectrometer actually measures:** the **mass/charge ratio (m/z),** not the mass alone.
- It is therefore a **unit derived from the Dalton,** taking into account the number of charges.

Examples:

Ion	Mass (Da)	Charge (z)	m/z ratio (Th)
H^+	1.008	+1	1.008 Th
Na^+	22,990	+1	22,990 Th
Protein M = 50,000 Da, z = +5	50,000	+5	10,000 Th

IV.4. Ionization methods

There are several **ionization methods,** adapted according to the type of sample (gas, liquid, solid) and the nature of the compound (small molecule, protein, polymer, etc.). Here are the main ones mentioned in your text:

(b) Electron Impact (EI)

- **Volatile** compounds (gases, vapors).
- A beam of electrons strikes **the molecules,** stripping them of an electron.
- Result: formation of a **positive ion (molecular cation),** often accompanied by characteristic **fragments.**

$M + e^- \rightarrow M^{+\bullet} + 2e^-$

(c) Electrospray (ESI – Electrospray Ionization)

- Used for **liquid solutions,** especially in biological chemistry (proteins, peptides…).
- The solution is sprayed under high voltage, creating fine **charged droplets** that evaporate, leaving **ions in the gaseous phase.**
- Advantage: **gentle method,** little fragmentation.

(d) Matrix-Assisted Laser Desorption (MALDI)

- Method used for **large biomolecules (proteins, polymers, etc.)** .

- The sample is mixed with a **matrix** that absorbs the energy from the **laser** .
- The laser vaporizes and ionizes the matrix, gently carrying away the compound molecules in ionic form.
- **fragile** molecules without destroying them.

IV.5. Analysis:

The ions produced are then **accelerated** in an electric or magnetic field. Depending on their **mass-to-charge ratio (m/z)** , they are **separated** from each other:

- Light or highly charged ions are deflected more strongly.
- while heavier ions are less so.

The type of analyzer used (quadrupole, time of flight, ion trap, etc.) determines the **resolution and accuracy** of the measurement.

IV.6. Detection and recording:

The separated ions reach a **detector, which measures their intensity and allows a mass spectrum** to be plotted. This spectrum represents the **signal as a function of the m/z ratio,** providing precise information on the **ionic species present** and their **relative abundance.**

Thus, from the **mass spectrum obtained,** it is possible to **determine the exact molecular mass** of a compound, to **identify its characteristic fragments,** and therefore to **deduce its chemical structure.**

IV. Mass Spectrum

The **mass spectrum** is the **graphical result** obtained during a mass spectrometry analysis. It represents the distribution of **detected ions** as a function of their **mass/charge ratio (m/z)** and their **relative abundance** .

- **The x-axis** indicates the **m/z ratio** of the detected ions. In electron impact ionization (EI), the charge z is generally **1,** so the m/z value corresponds directly to the **molecular mass in Daltons (Da).**
- **The y-axis** represents the **relative abundance** of the detected ions. The **most intense peak** is arbitrarily set at **100%** and called **the baseline peak.** The other peaks are expressed as a percentage of this baseline peak.

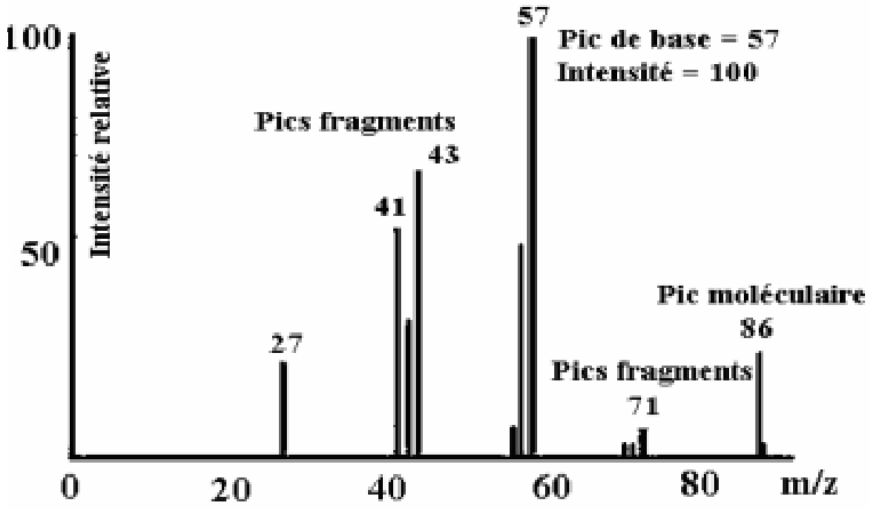
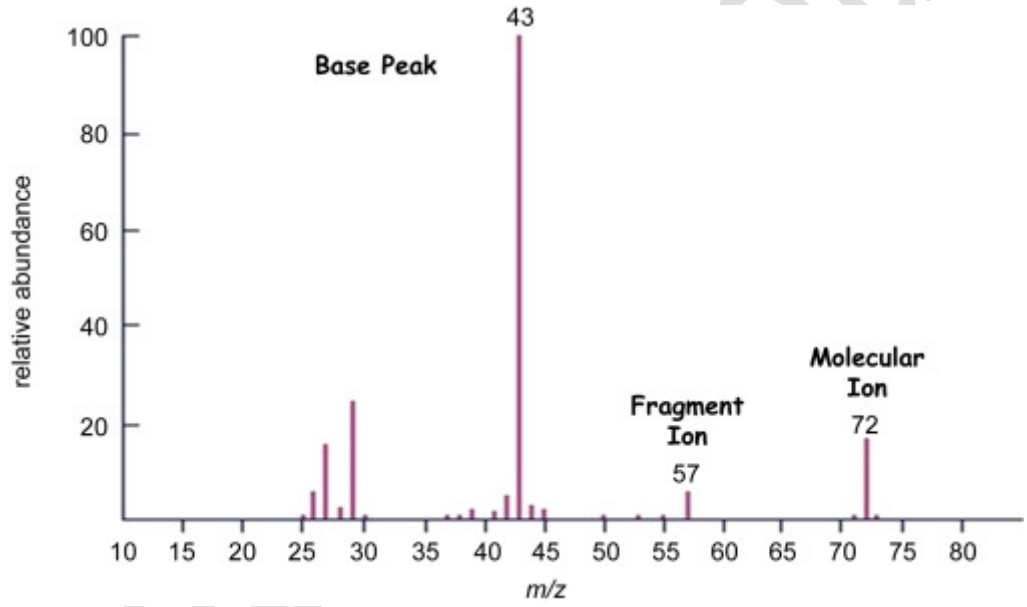


Fig. IV.1: Schematic example of a mass spectrum



IV.8. Types of peaks observed in a mass spectrum:

a) **The molecular peak (or parent peak, M^+):** This corresponds to the **molecular ion,** that is, the **ionized intact molecule.** Its position indicates the **molecular mass of the compound.** This peak is essential for the identification of a substance.

b) **The basal peak:** This is the **most intense peak** in the spectrum. It corresponds to the **most stable or most abundant ion** formed during ionization. By convention, its intensity is set at 100% and serves as a reference for expressing the relative abundance of other ions.

c) **Fragment peaks:** These result from the **fragmentation** of the molecular ion into smaller ions. Each fragment peak reflects a **specific break** in the molecule, thus providing valuable **structural information.** Analysis of

these fragments often allows **the chemical structure** of the compound under study to be determined.

d) **Metastable peaks:** These peaks sometimes appear at **non-integer masses** and do not correspond to **any real ion.** They originate from the **spontaneous disintegration** of a molecular ion into a fragment during its movement in the analyzer.

If an ion AB^+ (of mass m1) decomposes into an ion A^+ (of mass m^2) and a neutral fragment B, a **metastable peak** can appear at a mass m * given by the relation:

$$m^* = \frac{(m_2)^2}{m_1}$$

The presence of this peak **confirms the relationship** between the parent ion and the fragment formed.

IV.9. The isotopic profile and the P, P+1, P+2 peaks

In mass spectrometry, a molecule can exist in different forms called **isotopologues**, depending on the isotopes of the atoms that compose it. These forms give rise to several **closely spaced peaks** in the mass spectrum, forming what is called the **isotopic profile.**

(a) The monoisotopic peak (P)

- This is the **first peak** of the isotopic profile (the leftmost one).
- It corresponds to the **molecule formed solely with the lightest isotopes** :
  - ^{12}C, ^{1}H, ^{16}O , ^{14}N , etc.
- It represents the **monoisotopic mass** of the molecule (the smallest possible).

(b) Peak P+1

- Appears at a mass **greater than one unit** (m/z +1).
- It corresponds to the **same molecule** , but containing **a heavier isotope** :
  - for example, a ^{13}C instead of a ^{12}C, or a ^{2}H instead of a ^{1}H.
- Its intensity depends on the **number of carbon atoms** in the molecule, because the more carbons there are, the greater the probability of having a ^{13}C.

(c) The P+2 peak

- Located at **m/z +2.**
- It comes from a molecule containing **two heavy isotopes,** or a single even heavier isotope (such as ^{34}S or ^{18}O).

- This peak is more visible for molecules containing naturally occurring heavy isotopes.

IV.9. Average mass

- The **average mass** corresponds to the **weighted average** of all isotopic masses according to their natural abundance.
  - It is **slightly greater** than the monoisotopic mass, because it takes into account the presence of heavy isotopes.

In simple terms:

Peak	Meaning	Isotopic composition	Position in the spectrum
P	Monoisotopic peak	All light isotopes (^{12}C, ^{1}H, ^{16}O ...)	The leftmost
P+1	1 heavy isotope (^{13}C, ^{2}H, ^{15}N...)	Mass +1	After P
P+2	2 heavy isotopes or 1 isotope +2 (^{34}S, ^{18}O...)	Mass +2	After P+1
Average mass	Average of isotopes	—	Towards the center of the profile

Key points to remember:

The **P peak** indicates the **exact (monoisotopic) mass.** The **P+1, P+2… peaks** indicate the **presence of heavy isotopes.** And the **average mass** represents the **natural isotopic composition** of a molecule.

Monoisotopic peak **Average mass**

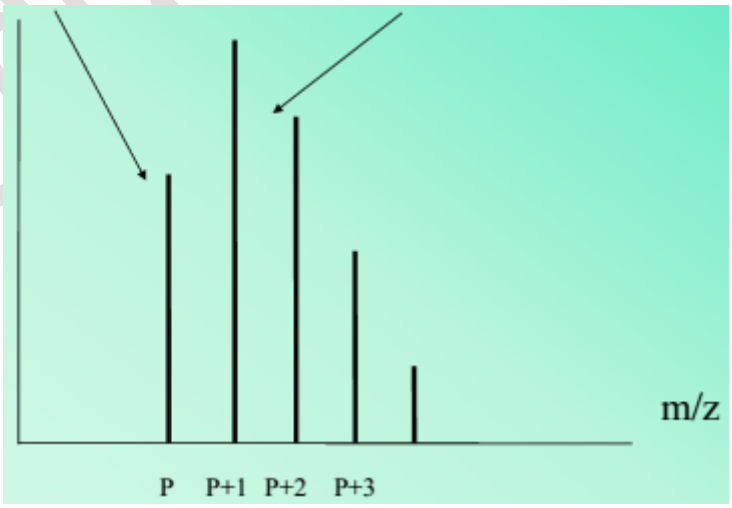


Fig.IV.2. Isotopic profile

IV.10. Monoisotopic mass :

(a) Definition :

The **monoisotopic mass** of a molecule is the **sum of the exact masses** of the **most abundant isotopes** of each of the elements that compose it.

In other words: We take **a single isotope (the most frequent)** for each element, and we add their exact atomic masses.

Why "monoisotopic"?

- "Mono" = one
- "Isotopic" = isotope

Therefore, we only consider **one isotopic form** of each atom (the most stable or most abundant one in nature).

Examples:

Example 1: Water (H_2O)

- H (most abundant isotope: ^{1}H) → 1.0078 u
- O (most abundant isotope: ^{16}O) → 15.9949 u

Monoisotopic mass (H_2O) = 2(1.0078) + 15.9949 = 18.0105 u

Example 2 : Ethanol (C_2H_6O)

- C (^{12}C) = 12.0000 u
- H (^{1}H) = 1.0078 u
- O ($^{1\ 6}O$) = 15.9949 u

Monoisotopic mass = 2(12.0000) + 6(1.0078) + 15.9949 = 46.0414 u

IV.11. Spectral Analysis :

The **interpretation of a mass spectrum** relies on the analysis of information contained in the various observed peaks. This analysis generally takes place in **two main stages** :

a) **Study of the molecular ion (or parent ion)** : Allows us to determine the **molecular mass, parity, isotopes present** and sometimes the **empirical formula** of the molecule.

b) **Fragment ion analysis:** Provides information on the **chemical structure** of the molecule, **broken bonds,** and **functional groups** present.

IV.12. Mass of the molecular ion

Mass spectrometry allows the determination of the **molecular mass** of an unknown substance using the **molecular peak (M^+),** corresponding to the **intact ionized molecule.** The position of this peak on the spectrum (m/z value) directly indicates the **molar mass** of the compound when the charge z=1.

Example: For benzene (C_6H_6), the molecular peak appears at m /**z = 78,** corresponding to its molecular mass.

IV.13. Molecular ion parity

The **parity (even or odd)** of the molecular mass of a compound gives a valuable indication of the **presence of trivalent atoms** such as nitrogen (N), phosphorus (P) or arsenic (As).

- If the **molecular mass is odd,** the molecule contains **an odd number of trivalent atoms** (N, P, etc.).
- If the **molecular mass is even,** it contains **an even or zero number** of trivalent atoms.

Example :

- **Ammonia (NH_3):** contains a nitrogen atom → odd molecular mass (m/**z = 17)**.
- **Methane (CH_4):** does not contain a trivalent atom → even molecular mass (m/**z = 16)**.

This rule, called **the parity rule** or **nitrogen rule,** is very useful for a quick initial identification.

IV.14. Isotopic cluster:

Chemical elements exist in different forms called **isotopes,** which are distinguished by their number of neutrons and therefore by their atomic mass. The presence of isotopes leads to the appearance, in the mass spectrum, of **secondary peaks** called **isotopic clusters.**

These peaks allow us to:

- Identify the **presence of isotopic elements** in the molecule (Cl, Br, S, etc.);
- Check the consistency of the proposed **chemical formula;**
- To recognize certain elements by their **characteristic isotopic signature.**

IV.15. Isotopic clusters and distribution law

The collection of **peaks due to the different isotopes** of the same chemical element forms what is called an **isotopic cluster.** Each peak corresponds to an **ion containing a different combination of isotopes,** and their **relative intensity** depends directly on the **natural abundances** of these isotopes.

(a) General principle

The **number** and **relative intensities** of the peaks in an isotopic cluster can be determined using the **binomial expansion** of the following relationship:

$(a+b)^2$

Or :

- **a: relative abundance of the lightest** isotope,
- **b: relative abundance of the heaviest** isotope, **normalized to 1,**
- **n:** number of atoms of the element considered in the molecule.

Each term in the development represents the **contribution of a particular ion** containing a certain number of heavy isotopes, and the associated **binomial coefficient** gives its **relative intensity.**

(b) Case of elements with significant abundance isotopes:

(**Chlorine** and **Bromine**)

Some elements, such as **chlorine (Cl)** and **bromine (Br),** have **heavy isotopes (+2)** in **notable abundance,** making their isotopic signature easily identifiable in a mass spectrum.

Element	Main isotopes	Relative abundance (%)	Peak intensity report
Cl	^{35}Cl (75.8%) / ^{37}Cl (24.2 %)	3:1	Peaks M and M+2
Br	^{79}Br (50.7%) / ^{81}Br (49.3%)	1:1	Peaks M and M+2

Example 1: Bromomethane (CH_3Br)

Bromomethane contains **a single bromine atom (n = 1).** Since bromine has two isotopes with masses of 79 and 81, the mass spectrum shows **two peaks**:

- a **peak M** corresponding to the isotope ^{79} **Br,**
- a **peak M+2** corresponding to the isotope $^{8\,1}$**Br.**

Since the two isotopes are practically equimolar (≈ 1:1), the **two peaks have similar intensities.**

Result: the CH_3Br spectrum shows two peaks of the same intensity at m/z = 94 (M) and m/z = 96 (M+2).

Dichloromethane **CH_2Cl_2** contains **two chlorine atoms →** therefore **n = 2.**

Chlorine has two natural isotopes:

- ^{35Cl} : light isotope → abundance ≈ 75 **%**
- ^{37Cl} **: heavy isotope →** **abundance** ≈ 25%

In your diagram, we simplify by taking the ratios: a=3 and b=1

(This corresponds approximately to the ratio 75% : 25% → or 3 : 1).

2. Relationship used

We apply the **binomial formula:**

$(a+b)^n$

Here, since there are two chlorine atoms:

$(a+b)^2 = a^2 + 2ab + b^2$

3. Interpretation of terms

Each term in the development corresponds to a **possible isotopic combination** :

Combination of isotopes in CH_2Cl_2	Term	relative intensity ratio	Corresponding peak
Two ^{35}cl	a^2	$3^2 = 9$	M
A ^{35}cl and a ^{37}cl	2ab	$2\times3\times1=6$	M+2
Two ^{37}cl	b^2	$1^2=1$	M+4

4. Spectral result and interpretation

You therefore obtain **three peaks** in the spectrum:

Peak	Isotopic composition	Relative intensity	Position (m/z)
M	$2(^{35}Cl)$	9	M
M+2	$^{35}cl + ^{37}cl$	6	M+2
M+4	$2(^{37}Cl)$	1	M+4

So the **intensity ratios** are approximately **9:6:1.**

5. In summary

CH_2Cl_2 gives an **isotopic cluster with three peaks** spaced 2 mass units apart (M, M+2, M+4), with relative intensities of **9 : 6:1** . This is due to the **presence of two chlorine atoms ,** each having two isotopes (^{35}Cl and ^{37}Cl).

IV.15. Exploitation of fragment ions

When a molecule is ionized in a mass spectrometer, it does not always remain intact. The molecular ion can break into several pieces called **fragment ions** . Analyzing these fragments is essential because it provides information about the **structure** and **stability** of the molecule being studied.

IV.16. Factors influencing fragmentation :

a) **The strength of chemical bonds:**
The **weakest bonds** (for example, tertiary C–C, C–O, or C–S bonds) break more easily during ionization.
➜ Thus, the molecule tends to fragment at the points where the bonds are weakest.

b) **The stability of the fragments formed :**
Stable fragments are favored; the more **electronically stable** a fragment ion possesses (radical, carbocation, anion, or resonant structure), the more likely it is to appear with a **high intensity** in the spectrum.
➜ Example: benzene or allylic ions often appear because they are stabilized by resonance.

c) **Fragmentation with Internal Rearrangement:**
Some molecules can undergo **internal rearrangement** before or during bond breaking.
This phenomenon is favored when **a six-center transition state** (i.e., involving six electrons delocalized over several atoms) can form.
➜ This type of mechanism leads to characteristic fragments, called **rearrangement ions** , which are very useful for identifying the structure of a molecule.

IV.16. McLafferty Rearrangement:

McLafferty rearrangement is a particular type of fragmentation observed in mass spectrometry, typical of **carbonyl compounds** (aldehydes, ketones, esters, carboxylic acids…).

(a) Principle of rearrangement:

During ionization, if the molecule contains:

- an **unsaturation,** that is to say a double bond (often the C=O function),
- and a **hydrogen atom in the γ position** (that is, on the third carbon atom relative to the carbonyl group),

then an **intramolecular transfer of a hydrogen atom** can occur, accompanied by a **breaking of the β–γ bond.**

This process forms:

- a **stable fragment ion** (often an enol ion or a radical ion),
- and a **neutral molecule** (often an alkene).

(b) McLafferty rearrangement mechanism:

The mechanism follows a **six-center reorganization** (6-electron delocalized ring):

$$[CH_2{-}CH_2{-}CH_2{-}C(=O){-}R]^+ \longrightarrow [CH_2=CH_2] + [R{-}C(OH)=CH_2]^+$$

In other words:

1. The hydrogen in the γ position migrates towards the oxygen atom of the carbonyl group.

2. Simultaneously, the β–γ bond breaks.
3. The result is the formation of a characteristic **McLafferty ion** and a **neutral alkene** .

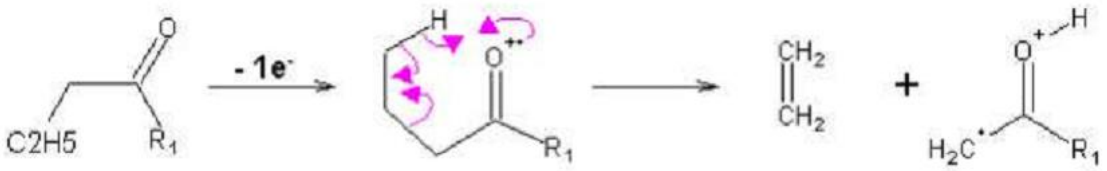
Features :

- This rearrangement only takes place **if a hydrogen atom in the γ position** is available.
- It is typical of **molecules containing a carbonyl function** .
- The fragment obtained is very **characteristic** and allows **the presence of a carbonyl group to be identified** in the molecule.

Example: 2-butanone ($CH_3–CO–CH_2–CH_3$)

2-Butanone has a hydrogen in the γ position. → It undergoes a McLafferty rearrangement giving an ion at **m/z = 58** , very typical of ketones.

Example:



If we denote the functional group or the reference atom as position **α** , then:

- **β-carbon** is the second carbon atom from this group.
- **γ carbon** is the third.

Thus, the **β–γ bond** directly links the **carbon in the β position** to the **carbon in the γ position** .

Example:

Let's take a carbon chain linked to a carbonyl group (C=O):

O = C – CH2 – CH2 – CH3

α β γ

- The **α–β bond** is that between C=O and CH2 (first C–C bond).
- The **β–γ bond** is that between the second and third carbon (**CH2–CH3**).

Note (useful in spectrometry or organic chemistry):

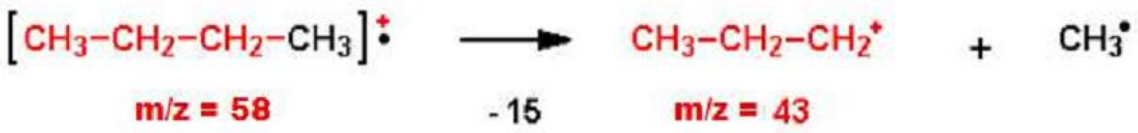
- In some **molecular rearrangements** (such as the **McLafferty rearrangement** in mass spectrometry), a **hydrogen in the γ position** migrates to an **atom of the functional group** via an **interaction through the β–γ bond** .

- This **β–γ bond** therefore plays a key role in the formation of the six-membered ring characteristic of this mechanism.

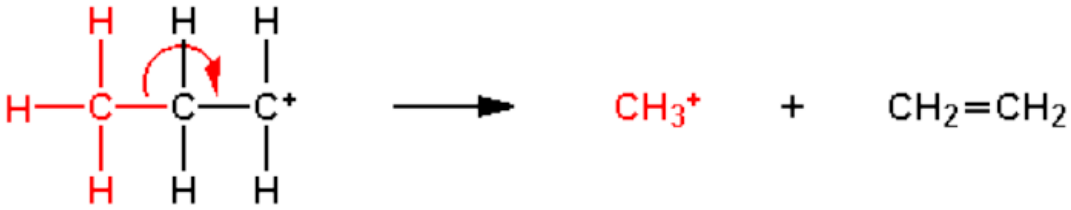
IV.17. Characteristic fragmentations of some chemical classes

V.17.1. Alkanes:

In linear alkanes, fragmentation is manifested by the loss of a methyl group; resulting in fragments of m/z = molar mass - 15.



In general, the fragments correspond to m/z = 29, 43, 57, 71... (or molar mass -15 - 14 xn). See the example of butane (fig). A neutral ethene molecule can also be formed.



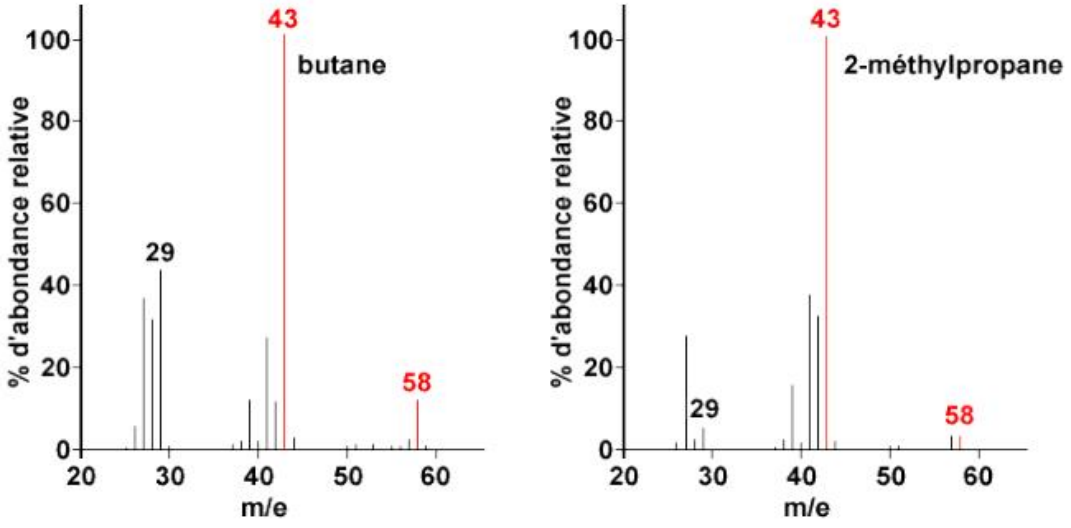
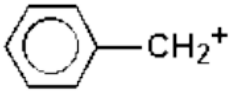


Fig. IV.2. Mass spectra of butane $CH_3CH_2CH_2CH_3$ and 2 -methylpropane $CH_3CH\ (CH_3)\ CH_3$, isomers with molar masses = 58 g/ mol.

For all linear hydrocarbons, ions 43 and 57 are the most intense peaks in the spectrum because they correspond to the most stable cations.

Ion R^+	CH_3^+	CH_3-CH_2^+	CH_3-CH_2-CH_2^+	CH_3-CH_2-CH_2-CH_2^+
m/z	15	29	43	57

Increasing order of stability:

$$CH_3^+ < RCH_2^+ < R_2CH^+ < R_3C^+ < CH_2{=}CHCH_2^+ <$$  $$CH_2^+$$

IV.4.2.2. Alkenes:

Alkenes very often give by ionization a fragment of m/z = 41 which corresponds to the allyl carbocation.

$$[R\text{-}CH_2{-}CH{=}CH_2]^{+\bullet} \longrightarrow R^\bullet + [{}^+CH_2{-}CH{=}CH_2 \longleftrightarrow CH_2{=}CH{-}CH_2^+]$$

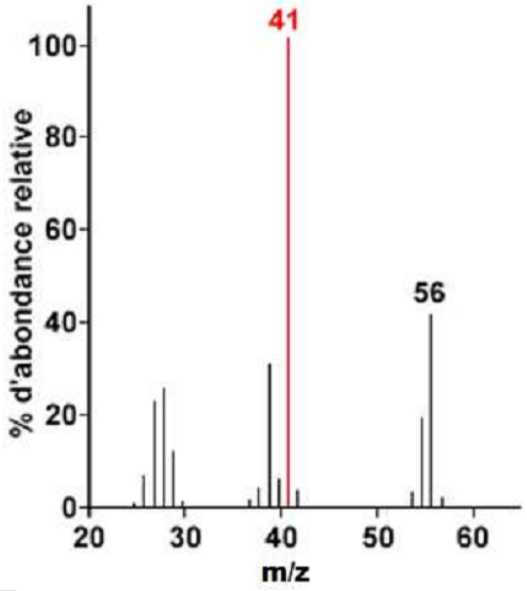


Fig.IV.3. Mass spectrum of 1-butene CH_3 - CH_2 - CH=CH_2 with a molar mass of 56 g/mol.

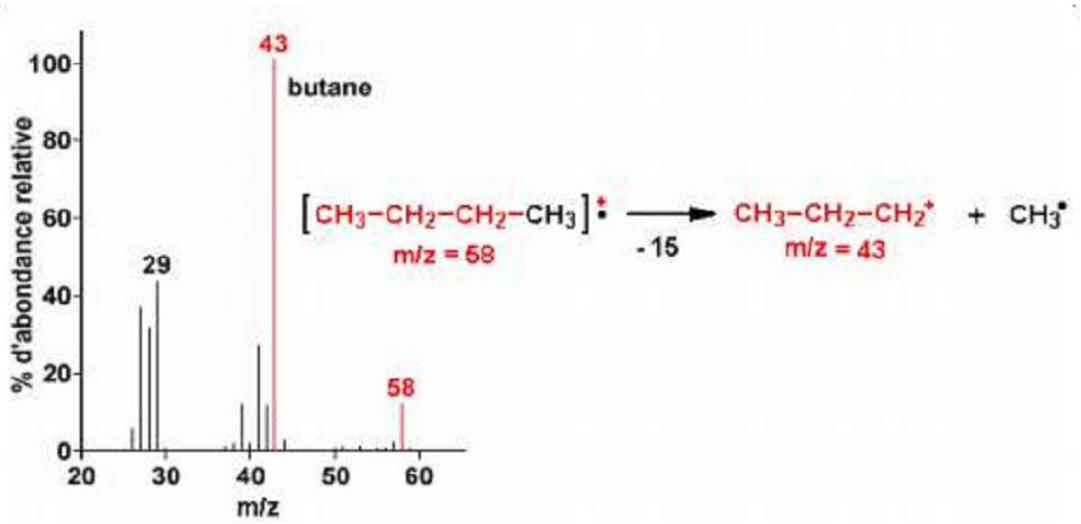
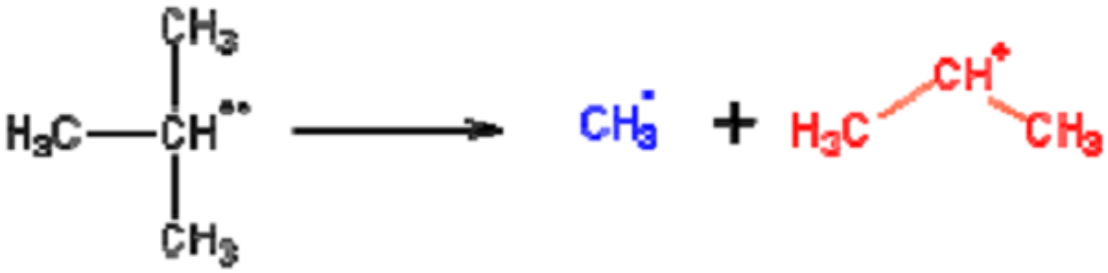


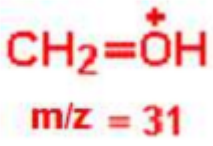
Fig. IV. 4. Mass spectrum of butane CH3 - CH2 - CH2 - CH3 with a molar mass of 58 g/ mol. In branched hydrocarbons, fragmentation occurs in the direction that yields the most stable carbocation.



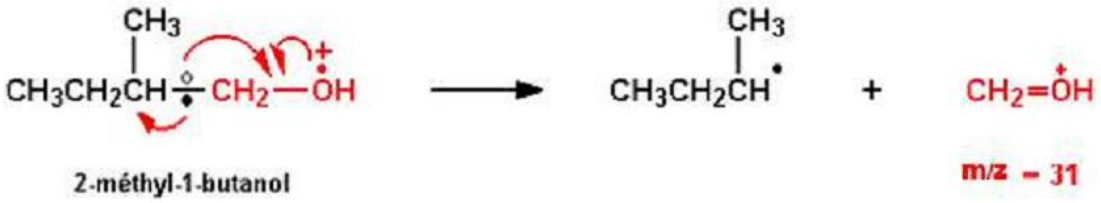
The isopropyl cation is more stable than the CH3+ cation.

IV.17.2. Alcohols:

The molecular ion peak of alcohols is almost nonexistent because they lose a water molecule very easily. This loss can even occur under the effect of heat before fragmentation. In this particular case, the spectral appearance will more closely resemble that of an alkene. The most common fragment is:



The case of branched alcohols is more difficult to analyze.



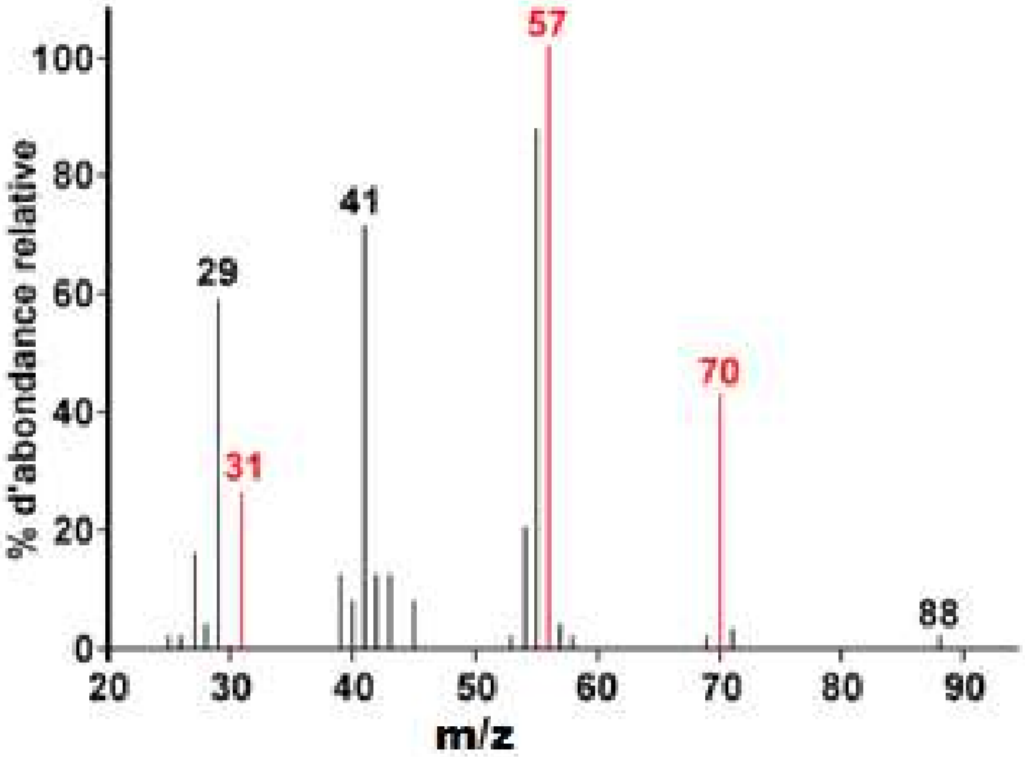
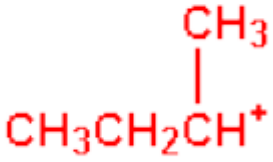
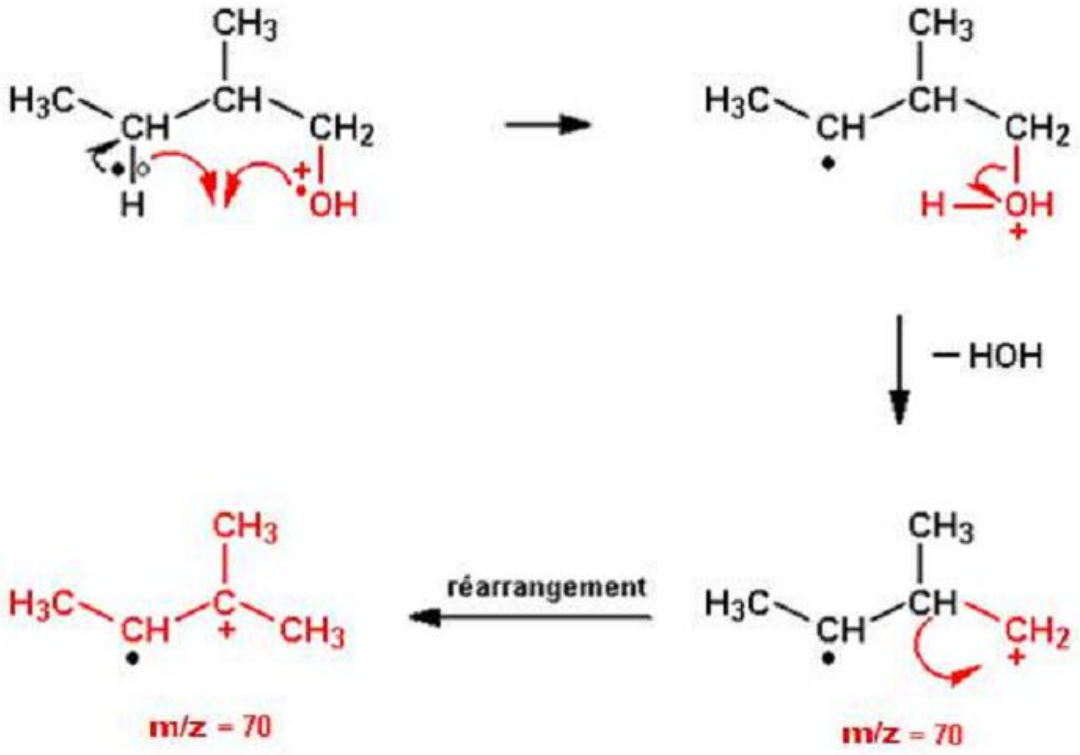


Fig. IV.5. Mass spectrum of 2-methyl-1-butanol CH3CH2CH (CH3) CH2OH with a molar mass of 88 g/mol. The peak at m/z = 57 would correspond to the fragment



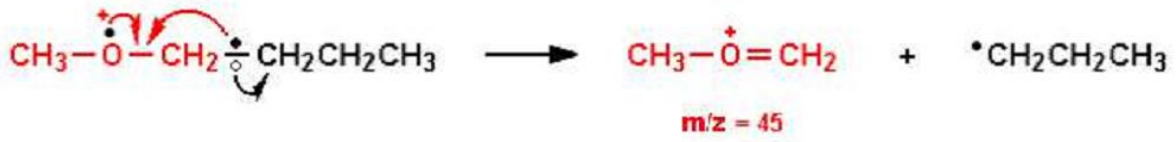
However, although fairly stable, its formation is quite difficult to explain. The peak at m/z = 70 corresponds to dehydration that would occur according to the following mechanism:



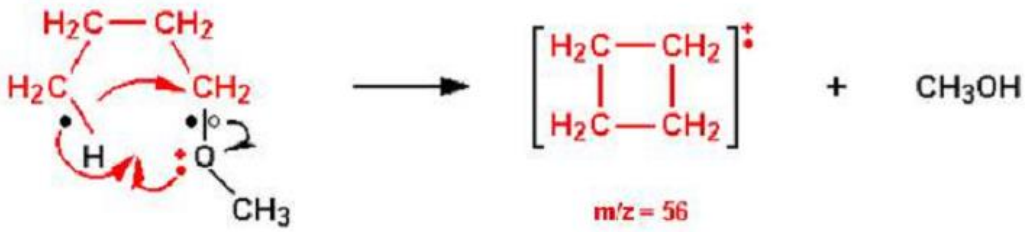
IV.17.3. Ethers:

The fragmentation of ethers occurs in much the same way as that of alcohols.

a. Rupture intoα methoxybutane.



b. Cyclization with formation of cyclobutane (in this particular case) and a neutral molecule.



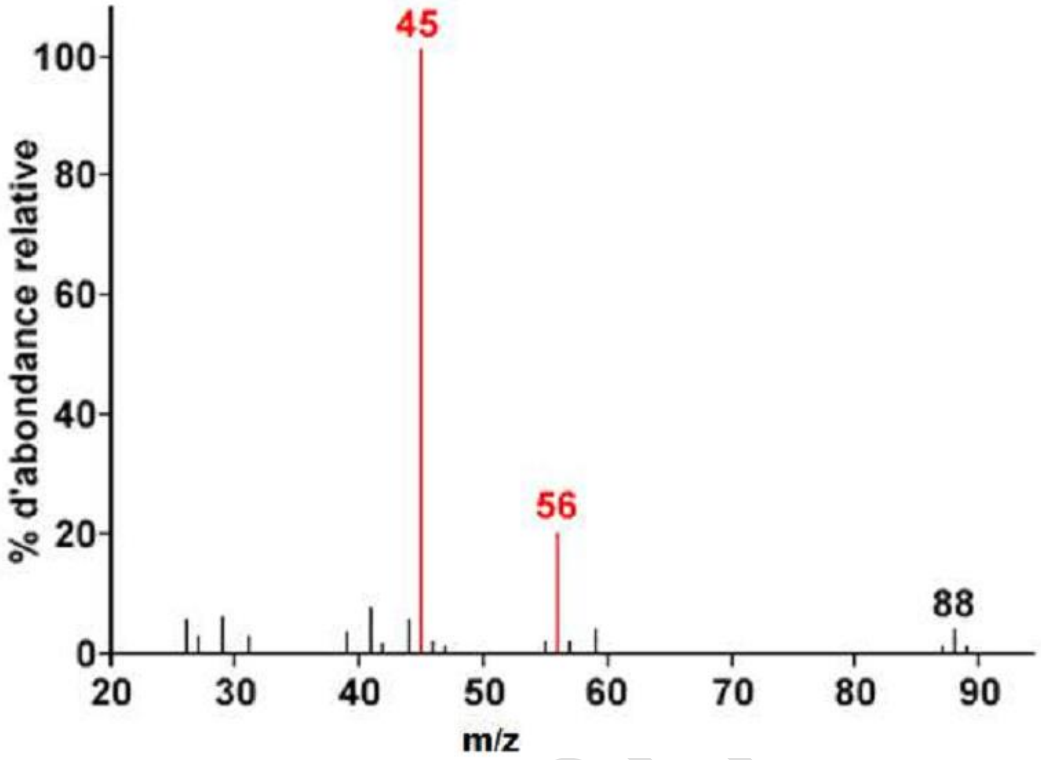


Fig. IV. 6. Mass spectrum of methoxybutane $CH_3OCH_2CH_2CH_2CH_3$ with a molar mass of 88 g / mol.

IV.17.4. Amines:

Amines behave like alcohols, and secondary amines like ethers. For example, the fragmentation of N-methylisopropylamine primarily yields:

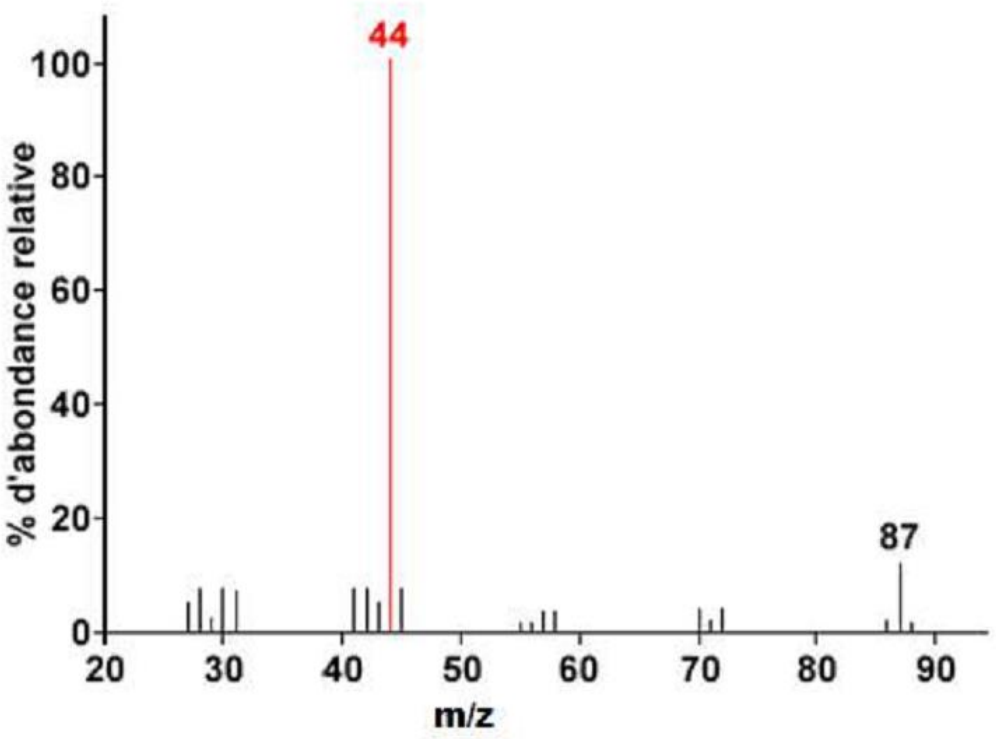


Fig. IV. 7. Mass spectrum of N-methyl-2-methylpropanamine $CH_3NCH_2CH(CH_3)CH_3$ with a molar mass of 87 g/mol.

IV.17.5. Benzene compounds:

These compounds produce easily interpretable mass spectra. The molecular peak is always intense because the molecular ion is strongly stabilized.

a. Benzene

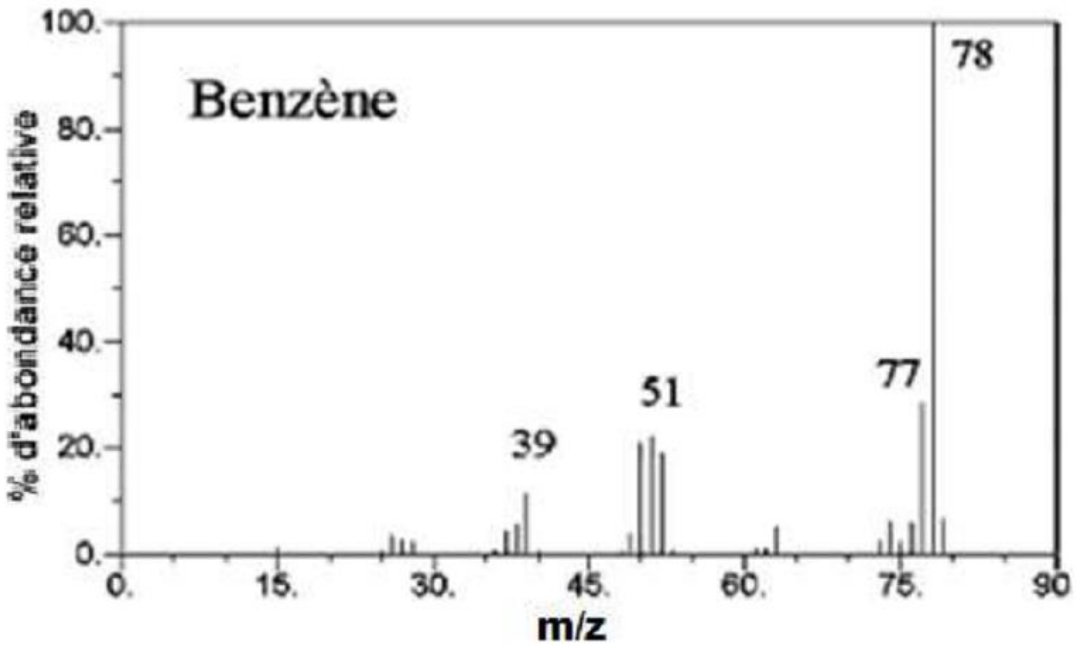


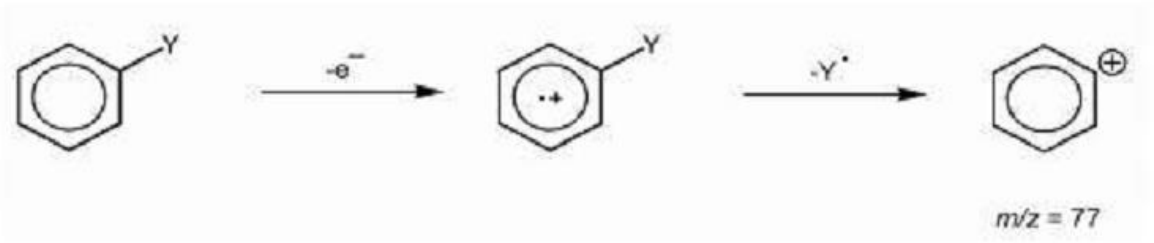
Fig. IV. 8: Mass spectrum of benzene C_6H_6 with a molar mass of 78 g/mol.

The fragmentations of benzene produce characteristic ions: m/z = 77 [MH]+, m/z = 51: C_4H_3+ [77 – 26 (acetylene)] and m/z = 39: cyclic ion C_3H_3+.

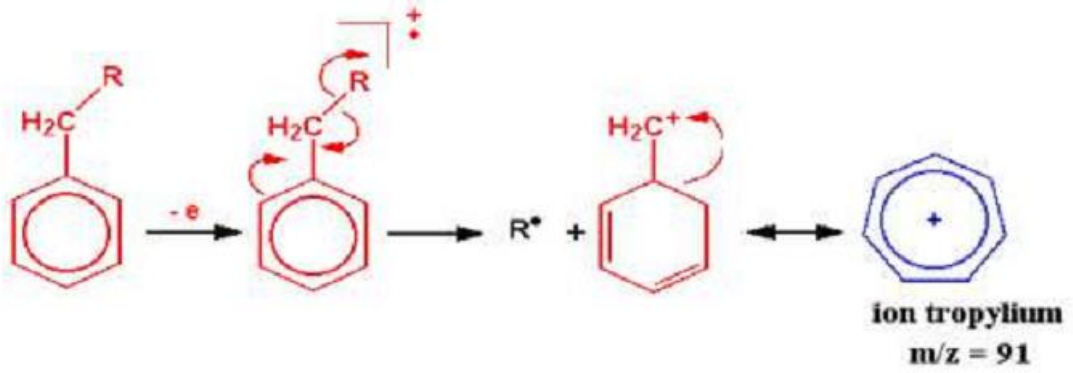
b. Monosubstituted benzenes:

α cleavage :

If the substituent is not an alkyl, monosubstituted benzenes frequently lose their substituent to form the phenyl cation at m/z = 77.



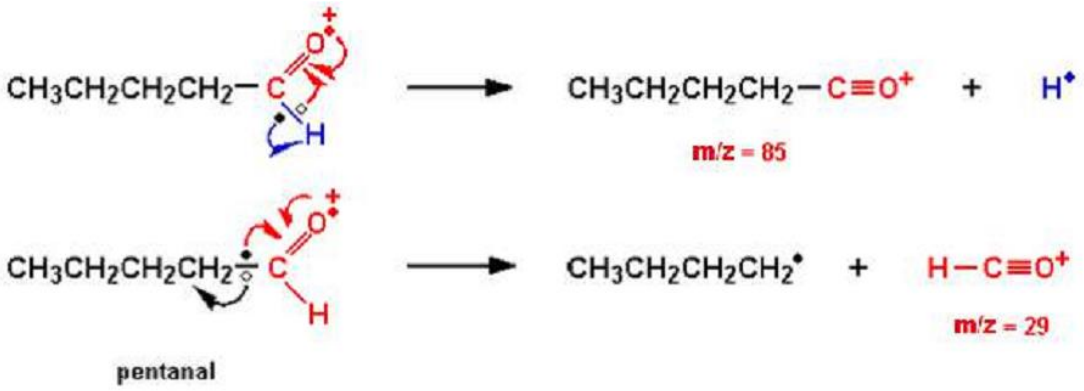
β-Cleavage: Benzenes substituted with an alkyl group undergo predominant fragmentation: β-cleavage of the aromatic ring, called benzyl cleavage. They lose a hydrogen or an alkyl group to form the aromatic tropylium cation at m/z = 91.



This ion is strongly stabilized and often constitutes the base peak of the mass spectrum.

IV.17.6. Aldehydes. There are four fragmentation "patterns" among aldehydes. Pentanal will serve as an example.

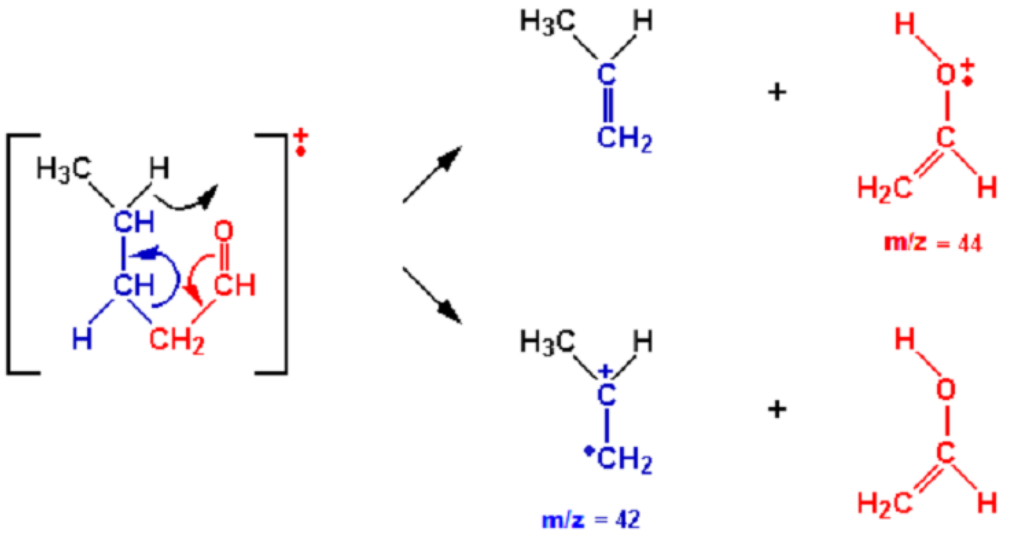
α cleavage:



❖ **Clivage β**

❖ **Rupture de la liaison en α**

McLafferty type rearrangement in this example, we have an H in γ of the unsaturation (aldehyde function).



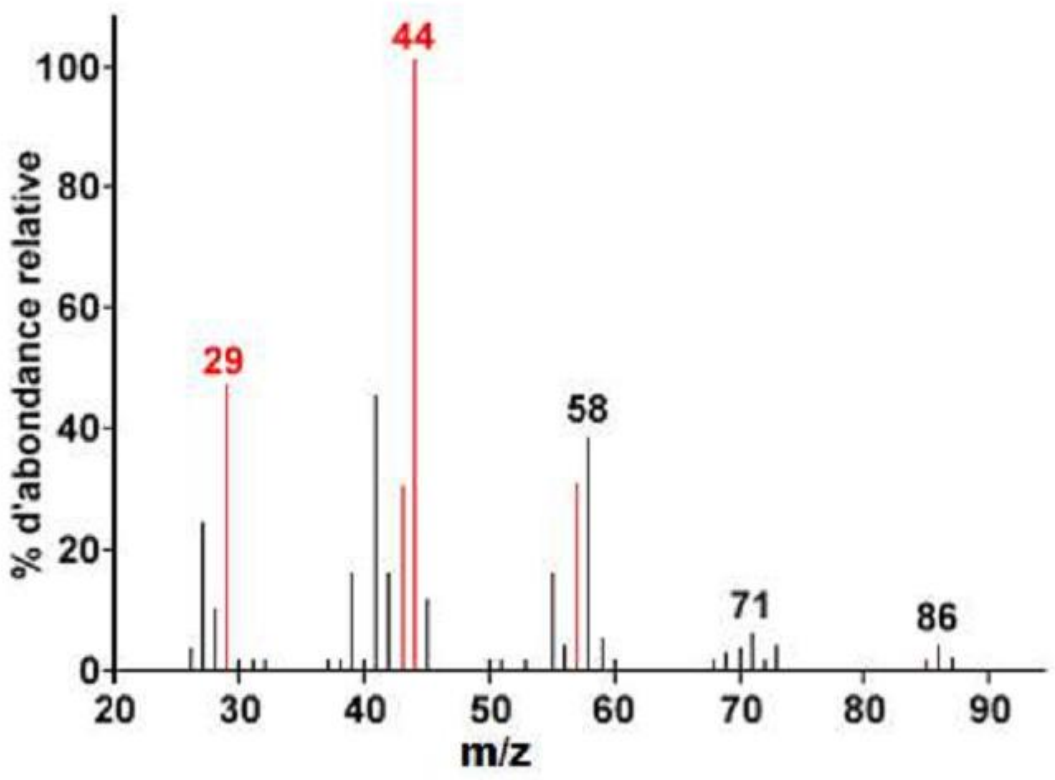
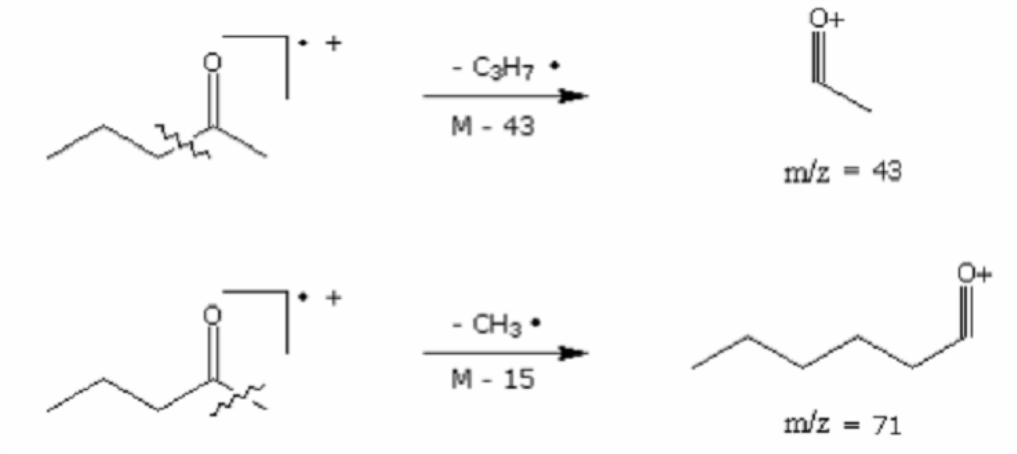


Fig. IV. 9. Mass spectrum of pentanal CH_3 (CH_2) $_3CHO$ with a molar mass of 86 g/mol.

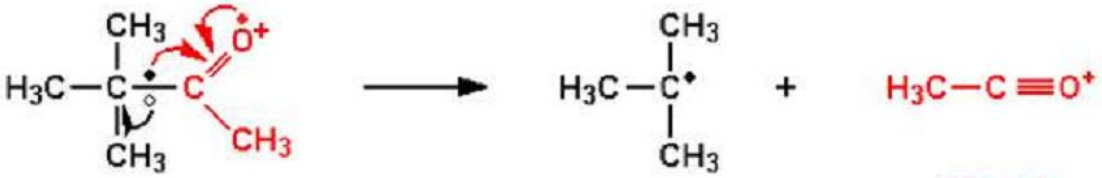
IV.17.7. Ketones and esters:

The most frequent mode of fragmentation of ketones R'COR" is the break at a which can give R'CO+ or R"CO+.

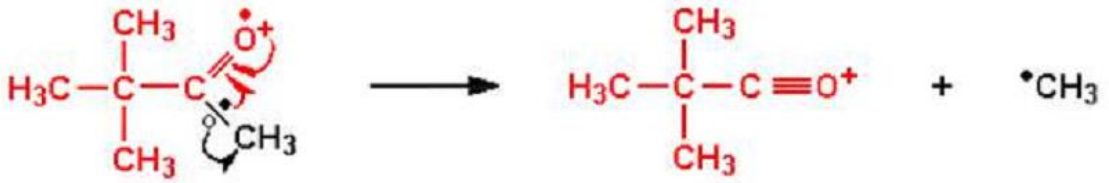
α cleavage:



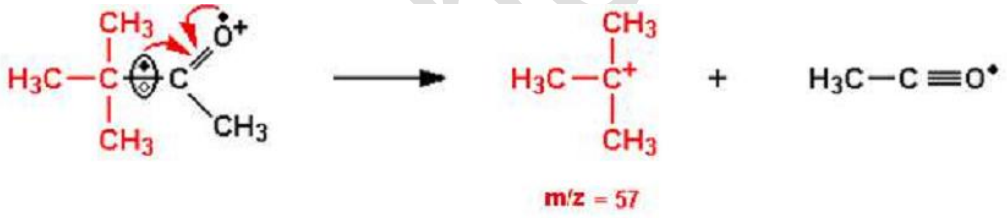
This gives the following results in the case of the ketone whose mass spectrum appears in Figure 10:



and to a lesser extent:



The peak at m/z = 57 corresponds to the fragment which is a very stable carbocation.



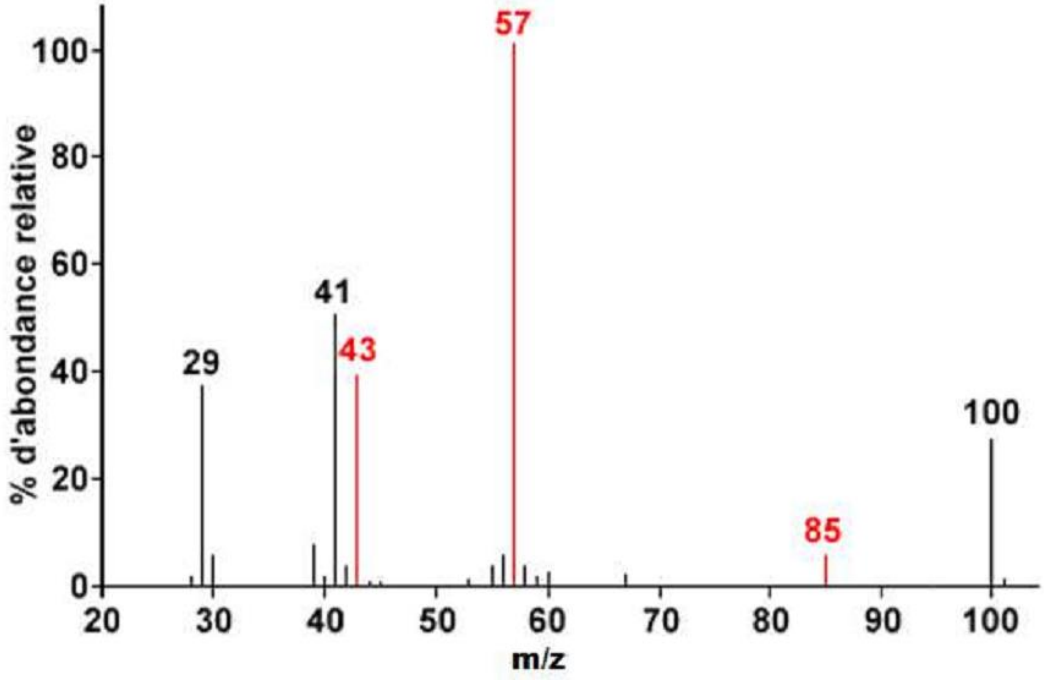
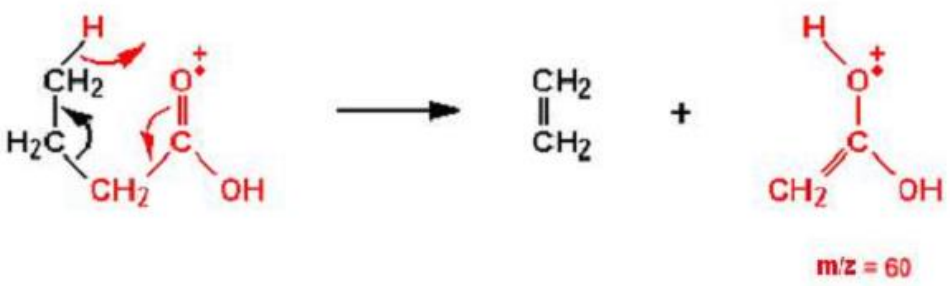


Fig. IV.10. Mass spectrum of 3,3-dimethyl-2-butanone CH3COC (CH3) 3 with a molar mass of 100 g/mol.

IV.17.8. Acids:

McLafferty-type rearrangement in butanoic acid



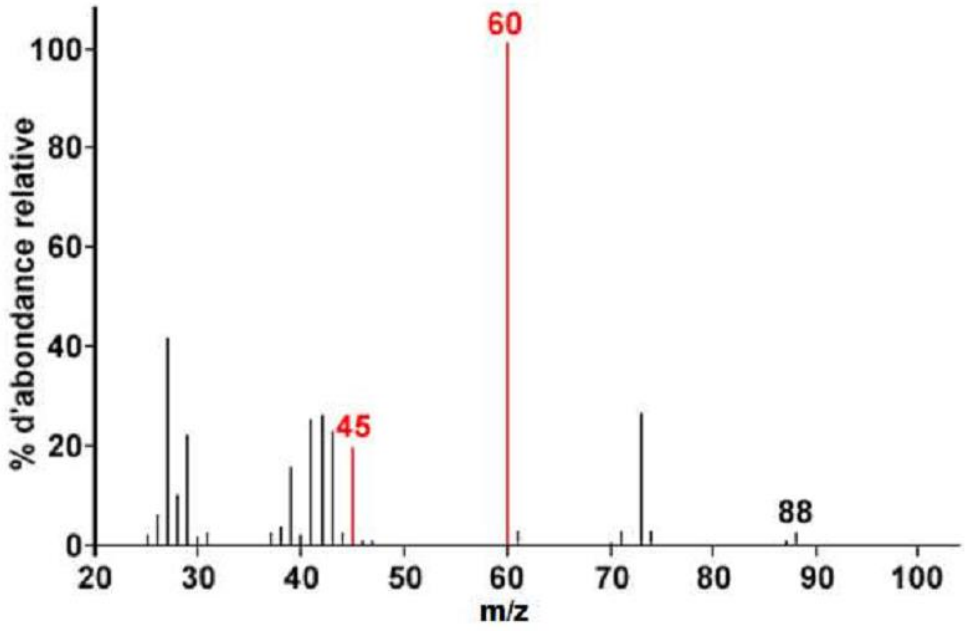
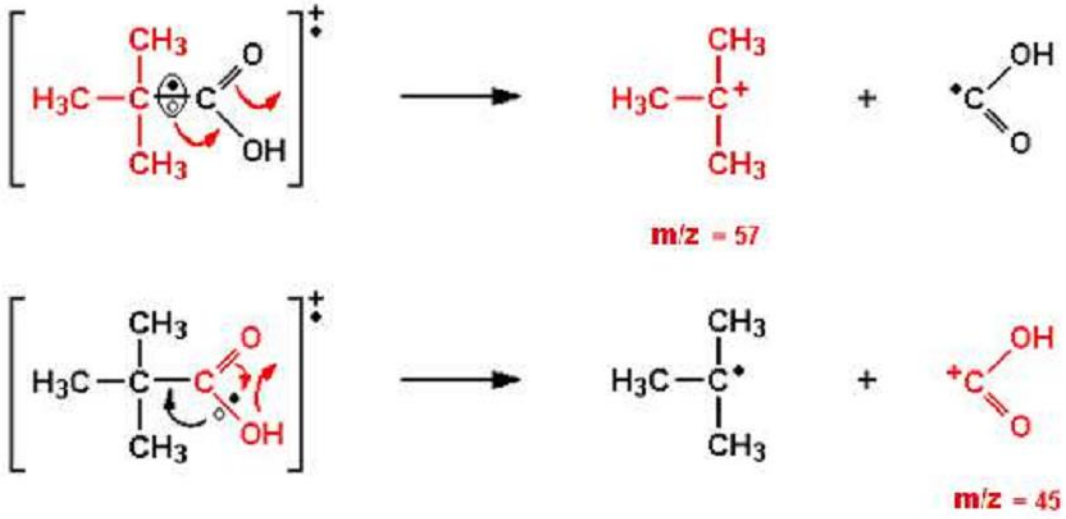


Fig. IV. 11. Mass spectrum of butanoic acid $CH_3CH_2CH_2COOH$ with molar mass = 88 g/ mol.

Fragmentation results in very stable carbocations at m/z = 45 and m/e = 57 in the case of 2,2-dimethylpropanoic acid. In the bottom row of these equations, there is cleavage of αthe acid function (m/z = 45).



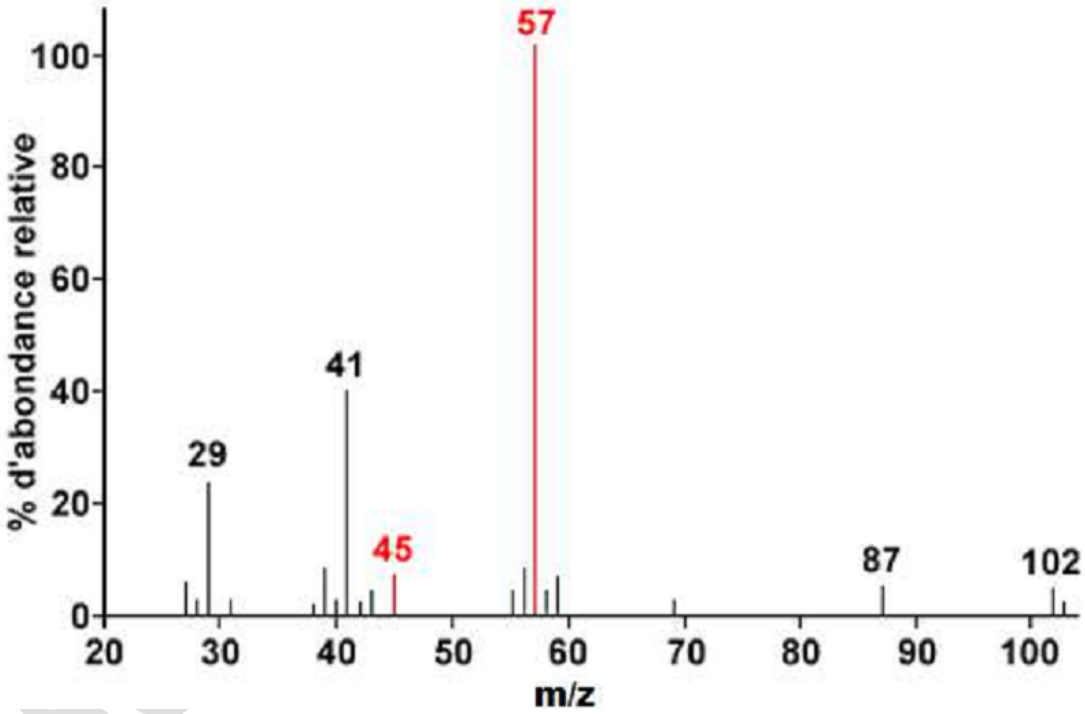


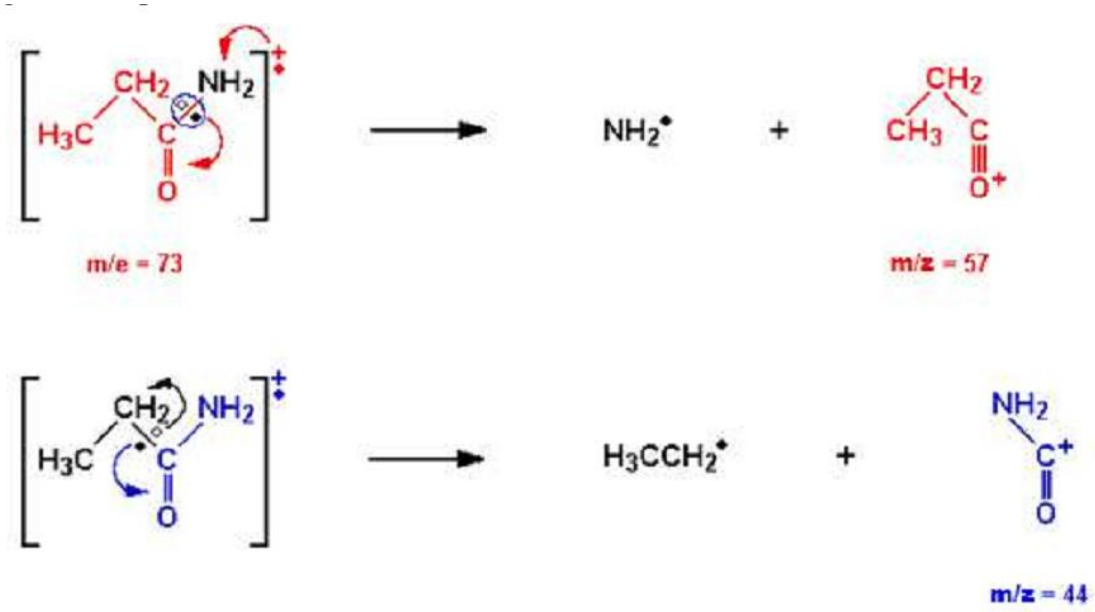
Fig. IV. 12. Mass spectra of 2,2-dimethylpropanoic acid $(CH_3)_3CCOOH$ with molar mass = 102 g/mol.

IV.17.9. Amides:

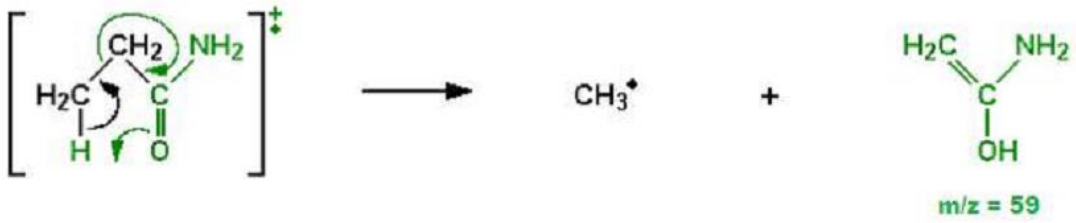
a. Propanamide :

The molecular ion is usually observable and provides a good indication of the presence of an amide.

An important fragmentation pattern involves bond breaking α(breakage of one or both of the C=O double bonds). Important fragments include:



The McLafferty rearrangement can involve amide groups on the alkyl chain side. Here is the rearrangement:



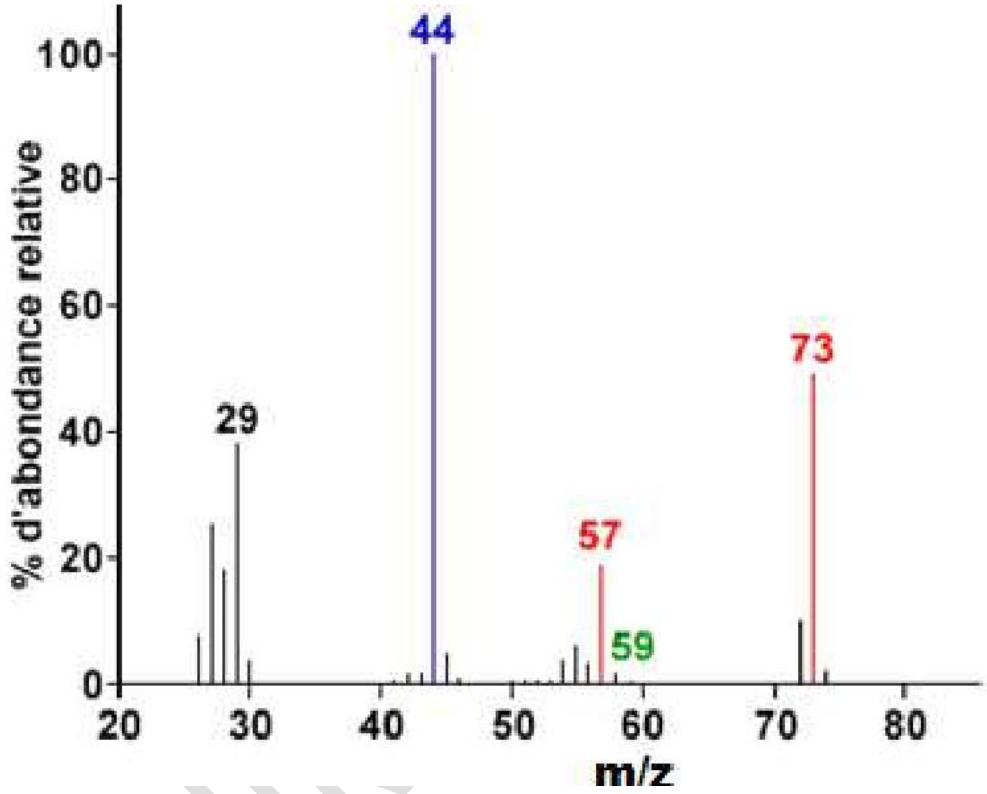
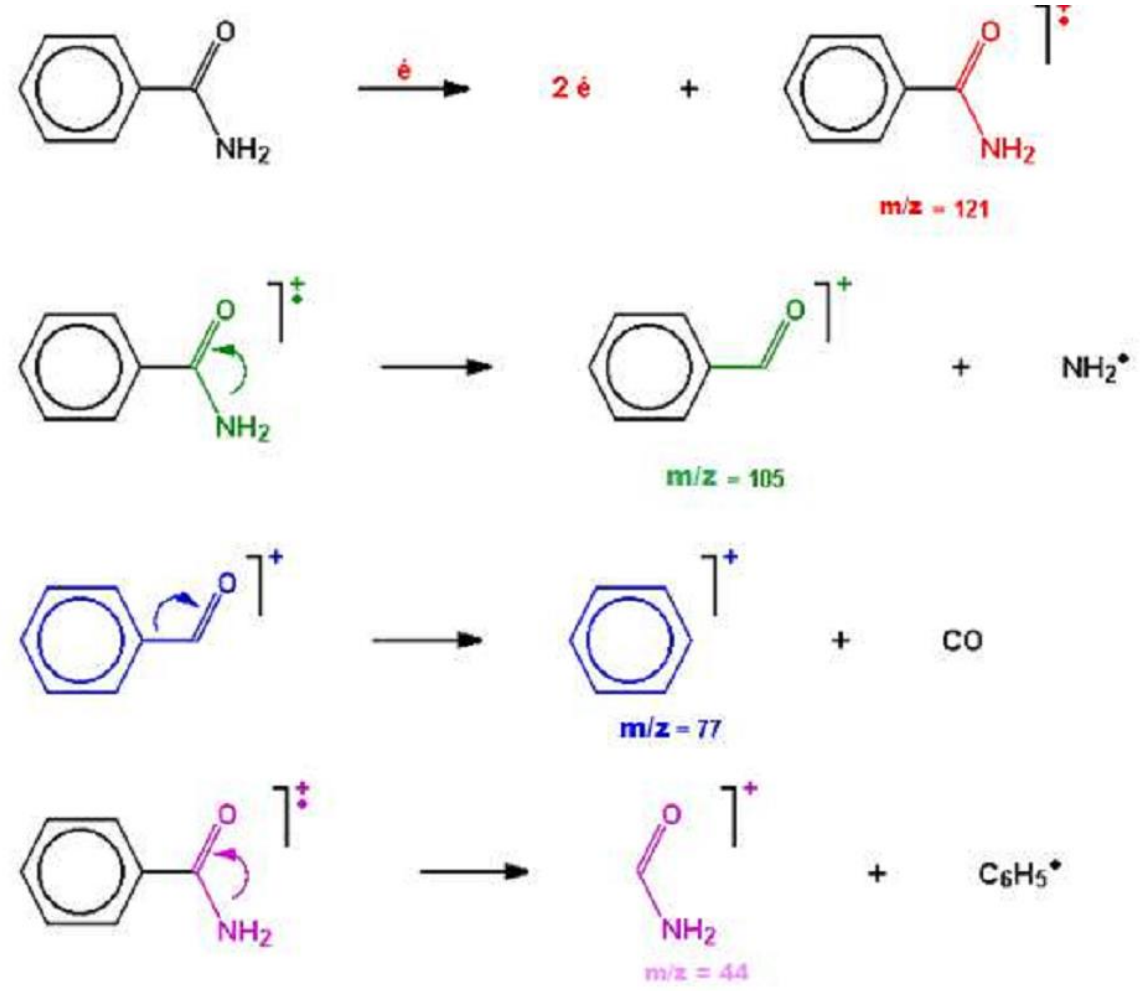


Fig. IV.13. Mass spectrum of propanamide $CH_3CH_2CONH_2$ of which M = 73 g / mol.

b. Benzamide:

Different types of ions are produced in the ionization process. Losing only one electron allows the entire molecule to reappear, especially if the molecule is stable, such as an aromatic compound. The important fragments are:



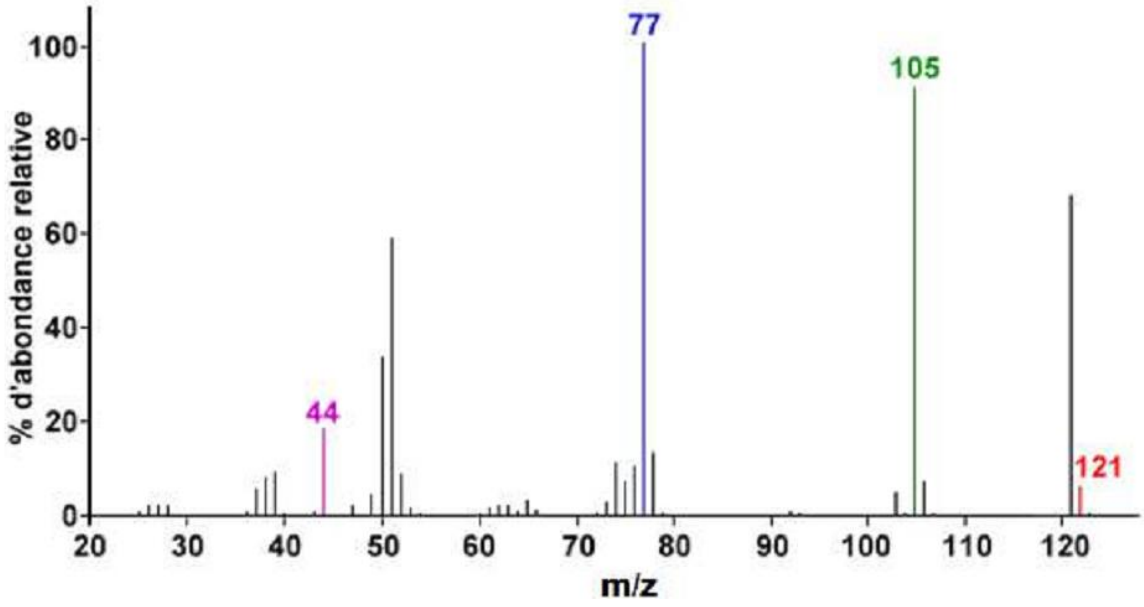


Fig. IV. 14. Mass spectrum of benzamide $ArCONH_2$ with M = 121 g/mol.

IV. 18. APPARATUS:

A mass spectrometer must be capable of performing at least the first three of the following operations:

IV. 18. 1. Ionization Techniques:

The large number of ionization methods, some of which are highly specialized, precludes an exhaustive study. The most common are gas-phase ionization, desorption, and evaporation, and are described below.

- **Gas-phase ionization techniques:**

Gas-phase ion generation techniques for mass spectrometry are the oldest and most popular among organic chemists.

- **Electron impact ionization** (EI):

Historically, ion production (IE) is the most widespread technique in mass spectrometry. It is also the one on which mass spectra are primarily interpreted for structure determination. Vapor-phase molecules in the sample are bombarded with highly energetic electrons (typically 70 eV), which eject an electron from a sample molecule, producing a cationic radical called a molecular ion. Furthermore, because this fragmentation process is partially predictable, it underlies the power of mass spectrometry in structure elucidation. Readily accessible **IE databases** contain the IE spectra of over 400,000 compounds and have efficient search engines.

- **Chemical ionization (CI):**

A reactive gas (usually methane, isobutane, ammonia, or others) is introduced into the ionization source and ionized. The sample molecules collide with the ionized molecules of the reactive gas ($C_2H_5^+$, $C_4H_9^+$, etc.) in the IC source, where the pressure is relatively high, leading to secondary ionization (i.e., chemical ionization) by proton transfer, producing an $[M^+]^-$ ion; by electrophilic addition, producing $[M^+_{15}]^+$, $[M^+_{29}]^+$, $[M^+_{41}]^+$, or $[M^+_{8}]^+$ ions (with NH_4^+ ions); or (more rarely) by charge transfer, producing an $[M]^+$ ion. Chemical ionization spectra sometimes show $[M^-_{1}]^+$ ion peaks resulting from the abstraction of a hydride. The ionization is sufficiently low, generally less than 5 eV, so that fragmentation is greatly reduced.

Example:

The IE mass spectrum of 3,4-dimethoxyacetophenone (***Fig. IV. 15***) shows, in addition to the molecular ion peak at m/z 180, numerous fragment peaks in the range m/z 15–167; among these are the base peak at m/z 165 and notable peaks at m/z 77 and 137. In the IC mass spectrum (reactive gas methane, CH_4), the base peak (100%) is the quasimolecular ion ([M + 1] +, m/z 181) and the only other peaks, each of low intensity, are from the molecular ion, at m/z 180, 209 ([M + 29] + or M + C_2H_5+) and 221 ([M + 41] + or M + C_3H_5+). These last

two, resulting from the electrophilic addition of carbocations, are particularly useful for identifying the molecular ion. The carrier gas, excess methane, is ionized during the electron impact to form CH_4+ and CH_3+ ions, which react with the excess methane to give secondary ions. CH_3+ + CH_4 → $C2H_5$+ and H_2 CH_4 + C_2H_5+ → C_3H_5+ and $2H_2$

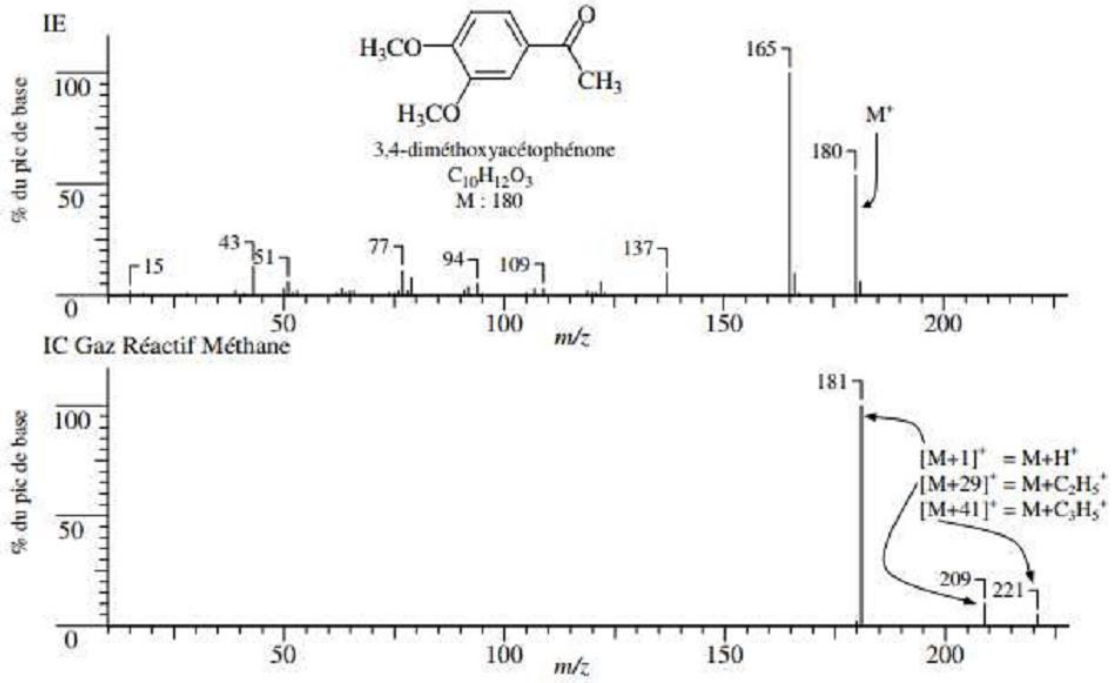


Fig. IV. 15. IE and IC spectra of 3,4-dimethoxyacetophenone.

IV.17.2. Desorption Ionization Techniques:

The molecules to be studied pass directly from a condensed phase (matrix) to the vapor phase in the form of ions. These techniques are mainly used for heavy, non-volatile, or ionic compounds. Often, the information obtained is limited. For unknown compounds, these techniques primarily serve to provide the molecular mass and sometimes an exact mass. This often results in spectra complicated by numerous ions from the matrix.

- **Field desorption** (FD) ionization:

The sample is placed on a metallic emitter equipped with carbon microneedles. The microneedles activate the surface, which is subjected to an accelerating voltage and acts as the anode. The very high voltage gradients at the tips of the needles remove an electron from the sample, and the resulting cation is expelled from the emitter. The generated ions have very little excess energy and therefore fragment minimally; the molecular ion is usually the only one observed in significant quantity. For example, no molecular ions are observed by IE or IC for cholest-5-ene-3,16,22,26-tetrol. Whereas the FD mass spectrum (Fig. **IV. 16)** shows primarily the molecular ion and virtually no fragmentation.

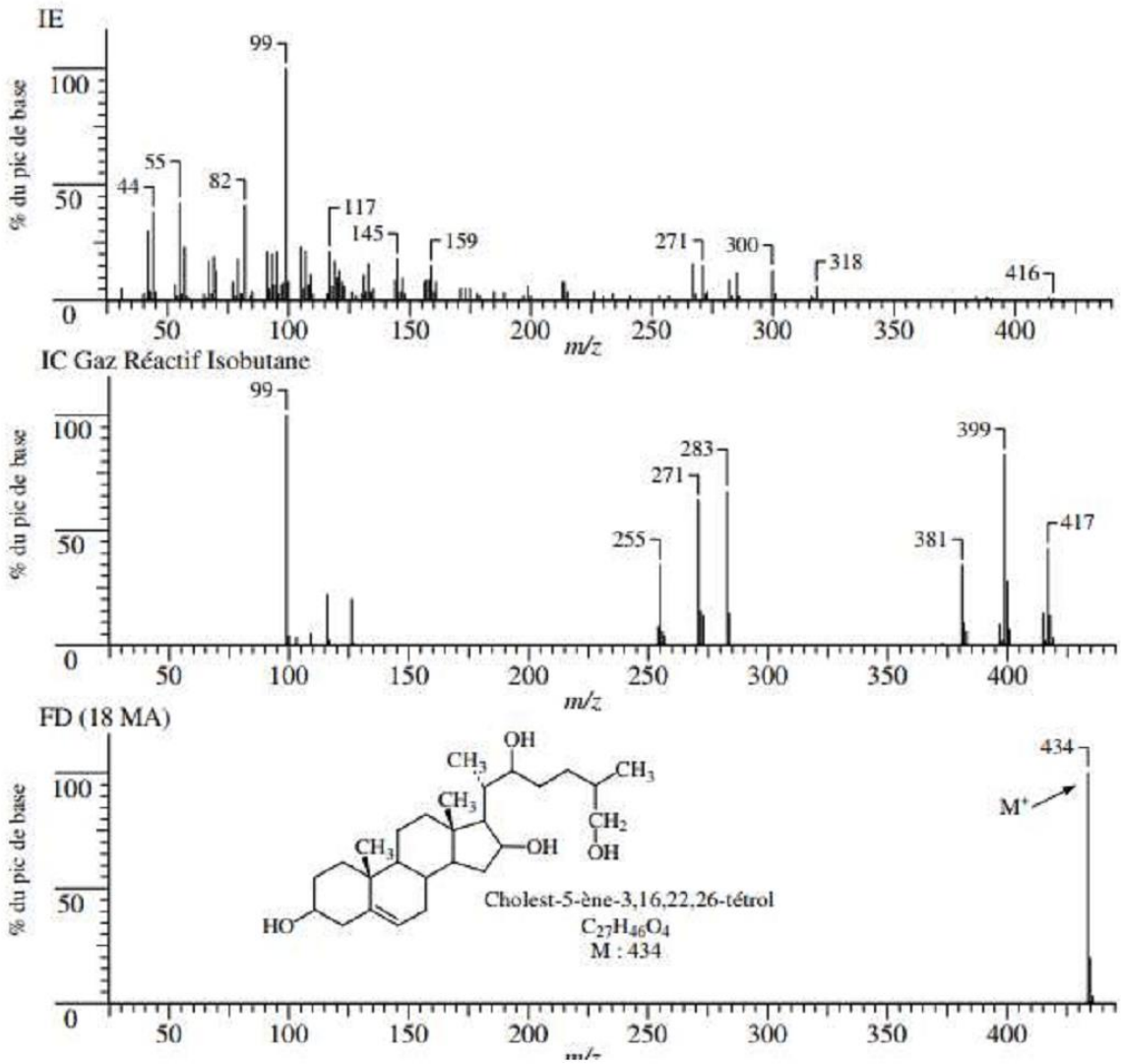


Fig. IV. 16. Mass spectra of cholest-5-ene-3,16,22,26-tetrol by electron impact (IE), chemical ionization (IC) and field desorption (FD).

- **Fast Atom Bombardment:** (FAB**)** ionization uses highly energetic xenon or argon atoms (6 to 10 keV) to bombard samples dissolved in a low vapor pressure liquid (e.g., glycerol). This matrix protects the sample from excessive radiation damage. A similar method, liquid secondary ionization mass spectrometry (LSIMS**)**, uses even more energetic cesium ions (10 to 30 keV). Both techniques produce positive ions (by capturing [M+1]+ or [M+23,Na]+ cations) and negative ions (by deprotonating [M-1]-). FAB is most often equipped with a dual-focusing magnetic sector instrument; however, it can be coupled to most analyzers.
- **Plasma Desorption-Ionization** This is a highly specialized technique almost exclusively used with a time-of-flight (TOF) analyzer. The fission products of californium-252 (252Cf) are used to bombard and ionize the sample. The instrument must be TOF-MS.
- **Laser desorption-ionization:**

A pulsed laser beam can be used in mass spectrometry for sample ionization. Because the ionization method is pulsed, it must be used with either a time-of-flight (TOF) or Fourier transform (FT-MS) mass spectrometer. The technique is much more powerful when matrix-assisted (Matrix-Assisted Laser Desorption/Ionization, or **MALDI)**. Two matrix materials, gentisic acid and sinapinic acid, whose adsorption bands coincide with the laser used, are commonly employed. Samples with molecular masses up to 200,000 to 300,000 Da have been successfully analyzed. A few picomoles of sample are mixed with the matrix before being irradiated with a pulse, which causes ion ejection. MALDI is most often used with a TOF-MS or a Fourier transform (FT-MS) mass spectrometer.

IV.17.3. Evaporation Ionization Techniques:

Coupled with liquid chromatography equipment, these methods have become extremely popular.

- **Thermos pray Mass Spectrometry.** The sample is introduced in solution into the mass spectrometer using a heated capillary tube. The tube nebulizes and partially evaporates the solvent to form a stream of fine droplets introduced into the ion source. When the solvent has completely evaporated, the ions can be analyzed.
- **Electrospray mass spectrometry (ES):**

The sample in solution (usually in a volatile polar solvent) enters the ion source via a stainless-steel capillary surrounded by a coaxial stream of nitrogen, called a nebulizer. The capillary tip is held at a high voltage relative to a counter electrode. The potential difference produces a field gradient of up to 5 kV/cm. An aerosol of charged droplets forms as the solution leaves the capillary. The nebulizer gas stream directs the effluent to the mass spectrometer (Fig. **17)**.

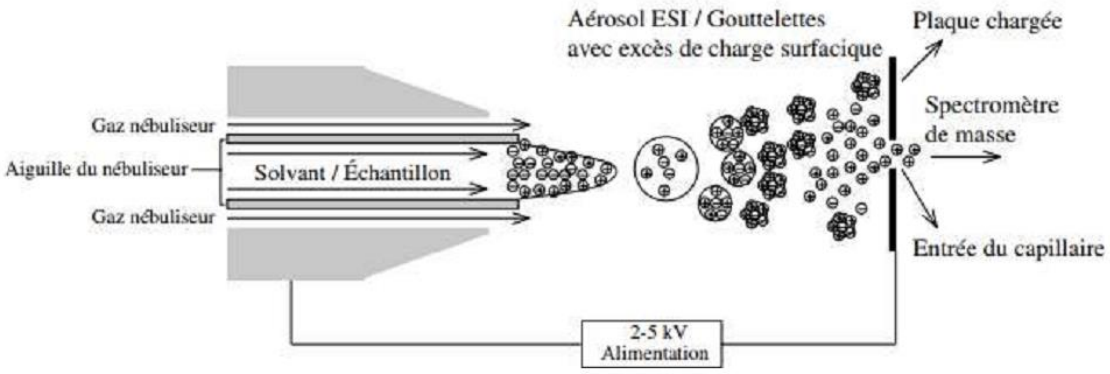


Fig. IV. 17. Diagram showing evaporation leading to isolated ions in an electrospray device.

Figure 18 compares the IE and ES mass spectra of lactose. The IE spectrum is completely unusable because lactose is relatively unvolatile and thermally

labile; the spectrum shows no characteristic peaks. The ES spectrum shows a low-intensity molecular peak at m/z 342 and a peak at [M + 23]+ characteristic of the sodium adduct. The ES spectrum of a tetrapeptide composed of valine, glycine, serine, and glutamic acid (VGSE) is shown in *Fig.IV.19*. For VGSE: The basal peak is the [M + 1]+ ion at m/z 391, and the intensity of the sodium adduct, [M + 23]+, is almost 90% of this. Furthermore, useful information on fragmentation, characteristic of each amino acid, is obtained. For small peptides, interesting fragmentation is not uncommon, but this is less true for proteins.

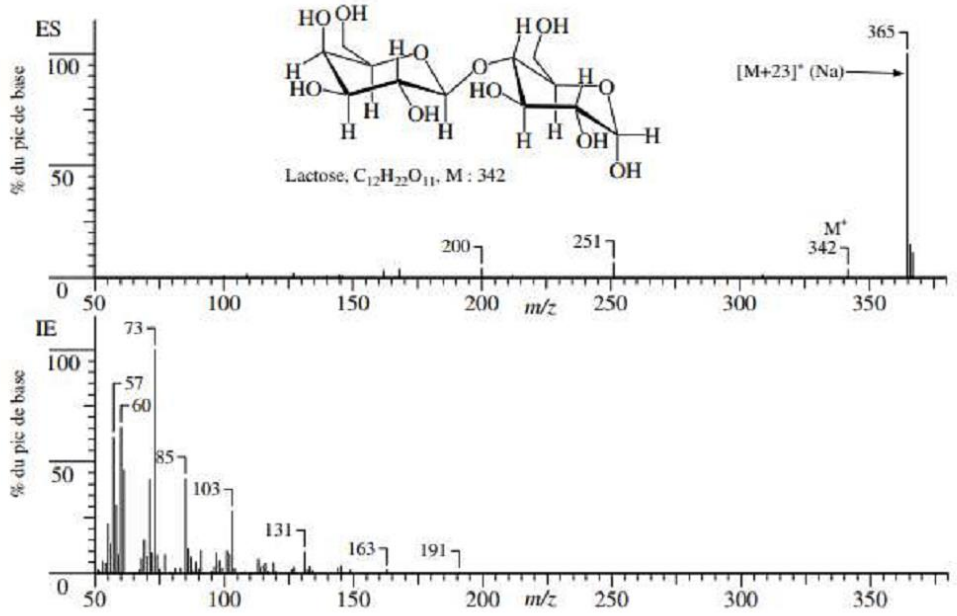


Fig. IV. 18. IE and ES spectra of lactose.

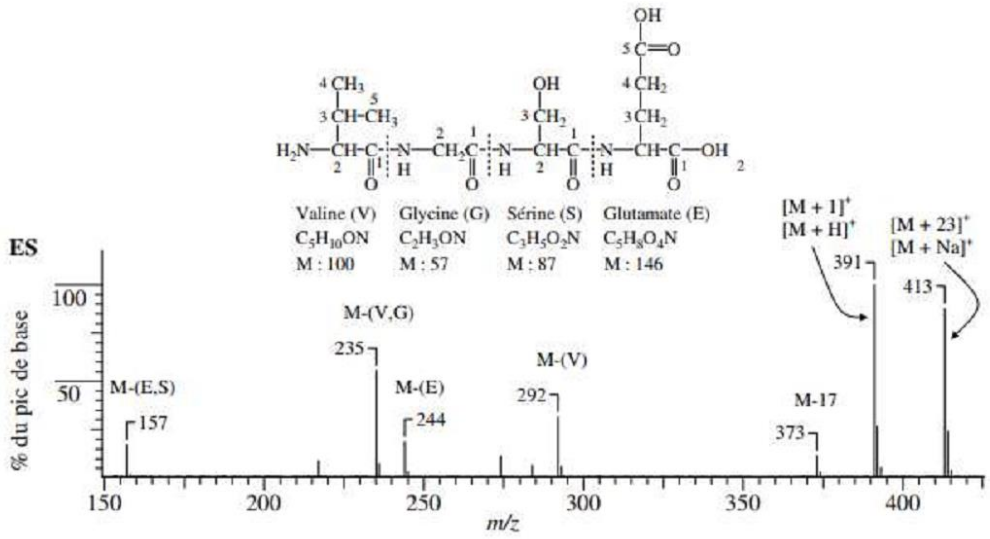


Fig. IV. 19. Electrospray (ES) spectrum of the tetrapeptide VGSE, the structure of which is shown in the figure. See explanations in the text.

IV.17. 3. Mass Analyzers:

The analyzer, which separates the ion mixture generated in the ionization step by m/z order to obtain a spectrum, is the heart of the spectrometer. Different types exist, each with its own characteristics.

- **Magnetic sector mass spectrometers:**

A magnetic field circularly deflects the trajectory of the ions (Fig. 20). The separation of the ions is based on the m/z ratio; lighter ions are deflected more than heavier ions, according to the equation: