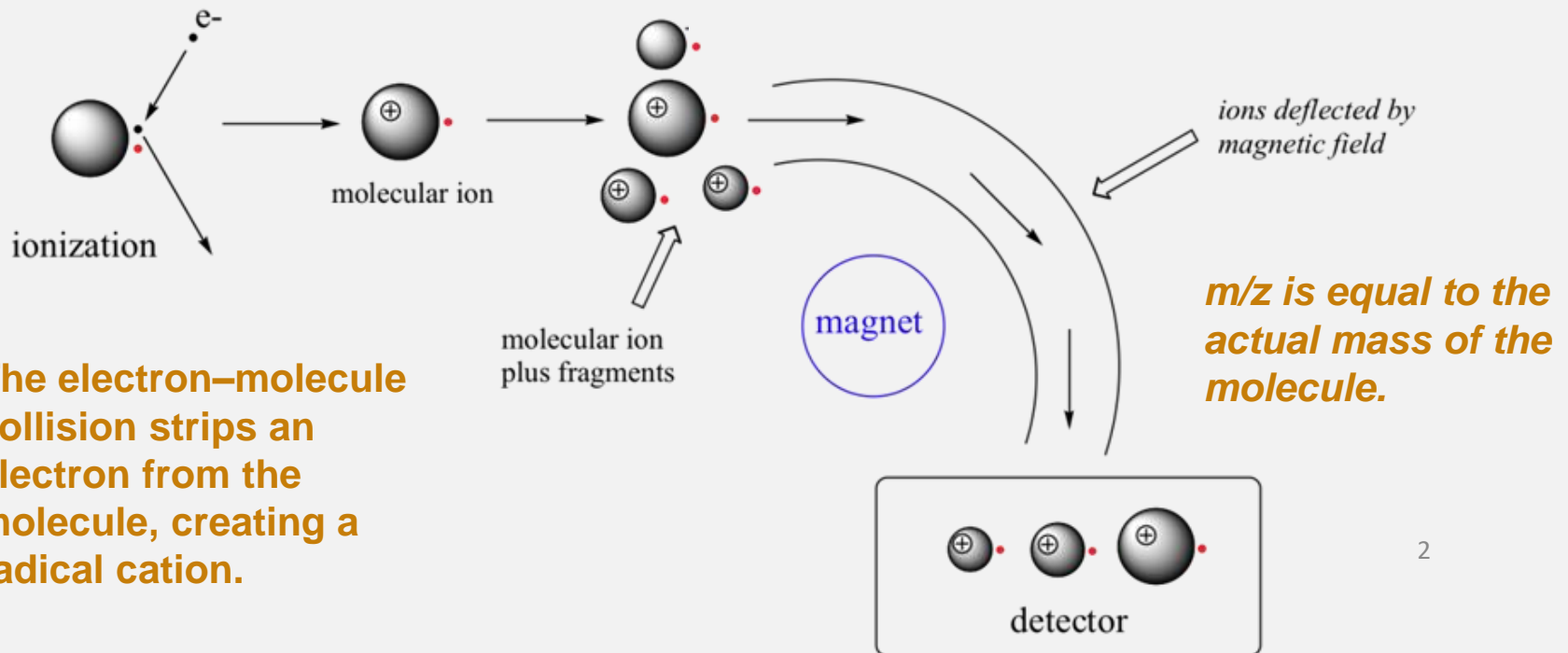# MASS SPECTROMETRY

○The mass spectrometer is a device developed for evaporation, ionization of the analysis sample and recording of that ions based on their mass-to-charge (*m/z*) ratio.
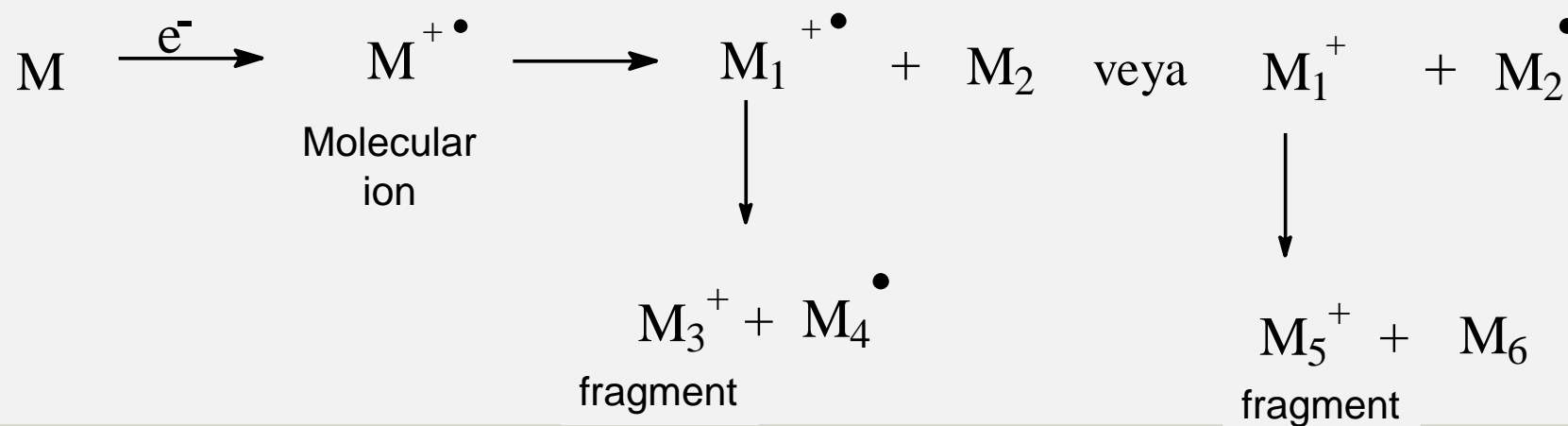
Electron (*e*⁻) beam radiate from a heated tungsten or rhenium filament

e-

ionization

molecular ion

molecular ion plus fragments

magnet

ions deflected by magnetic field

m/z is equal to the actual mass of the molecule.

The electron–molecule collision strips an electron from the molecule, creating a radical cation.
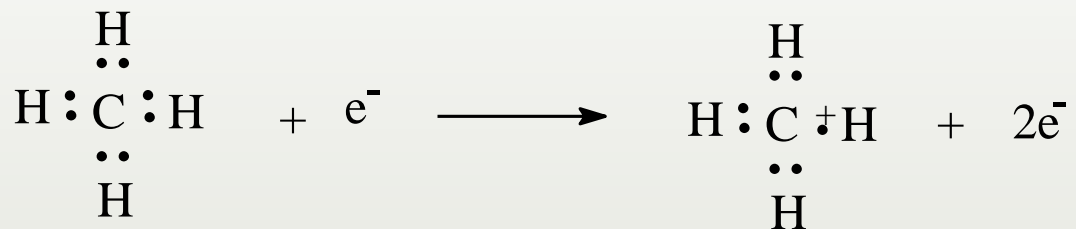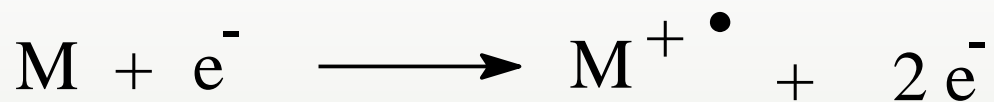
detector

o The energy required to remove an electron from an atom or molecule is its **ionization potential** or **ionization energy**.

o The simple removal of an electron from a molecule yields an ion with weight that is the actual molecular weight of the original molecule. This is the **molecular ion,** which is usually represented by **$M^{+\cdot}$**

o The molecular ion is a radical cation since it contains an unpaired electron as well as a positive charge.

$$M + e^- \longrightarrow M^{+\bullet} + 2\,e^-$$
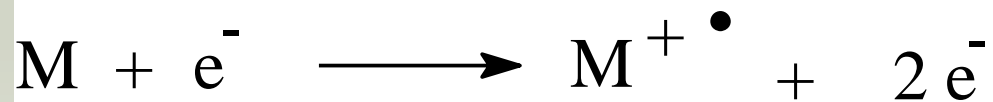
o If ionization energy is increased, the molecular ion undergoes further fragmentation and small organic molecules, called **fragment**, occur.

o Ionization and fragmentation result in a mixture of various radical cations, cations, neutral or radical particles.

$$M \xrightarrow{\ e^- \ } M^{+\bullet} \longrightarrow M_1^{+\bullet} + M_2 \quad \text{veya} \quad M_1^+ + M_2^{\bullet}$$

Molecular ion

$$M_3^+ + M_4^{\bullet}$$

fragment

$$M_5^+ + M_6$$

fragment

$$M + e^- \longrightarrow M^{+\bullet} + 2\,e^-$$

$$
\begin{array}{c}
\overset{\displaystyle H}{} \\[-2pt]
H:\underset{\displaystyle H}{\overset{..}{\underset{..}{C}}}:H \quad + \; e^-
\end{array}
\longrightarrow
\begin{array}{c}
\overset{\displaystyle H}{} \\[-2pt]
H:\underset{\displaystyle H}{\overset{..}{\underset{..}{C}}}\,\overset{\bullet}{:}H \quad + \; 2e^-
\end{array}
$$

radical cation

Purpose of mass spectrometry ;

Positive particules formed by a beam of electrons with high energy are plotted to the relative abundances of mass to charge (m/z) value. The structure of the molecule is determined by obtaining **the mass spectrum**.
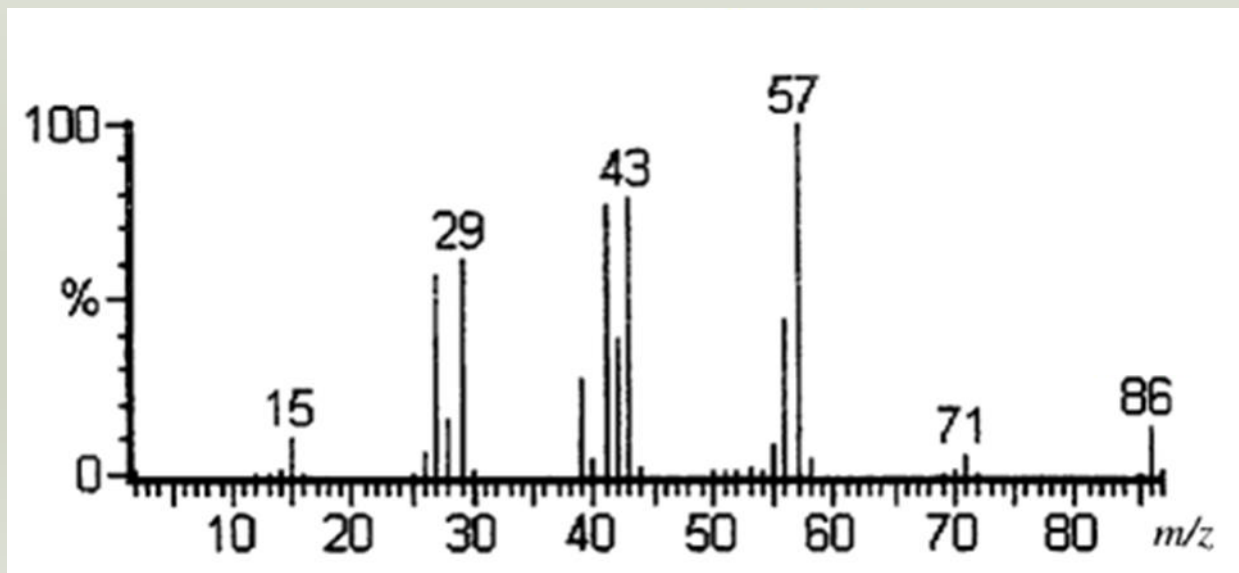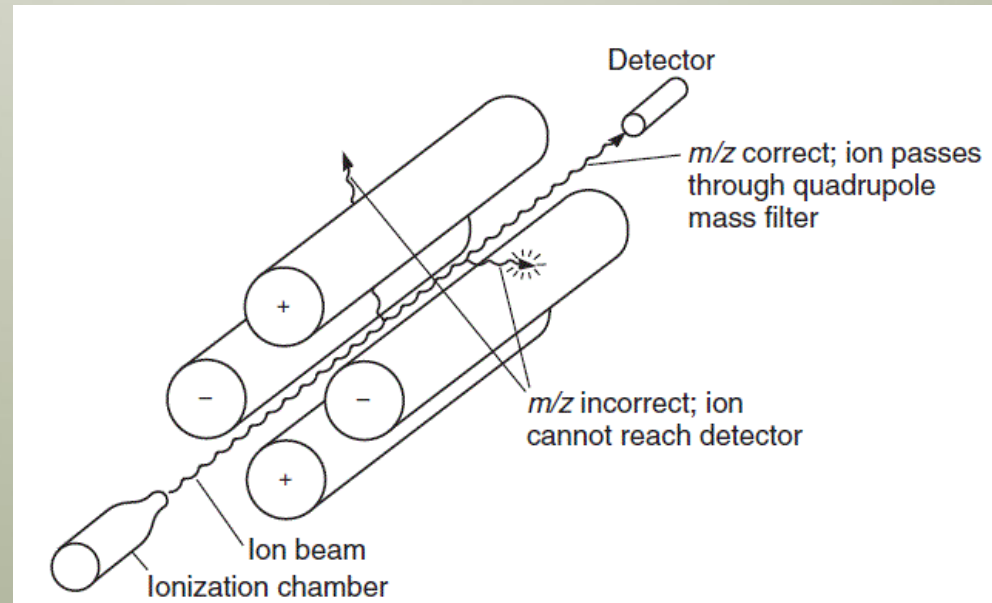
MASS SPECTROMETERS

- <u>The Magnetic Sector Mass Spectrometers</u>

  -Single-focusing mass spectrometer: (Most used in practice)

  Contains only magnetic field.

  -Double-focusing mass spectrometer:

  Contains magnetic field and electrical field.

- <u>Quadrupole Mass Spectrometer</u>

- <u>Time-of-flight Mass Spectrometer</u>

- <u>Ion-cyclotron Rezonance Mass Spectrometer</u>

Quadrupole Mass Spectrometer

- In a quadrupole mass spectrometer, a set of four solid rods is arranged parallel to the direction of the ion beam. A direct-current (DC) voltage and a radiofrequency (RF) is applied to the rods, generating an oscillating electrostatic field in the region between the rods. Ions of an incorrect m/z ratio (too small or too large) undergo an unstable oscillation. Ions of the correct mass-to-charge ratio pass through the analyzer to reach the detector.

Detector

m/z correct; ion passes through quadrupole mass filter

m/z incorrect; ion cannot reach detector

Ion beam
Ionization chamber

Time-of-flight Mass Spectrometer

• The time-of-flight (TOF) mass analyzer is based on the velocities of two ions, created at the same instant with the same kinetic energy, will vary depending on the mass of the ions—the lighter ion will have a higher velocity.

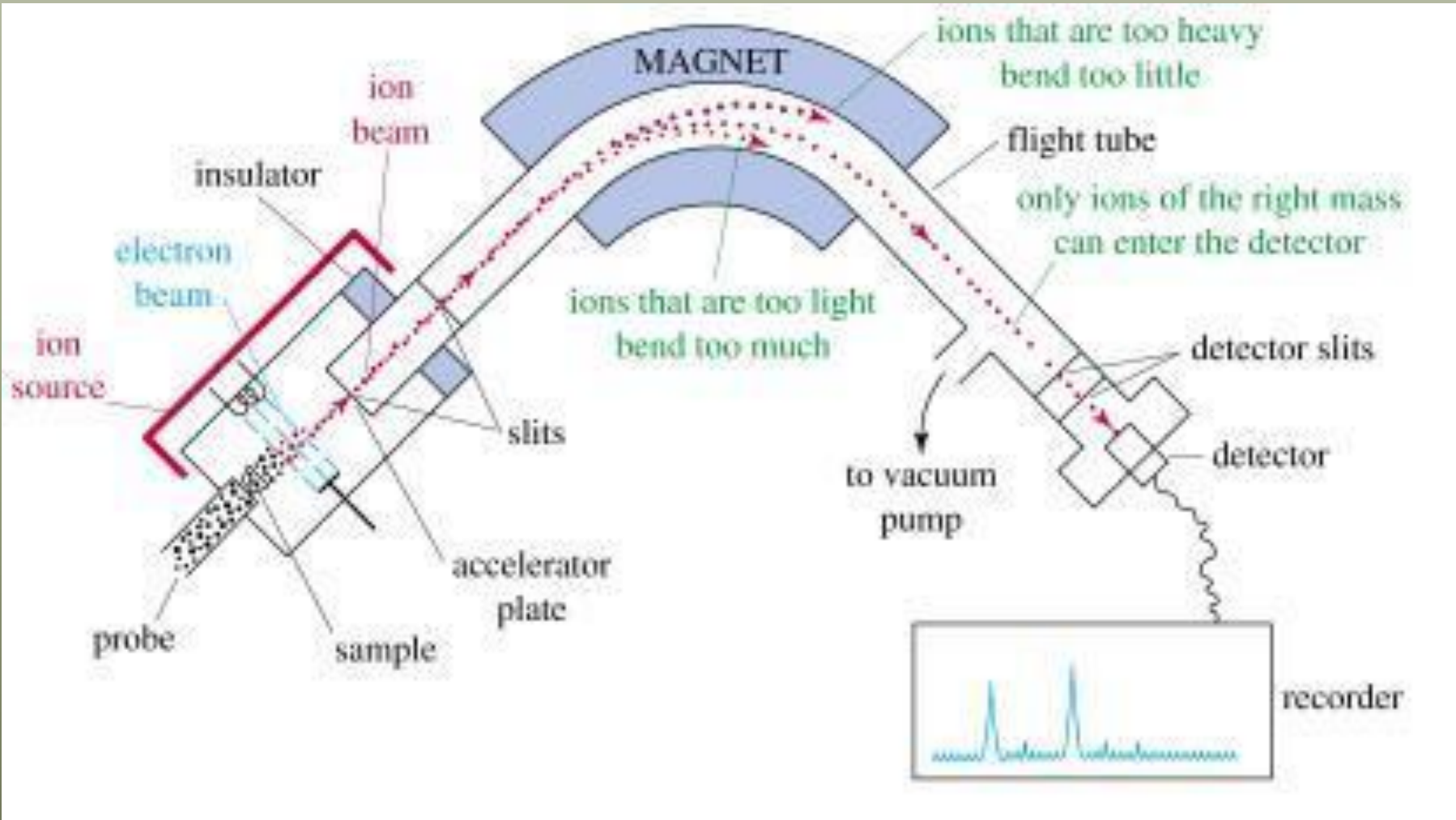• Ions are collected at different times according to the m/z values. This spectrometer is not highly distinguished.
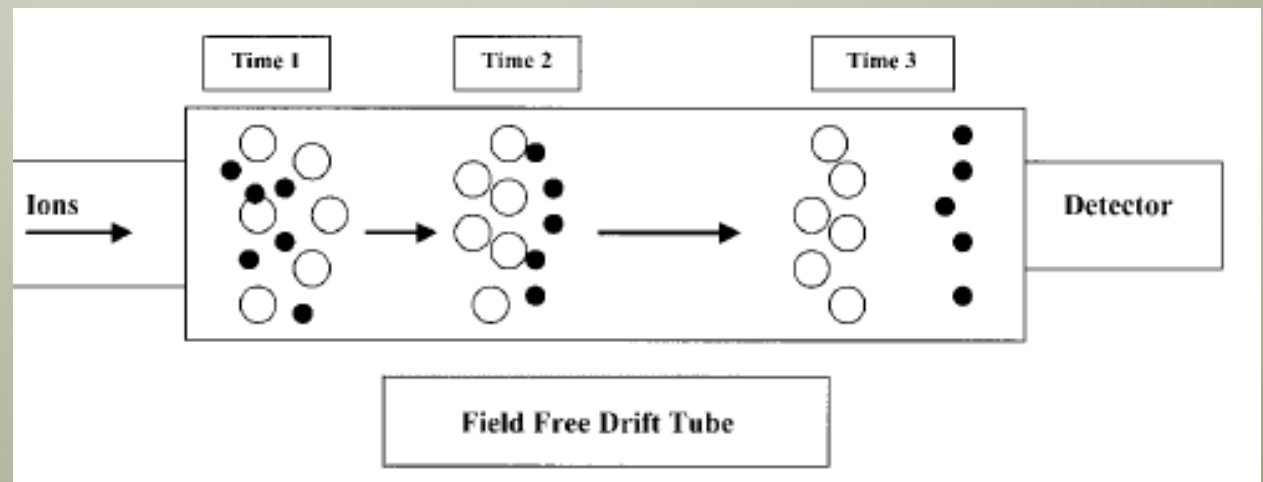
Single-focusing Mass Spectrometer

Consists of 5 parts ;

⭕Sample introduction

⭕Ion source and accelerating plates

⭕Analysis tube

⭕Ion collector and amplifier

⭕Detector (recorder)

Tek Odaklı Kütle Spektrometresi

1) Sample introduction (Sample inlet): A sample studied by mass spectrometry may be a gas, a liquid, or a solid. Enough of the sample must be converted to the vapor state at a certain temperature and low pressure without degradation.

2) Ion source and accelerating plates: Vapor phase sample molecules entering the ionization chamber are bombarded with high energy electrons (generally 70 eV) by heated tungsten or rhenium filament **(Electron Impact-EI)**. As a result of bombardment, an electron is ejected from the sample molecule to produce a radical cation, known as molecular ion. Although 15 eV is sufficient for ionization potential, 70 eV is used for fragmentation along with ionization.

Positive ions produced by electron bombardment reach their final velocities by passing through the accelerating plates. These ions arrive to the **mass analyzer,** the region of the mass spectrometer where the ions are separated according to their mass-to-charge (*m/z*) ratios.

3) Analysis tube: It is a metal tube which is evacuated inside and bended in a semicircle, the pressure inside is 10^{-7}–10^{-8} mmHg. It must be deflected in a strong magnetic field to separate the **positive** ions passing through the accelerator by mass differences. When the ions enter the magnetic field, they follow a circular path, perpendicular to the area. The voltage difference of the ion-accelerating plates (V), the strength of the magnetic field (H,B) and the radius of curvature of the path (r) are proportional to the mass/charge (*m/e*, *m/z*) value.

Particules with m/z ratios that are small have smaller circle, they deviate more.

Since ions with the same mass follow the same path, they are recorded in the same place in the spectrum.

m= the mass of the ion

z,e= the charge on the ion

H,B= the strength of the magnetic field

$$\frac{m}{e} = \frac{H^2 r^2}{2\,V}$$

r= the radius of curvature of the path

V= the potential (voltage) diffirence of the ion-accelerating plates

4) Ion collector and amplifier: In the magnetic field, the ions separated according to their *m/z* ratios pass through regulator, strike to collector as ion beam and are counted here. Faraday cylinder, vacuum-tube and electron multiplexers are used for this counting process.

5) Detector (recorder): The mass spectrum is recorded by scanning from small to large or from large to small. The height of the peaks is proportional to the number of ions (relative abundances) in each *m* value. The result in mass spectrometry is shown either in graphical form (mass spectrum) or as a list of relative abundances of particules.
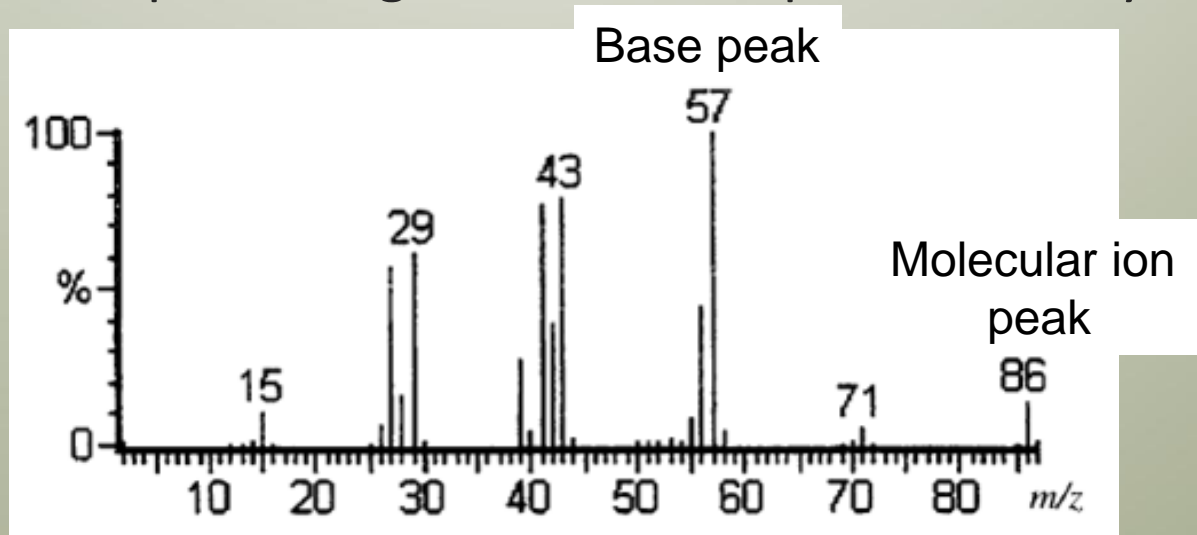
Mass Spectrum

The mass spectrum is a graphical form of the relative abundances of positive ions peaks (cations and radical cations formed by electrons with 70 eV energy) according to the their mass-to-charge values.

Base peak
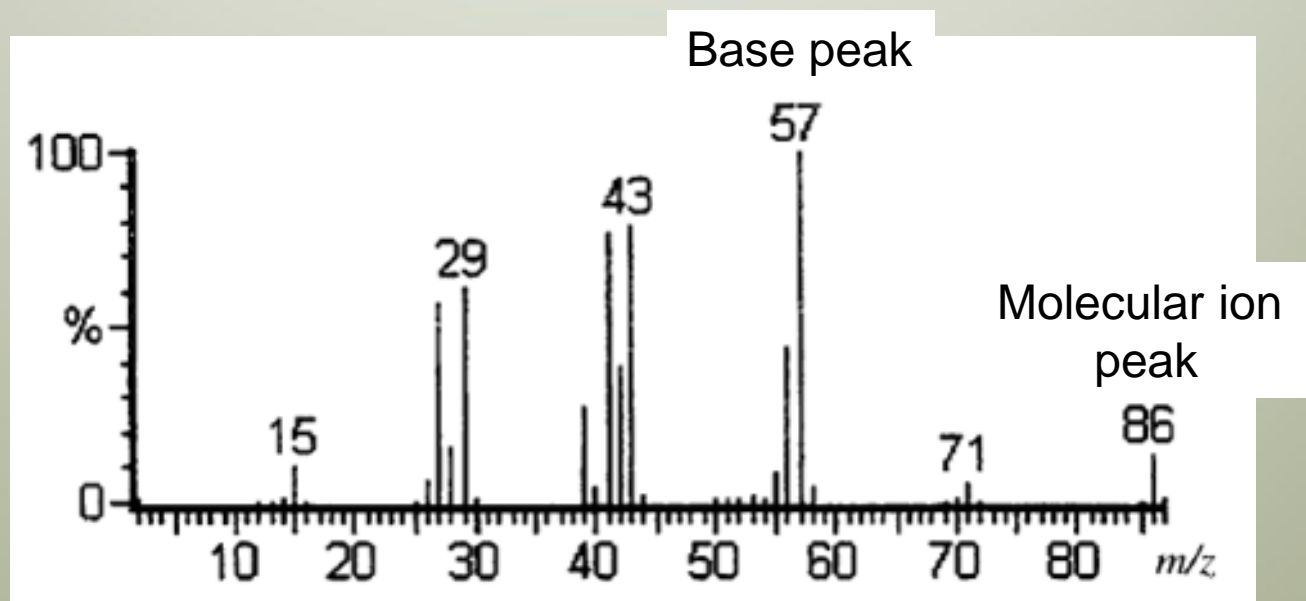
Molecular ion peak

Mass spectrum of hexane

- If **the molecular ion** (**M$^{+\cdot}$**, parent ion) formed by the separation of an elektron from the molecule can remain without fragmentation for 10^{-6} sec and reach the detector, **the molecular ion peak** is observed in the spectrum.

- The most abundant ion formed in the ionization chamber gives rise to the tallest peak in the mass spectrum, called the **base peak.** The spectral intensities are normalized by setting the base peak to relative abundance 100, and the rest of the ions are reported as percentages of the base peak intensity.

Mass spectrum of hexane

Natural Abundances of Common Elements and Their Isotopes

Element	Isotope	Natural Abundances	Atomic Weight
Hydrogen	^{1}H	**100**	**1.0078**
	^{2}H	0.016	2.01410
Carbon	^{12}C	**100**	**12.0000**
	^{13}C	1.08	13.0034
Nitrogen	^{14}N	**100**	**14.0031**
	^{15}N	0.37	15.0001
Oxygen	^{16}O	**100**	**15.9949**
	^{17}O	0.04	16.9991
	^{18}O	0.20	17.9992
Flourine	^{19}F	100	18.9984
Chlorine	^{35}Cl	**100**	**34.9689**
	^{37}Cl	32.5	36.9659
Bromine	^{79}Br	**100**	**78.91 83**
	^{81}Br	98	80.91 63

Molecular Ion

1) The mass of the molecular ion is equal to the molecular mass calculated according to the masses of the most abundant isotopes of the relative abundance of atoms in the molecule.

Molecular ion has the lowest ionization energy.

2) In addition to molecular ion peak, isotopic peaks can also be observed.

3) More or less relative abundance of the molecular ion peak depends on the stability of the molecular ion. If the molecular ion, once it is formed, is so unstable that it disintegrates before it can pass into the accelerating region of the ionization chamber. And the molecular ion peak can be too small or cannot be observed.

Molecular Ion

4) Nitrogen Rule: This rule states that if a compound has an even number of nitrogen atoms (zero is an even number), its molecular ion will appear at an even mass value. On the other hand, a molecule with an odd number of nitrogen atoms will form a molecular ion with an odd mass.

Question 1:

- How is the molecular mass of compound CH_4 calculated?

- What is the value of the Molecular Ion peak in the mass spectrum?

- Are the peaks composed of other isotopes of carbon and hydrogen atoms (^{13}C ve ^{2}H) observed in the mass spectrum?

When calculating the molecular mass, the masses of atoms with the highest abundance are used.

Molecular weight of CH_4 =(1x12,0000)+(4x1,0078)= 16,0312

Molecular Ion (**M$^{+\cdot}$**) weight of CH_4 = 16,0312

If a high resolution spectrometer is used, the exact mass of the peaks can be determined.

For CH_4, M+1 peak is observed due to ^{13}C and ^{2}H isotopes with low abundance. The percentage of this peak relative to the **M$^{+\cdot}$** peak is calculated as follows.
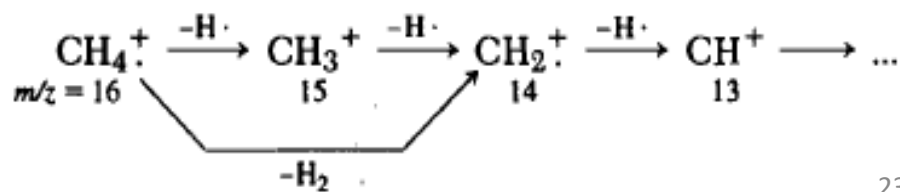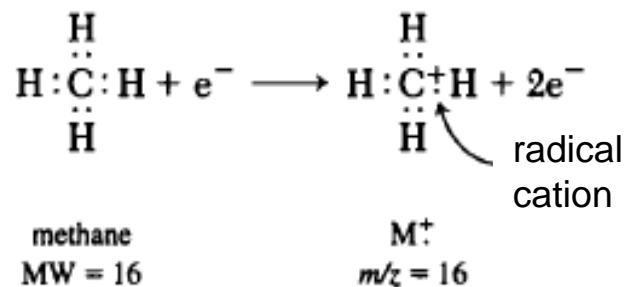
%M+1= (Natural relative abundance of ^{13}C x C number in the molecule) + (Natural relative abundance of ^{2}H x H number in the molecule)

%M+1= (1,08x1) + (0,016x4)= 1,14

Relative abundance
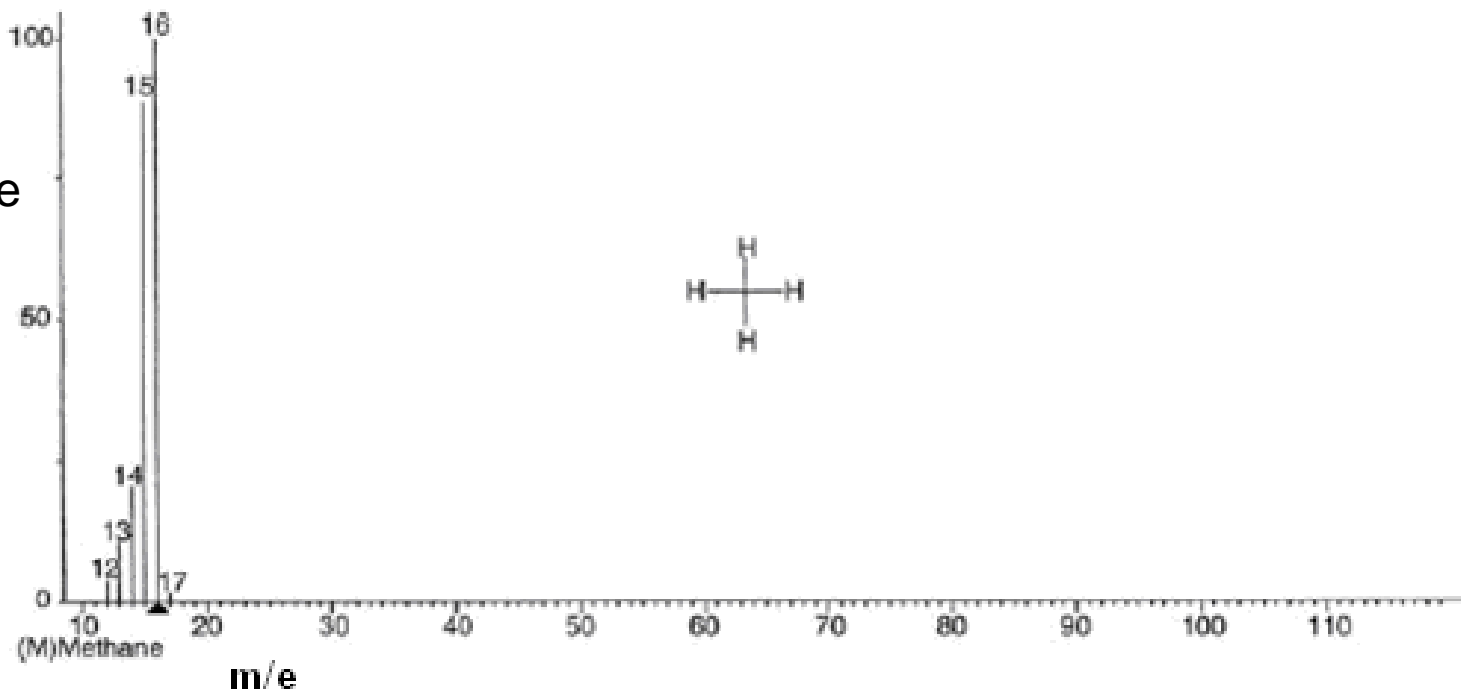
m/z	Relative abundance
1	3.1
2	0.17
12	1.0
13	3.9
14	9.2
15	85
16	100
17	1.11

$$H:\overset{\overset{H}{..}}{\underset{\overset{..}{H}}{C}}:H + e^- \longrightarrow H:\overset{\overset{H}{..}}{\underset{\overset{..}{H}}{C^+}}H + 2e^-$$

radical cation

methane
MW = 16

M$^+$
m/z = 16

$$CH_4^{+} \xrightarrow{-H\cdot} CH_3^{+} \xrightarrow{-H\cdot} CH_2^{+} \xrightarrow{-H\cdot} CH^{+} \longrightarrow \dots$$

m/z = 16   15   14   13

$-H_2$

Besides molecular masses for compounds containing C, H, N and O in molecular structure, percentages of M+1 and/or M+2 peaks originating from isotopes with low relative abundance in atoms are given in various sources as **Beynon Table**.

Relative abundances of molecular ions are not always very high. In this case, it is not possible to observe M+1 and/or M+2 peaks due to isotopes with very low abundance.

In molecules containing Cl and/or Br, the relative abundances of peaks resulting from different isotopes of atoms are comparable to the molecular ion peak and should be taken into account when evaluating the mass spectrum.
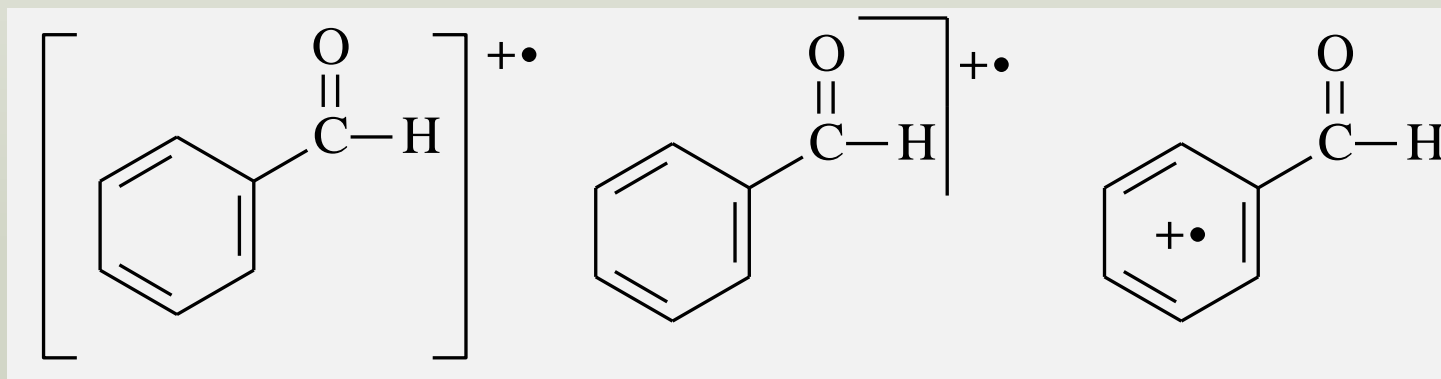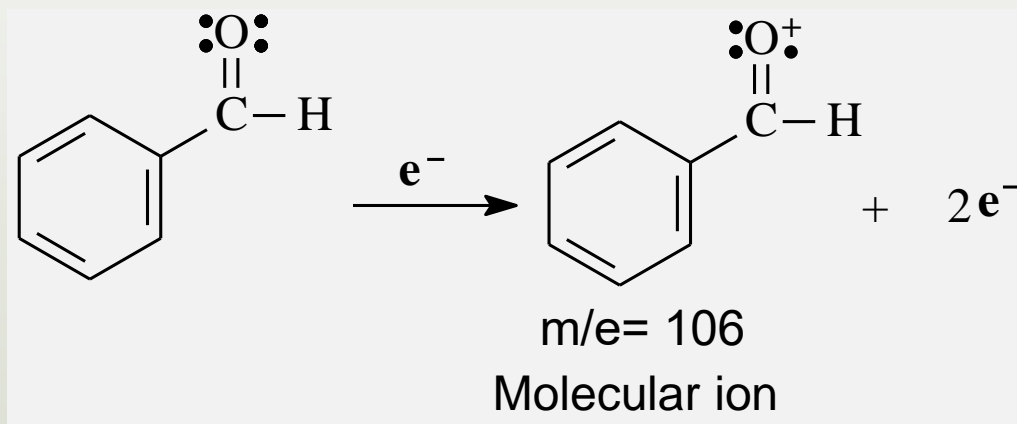
Question 2:

- Which m/e value is observed in the mass spectrum of benzaldehyde?

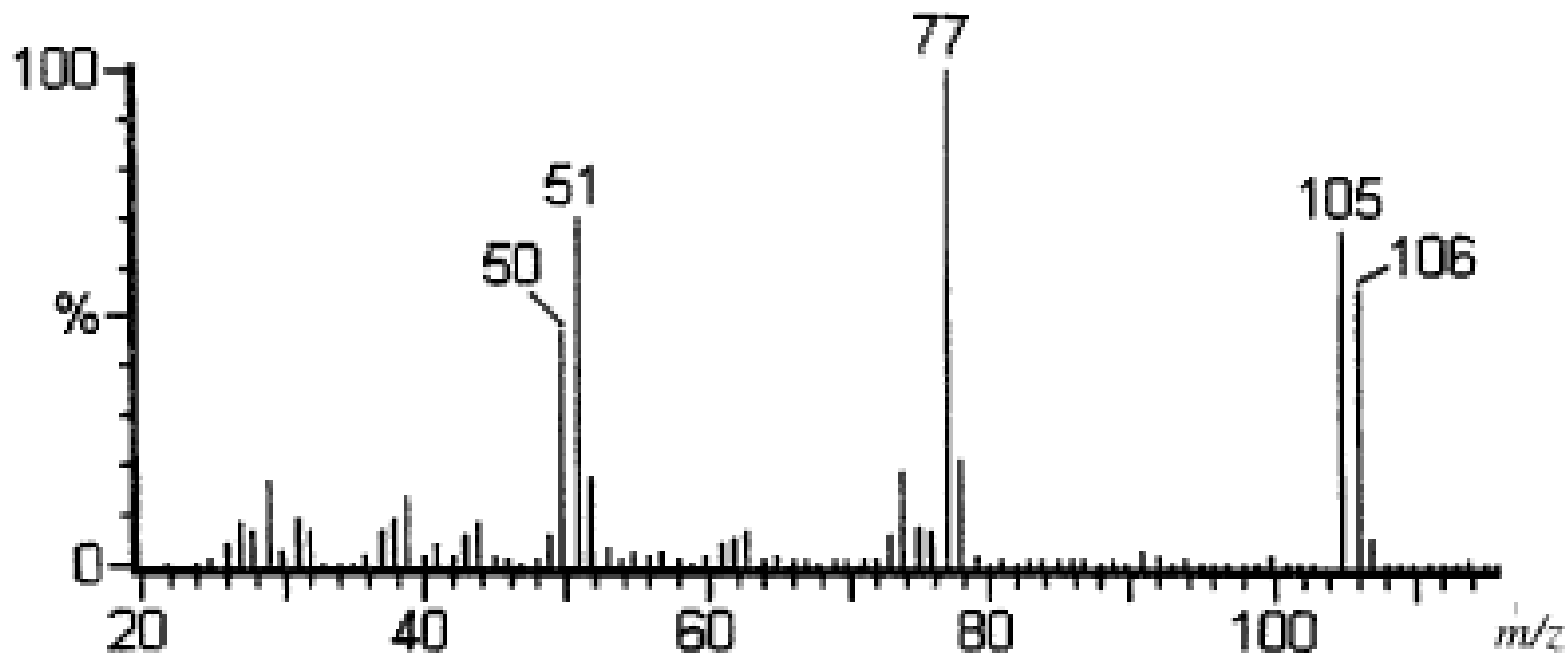- How can the formula of the molecular ion be shown?
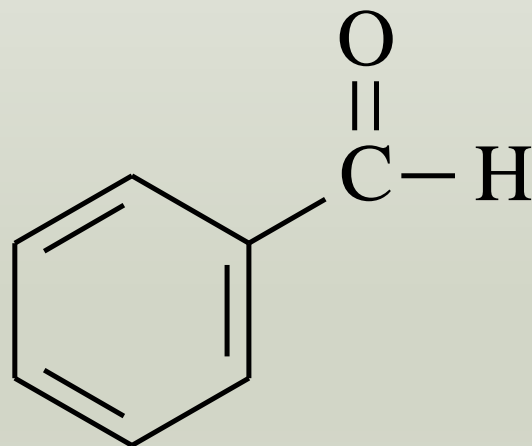
Molecular formula= $C_7H_6O_1$

Molecular weight= (7.12)+(6.1)+(1.16)=106

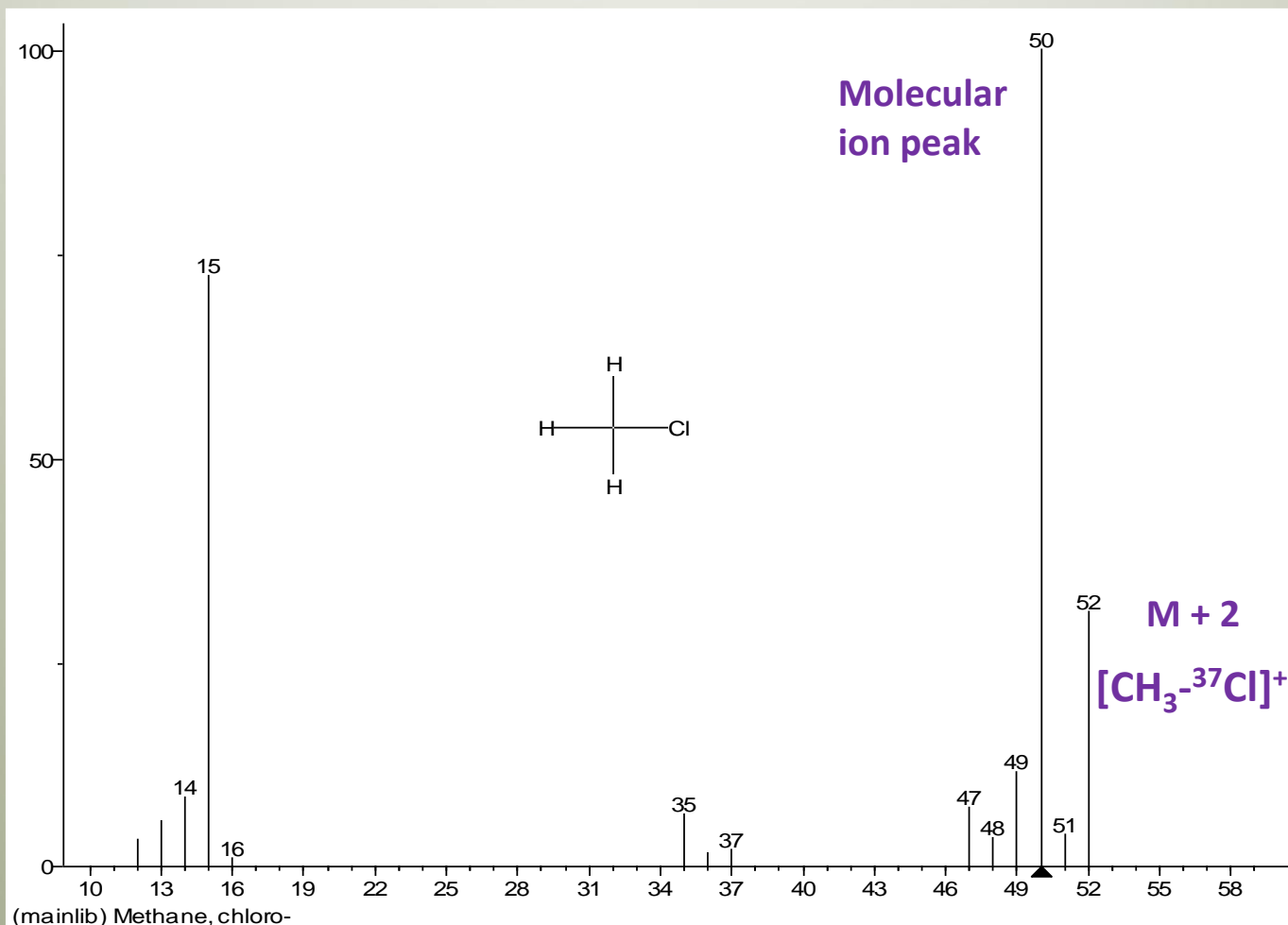*m/e* for molecular ion= 106

m/e= 106
Molecular ion

Different representations of molecular ion

Mass Spectrum of Benzaldehyde

Question 3: The mass spectrum of the Chloromethane compound is given below. Which is the molecular ion peak? Write the formula of ion with *m/z* value 52.

$$CH_3Cl = 12+(3 \times 1)+35 = 50$$

Molecular ion peak

M + 2

$$[CH_3-^{37}Cl]^{+\cdot}$$

(mainlib) Methane, chloro-

If a compound has more than one chlorine atom, the relative abundances of isotope peaks can be found using the formula $(a+b)^n$. a and b are the relative abundance of isotopes and n is the number of chlorine atoms in the iodine.

If there is one chlorine, a≈3, b≈1, n=1 and the relative abundance of peaks is 3: 1, so if M peak is three units and M+2 is one unit.

If there are two chlorides, $(a+b)^2=a^2+2ab+b^2$ equation is used, which is $(3+1)^2$. The relative abundance ratio of M, M + 2 and M + 4 peaks is 9:6:1, respectively.

If there is one bromine in a compound, a=1, b=1, n=1, the relative abundance ratios of M and M+2 peaks are 1:1, and if there are two bromine M, M+2 and M+4 peaks are 1:2:1, respectively.

Metastable peak (m*)

- If any (a) ion passes through the acceleration plates of the spectrometer without fragmentation, it is detected as (a) ion in the detector.

- In contrast, if (a) ion fragmentise to (b) ion before pass to acceleration field and pass through the acceleration field without fragmentation, it will be detected as (b).

- If (a) ion fragmentise into (b) ion within the acceleration field, then neither (a) nor (b) will be detected in the detector; instead, a very small and broad peak which called metastable peak will be observed.
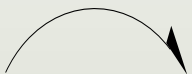
$$m^* = \frac{b^2}{a}$$

Although the presence of a metastable peak in the spectrum is evidence of a fragmentation of a → b, the absence of this peak doesn't mean that (a) definitely doesn't fragmentise to (b).

Fragmentation of the Molecule

o Fragmentation can be expressed as a bond-breaking, and the possibility of a bond break is related to the strength of that bond.

o When evaluating the mass spectra of organic compounds, it can be estimated how and where from the fragmentation is going to be in the molecule, considering the effects such as inductive effect, mesomeric effect, carbocation stability, conjugation.

o In the mass spectrum, the peaks of the (+) charged ion and/or radical cations formed as a result of the homolitic or heterolitic cleavage in the molecule can be observed as well as the peaks of cations formed by transposition reactions.
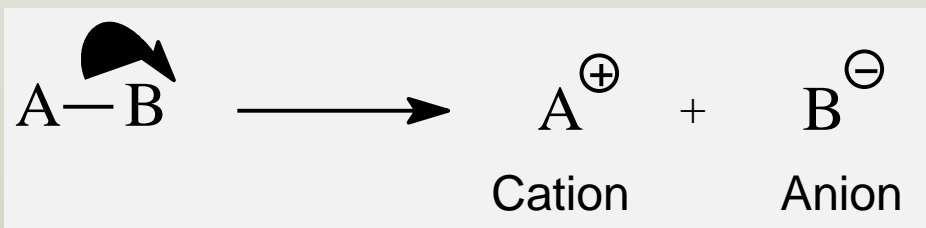
Homolytic Cleavage

In a neutral compound, two radicals are formed by homolytic cleavage of the bond electrons between the two atoms. A single-headed arrow (fishhook) ⌒➘ is used to show movement of a single electron.

$$A - B \longrightarrow A^{\bullet} + B^{\bullet}$$
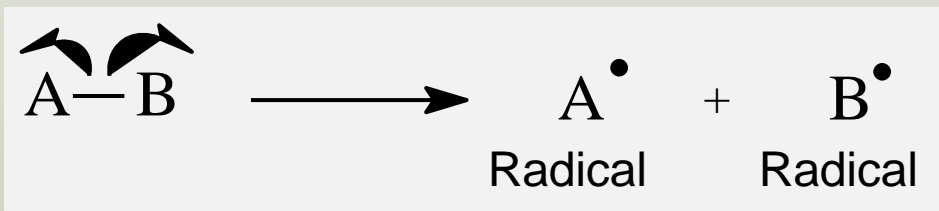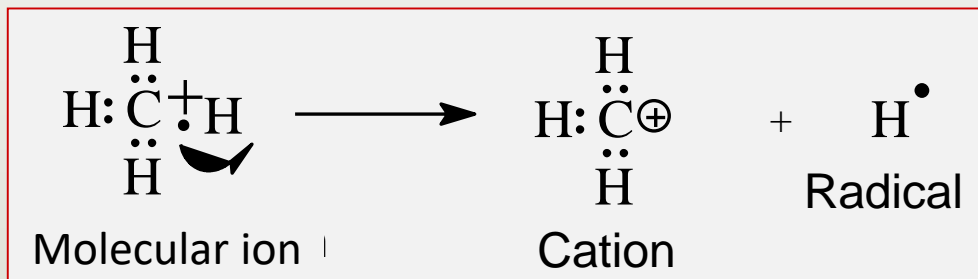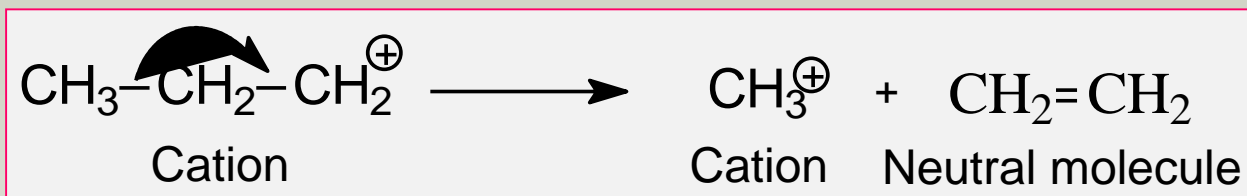
Radical   Radical

Heterolytic Cleavage

In a neutral compound, heterolytic cleavage results in a (+) charged ion (cation) and a (-) charged ion (anion). A double-headed arrow ⤻ is used to show movement of two-electron.

$$A{-}B \longrightarrow A^{\oplus} + B^{\ominus}$$

Cation   Anion

The molecular ion is a radical cation, which can be converted into a cation by cleavage of a radical group.

$$H:\overset{\overset{\displaystyle H}{}}{\underset{\overset{\displaystyle H}{}}{\ddot{C}}}\overset{+}{\cdot} H \longrightarrow H:\overset{\overset{\displaystyle H}{}}{\underset{\overset{\displaystyle H}{}}{\ddot{C}}}\oplus \quad + \quad H^{\bullet}$$

Molecular ion   Cation   Radical

A new cation may be formed from a cation by separating a neutral group with a heterolytic cleavage.

$$CH_3-CH_2-CH_2^{\oplus} \longrightarrow CH_3-CH_2^{\oplus} \quad + \quad :CH_2$$

Cation   Cation   Carbene

$$CH_3-CH_2-CH_2^{\oplus} \longrightarrow CH_3^{\oplus} \quad + \quad CH_2{=}CH_2$$
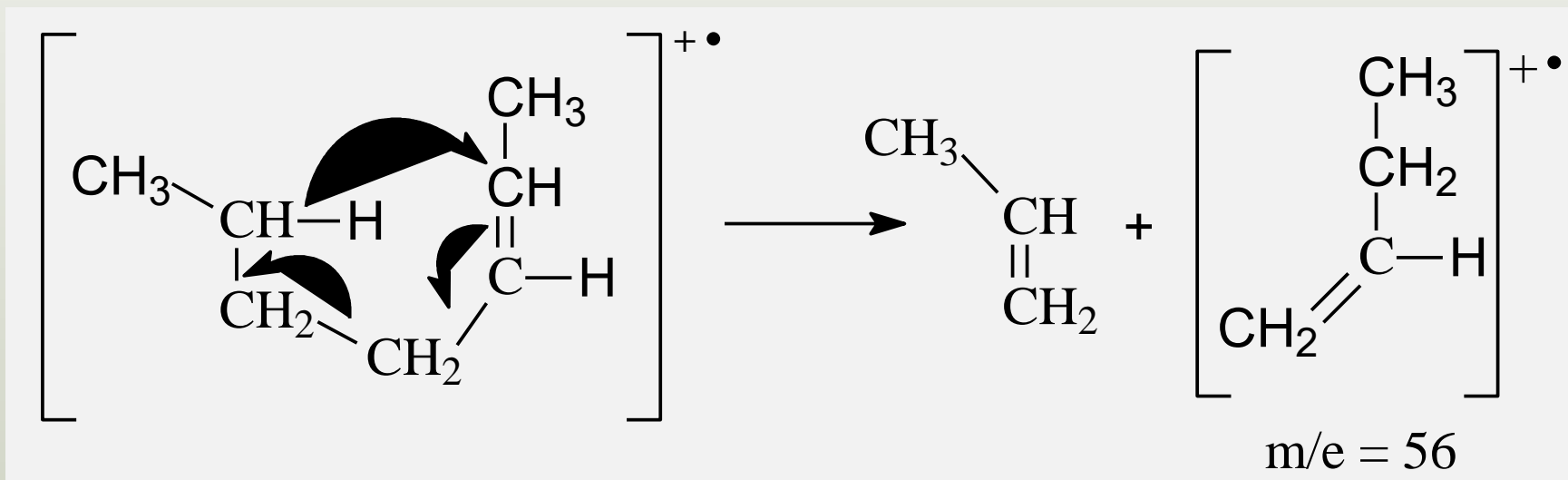
Cation   Cation   Neutral molecule

○When the mass fragmentation of the molecules is written, first, it is determined which type of cleavage.

○The movements of the electron or electrons are indicated using appropriate arrows.

○Charges and electrons on fragmentation products must be written correctly

Mc Lafferty Rearrangements

In order for this rearrangement to occur, there must be a double or triple bond and a substituent H attached to the carbon at the gamma (γ) position.

Ethylenic structure
Neutral molecule

Enolic structure
Radical cation

X = H, R, OH, OR, NH$_2$, Cl
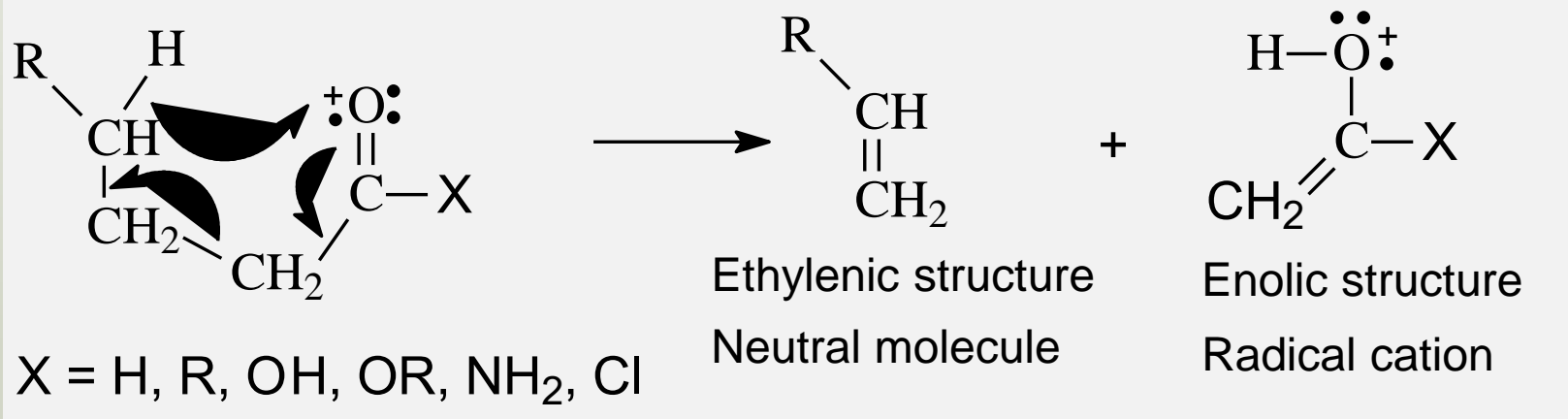
Mc Lafferty rearrangement can also be observed in alkynes and alkynes.


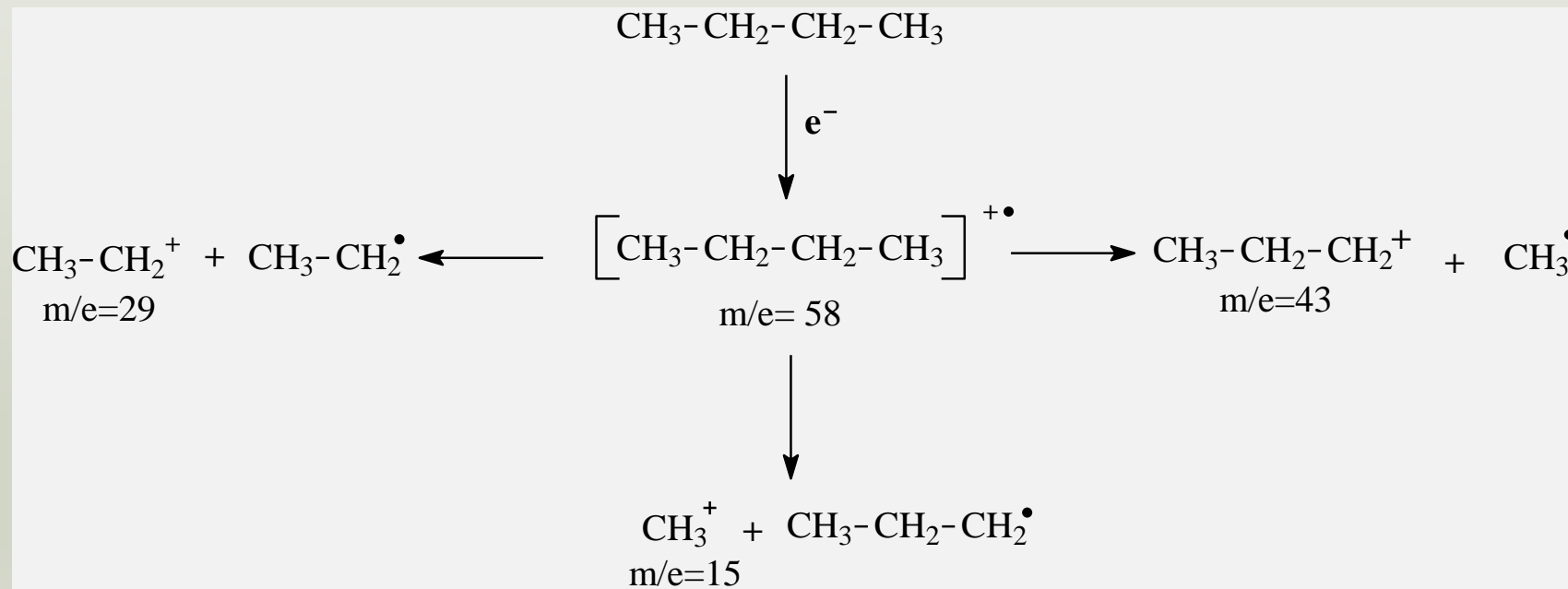$$m/e = 56$$

FRAGMENTATION OF VARIOUS FUNCTIONAL GROUPS

The fragmentation products and their relative abundance in the mass spectrometer are determined by considering the carbocation stability.

Hydrocarbons

- In a homologous series, the relative abundance of the molecular ion decreases as the molecular weight increases.

- Relative abundance of molecular ion in straight-chain hydrocarbons,

(resistance, stability) is larger than branched hydrocarbons.

- The relative abundance of the molecular ion decreases as the branching increases.

- The stability of cations formed as a result of fragmentation of hydrocarbons is as follows.

$$(CH_3)_3C^+ > \text{[phenyl-}CH_2^+\text{]} \approx CH_2=CH-CH_2^+ > R_2CH^+ > RCH_2^+$$
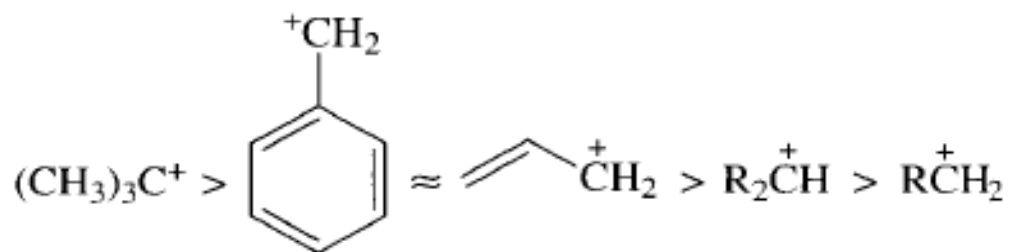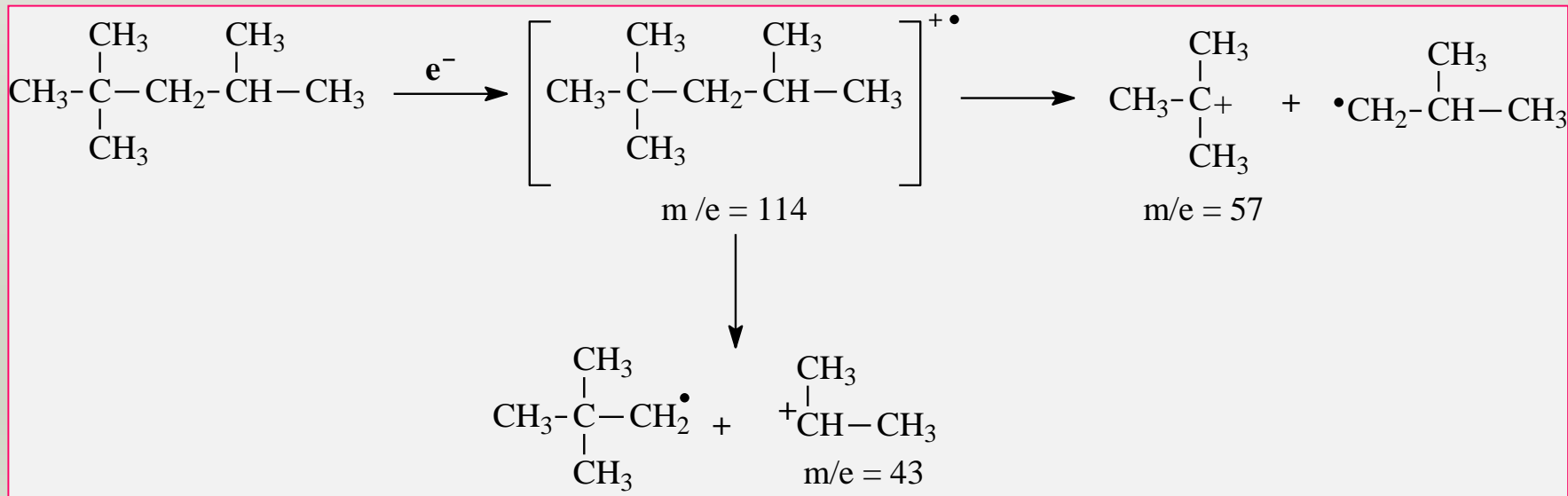
The major mass fragmentation of the butane compound is given below. The relative abundance of the molecular ion peak is observed as 10%. The m/e value of the base peak is 43.

$$CH_3-CH_2-CH_2-CH_3$$

$$\downarrow e^-$$

$$CH_3-CH_2^+ \;+\; CH_3-CH_2^{\bullet} \longleftarrow \left[CH_3-CH_2-CH_2-CH_3\right]^{+\bullet} \longrightarrow CH_3-CH_2-CH_2^+ \;+\; CH_3^{\bullet}$$

m/e=29   m/e= 58   m/e=43

$$\downarrow$$

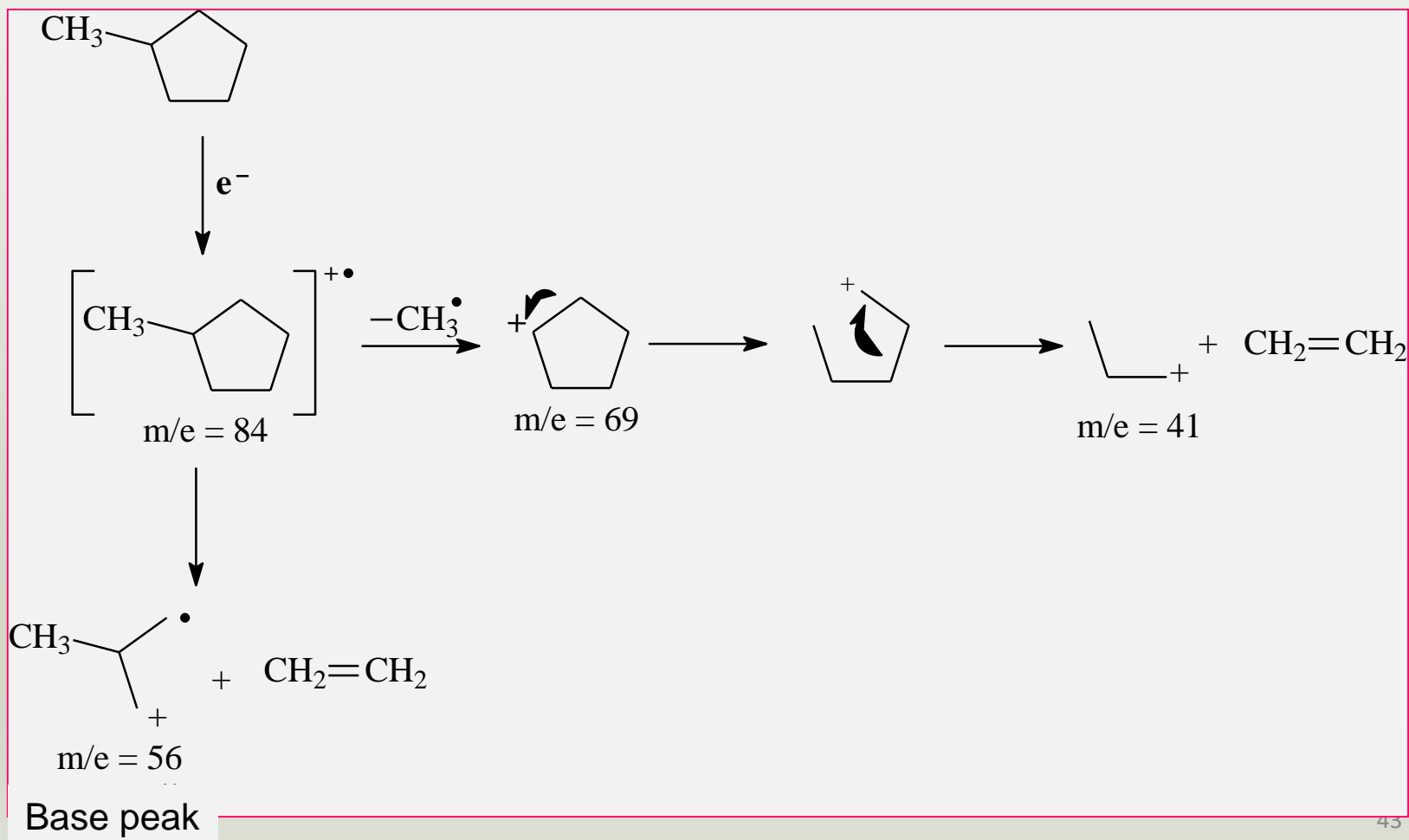$$CH_3^+ \;+\; CH_3-CH_2-CH_2^{\bullet}$$

m/e=15

In the 2,2,4-trimethylpentane compound, no molecular ion peak is observed in the spectrum, the tertiary carbocation peak with the value m/e=57 is the base peak. The relative abundance of secondary carbocation with m/e=43 is about 20%.

$$CH_3-\underset{\underset{CH_3}{|}}{\overset{\overset{CH_3}{|}}{C}}-CH_2-\underset{}{\overset{\overset{CH_3}{|}}{CH}}-CH_3 \xrightarrow{e^-} \left[CH_3-\underset{\underset{CH_3}{|}}{\overset{\overset{CH_3}{|}}{C}}-CH_2-\overset{\overset{CH_3}{|}}{CH}-CH_3 \right]^{+\bullet}$$

m /e = 114

$$\longrightarrow CH_3-\underset{\underset{CH_3}{|}}{\overset{\overset{CH_3}{|}}{C}}+ \quad + \quad {}^\bullet CH_2-\overset{\overset{CH_3}{|}}{CH}-CH_3$$

m/e = 57

$$CH_3-\underset{\underset{CH_3}{|}}{\overset{\overset{CH_3}{|}}{C}}-CH_2^\bullet \quad + \quad {}^+CH-CH_3 \text{ (with } CH_3 \text{)}$$

m/e = 43

The most important fragmentation product in cycloalkanes is the radical cation remaining by the cleavage of an ethene group from the molecule and the base peak belongs to this ion.
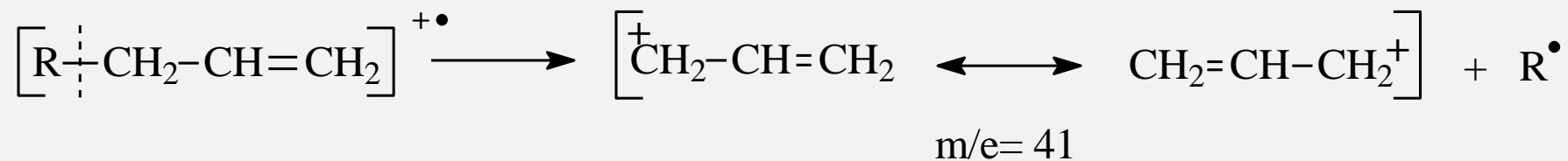

m/e=70

m/e=42
Temel pik
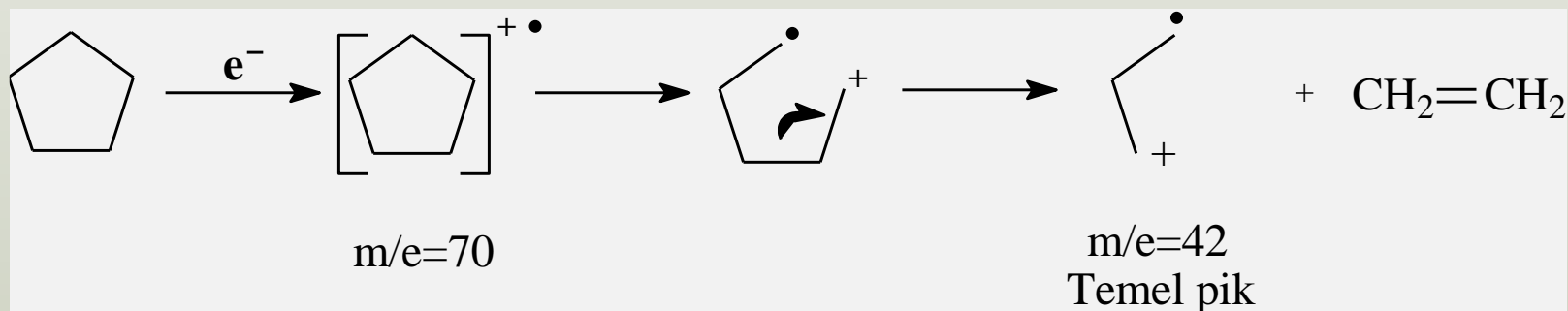
Mass Fragmentations of Methyl Cyclopentane

CH_3—⬠

$\downarrow e^-$

$\left[CH_3-\text{⬠} \right]^{+\bullet}$

m/e = 84

$\xrightarrow{-CH_3^\bullet}$ $+\text{⬠}$

m/e = 69

$\longrightarrow$ ⬠ $\longrightarrow$ $+$ $CH_2=CH_2$

m/e = 41

$CH_3-\overset{\bullet}{\underset{+}{\diagdown}}$ $+$ $CH_2=CH_2$

m/e = 56

Base peak

43

Alkenes

In the terminal alkenes, the allyl cation *m/e* =41 is formed. This cation is extremely stable.

$$\left[R \vdots CH_2-CH=CH_2 \right]^{+\bullet} \longrightarrow \left[\overset{+}{C}H_2-CH=CH_2 \longleftrightarrow CH_2=CH-CH_2^{+} \right] + R^{\bullet}$$
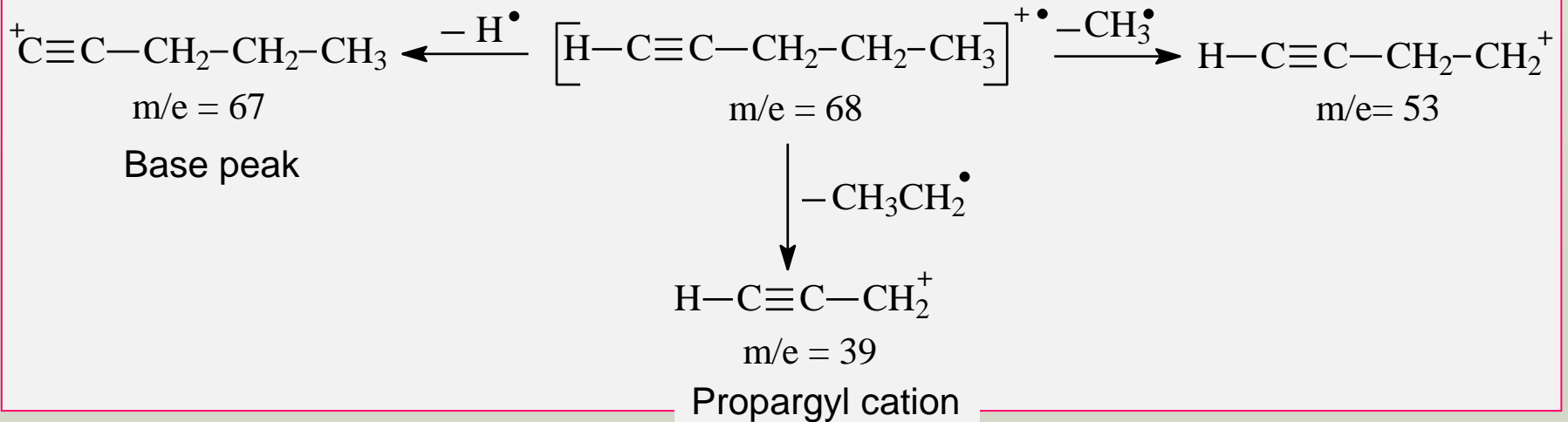
m/e= 41

Characteristic fragmentation of cyclo alkenes is compatible with **Retro-Diels Alder** reaction.
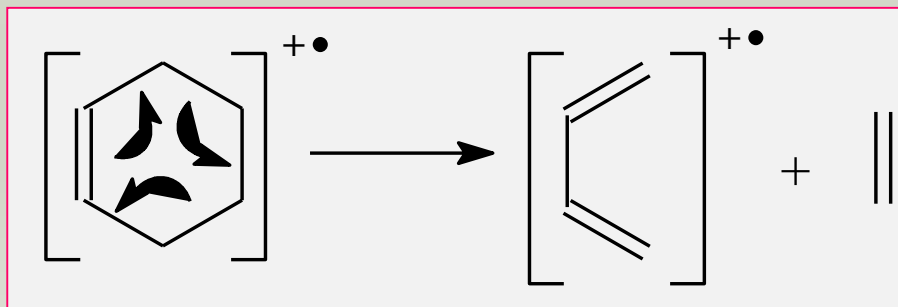
Alkynes

M-1 peak is observed by radical cleavage of terminal hydrogen in the terminal alkynes. Propargyl cation may occur depending on the structure of the alkyne. This cation is not as stable as the allyl cation.

$$\overset{+}{C} \equiv C - CH_2 - CH_2 - CH_3 \xleftarrow{-H^\bullet} \left[H - C \equiv C - CH_2 - CH_2 - CH_3 \right]^{+\bullet} \xrightarrow{-CH_3^\bullet} H - C \equiv C - CH_2 - CH_2^+$$

m/e = 67   m/e = 68   m/e= 53

Base peak

$$\Big\downarrow -CH_3CH_2^\bullet$$

$$H - C \equiv C - CH_2^+$$

m/e = 39

Propargyl cation

Aromatic Hydrocarbons

In alkyl benzenes, benzyl carbocation is formed by benzylic cleavage.

Then this carbocation rearranges to form aromatic **Tropylium ion**. The peak observed in the mass spectrum at m/e=91 belongs to the Tropylium cation ($C_7H_7^+$), not to the benzyl cation.

$m/e = 91$
Benzyl cation

$m/e = 91$
Tropylium cation
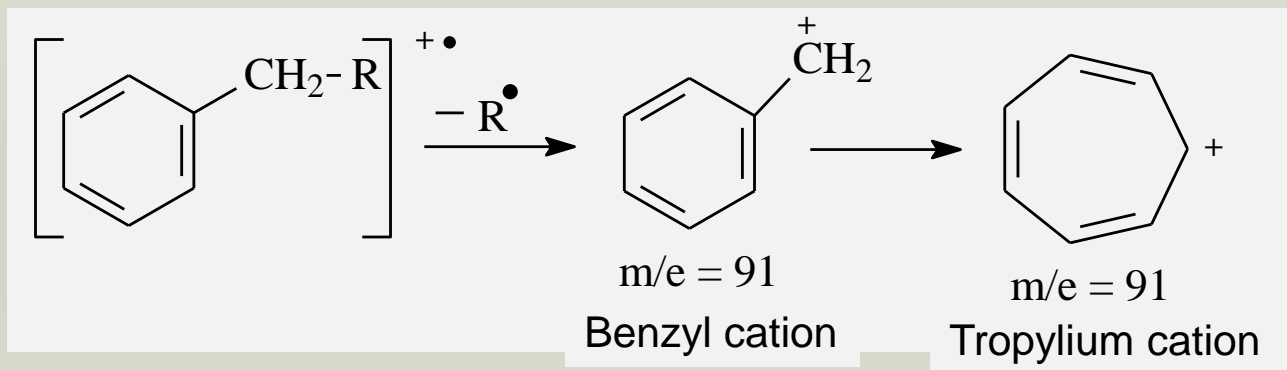
The peak of the cation formed as a result of the McLafferty Rearrangement can also be observed in the mass spectrum of benzene derivatives with propyl and a larger alkyl chain (having hydrogen in the γ position).

$$m/e = 92$$
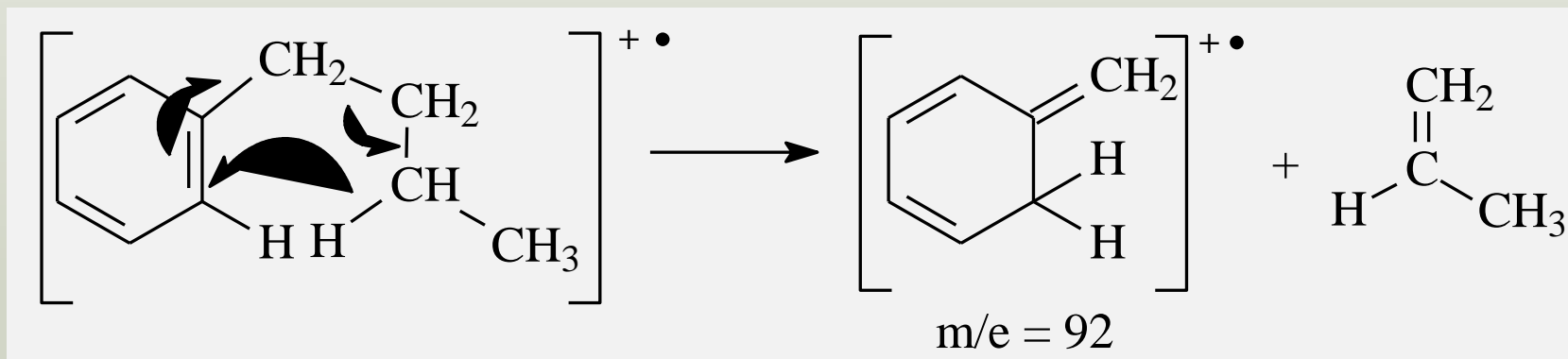
In the isopropyl benzene compound, the cation formed by the cleavage of a methyl radical from the molecular ion first converts into a methyl-substituted Tropylium ion by the rearrangement reaction. This ion was observed as the base peak because its stability was higher than the Tropylium cation with hydrogen instead of methyl.


m/e = 105
Methyl-substituted Tropylium ion
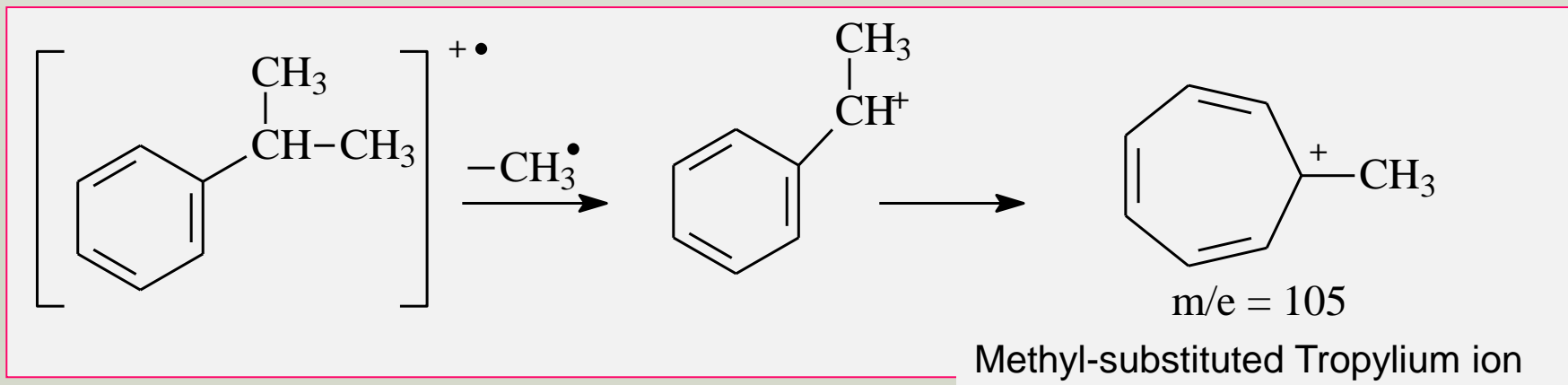
Alcohols

o The intensity of the molecular ion peak in the mass spectrum of a primary or secondary alcohol is usually rather low, and the molecular ion peak is often entirely absent in the mass spectrum of a tertiary alcohol.

o Common fragmentations of alcohols are α-cleavage adjacent to the hydroxyl group and dehydration.

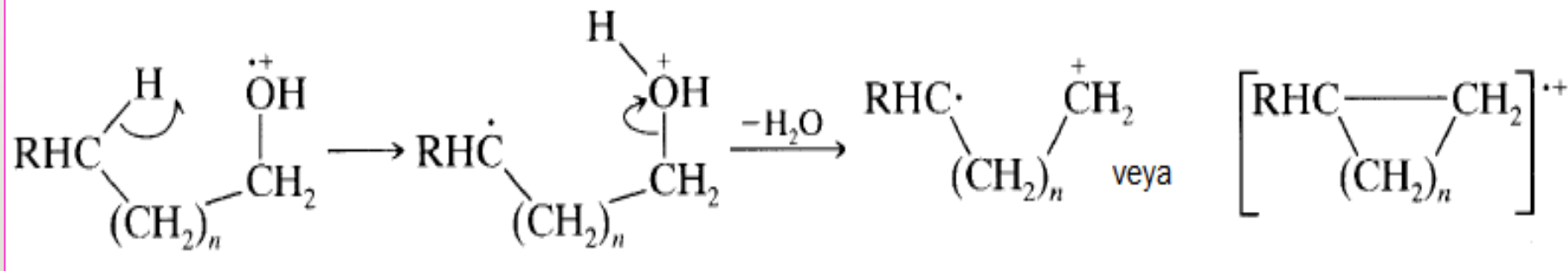o Peaks formed by cleavage of other alkyl groups can also be observed in the spectrum.

In primary alcohols, the oxonium cation with the value of m/e=31 is characteristic.
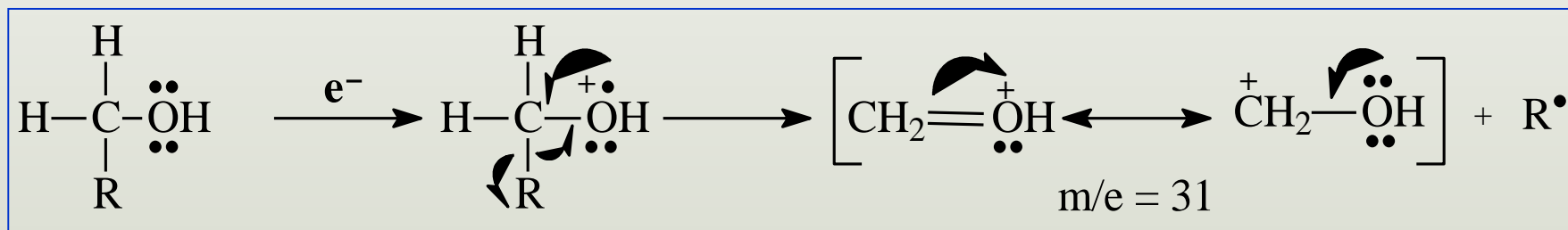
M-1, M-2 and rarely M-3 peaks can be observed.
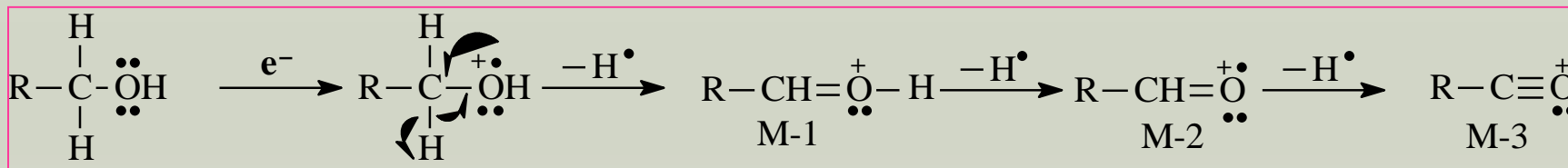
The M-18 peak formed by the cleavage of H_2O from the molecular ion should also be considered when evaluating the spectrum.
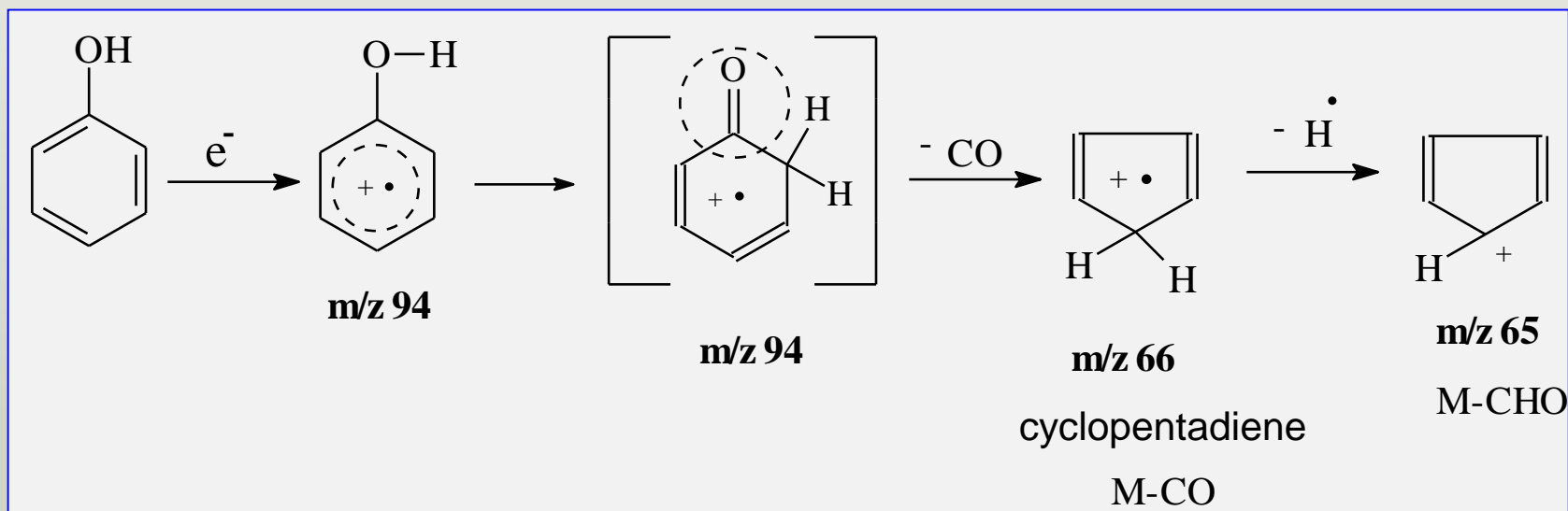

In cyclic structures, the M-1 ion can be observed by cleavage of the hydrogen in the carbon to which the hydroxyl group in the molecular ion is bound. The M-18 peak can also be formed by the separation of water.
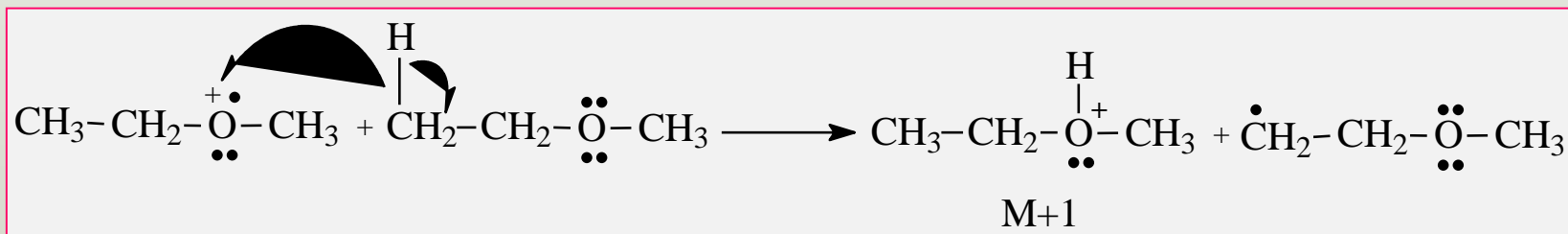

m/e = 100   m/e = 99
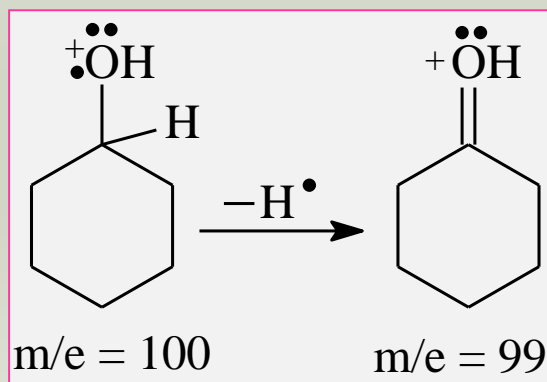
Phenols

The molecular ion peak is observed as a significant peak in the spectrum due to its stability. In addition, M-CO and M-CHO peaks are also seen.
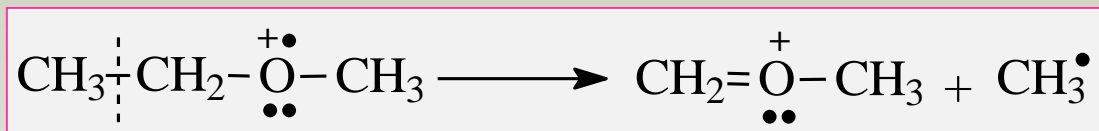
Ethers

The molecular ion in the dialkylether is formed by the loss of one of the unpaired electrons in oxygen. M+1 peak can be observed by transferring 1 H$^{\bullet}$ from neutral molecule to molecular ion.

$$CH_3-CH_2-\overset{+}{\overset{\bullet}{O}}-CH_3 \; + \; \overset{}{CH_2}-CH_2-\ddot{\ddot{O}}-CH_3 \longrightarrow CH_3-CH_2-\overset{H}{\overset{|}{\underset{\bullet\bullet}{O}}}\!\!\!{}^{+}-CH_3 \; + \; \overset{\bullet}{CH_2}-CH_2-\ddot{\ddot{O}}-CH_3$$

M+1

The C-C bond adjacent to the oxygen atom may separate.

$$CH_3\!\!\mid\!\!CH_2-\overset{+}{\overset{\bullet}{O}}-CH_3 \longrightarrow CH_2=\overset{+}{O}-CH_3 \; + \; \overset{\bullet}{CH_3}$$

If the alkyl chain contains two or more carbons, the oxonium ion may form with the cleavage of the C-O bond and the H shift.

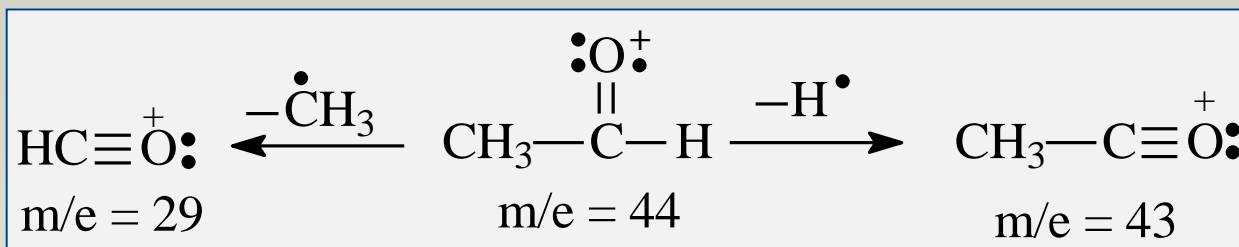$$CH_2-CH_2-\overset{+\bullet}{\underset{\bullet\bullet}{O}}-CH_3 \longrightarrow H-\overset{+\bullet}{\underset{\bullet\bullet}{O}}-CH_3 + CH_2=CH_2$$
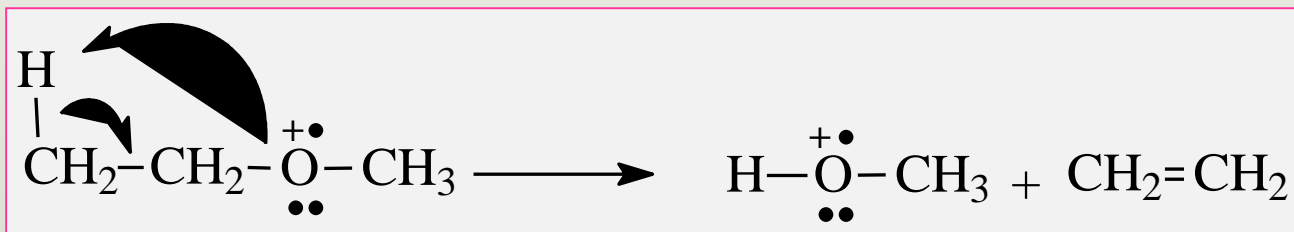
It can be heterolytic (inductive cleavage) with the cleavage of C-O bond.

$$CH_3-CH_2-\overset{+\bullet}{\underset{\bullet\bullet}{O}}-CH_3 \longrightarrow CH_3-CH_2-\overset{\bullet\bullet}{\underset{\bullet\bullet}{O}}\bullet + \overset{+}{C}H_3$$

$$CH_3-CH_2-\overset{+\bullet}{\underset{\bullet\bullet}{O}}-CH_3 \longrightarrow \bullet\overset{\bullet\bullet}{\underset{\bullet\bullet}{O}}-CH_3 + CH_3-\overset{+}{C}H_2$$
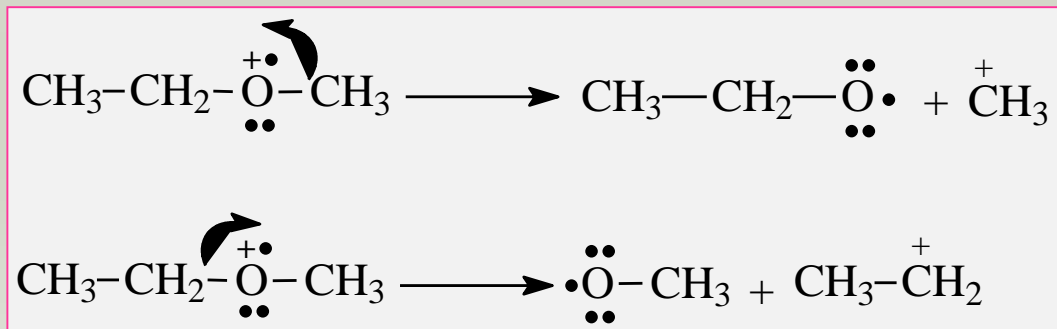
Aldehyde, Ketone and Esters

α- cleavage

$HC\equiv\overset{+}{O}:$ $\xleftarrow{\;-R^{\bullet}\;}$ $R-\overset{\overset{\displaystyle :\overset{+}{O}:}{\|}}{C}-H$ $\xrightarrow{\;-H^{\bullet}\;}$

$R_1-C\equiv\overset{+}{O}:$ $\xleftarrow{\;-R^{\bullet}\;}$ $R-\overset{\overset{\displaystyle :\overset{+}{O}:}{\|}}{C}-R_1$ $\xrightarrow{\;-R_1^{\bullet}\;}$ $R-C\equiv\overset{+}{O}:$

$R_1O-C\equiv\overset{+}{O}:$ $\xleftarrow{\;-R^{\bullet}\;}$ $R-\overset{\overset{\displaystyle :\overset{+}{O}:}{\|}}{C}-OR_1$ $\xrightarrow{\;-R_1\overset{\bullet\bullet}{\underset{\bullet\bullet}{O}}^{\bullet}\;}$

$HC\equiv\overset{+}{O}:$ $\xleftarrow{\;-\overset{\bullet}{C}H_3\;}$ $CH_3-\overset{\overset{\displaystyle :\overset{+}{O}:}{\|}}{C}-H$ $\xrightarrow{\;-H^{\bullet}\;}$ $CH_3-C\equiv\overset{+}{O}:$

m/e = 29   m/e = 44   m/e = 43
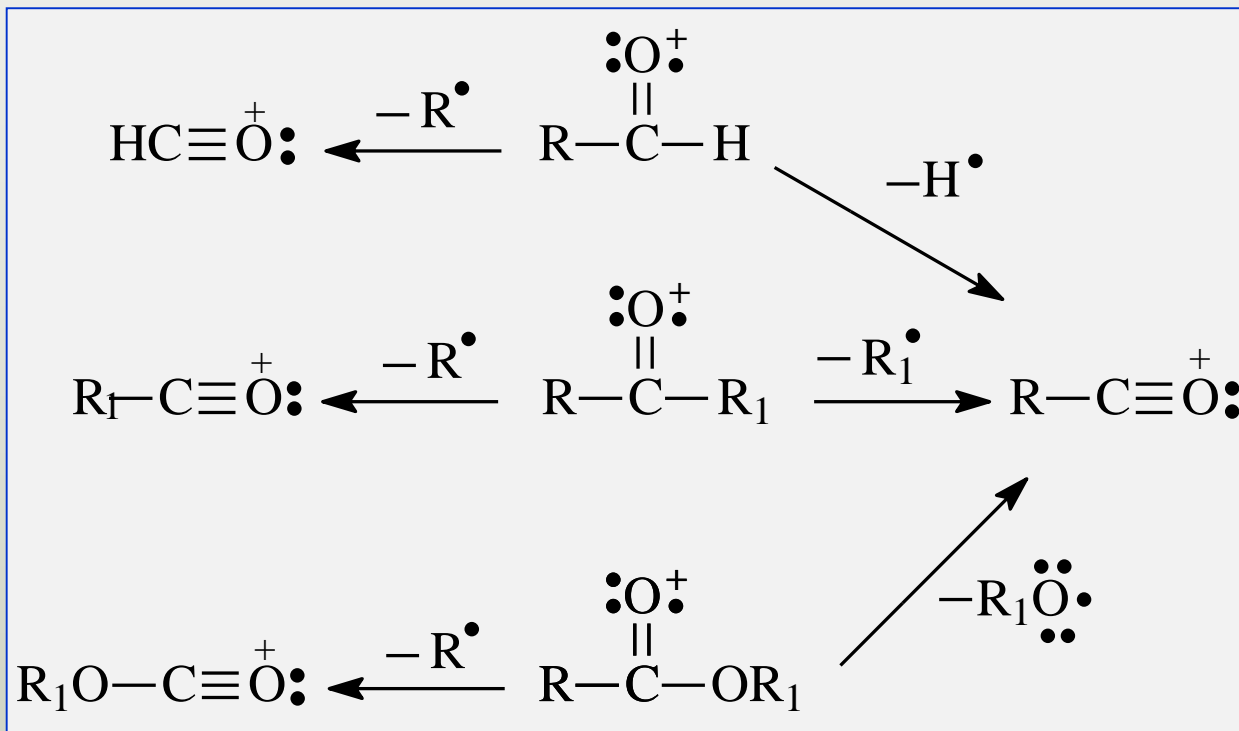
β- cleavage

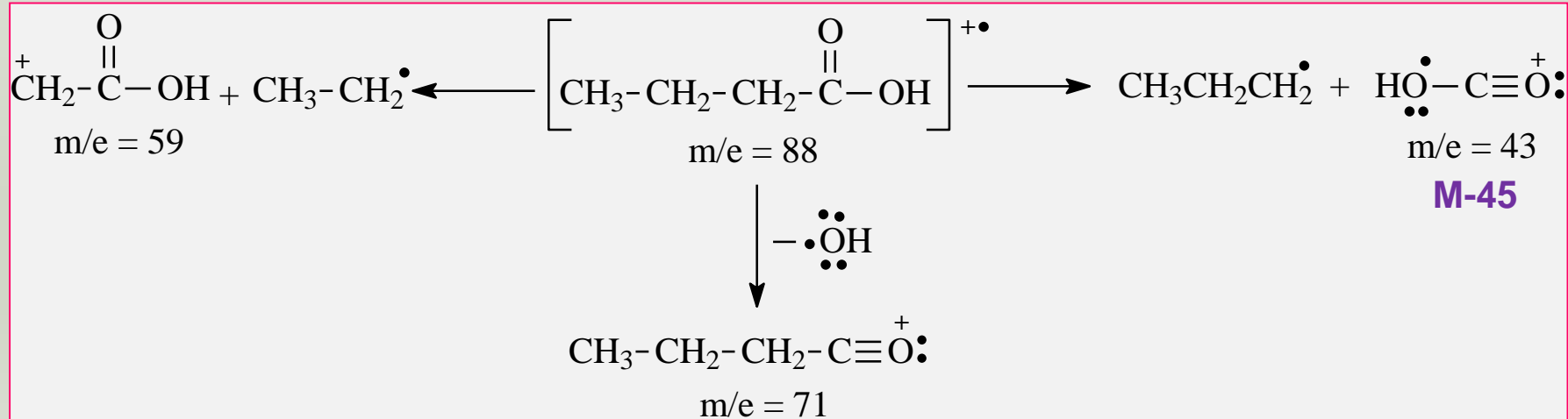# Mc Lafferty Rearrangment

$$m/e = 44$$
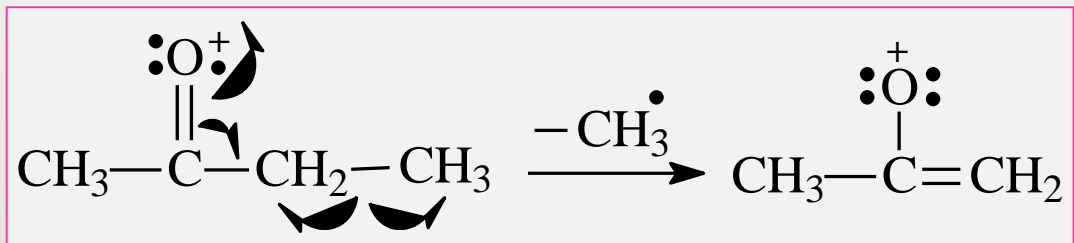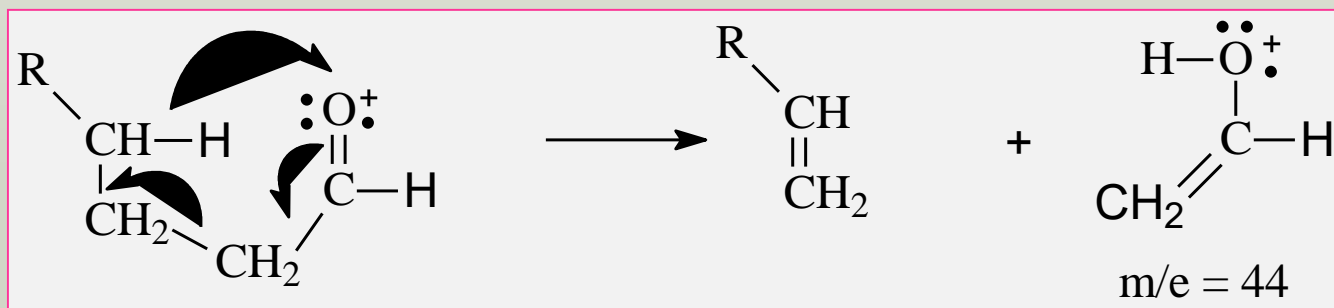
Carboxylic Acids

The *m/e*=60 peak formed by Mc Lafferty Rearrangement in the spectrum of straight-chain aliphatic carboxylic acids is highly characteristic.
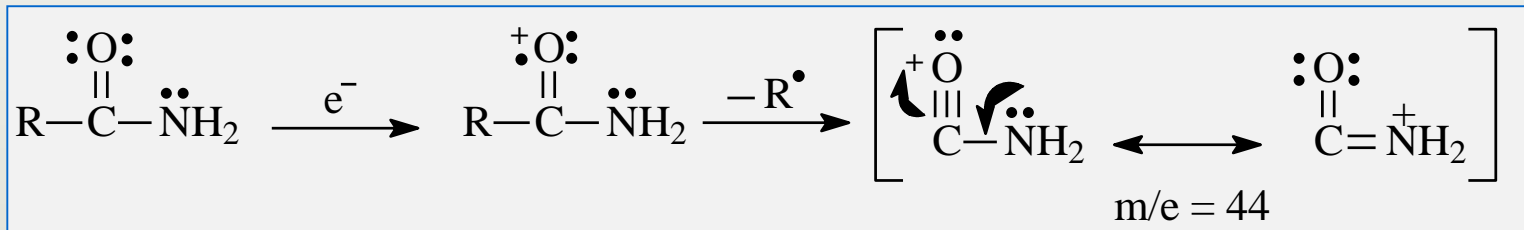
The M-17 peak formed by separation of the hydroxyl group from the molecule and the M-45 peak formed by separation of the carboxyl group are characteristic.
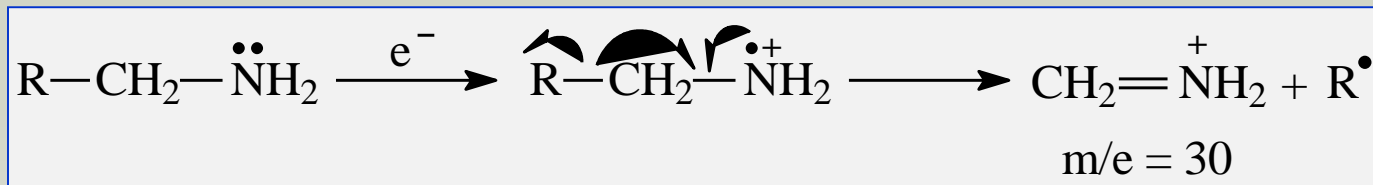
Amides

*m/e*= 44 peak is characteristic and has resonance stability.

Amines

M-1 peak can be observed. *m/e*=30 peak is characteristic in primary amines.
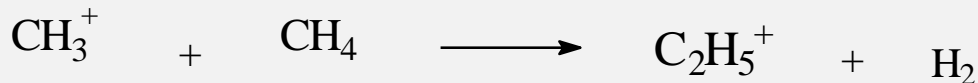
Nitro Compounds and Phenyl Ring

o In aliphatic nitro compounds, the molecular ion peak is usually not observed.

o In addition to the peaks given by the alkyl cations, the $^+NO_2$ peak with m/e=46 and ^+NO peak with m/e=30 are characteristic for the nitro derivative compounds.

o The molecular ion peaks in aromatic nitro compounds are large. In nitrobenzene, M-46 ion formed by the cleavage of the nitro radical from the molecule constitutes the base peak. In addition, the phenoxy cation (M-30) is obtained by cleavage of the neutral NO from the molecule.
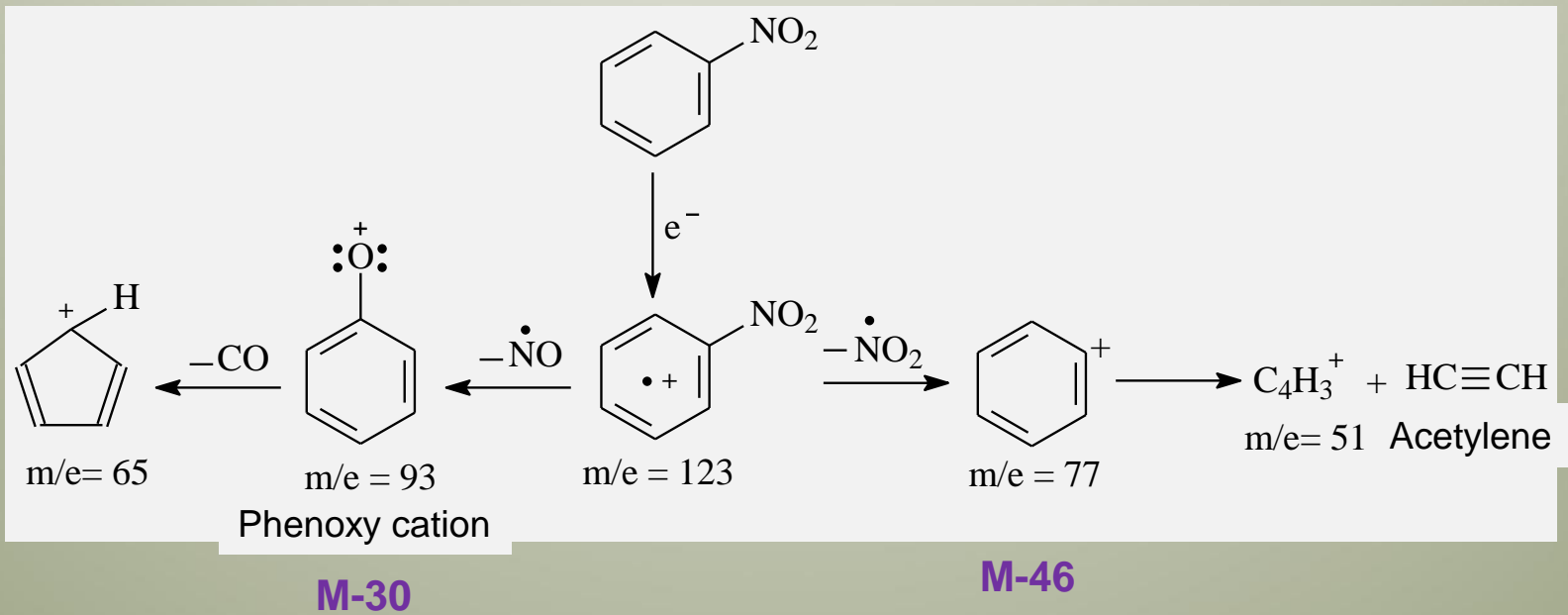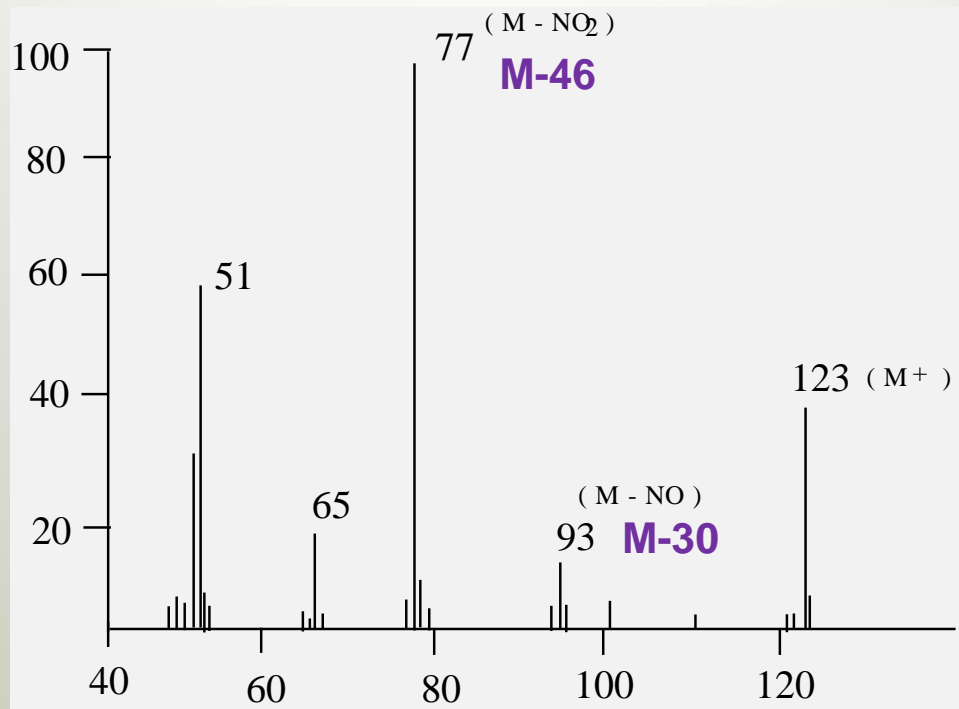
IONIZATION METHODS

○ **Electron Impact Ionization – EI**

○ **Chemical Ionization – CI** : The subtance is bombarded with a gas such as methane, ethane, propane and ammonia under pressure of 10^{-4} mmHg. For example, prior to substance, methane, a small molecule gas, is ionized to form charged particles such as CH_4^+, CH_3^+.

$$CH_4 \quad + \quad e^- \quad \longrightarrow \quad CH_4^{+\bullet} \quad + \quad CH_3^{+\bullet} \quad vb.$$

$$CH_4^{+\bullet} \quad + \quad CH_4 \quad \longrightarrow \quad CH_5^+ \quad + \quad CH_3^{\bullet}$$

$$CH_3^+ \quad + \quad CH_4 \quad \longrightarrow \quad C_2H_5^+ \quad + \quad H_2$$
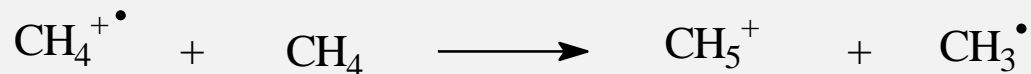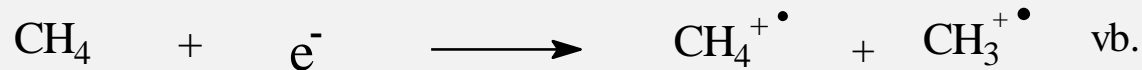
○ Here, CH_5^+ acts as the **Bronsted acid** and $C_2H_5^+$ acts as the **Lewis acid** against the sample.

○ When the sample molecules collide with ions, ionization occurs via charge transfer. Since the charge transfer energy is low, the substance molecules do not fragmentise and the molecular peak are easily observed in spectrum.

○ Compounds, which are basic character, nitrogen-bearing and not given molecular peak via other techniques, protonize with **CI** easily and give M+1 ions.

$$\text{Pyrrolidine-2-carboxylic acid} \quad + \quad CH_5^+ \quad \longrightarrow \quad \text{M+1 molecular ion} \quad + \quad CH_4$$

Pyrrolidine-2-carboxylic acid
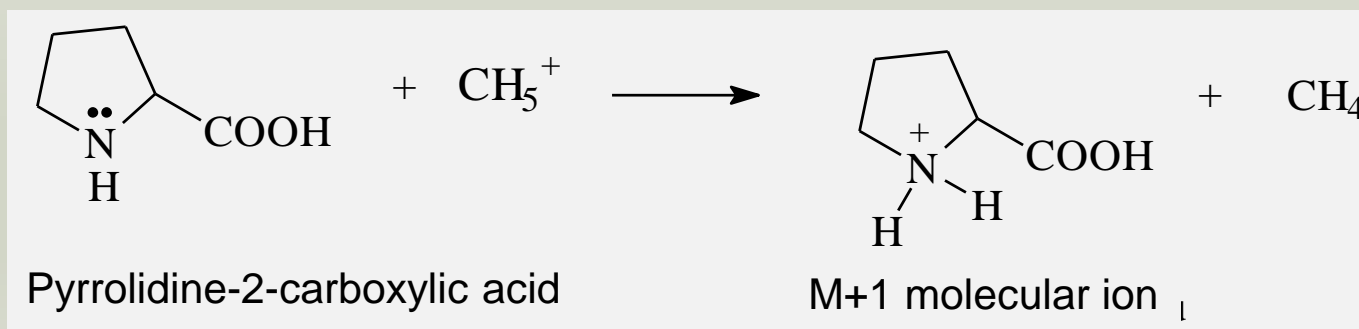
M+1 molecular ion

Field Ionization – FI

- An analysis sample on gaseous state is ionized by a strong electrical field of 10^8 Volt/cm. The duration of the substance in the ion source is 10^{-12} sec.

- Because the internal energies of the molecular ions formed at this source are low, the relative abundance of the resulting ions is low and the intensity of the molecular ion peak is high.

- These three methods are intended to ionize substances in gaseous phase or turning into gas phase. <u>For thermal stable and non-volatile substances, some desorption methods have been developed.</u>

○**Field Desorption** - **FD**

If the sample is less volatile or heat-labile, the substance molecules are converted into (+) ions by the anode and removed. M^+ and more M+1 ions are obtained with this method.

○**Laser Desorption (LD)**

○**Fast Atom Bombardment (FAB)**

○**Secondary Ion Mass Spectrometry (SIMS)**

○**Californium (^{252}Cf) Plasma Desorption (PD)**

The last four techniques have been developed from the late 1970s to the present in order to find the molecular mass of polar compounds with molecular mass 300-25000.

Electrospray Ionization (ESI)

- This method was first used in 1984 for the analysis of biomolecules such as proteins, polypeptides and oligonucleotides. For determining the molecular ion peak of many organic compounds and drug molecules is also quite convenient method.

- ESI takes place at atmospheric pressure and at room temperature. The advantage of this method is the determination of the molar masses of large and easily fragmentised substances.

- ESI method is especially used with liquid chromatography/mass spectrometry (LC/MS).

Gas Chromatography - Mass Spectrometry (GC/MS)

- After the organic compounds in a mixture separate readily by gas chromatography, it is possible to identify these compounds.

- Mass spectra can be obtained even with nanogram amounts of compounds separated by gas chromatography.

Liquid Chromatography-Mass Spectrometry (LC/MS = HPLC/MS)

- Mass spectrometry is also combined with liquid chromatography for **the analysis of samples containing non-volatile components**.

- After substance or substance mixture is dissolved in suitable solvents, it is placed in the device. Each substance in the sample is separated in the high-pressure liquid chromatography section and comes to the mass spectrometer section, the mass spectrum of each substance is obtained separately.

- Many modern mass spectrometers have computer-aided scanning libraries. The spectra loaded on the computer can be used for diagnostic purposes by comparing with the mass spectrum of the sample.
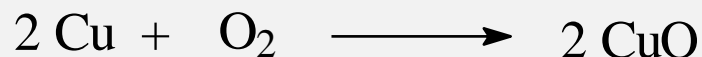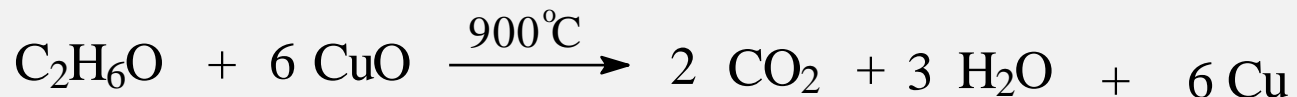
ELEMENTAL ANALYSIS

1772-1777 Lavoisier

Lavoisier studied the combustion products of various compounds and was able to reveal which elements were present in a burned substance. <u>For example</u>, carbon dioxide and water are formed by the combustion of methane, and thus methane is composed of carbon and hydrogen.

$$Methane + O_2 \longrightarrow CO_2 + H_2O$$
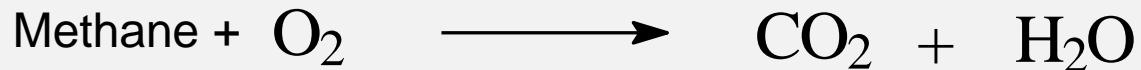
In 1831, Justus von Liebig developed Lavoisier's method thus it was possible to identify the exact empirical formulas of organic compounds for the first time. An amount of sample is weighed and burned in the presence of red-hot copper oxide.

$$C_{10}H_{14} \;+\; 27\;CuO \xrightarrow{900^{o}C} 10\;\;CO_2 \;+\; 7\;H_2O \;+\; 27\;Cu$$

$$C_2H_6O \;+\; 6\;CuO \xrightarrow{900^{o}C} 2\;\;CO_2 \;+\; 3\;H_2O \;+\; 6\;Cu$$

$$2\;Cu \;+\; O_2 \longrightarrow 2\;CuO$$

The amount of H_2O and CO_2 formed as a result of combustion is determined and the percentages of hydrogen and carbon in the sample are calculated.

$$\text{Amount of H in the sample} = \text{Mass of } H_2O \times \frac{2.016}{18.016}$$

$$\text{Amount of C in the sample} = \text{Mass of } CO_2 \times \frac{12.01}{44.01}$$

$$\%H \text{ in the sample} = \frac{\text{Amount of H in the sample}}{\text{Mass of the sample}} \times 100$$

$$\%C \text{ in the sample} = \frac{\text{Amount of C in the sample}}{\text{Mass of the sample}} \times 100$$

Example: 0.550g sample consisting of C,H and O is burned with oxygen in the presence of CuO by elemental analysis method. As a result, 0.660 g H_2O and 1.037 g CO_2 is obtained.

The amount of hydrogen and carbon in the sample is calculated.

18,016 g H_2O 2,016 g H

0,660 g H_2O x g H

X= 0,074 g H

44,01 g CO_2 12,01 g C

1,037 g CO_2 x g C

X= 0,283 g C

The percentages of hydrogen and carbon in the sample are calculated.

0,55 g sample   0,074 g H

100   x

X= 13,44 = % H

0,55 g sample   0,283 g C

100   x C

X= 51,47 = % C

Since the total percentage of carbon and hydrogen is 64.91%, it is assumed that the sample contains 35.09% oxygen. Then, in order to find the proportion of atoms, the percentage of each element is divided by the atomic weight.

$$C = \frac{\% \ 51.47}{12.01} = 4.29$$

$$H = \frac{\% \ 13.44}{1.008} = 13.33$$

$$O = \frac{\% \ 35.09}{16.00} = 2.19$$

The atomic ratios of the elements in the sample are C=4.29, H=13.33 and O=2.19. If all values are divided into the smallest (2.19) of these values, then the results will be C=1.95, H=6.1 and O=1. When these ratios are rounded up, the empirical formula is obtained as C_2H_6O.

It should not be forgotten that this analysis gives only atomic ratios. It is also necessary to know the molecular weight in order to determine the molecular formula. (The molecular weight of the sample, which we do not know its structure but we are sure of its purity, can be determined by **Mass Spectrometer**). In this example, multiples of C_2H_6O formula cannot be considered due to valence rules. Because the carbon has 4 valence electrons, the n number of carbon cannot carry more than 2n+2 hydrogen. A formula such as $C_4H_{12}O_2$ is not possible. This may be ethyl alcohol (CH_3CH_2OH).

Example: The percentages of C, H, O and N obtained by elemental analysis are given below. Calculate the empirical and molecular formula of the compound that its molecular weight 414.

C= 69.56; H= 4.35; O= 19.33; N= 6.76

Answer:

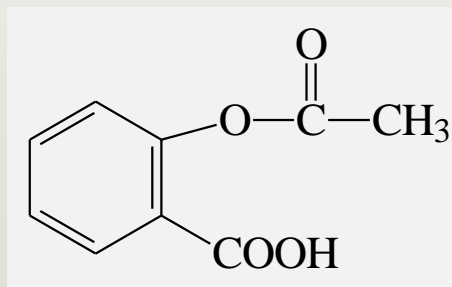C = 69.56 / 12 = 5.79 / 0.48 = 12

H = 4.35 / 1 = 4.35 / 0.48 = 9

O = 19.33 / 16 = 1.208 / 0.48 = 2.5

N = 6.76 / 14 = 0.48 / 0.48 = 1

$C_{12}H_9O_{2,5}N$ Empirical formula

$C_{24}H_{18}O_5N_2$ Molecular formula

Example: %C: 60.02 %H: 4.51 %O: 35.72 are found as a result of analysis for an aspirin sample. Calculate the percentage of these atoms for aspirin, compare these calculations with the analysis results and comment on the purity of the sample.

180	9 x 12 = 108	180	4 x 16 = 64	180	1 x 8 = 8
100	X	100	X	100	X
X = % 60.00 C		X = % 35.55 O		X = % 4.44 H	

The sample is pure because the analysis percentages are found to be less than 0.4% off from the calculated value.