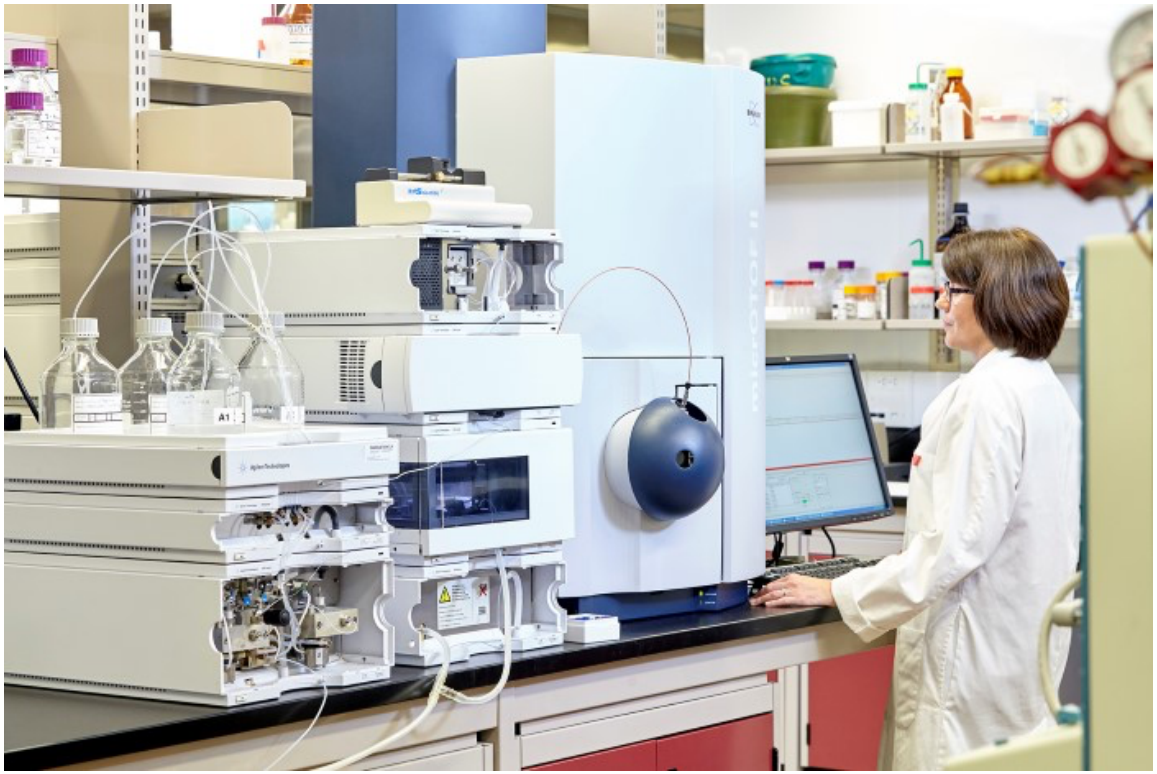
Mass Spectrometry (MS)



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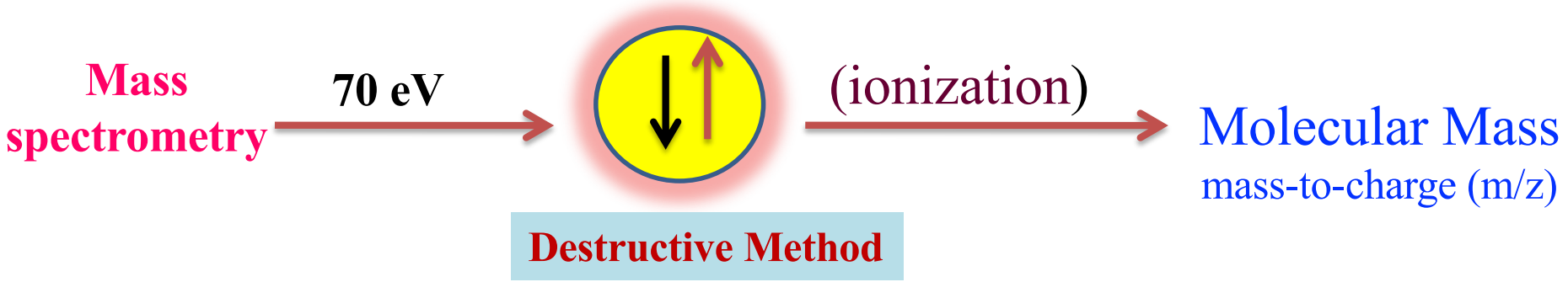
Syllabus M. Sc. II

- Introduction
- Ion production- EI, CI, FD and FAB
- Factors affecting fragmentation,
- Ion analysis, Ion abundance.
- High resolution mass spectrometry (HRMS).
- Mass spectral fragmentation of organic compounds,
- Common functional groups,
- Molecular ion peak,
- Metastable peak,
- Mc-Lafferty rearrangement,
- Nitrogen rule.
- Examples of mass spectral fragmentation of organic compounds with respect to their structure determination.

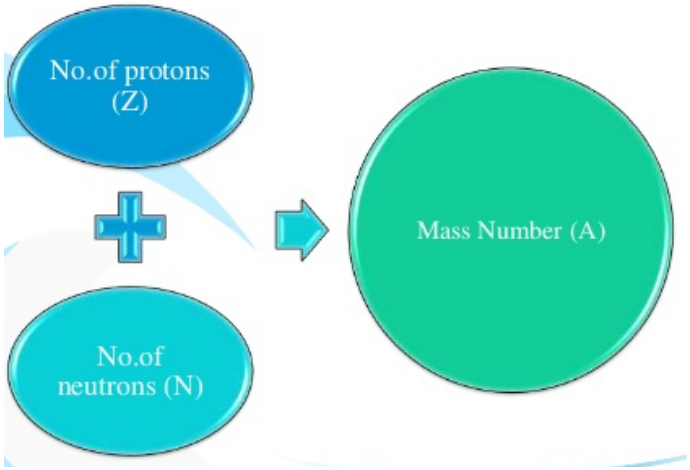
What is Mass Spectrometry ?

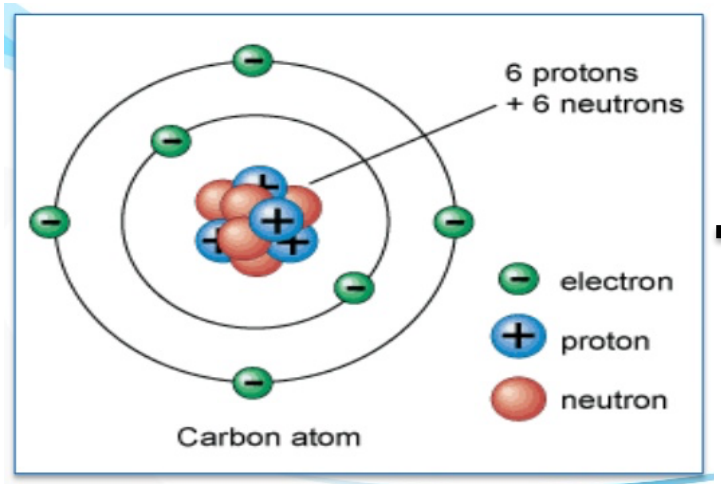
Mass spectrometry (MS) is an analytical technique that ionizes chemical species and separates the ions based on their mass-to-charge ratio (m/z).



- **MS is quantitative technique (Process of measuring)**
- **MS is most accurate method for determining the molecular mass of the compound.**
- **In simpler terms, a mass spectrum measures the masses within a sample.**
- **MS is used in many different fields and is applied to pure samples as well as complex mixtures.**

Why molecular mass in Mass Spectrometry ?

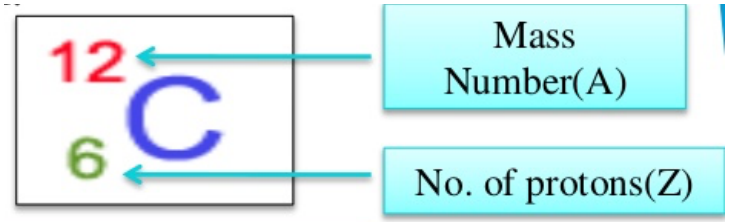




e (70 eV)

-2e

(ionization)



Molecular Mass

mass-to-charge (m/z)

History of Mass Spectrometry

- ✓ **In 1886, E. Goldstein** observed that, cathode rays are negatively charged and anode rays positively charged.

- ✓ **In 1899, Wilhem Wien** constructed a device with perpendicular electric and magnetic fields that separated the positive anode rays according to their charge-to-mass ratio (Q/m).
    - He 1st identified a positive particle equal in mass to the hydrogen atom.

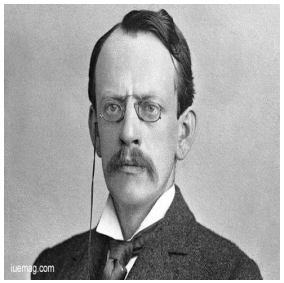
- ✓ **In 1890,** English scientist **J. J. Thomson** later improved on the work of **W. Wien** by reducing the pressure to create the mass spectrograph and mass-to-charge ratio of electron.
    - In 1912-13 **J. J. Thomson** studied the mass spectra of atmospheric gases and used to demonstrate the existence of Neon-22 from Neon-20, thereby establishing that elements could have isotopes.

- ✓ The earliest Modern techniques of mass spectrometry were devised by **A. J. Dempster** and **F. W. Aston** in 1918 and 1919 respectively.

Nobel Prizes for Mass Spectrometry

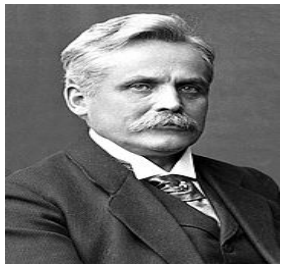
To date, no fewer than five Nobel prizes have been awarded for work directly related to mass spectrometry:

J. J. Thomson, 1906, Physics



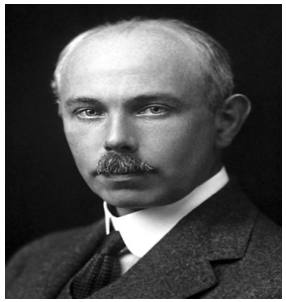
for theoretical and experimental investigation on the conduction of electricity by gases.

Wilhem Wien, 1911, Physics



for his discoveries regarding the laws governing the radiation of heat.

F. W. Aston, 1922, Chemistry



for discovery by means of a mass spectrograph, of isotopes, in a large number of non-radioactive elements.

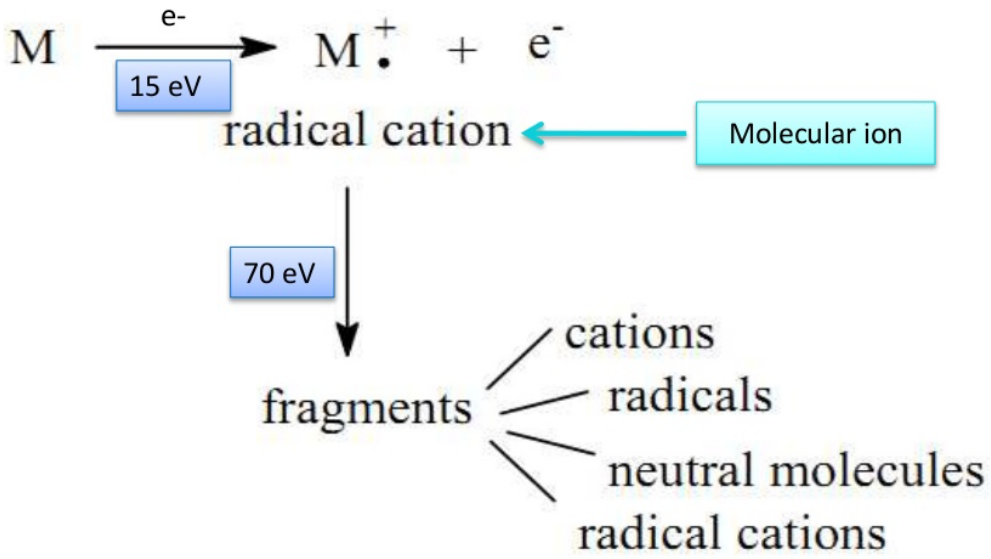
Wolfgang Paul, 1989, Physics

for the development of the ion trap technique.

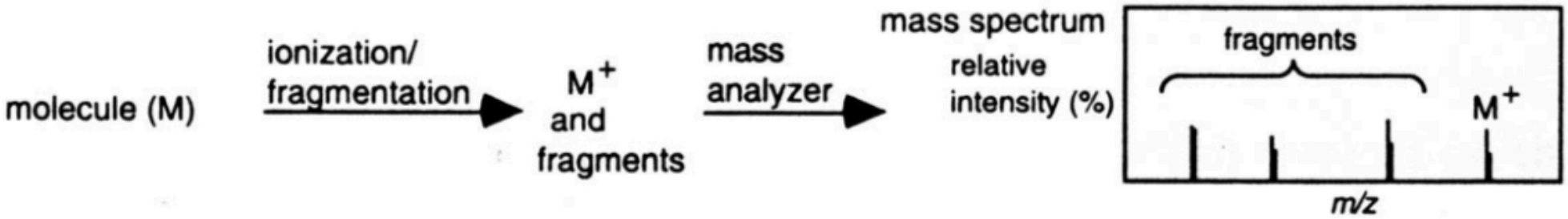
J. B. Fenn and K. Tanaka, 2002, Chemistry

For development of electrospray (soft desorption) ionization, now a commonly used technique for large molecules i.e. biological macromolecules and routine liquid chromatography-tandem mass spectrometry.

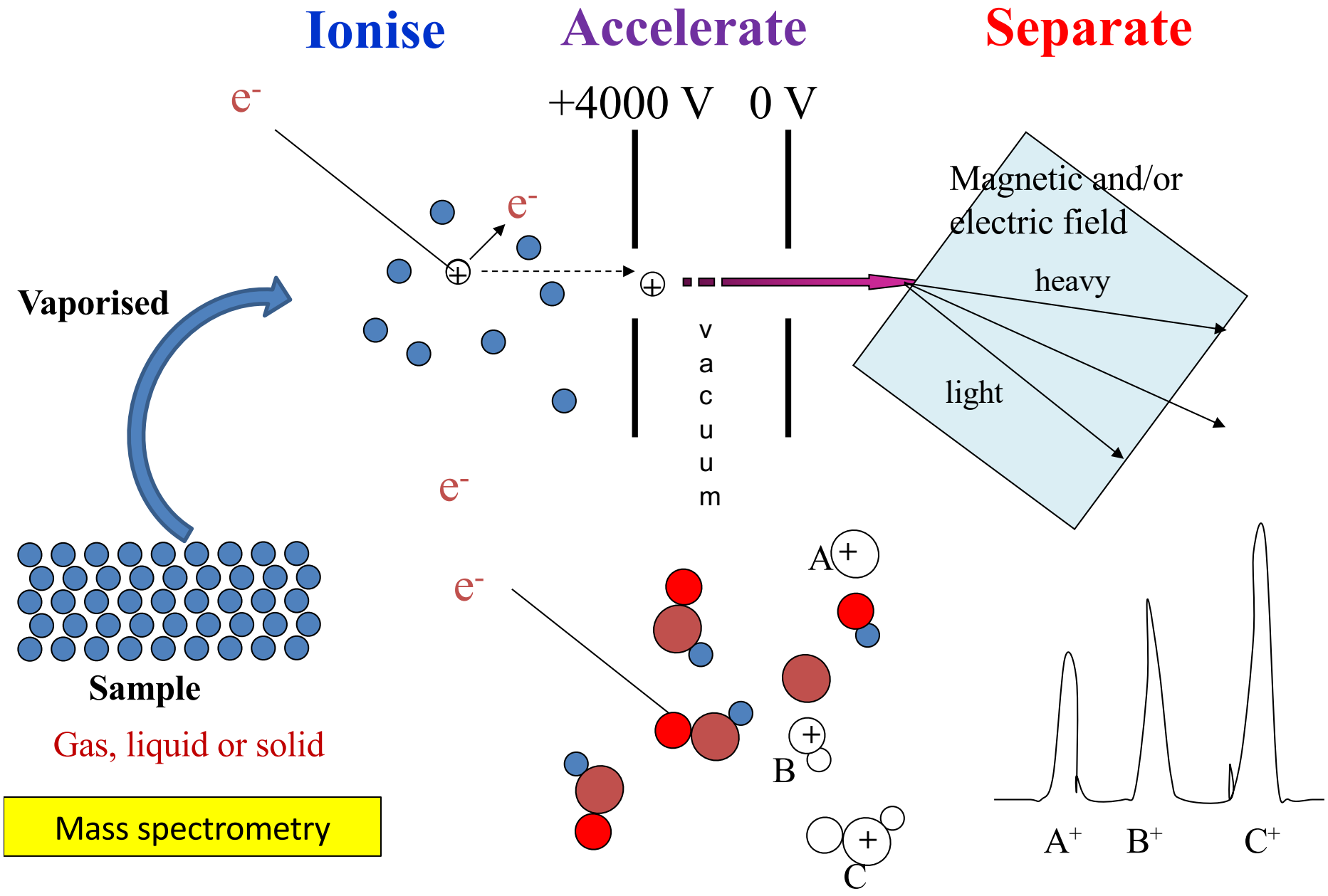
Basic Principle



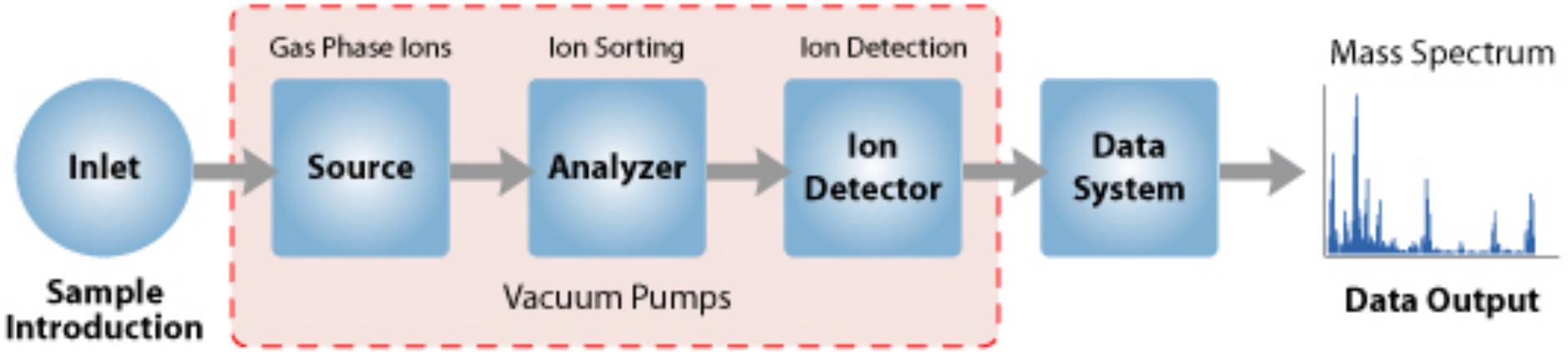
A technique for measuring and analyzing molecules, that involves introducing enough energy into a (neutral) target molecule to cause its ionization and disintegration. The resulting primary ions and their fragments are then analyzed, based on their mass-to-charge (m/z) ratios, to produce a "molecular fingerprint."

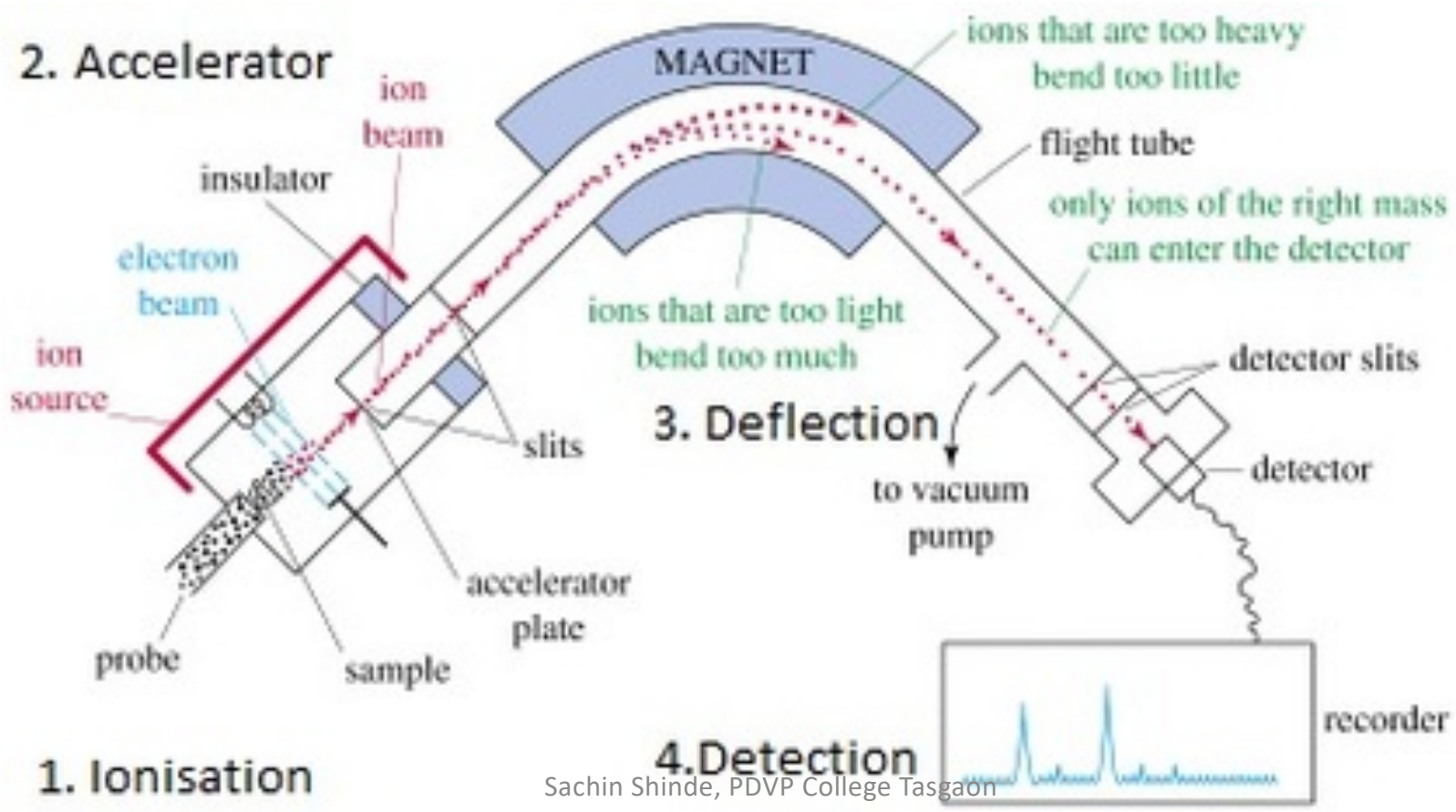


How does it work ?

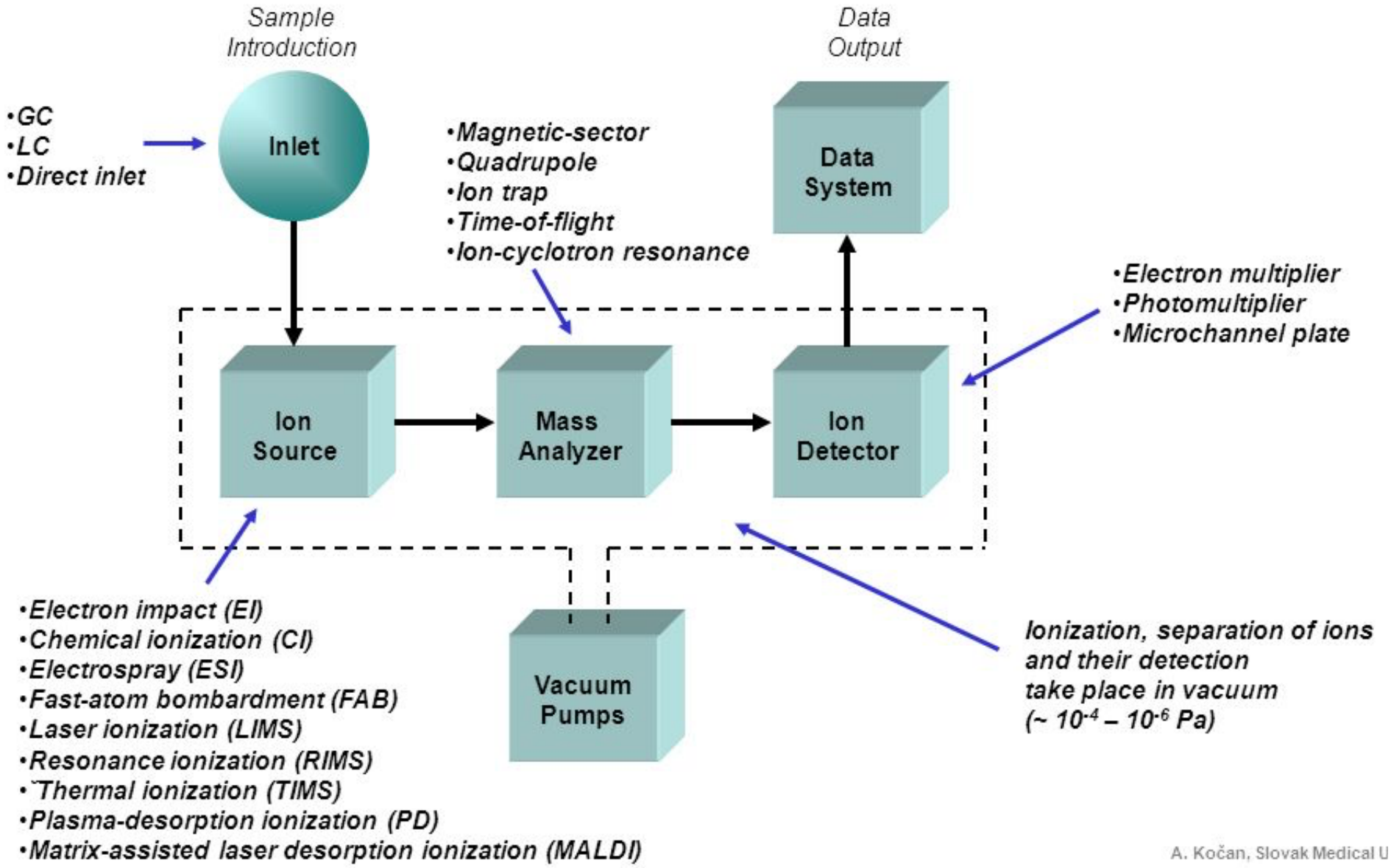


Instrumentation





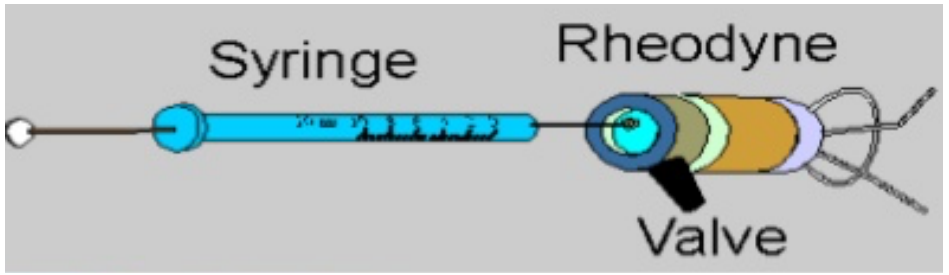
Instrumentation



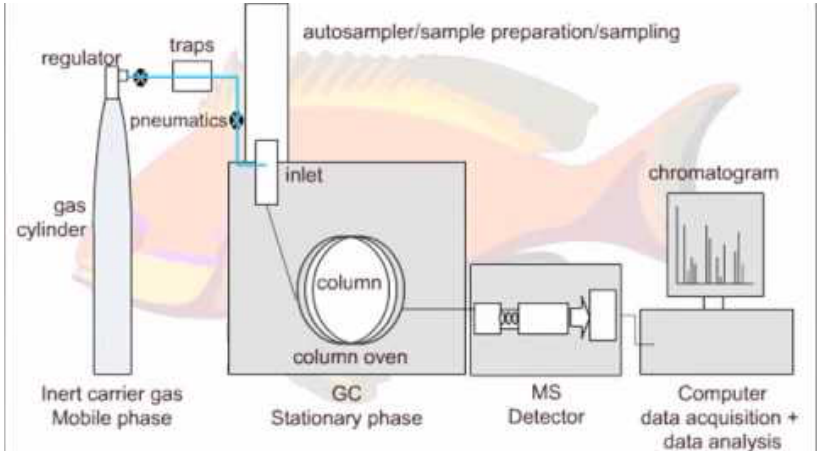
Sample Inlet system

Sample introduction system

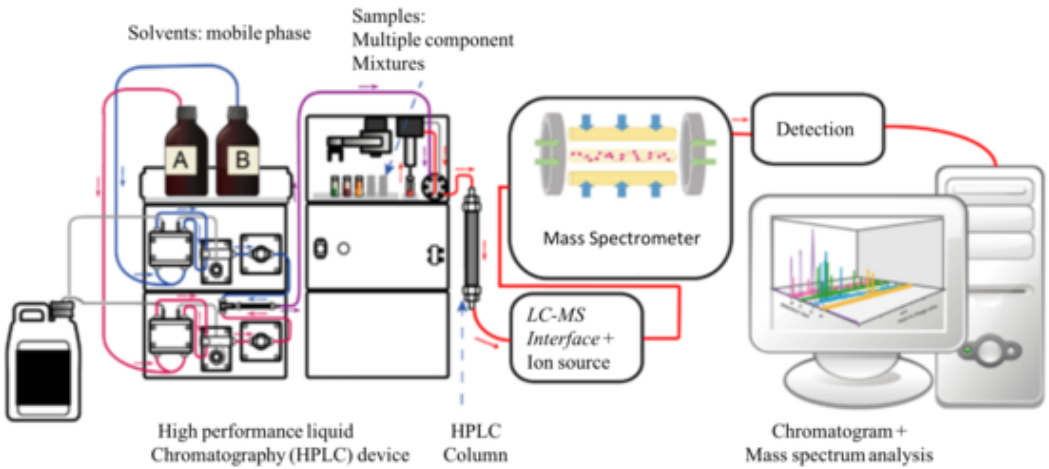
1. **Direct probe method**
   - **i) Syringe for direct infusion**
   - **ii) MALDI probe**



2. **Gas Chromatography-Mass Spectrometry (GC-MS)**
3. **Liquid Chromatography-Mass Spectrometry (LC-MS)**
4. **High Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS)**
5. **Ultra High Performance Liquid Chromatography-Mass Spectrometry (UPLC-MS)**



GC-MS



HPLC-MS

Components of Mass Spectrometer

Ionisation ***Ion Separation*** ***Ion Detection***

Ion Source

Electron Ionisation (EI)

Chemical Ionisation (CI)

Electrospray Ionisation (ESI)

Fast Atom Bombardment (FAB)

Matrix-Assisted Laserdesorption/ Ionisation (MALDI)

Mass Analyser

Quadrupole

Magnetic Sector Field

Electric Sector Field

Time-Of-Flight (TOF)

Ion Trap

Detector

Electron Multiplier

Multichannel plate

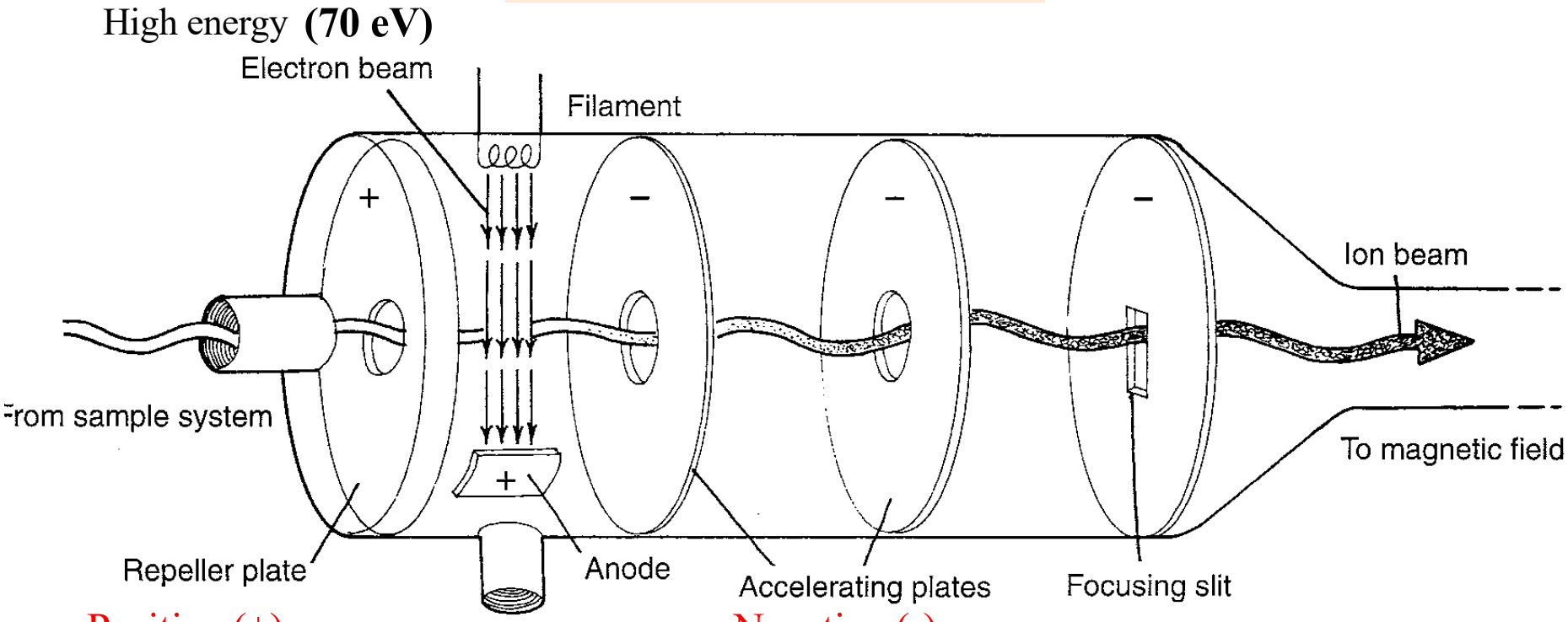
Faraday Cup

Ionization Chamber

Electron Ionization:

$$M + e^- \rightarrow (M^{+\cdot})^* + 2e^-$$

•Removal/addition of electron(s)



Most organic compounds have ionization potential ranging between 8 and 15 eV.

Ionization Methods

Neutral species → Charged species

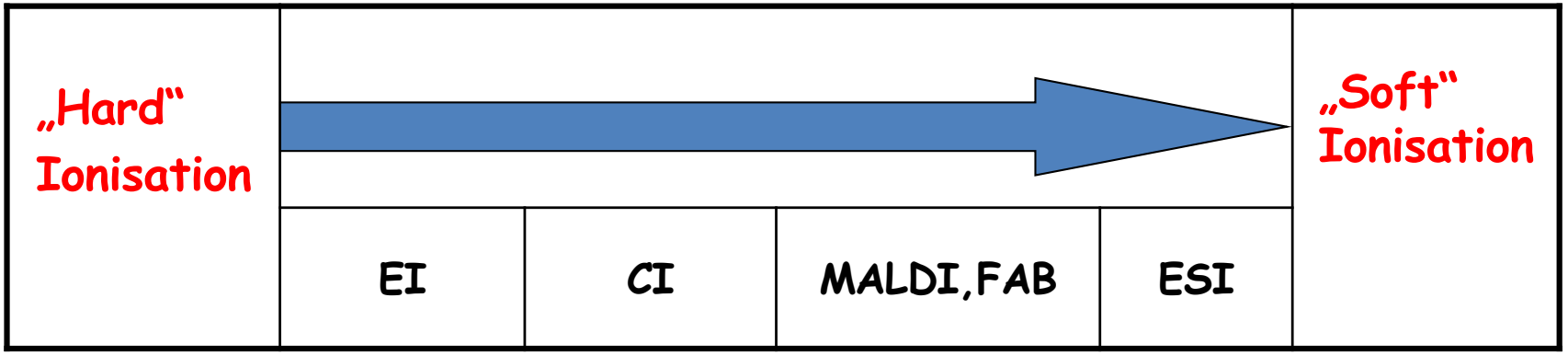
1. Removal/addition of electron(s)

$M + e^- \rightarrow (M^{+\cdot})^* + 2e^-$ • Electron Ionization (EI)

2. Removal/addition of proton(s)

$M + (Matrix)\text{-}H \rightarrow MH^+ + (Matrix)^-$

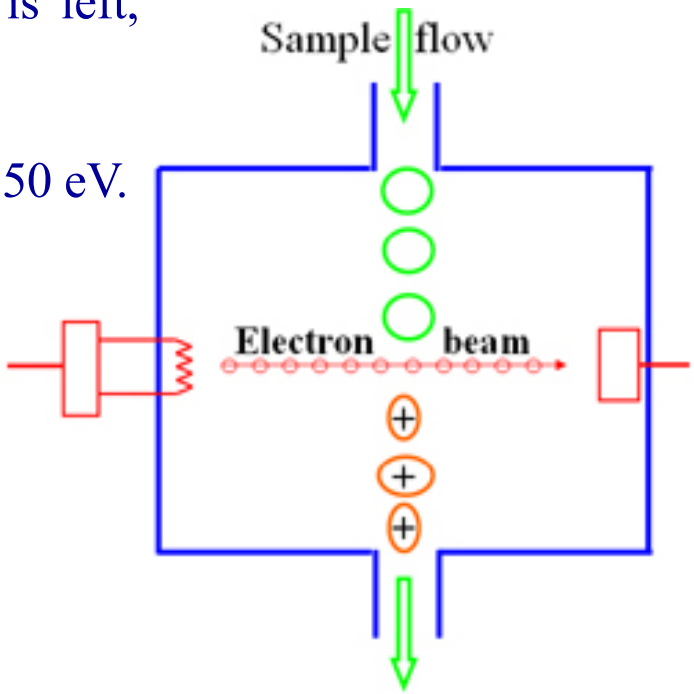
- Chemical Ionisation (CI)
- Atmospheric Pressure Chemical Ionization CI (APCI)
- Fast Atom Bombardment (FAB)
- Electrospray Ionization (ESI)
- Matrix Assisted Laser Desorption/ionization (MALDI)



1. Electron Ionization (EI-MS)

Probably the most widely used method

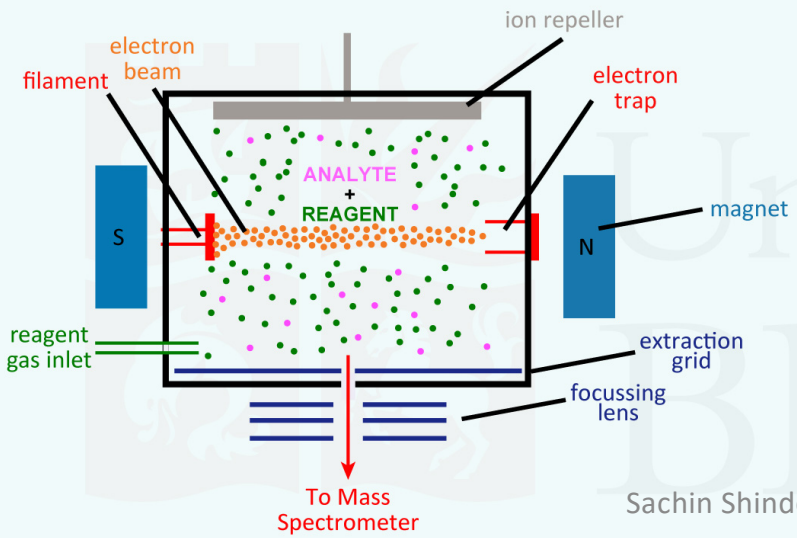
- Vapor phase samples → from GC
- Sample bombarded with high energy electrons 25-80 eV (2.4-7.6 MJ/mol, 573–1,816 kcal/mol) → 70 eV typical
- Ejects 1 electron from molecules positively charged ion is left, odd electron ion, radical cation.
- Typical ionization energies for organic molecules ~15 eV~50 eV.
- Excess energy dissipated by breaking covalent bonds
- Sample must be relatively volatile.
- Lots of fragment ions.
- Fragment pattern is unique to a given molecule.
- Many times molecular ion (M^+) is not observed.
- Difficult to analyze high molecular weight compounds and biomolecules.



EI is most useful for organic compounds which have a molecular weight below 600

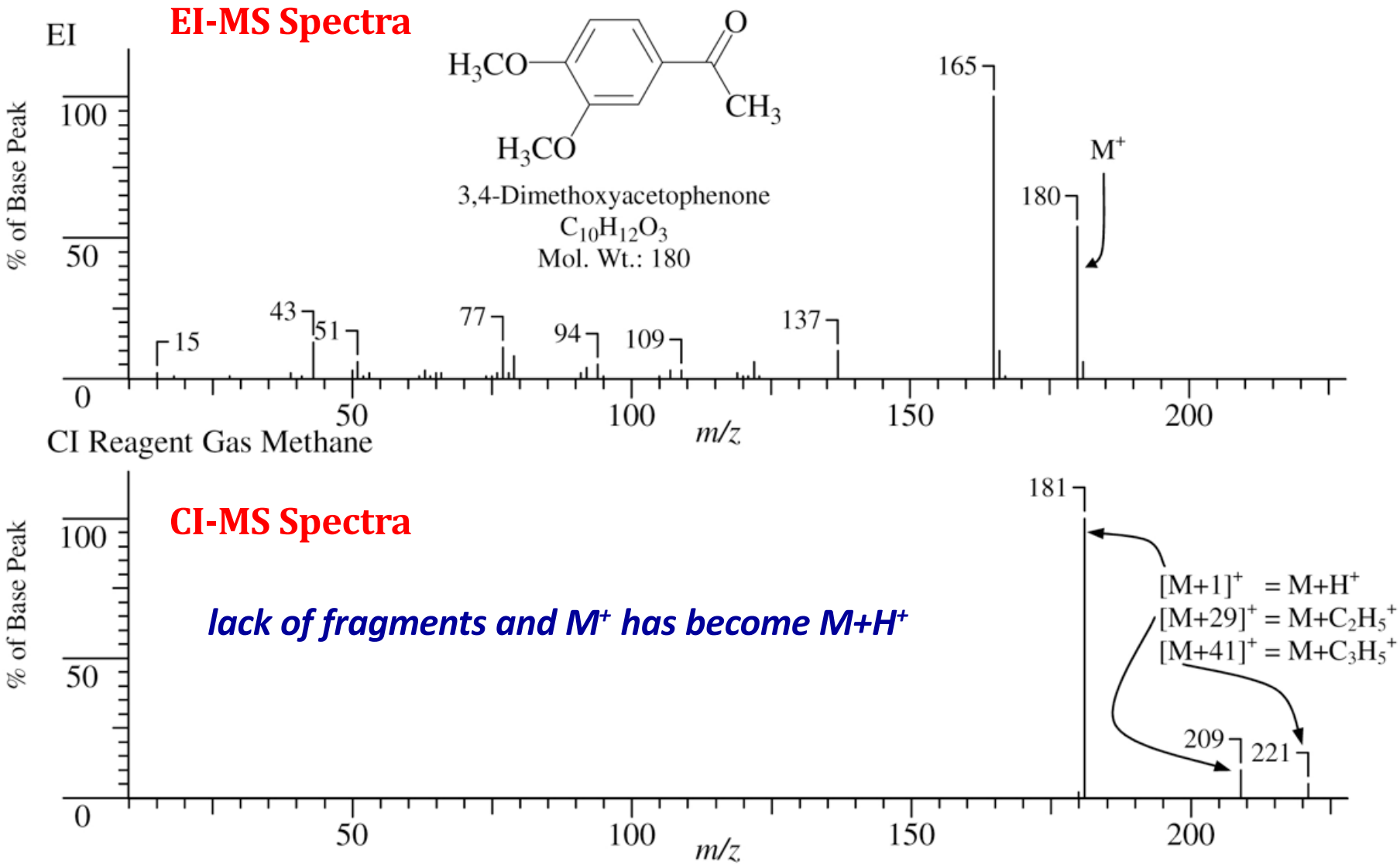
2. Chemical Ionization (CI-MS)

- It is “Soft Ionization” method.
- Vapor phase samples (Much less energy transferred < 5 eV).
- Sample introduced to **ionized reagent gas** (Highly volatile liquids, Methane, Isobutane, Ammonia, methanol others)
- Collisions between sample & gas ions cause H transfers, electron transfer and adduct (+ ve or –ve) formation which → produces [M+H]+ ions, *not M+ ion.*
- Less fragmentation and Less information about structure.
- High abundance of molecular ions means good for molecular weight determination.
- Analysis of high molecular weight compounds and many biomolecules.



$CH_4 + e^{-*}$	$\rightarrow CH_4^{+\bullet} + 2e^-$	methane molecular ion formation
$CH_4^{+\bullet} + CH_4$	$\rightarrow CH_5^+ + CH_3^\bullet$	methanium formation
$CH_5^+ + M$	$\rightarrow CH_4 + [M+H]^+$	protonated analyte formation
$CH_4^{+\bullet}$	$\rightarrow CH_3^+ + H^\bullet$	side reaction - fragmentation
$CH_3^+ + CH_4$	$\rightarrow C_2H_5^+ + H_2$	side reaction - carbocation formation
$C_2H_5^+ + M$	$\rightarrow [M+C_2H_5]^+$	analyte adduct ion formation

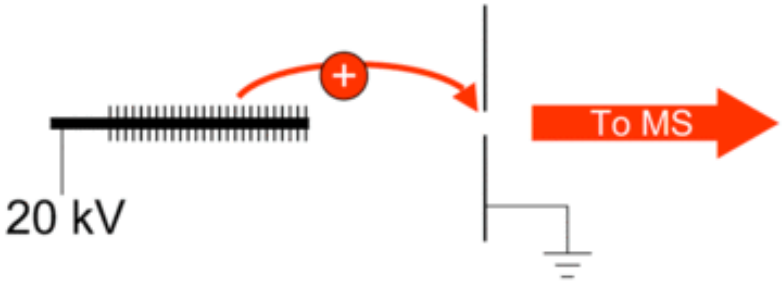
Comparison of EI-MS and CI-MS Spectra



3. Field Desorption (FD-MS)

- It is a soft ionization method.
- A high-potential electric field is applied to an *emitter* with a sharp surface, such as a razor blade, or more commonly, a filament from which tiny "whiskers" have formed.
- Little or no fragmentation.
- The technique was first reported by Beckey in 1969.
- It is also the first ionization method to ionize nonvolatile and thermally labile compounds.
- One major difference of FD with other ionization methods is that it does not need a primary beam to bombard a sample.

In FD, the analyte is applied as a thin film directly to the emitter, or small crystals of solid materials are placed onto the emitter. Slow heating of the emitter then begins, by passing a high current through the emitter, which is maintained at a high potential (e.g. 5 kilovolts). As heating of the emitter continues, low-vapor pressure materials get desorbed and ionized by alkali metal cation attachment.



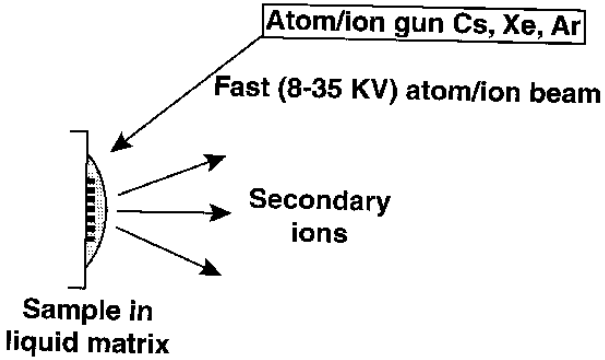
Ion formation mechanisms;

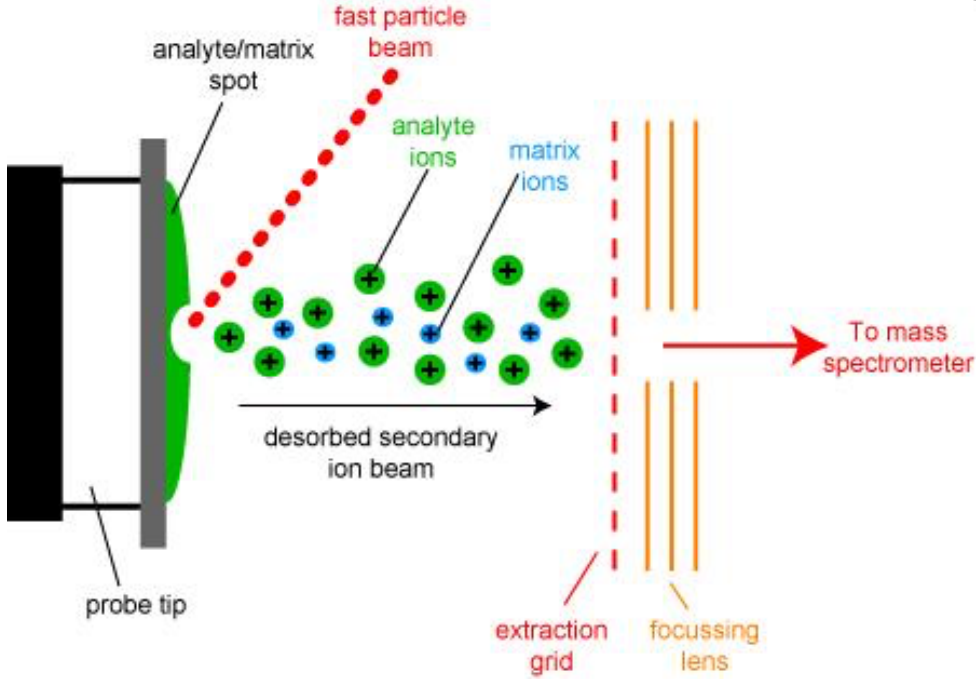
Field ionization, Cation attachment, Thermal ionization, Proton abstraction

- A major application of FD is to determine the molecular mass of a large variety of thermally labile and stable nonvolatile, nonpolar, and polar organic and organometallic compounds, and of molecules from biochemical and environmental sources.
- The FD methods are good for qualitative analysis but less suitable for quantitative analysis of complex mixtures

4. Fast Atom Bombardment (FAB)

- It is Soft Ionization method for large and non-volatile molecules.
- Neutral atoms to ionize the sample. Sample is mixed with a condensed phase matrix.
- Mixture is ionized with high energy (6-10 keV) Xe or Ar atoms.
- Matrix protects sample from excess energy (glycerol, thioglycerol triethanolamine).
- Ionization from protonation ([M+H]+), cation attachment ([M+23(Na)]+), or deprotonation ([M-H]+).
- High resolution masses are possible (peptides)
- Exact mass determination upto 20,000.
- Can be complicated by ions from matrix





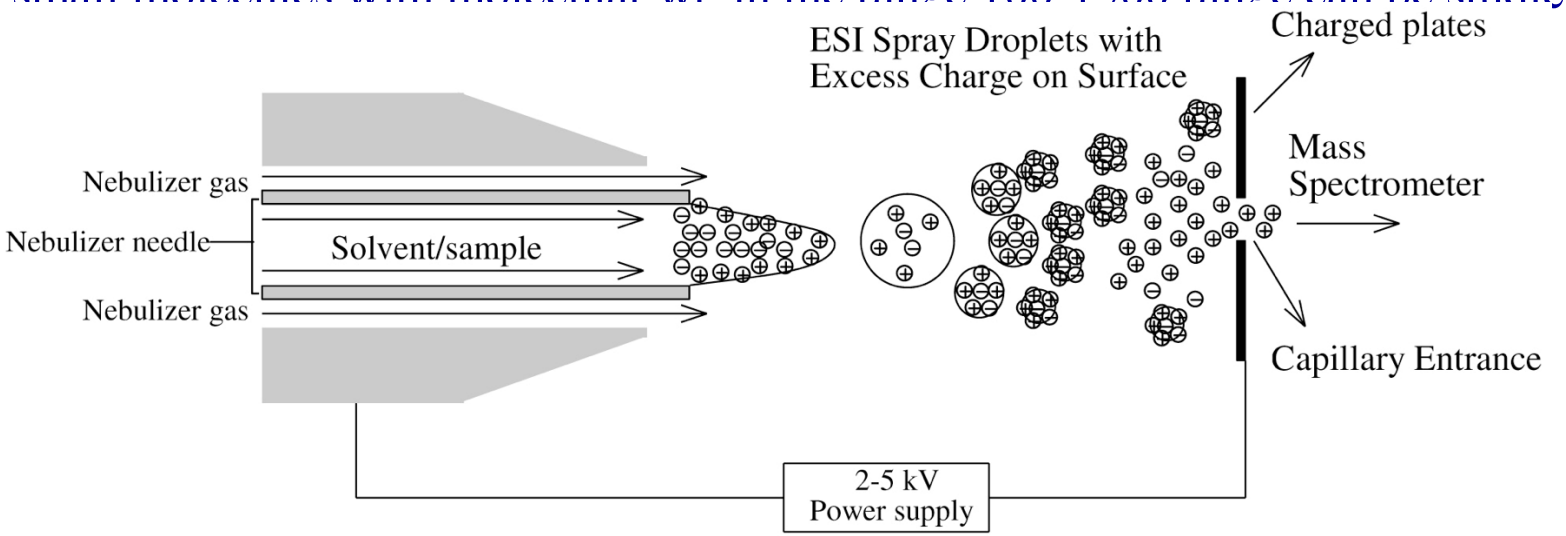
5. Electrospray Ionization (ESI-MS)

ESI and Atmospheric Pressure Chemical Ionization (APCI) two methods are common.

- More useful for studding high molecular wt. biomolecules and non-volatile compounds.

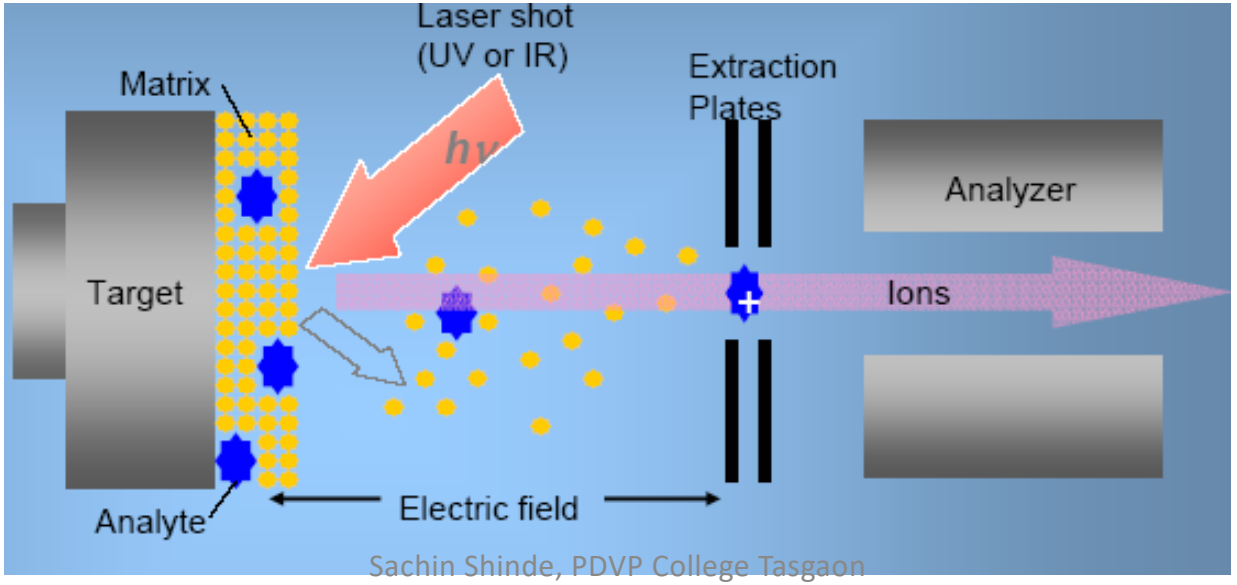
Do not require vacuum pressures. Same working as old **Thermospray ionization (TSI)**

- Ideal for study by both can be coupled to an GC and LC system (GC-MS and LC-MS).
- Multiply charged ions are possible and can be useful for large molecules (e.g. proteins).
- *m/z = 100,000 Da/40 charges 2,500 m/z,* used for basic amino acid side chain.
- The charged droplet evaporates and ionizes the analyte.
- Liquid phase samples of large size (biomolecules) can be ionized using ESI.
- Many small molecules with molecular wt. in the range 100-1500 range can be studied.



6. Matrix-Assisted Laser Desorption Ionization (MALDI)

- It is Soft Ionization method for wide range molecular wt. from small polymers to 3,00,000 amu.
- Sample is mixed with a condensed phase matrix.(Nicotinic, picolinic cinnamic acid derivatives)
- MALDI requires only a few femtomoles (1 X 10^{-15} mole) of sample.
- Ability to absorb the UV light from laser pulse (337nm for N2 laser).
- Matrix absorb most of the energy from the laser pulse and mixture is ionized with a laser.
- Charged molecules are ejected from matrix.
- Little excess energy → little fragmentation.
- Good for large molecules (e.g. proteins, oligonucleotides, antibodies,carbohydrates, polymers).

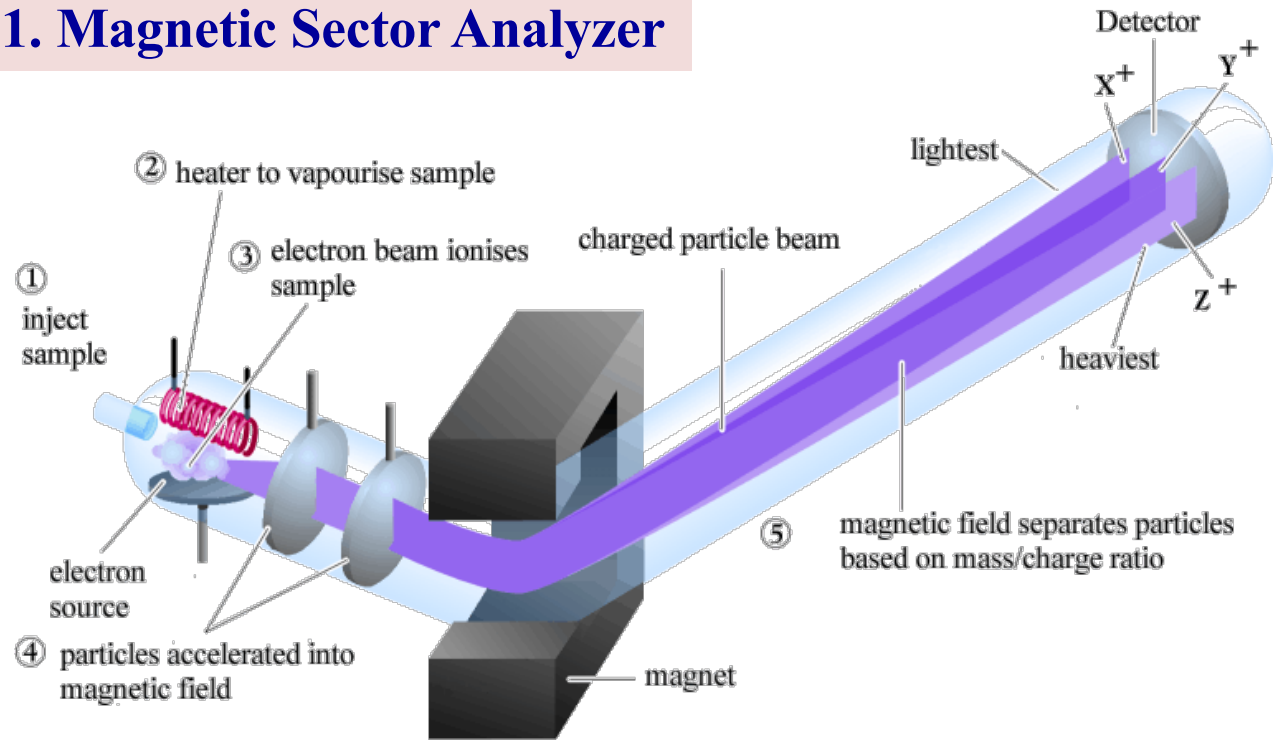


Advantages and Disadvantages of Matrix-Assisted Laser Desorption/Ionization (MALDI)

Advantages	Disadvantages
Practical mass range of up to 300,000 Da. Species of much greater mass have been reported.	Low resolution (see Chapter 2). Some MALDI instruments are capable of higher resolution; however, this is only in a relatively low mass range and is accomplished at the expense of sensitivity.
Typical sensitivity on the order of low femtomole to low picomole. Reports have indicated that attomole sensitivity is possible.	Matrix background, which can be problem for compounds below a mass of 1000 Da. This background interference is highly dependent on the matrix material.
Soft ionization with little to no fragmentation observed.	Possibility of photodegradation by laser desorption/ionization.
Tolerance of salts in millimolar concentrations.	
Suitable for the analysis of complex mixtures.	

Ion Separation (Mass Analyzers)

1. Magnetic Sector Analyzer



Mass Spec Equation (Magnet Sector)

$$\frac{m}{z} = \frac{B^2 r^2}{2V}$$

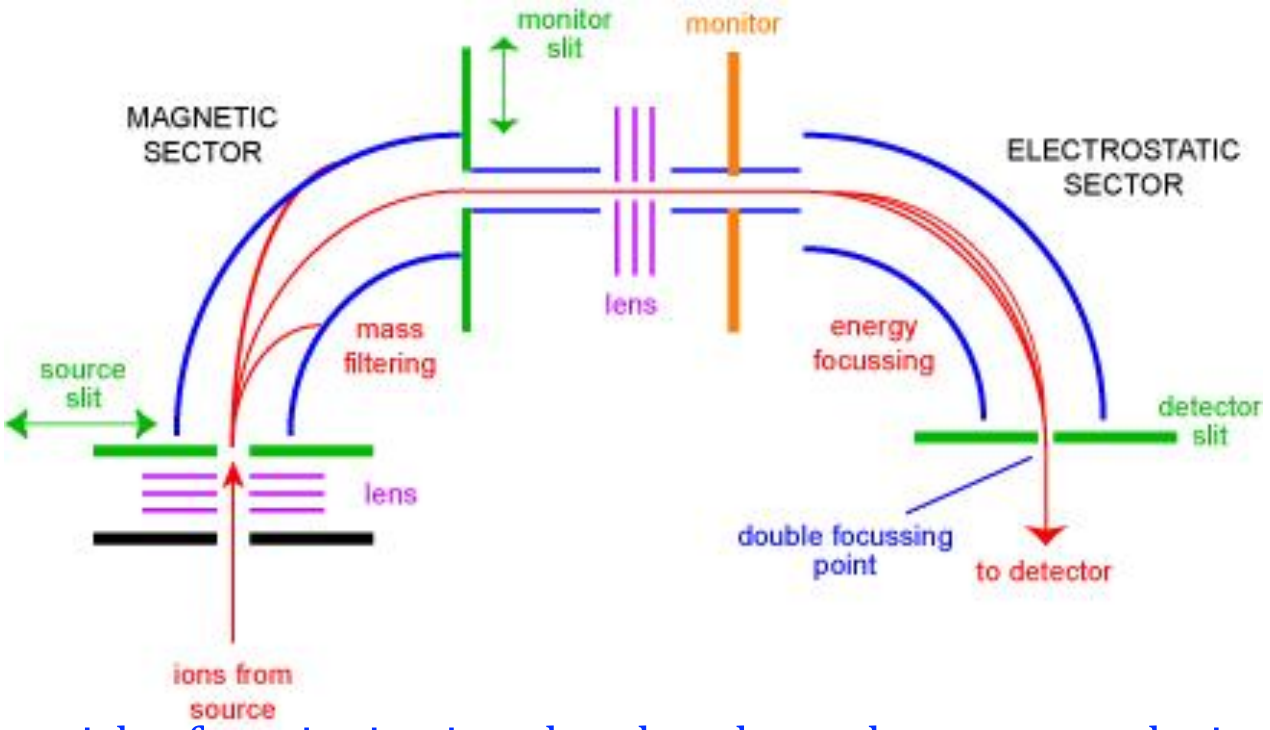
M = mass of ion
z = charge of ion
V = voltage
B = magnetic field
r = radius of circle

The greater value of m/z, larger the radius of the curved path.

- A particle with the correct m/z ratio can pass through the curved analyzer tube and reach the detector.
- A particle with m/z ratios that are either too large or too small strike the sides of the analyzer tube and do not reach the detector.
- Therefore, magnetic field strength is continuously varied (*magnetic field scan*) so that all of the ions produced in the ionization chamber can be detected.
- The detector plot of the number of ions versus their m/z values.

2. Magnetic Sector and Electric Sector Analyzer

Double beam mass spectrometer



- All leaving particles from ionization chamber do not have same velocity.
- The introduction of an electric field after (or before) the magnetic field is called double beam mass spectrometer. So ions travels in constant velocity.
- It permits high resolution so that called High resolution mass spectrometry (HRMS).
- It provides the mass of ion in four decimal places. (i.e M^+= 132.1234).
- The resolution of the mass improves by a factor of 10 or more over the magnetic sector alone.

High Resolution Mass Spectrometer (HRMS)

Determination of Molecular Formula

High Resolution Mass Spectrometry (HRMS)

CO
N_2
C_2H_4
CH_2N
} all show m^+ at 28

	exact mass
CO	27.9949
N_2	28.0062
C_2H_4	28.0312
CH_2N	28.0187

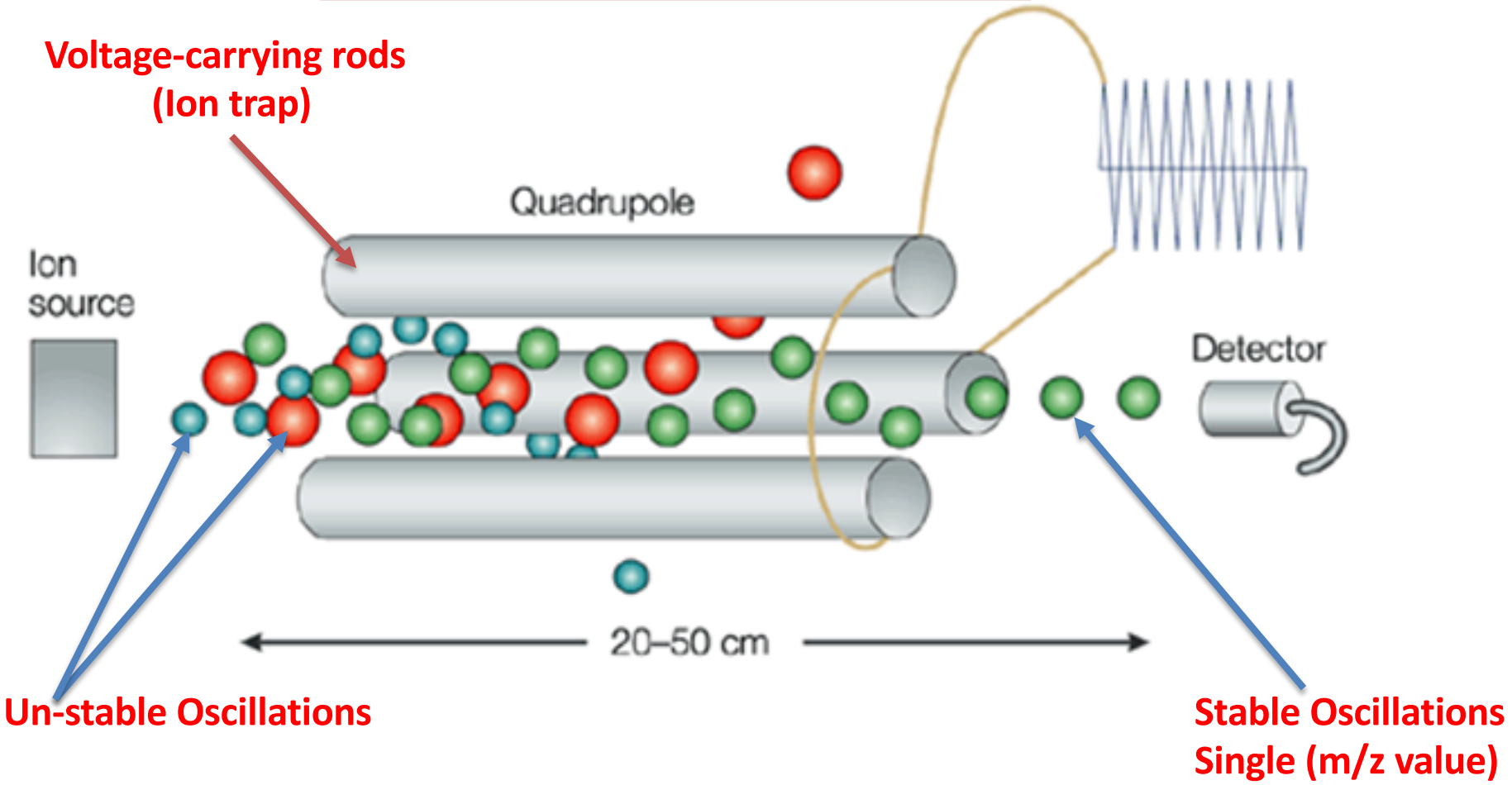
Comparisons of Molecular Weights and Precise Masses

MF	MW	exact mass
C_3H_8O	60.1	60.05754
$C_2H_8N_2$	60.1	60.06884
$C_2H_4O_2$	60.1	60.02112
CH_4N_2O	60.1	60.03242

TABLE 1.4 Exact Masses of Isotopes.

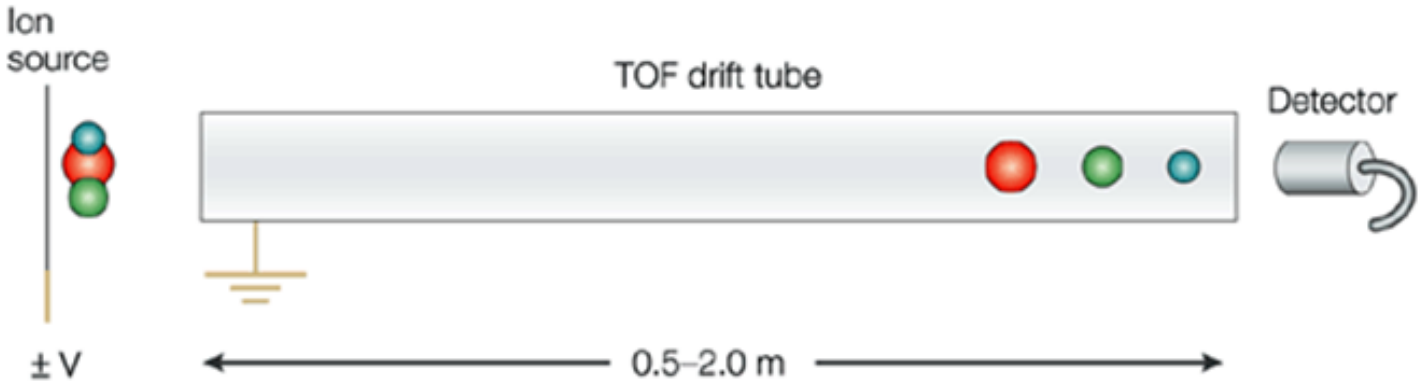
Element	Atomic Weight	Nuclide	Mass
Hydrogen	1.00794	^{1}H	1.00783
		D(^{2}H)	2.01410
Carbon	12.01115	^{12}C	12.00000 (std)
		^{13}C	13.00336
Nitrogen	14.0067	^{14}N	14.0031
		^{15}N	15.0001
Oxygen	15.9994	^{16}O	15.9949
		^{17}O	16.9991
		^{18}O	17.9992
Fluorine	18.9984	^{19}F	18.9984
Silicon	28.0855	^{28}Si	27.9769
		^{29}Si	28.9765
		^{30}Si	29.9738
Phosphorus	30.9738	^{31}P	30.9738
Sulfur	32.0660	^{32}S	31.9721
		^{33}S	32.9715
		^{34}S	33.9679
Chlorine	35.4527	^{35}CI	34.9689
		^{37}CI	36.9659
Bromine	79.9094	^{79}Br	78.9183
		^{81}Br	80.9163
Iodine	126.9045	^{127}I	126.9045

3. Quadrupole Analyzer



- There is a stable oscillation that allows a particular ion to pass from one end of the quadrupole to the other without striking he poles.
- This oscillation dependent on the m/z ratio of an ion.
- Therefore, ion of only a single m/z value will traverse the entire length of the filter.
- All other ions of unstable oscillations will strike the poles and be lost.

4. Time of Flight (TOF) Analyzer



In the time-of-flight (TOF) mass spectrometers, all singly charged particles subjected to a potential difference V attain the same translational energy in electron volts (eV). Thus lighter particles have the shorter TOF over a given distance. The accelerated particles are passed into a field-free region where they are separated in time by their m/z values and collected. Since arrival times between successive ions can be less than 10^{-7} s, fast electronics are necessary for adequate resolution. Time-of-flight devices are used with sophisticated ionizing meth-

Detectors

- Measure one (single) m/z value at a time (single channel detectors)
- Multiple detectors are used for multiple ion detection
- High resolution magnetic sector instruments use multiple detectors (called multicollectors)

Electron Multiplier (EM)

- The most common detector in MS for ions
- Similar to PMT
- Very sensitive and has fast response

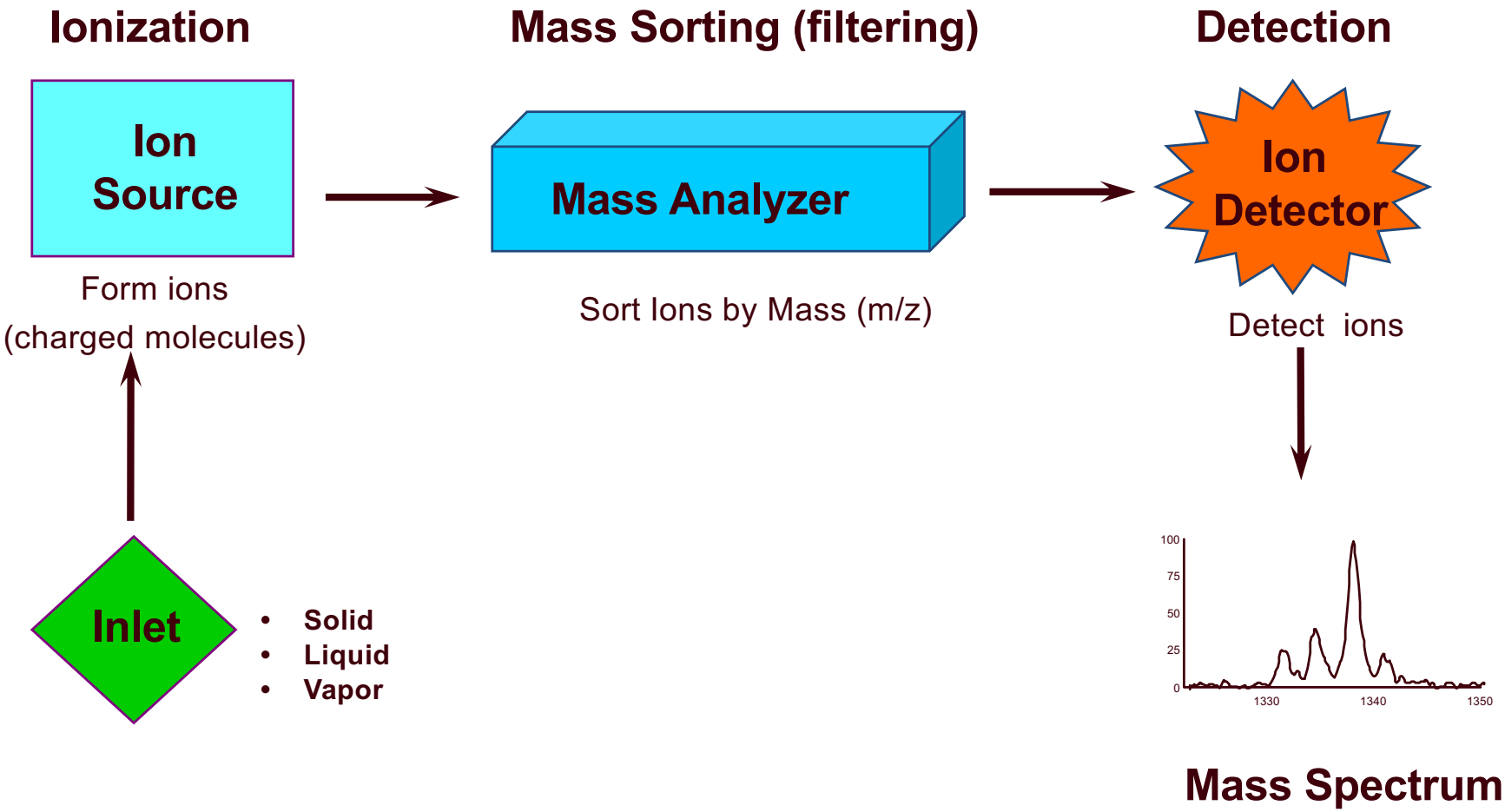
Faraday Cup

- A metal or carbon cup serves to capture ions and store the charge
- Cup shape decreases loss of electrons
- Least expensive detector for ions
- Has long response time

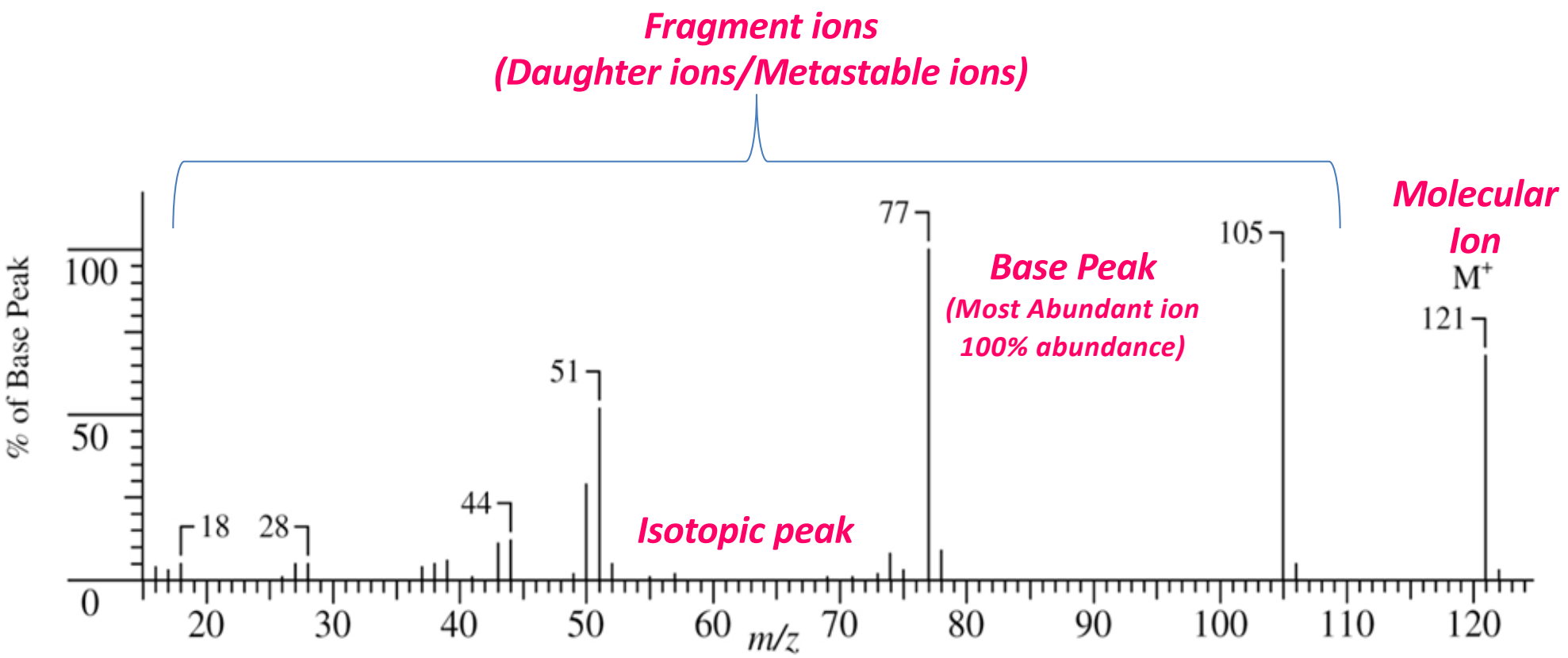
Array Detectors

- Used in TOF MS instruments
- Employs a focal plane camera (FPC) consisting of an array of 31 Faraday Cup
- Up to 15 m/z values can be measured simultaneously
- Exhibits improved precision compared with single channel detectors

Summary: acquiring a mass spectrum

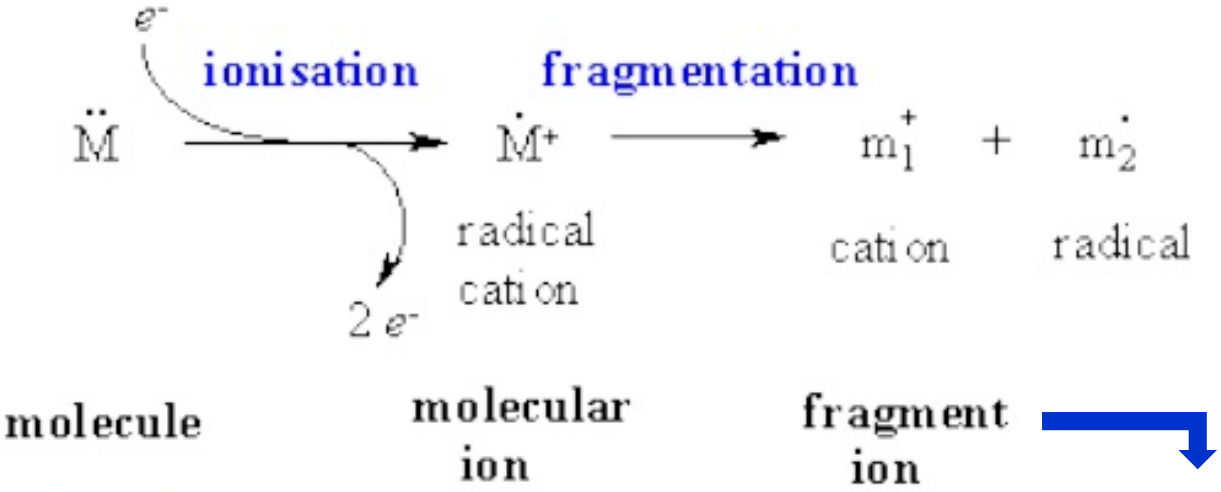


Nature of Mass Spectrum



- ✓ Mixture of ions of different mass gives separate peak **(each m/z)** .
- ✓ Separation of peaks depends on relative mass to charge ration **(m/z or m/e ratio).**
- ✓ Intensity of peak proportional to percentage of each ion of different mass in mixture **(% Abundance).**

Various ions produced in mass spectrum



Fragmentation process:

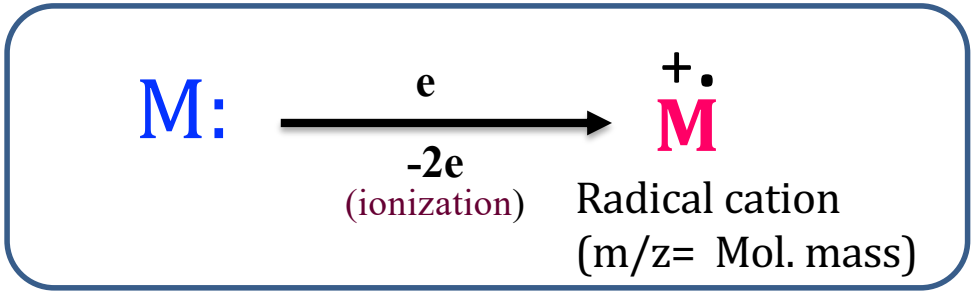
❖Bombardment of molecules by an electron beam with energy between 10-15ev usually results in the ionization of molecules by removal of one electron (Molecular ion formation).

❖When the energy of electron beam is increased between 50-70ev, these molecular ions acquire a high excitation resulting in their break down into various fragments. This process is called " Fragmentation process".

1. Molecular ion or Parent ion.
2. Fragment ions.
3. Rearrangement ions.
4. Multicharged ions.
5. Negative ions.
6. Metastable ions.

Molecular ion peak (M^+)

❖Bombardment of molecules by an electron beam with energy between 10-15ev usually results in the ionization of molecules by removal of one electron (Molecular ion formation).



The simple/single removal of electron from a molecule to give radical cation is known as molecular ion peak.

Characteristics:

- Also called as parent ion.
- The ion of highest mass (m/z) in spectrum. (Heaviest peak in mass spectrum.)
- It is indicated by M^+ or Radical cation ($M^{+\cdot}$).
- Lifetime was 10^{-5} sec before fragmentation.
- The mass (m/z) of M^+ gives molecular mass of the sample.
- The abundance of M^+ increases at low ionization potential.
- The M^+ is sometimes base peak in the spectrum.
- The M^+ is sometimes absent. The decomposition of the M^+ increases with increasing molecular size of compounds in homologous series. (highly substituted alkanes and tertiary alcohols).

Molecular ion peak (M^+)

The order of energy required to remove electron is as follows—

σ electrons > non-conjugated π > conjugated π > non bonding or lone pair of electrons.

Loss of e^- =Non-bonding electron > pi conjugated > pi non-conjugated > Sigma electrons

❖ Most molecules show a peak for the molecular ion, the stability of which is usually in the order—

Aromatic > Conjugated acyclic polyenes > Alicyclics > n-hydrocarbons > ketones > ethers> Branched chain hydrocarbons > Alcohols.

Nature of molecular ion (M^+):

- Exact mass (HRMS): Identification of isobaric structures
- Nitrogen Rule: No. of Nitrogen atoms in molecule.
- Base peak: Most stable ion formed in MS
- Isotopic peaks: Presence of isotopes and their ratio.

Application of molecular ion (M^+):

- Determination of molecular weight and molecular formula.

Determination of Exact Mass (HRMS)

Comparisons of Molecular Weights and Precise Masses (Isobaric Species)

MF	MW	Exact mass
C_3H_8O	60.1	60.05754
$C_2H_8N_2$	60.1	60.06884
$C_2H_4O_2$	60.1	60.02112
CH_4N_2O	60.1	60.03242

Rule of Thirteen: (Empirical molecular formula)

$$\frac{M}{13} = n + \frac{r}{13}$$

M= Exact Mass of compound
n= numerator
r= remainder

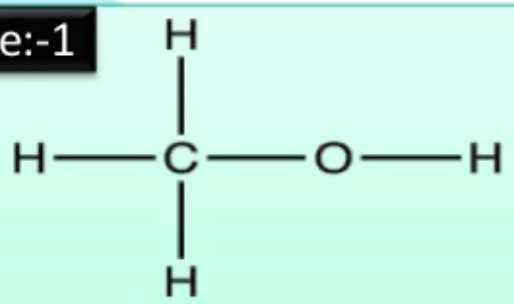
Base formula = C_nH_{n+r}

Index of Hydrogen Deficiency $U = \frac{(n - r + 2)}{2}$

Nitrogen Rule

The nitrogen rule states, that a molecule that has no or even number of nitrogen atoms has an even nominal mass, whereas a molecule that has an odd number of nitrogen atoms has an odd nominal mass.

Example:-1



molecular formula = CH_4O

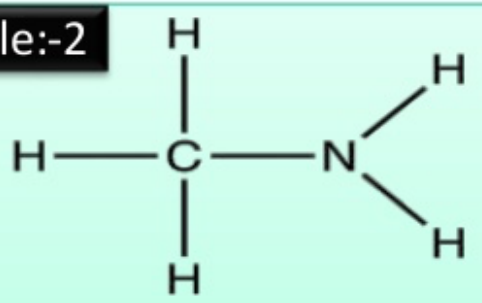
nominal mass = (1x12) + (4x1) + (1x16)

= 32 ←

N atoms = 0

nominal mass = 32 (even #)

Example:-2



molecular formula = CH_5N

nominal mass = (1x12) + (5x1) + (1x14)

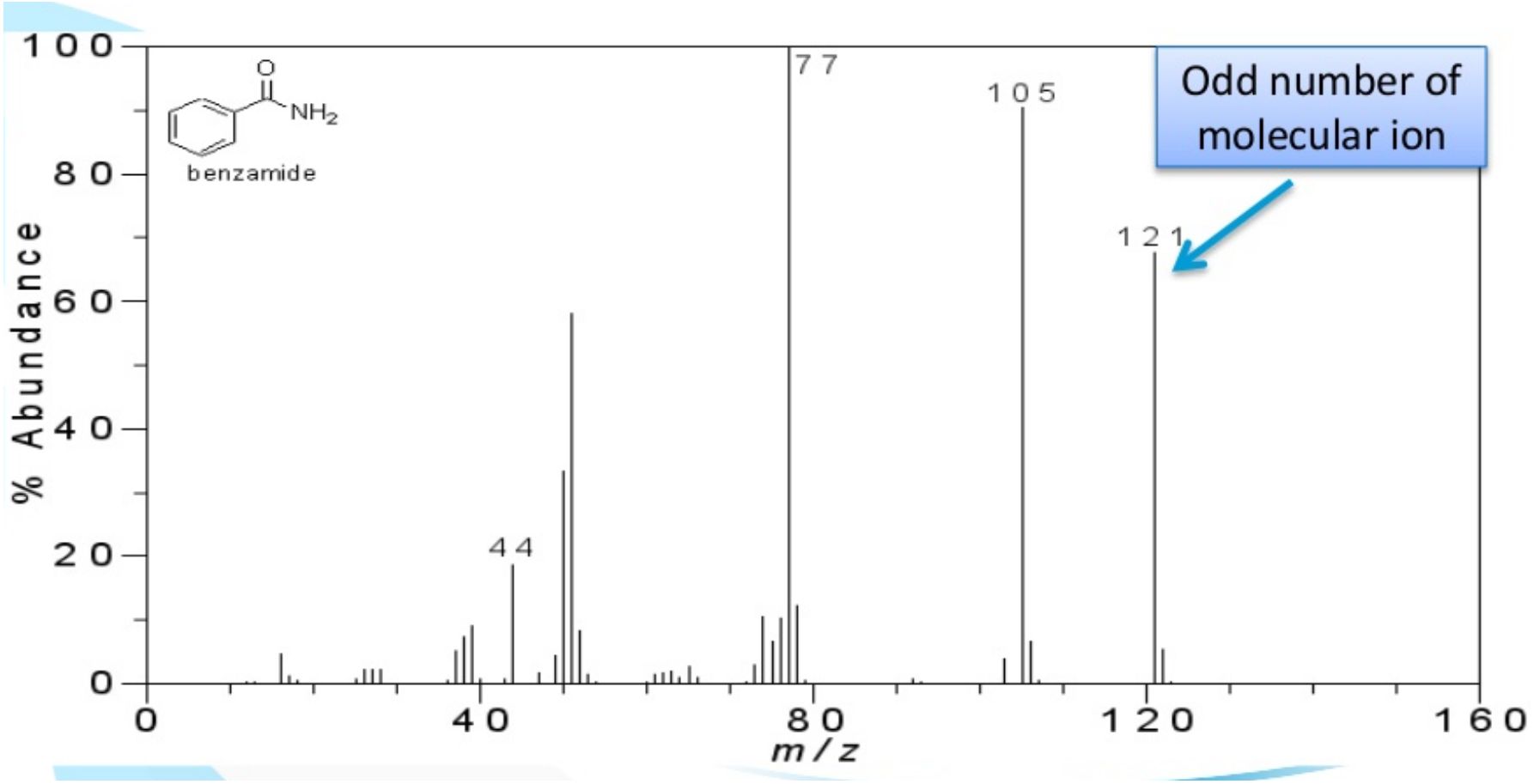
= 31 ←

N atoms = 1 (odd #)

nominal mass = 31 (odd #)

Nitrogen Rule

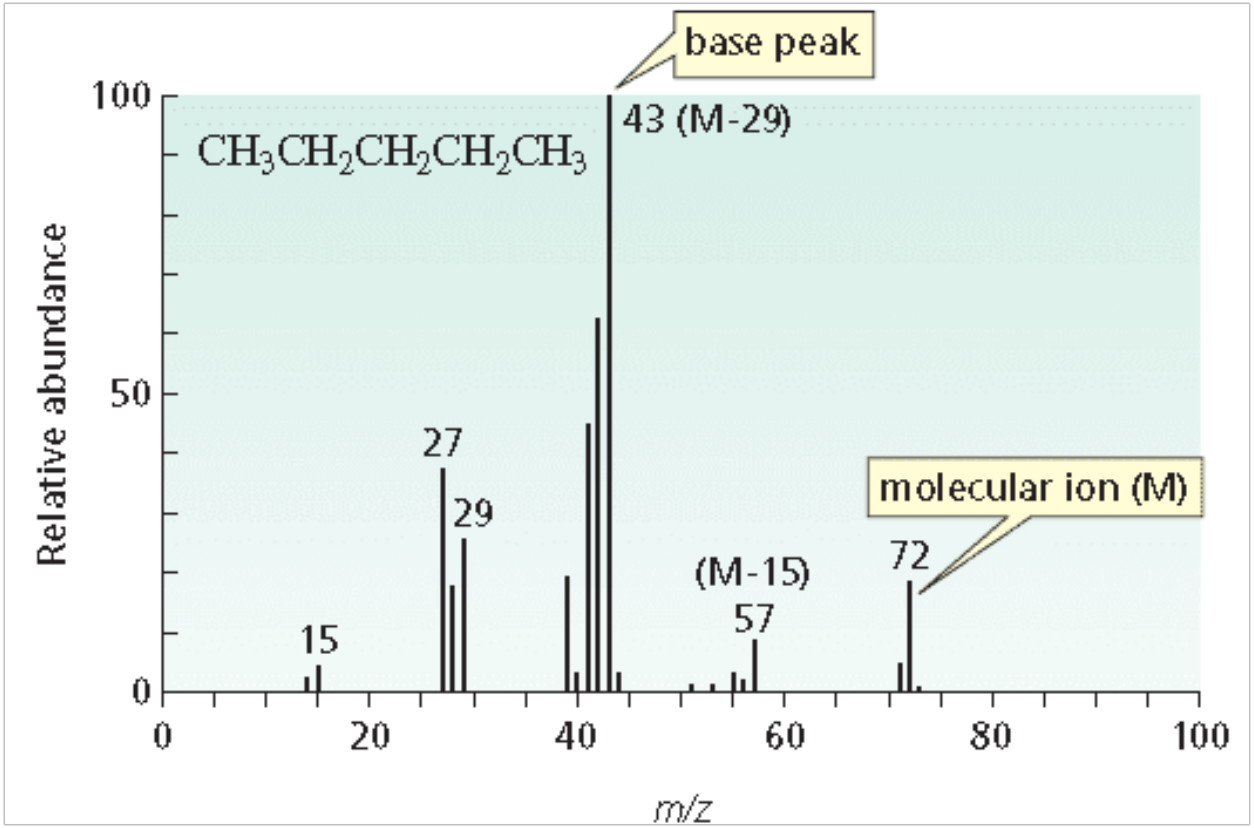
The molecular ion appears at m/z 121, indicating an odd number of nitrogen atoms in the structure.



Base peak (100 % abundance)

The most abundant ion formed in ionization chamber gives tallest peak in mass spectrum is called as base peak.

- Most stabilized ion in ionization chamber.
- Most intense peak in mass spectrum.
- Base peak indicated as 100 % abundance.
- Relative abundance of other peak reported from % of base peak.
- Base peak sometimes molecular ion peak (M^+).



Isotopic peak

- ✓ **Mass spectrometers are capable of separating and detecting individual ions even those that only differ by a single atomic mass unit (Isotopes).**
- ✓ **As a result molecules containing different isotopes can be distinguished.**

	Relative Intensities			
Halogen	*M*	*M* + 2	*M* + 4	*M* + 6
Br	100	97.7		
Br_2	100	195.0	95.4	
Br_3	100	293.0	286.0	93.4
Cl	100	32.6		
Cl_2	100	65.3	10.6	
Cl_3	100	97.8	31.9	3.47
BrCl	100	130.0	31.9	
Br_2Cl	100	228.0	159.0	31.2
Cl_2Br	100	163.0	74.4	10.4

M+ : Mass of Molecular ion Peak

M+1: Mass is One unit higher than M+

M+2: Mass is Two unit higher than M+

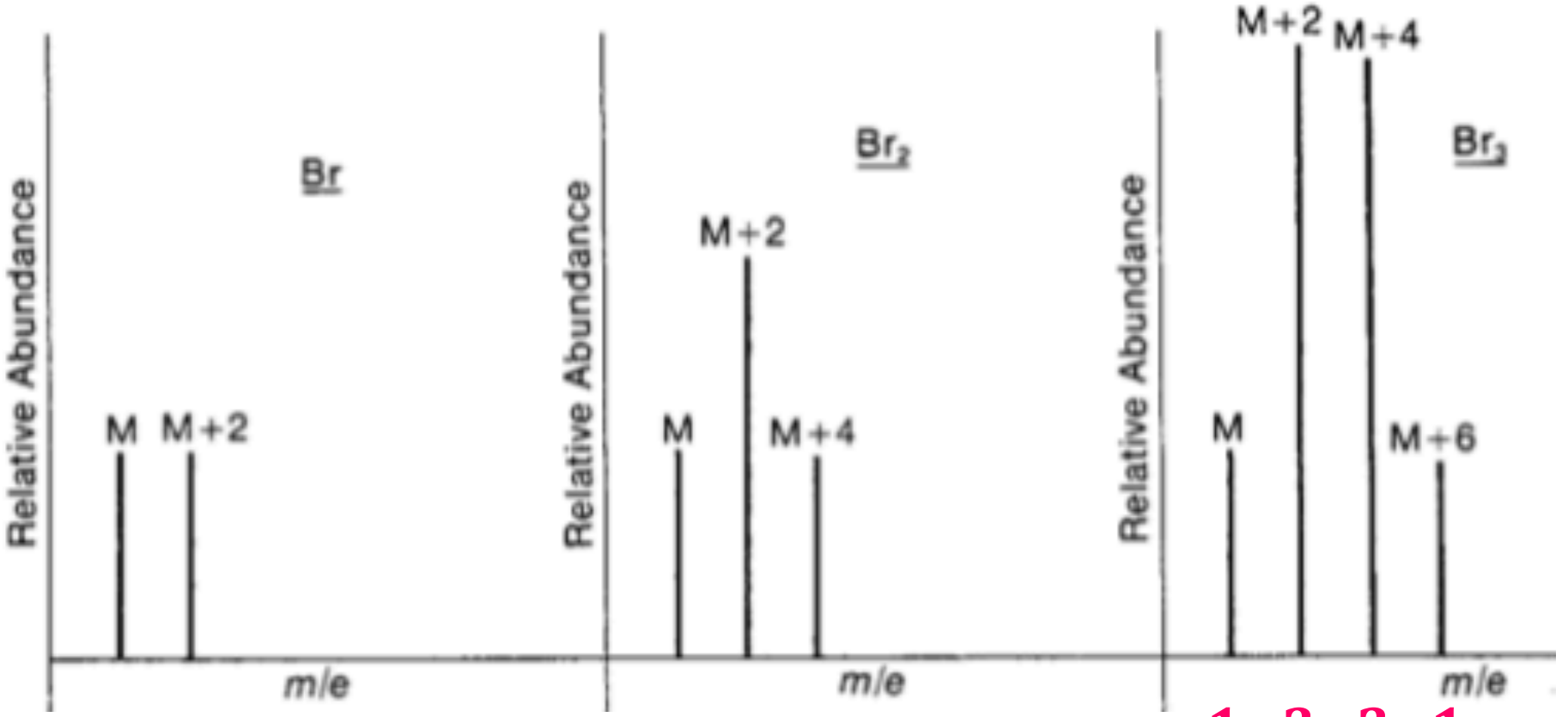
M+4: Mass is Four unit higher than M+

M+6: Mass is Six unit higher than M+

- **The intensity ratios in the isotope patterns are due to the natural abundance of the isotopes.**
- **"M+1" peaks are seen due the the presence of ^{13}C in the sample.**
- **This is most apparent when atoms such as bromine or chlorine are present (^{79}Br : ^{81}Br, intensity 1:1 and ^{35}Cl : ^{37}Cl, intensity 3:1) where peaks at "M" and "M+2" are obtained.**

Isotopic peaks of Bromine

The intensity ratios in the isotopic patterns are due to the natural abundance of the isotopes.

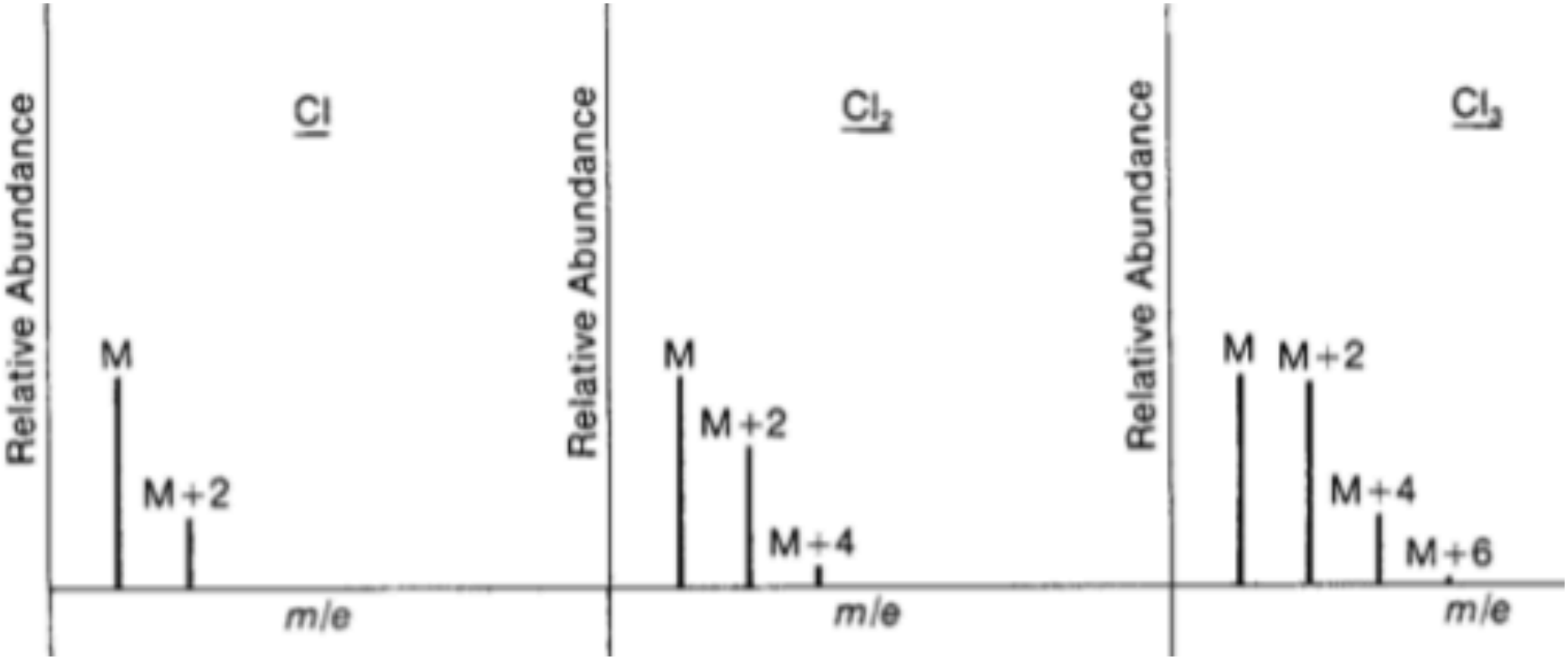


Intensity **1 : 1** **1 : 2 : 1** **1 : 3 : 3 : 1**

Abundance **100:97.7** **100 : 195 : 95.4** **100: 293.0 : 286.0 : 93.4**

Isotopic peaks of Chlorine

The intensity ratios in the isotopic patterns are due to the natural abundance of the isotopes.



Intensity 3 : 1 | 9 : 6 : 1 | : : :

Abundance 100 : 32.6 | 100 : 65.3 : 10.6 | 100: 97.8 : 31.9 : 3.47

Calculation of intensity of Isotopic peak

[1] The formula for calculating the intensity of the $M+1$ peak is as follows.

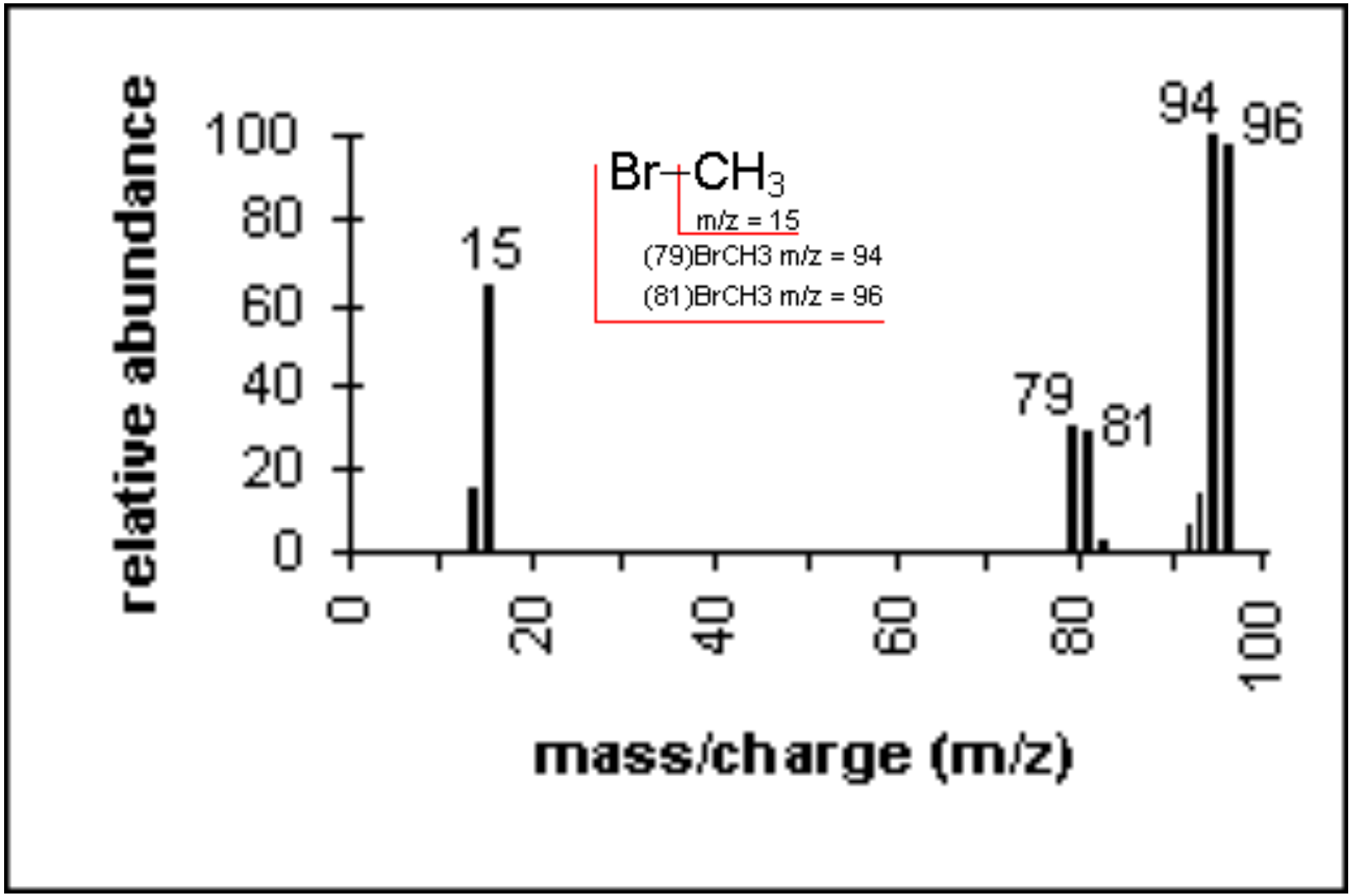
$$\%(M+1) = 100\,\frac{(M+1)}{M} = 1.1 \times \text{number of C atoms} + 0.016 \times \text{number of H atoms} + 0.38 \times \text{number of N atoms} + \ldots$$

The formula for calculating the approximate intensity of the $M+2$ peak is as follows.

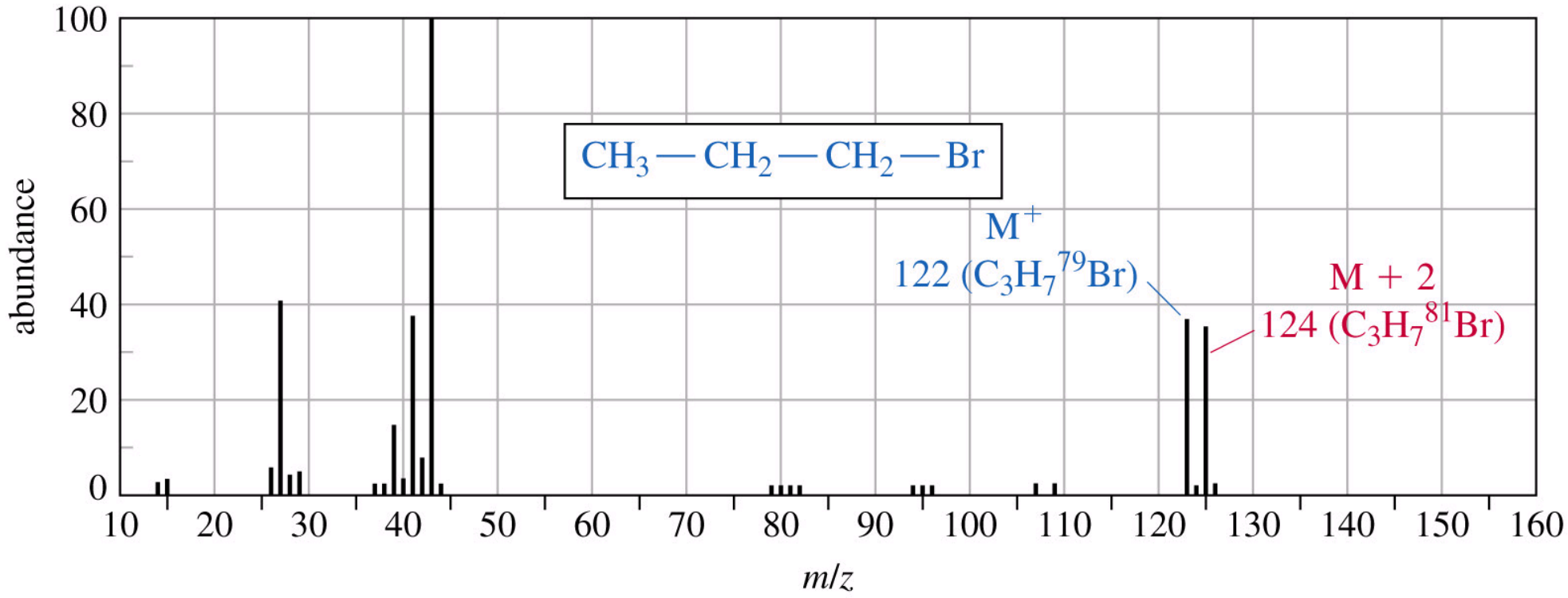
$$\%(M+2) = 100\,\frac{(M+2)}{M} \cong \frac{(1.1 \times \text{number of C atoms})^2}{200} + \frac{(0.016 \times \text{number of H atoms})^2}{200} + 0.20 \times \text{number of O atoms}$$

Compound	Molecular Mass	Relative Intensities		
		M	$M+1$	$M+2$
C_3H_6	42	100	3.34	0.05
CH_2N_2	42	100	1.87	0.01

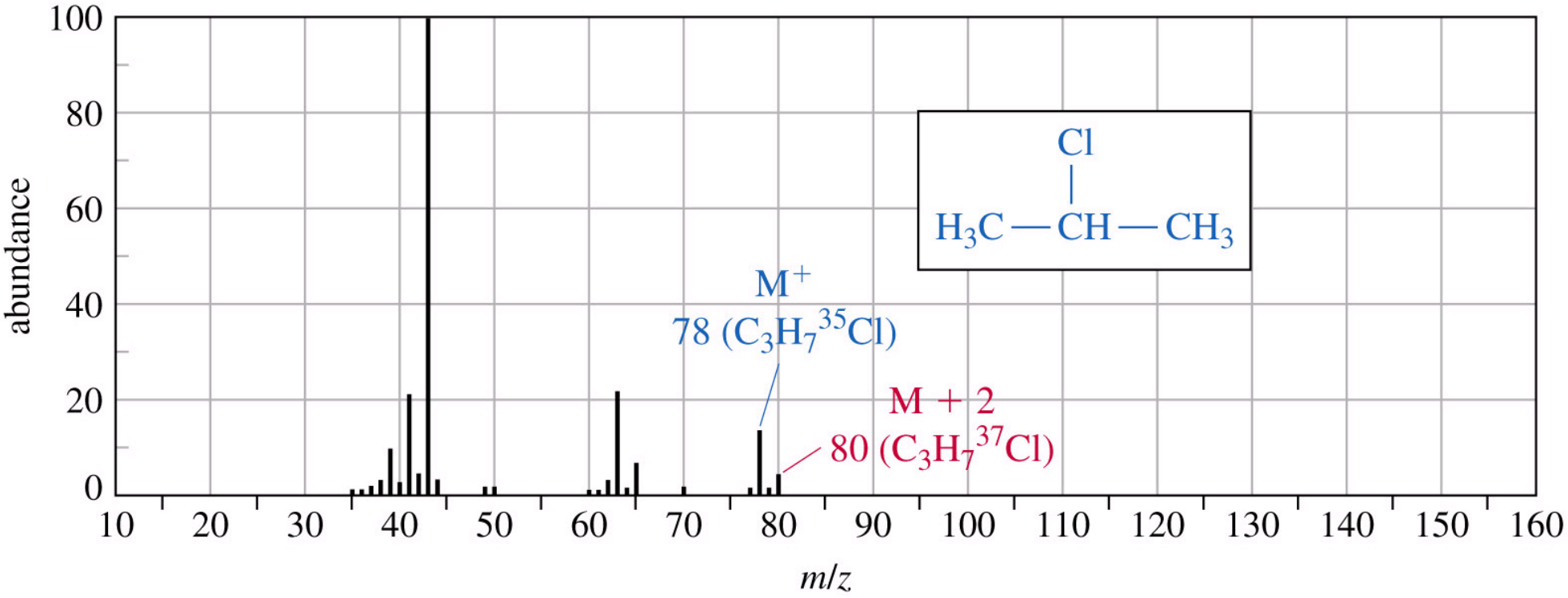
Bromomethane



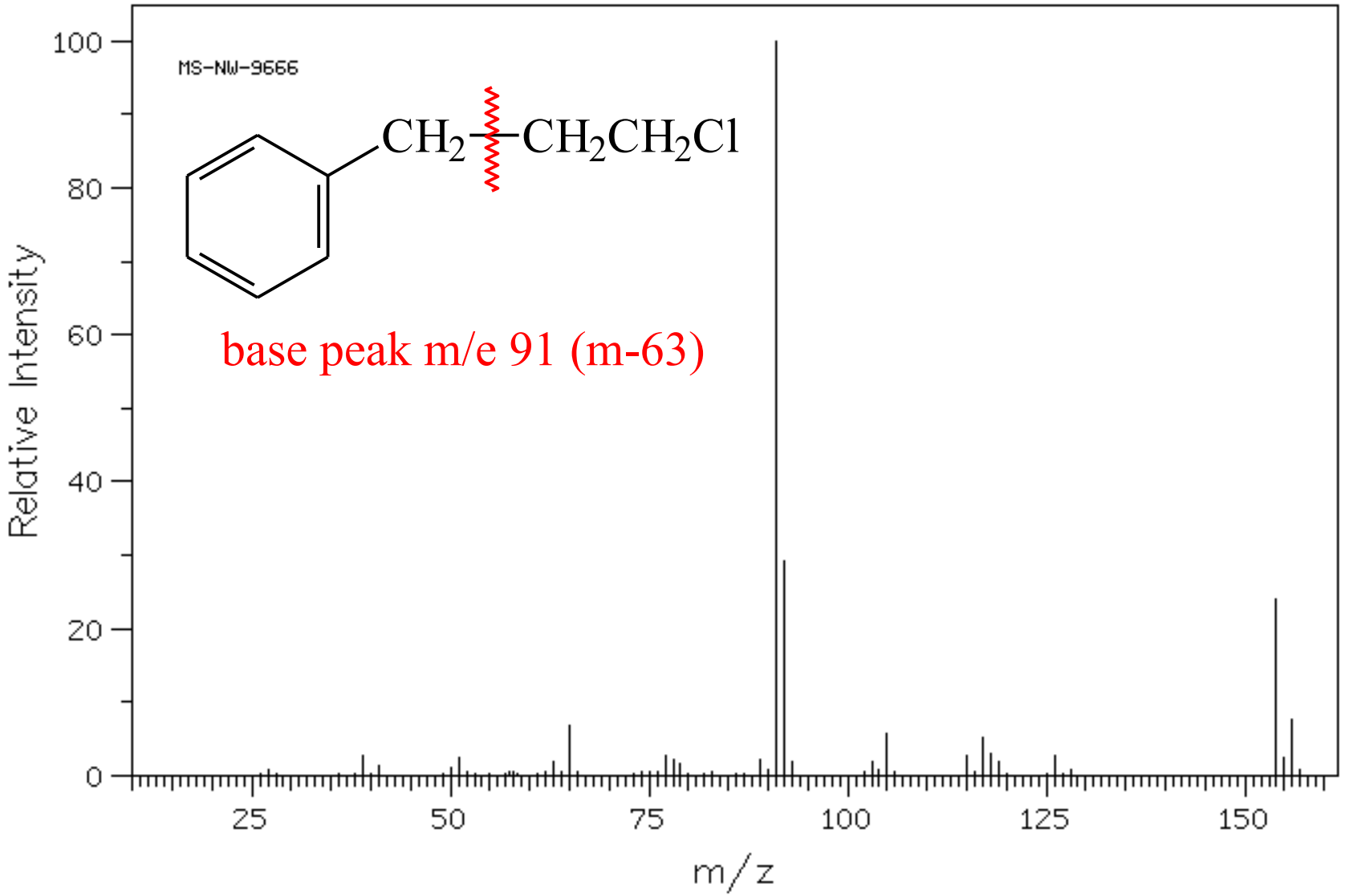
1-Bromopropane



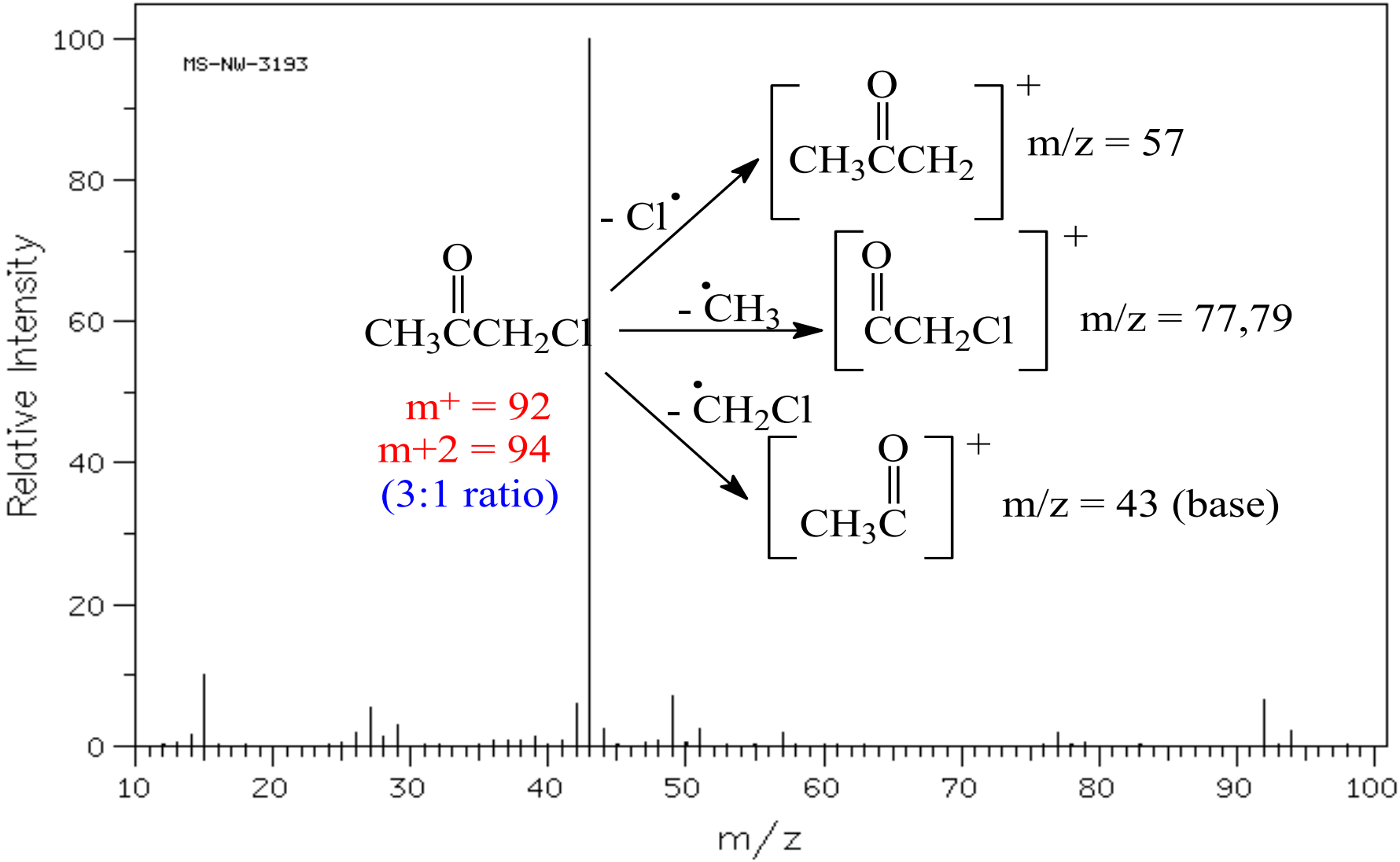
2-Chloropropane



(3-Chloropropyl)benzene



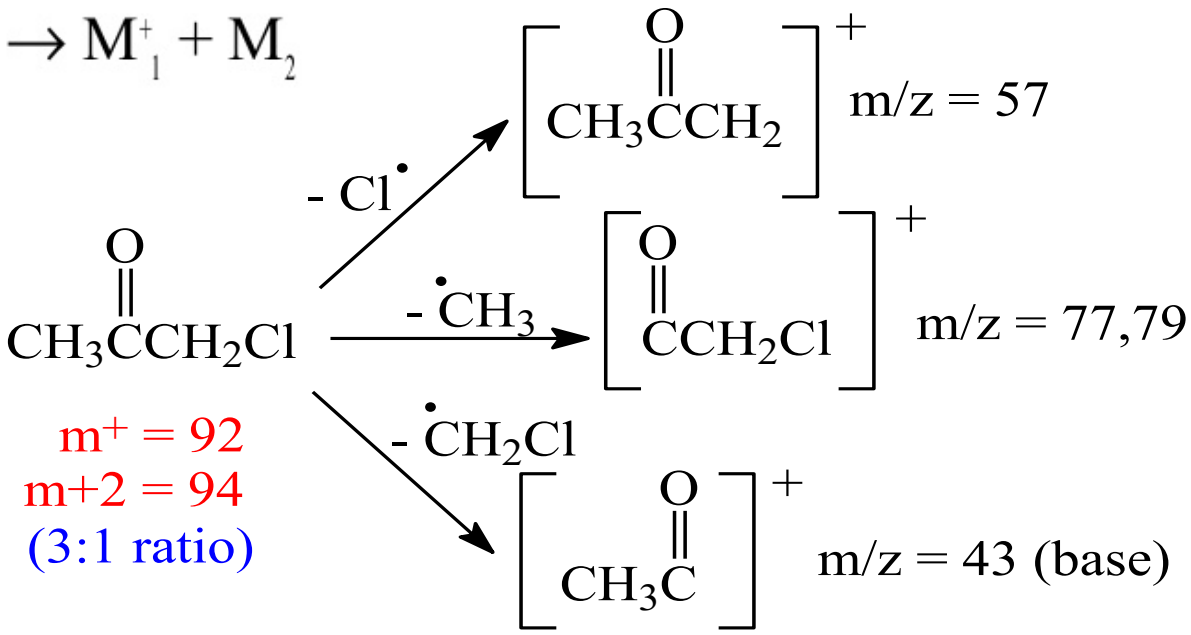
Chloroacetone



2. *Fragment ions:*

When the energy is given to Molecular ion during electron impact, further cleavage takes place and ions of lower mass number known as Fragment ions are produced.

$$M^{+\bullet} \rightarrow M_1^{+} + M_2$$

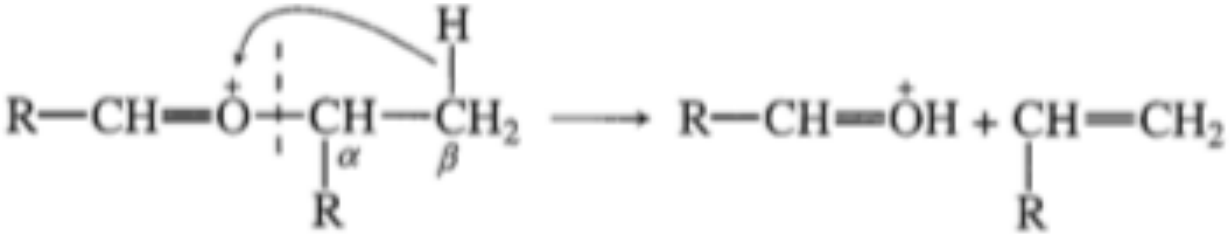


Typical EI-MS gives both molecular ion and fragment ions

❖When the energy of electron beam is increased between 50-70ev, these molecular ions acquire a high excitation resulting in their break down into various fragments. This process is called " Fragmentation process".

3.Rearrangement ions:

Rearrangement ions are the fragments whose origin cannot be described by simple cleavage of bonds in the parent ion, but are result of intramolecular atomic rearrangement during fragmentation. 25



These are probably due to recombination of fragment ions and known as rearrangement peaks.

Ex: Prominent peak in spectrum of diethyl ether occurs at m/e 31. This is due to the ions CH_3O^+, which is formed by rearrangement of $C_2H_5O^+$ ions.

4. Multi charged ions:

Some times ions may also exist with two or three charges instead of usual single charge in the mass spectrum. These are known as doubly or triply charged ions. They are created as follows:

$$M^{+\circ} + e^- \rightarrow M^{++} + 3e^-$$

But under normal operating conditions, most of the ions produced are single charged. The doubly or triply charged ions are recorded at a half or one third of the m/e value of the single charged ions.

Formation of these multiple charged ions is more common in hetero aromatic compounds. They are also common in inorganic mass spectrum. Gases such as CO, N_2,CO_2 and O_2 have measurable peaks corresponding to CO^{+2},N^{+2},and O^{+2}.

5. Negative ions:

The positive ions predominate in electronic impact ionization because of greater stability. The Negative ions are not very useful in structural determinations. The formation of Negative ions is very rare but these can be produced in three ways:

1. $AB + e \rightarrow A^+ + B^-$

2. $AB + e \rightarrow AB^-$

3. $AB + e \rightarrow A^+ + B^- + e^-$

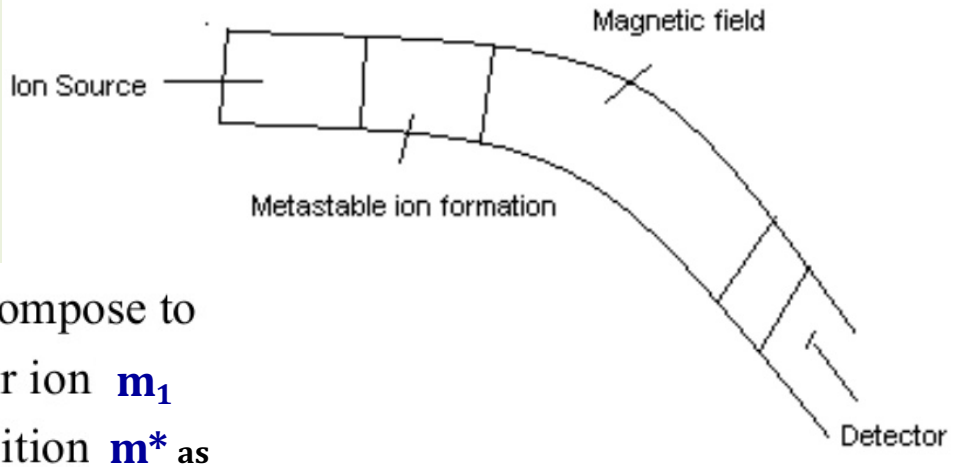
6. *Metastable Ions:* Fragment of a parent ion will give rise to a new ion (daughter) plus either a neutral molecule or a radical.

$M^+ \longrightarrow m_1 + m^*$ (Daughter ion of fragment ion)

The ion appears at an m/z ratio that depends on its own mass as well as the mass of the original ion from which it formed. Such ion is called metastable ion peak.

$$m^* = (m_1)^2/M^+$$

m^*= Apparent mass of metastable ion.
m_1= Mass of new fragment ion.
M^+= Mass of original ion from which fragment is formed.



An intermediate situation is possible; M^+ may decompose to m_1 while being accelerated. The resultant daughter ion m_1 will not be recorded at either M^+ or m_1 but at a position m^* as a rather broad, poorly focused peak. Such an ion is called a metastable ion.

Nature of Metastable ion:

- Lower kinetic energy.
- Much broader than normal ion.
- Relatively low abundance.
- Arise by flight down through ions rather than IC.
- Frequently occurs at non integral m/z values.
- Linear peak on the mass spectrum.

Molecular ions formed in the ionization chamber do one of the following things:

1. Either they decompose completely and very rapidly in the ion source and never reach the collector (as in case of highly branched molecular ions with life times less than 10^{-5} seconds).
2. Or else they survive long enough to reach the collector and be recorded there (life times longer than 10^{-5}).

Advantages/ Importance/ Significance:

1. It gives definitely/responsive link of two fragment ions together.
2. To prove a proposed fragmentation pattern.
3. Gives more correct structural proof problem.
4. Always distance below than fragment ion on the mass scale which is lies below than parent ion.
5. Distance is approximately similar to the distance that fragment ion lies below parent ion.

Calculations of position of metastable ion peak

$$m^* = (m_1)^2/M^+$$

m^*= Apparent mass of metastable ion.
m_1= Mass of new fragment ion.
M^+= Mass of original ion from which fragment is formed.

Examples:

1. Toluene (m/z): 92, 91, 65 (46.64).

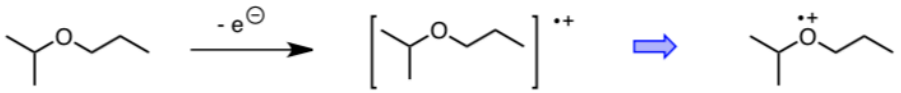
2. p-Methoxyaniline (m/z): 128, 108 (94.8), 80 (59.2)

Generation of Molecular ion (M^+)

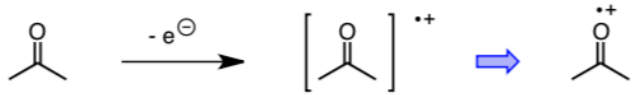
Initial Loss of Electron

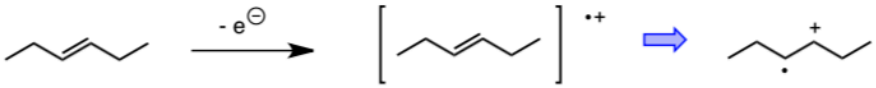
non-bonding orbital > π-orbital > σ-orbital

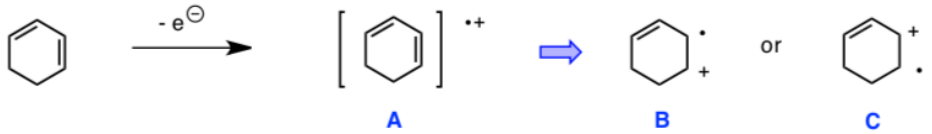
from a non-bonding orbital



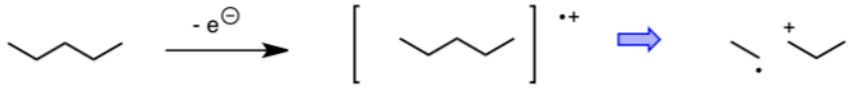
from a π orbital





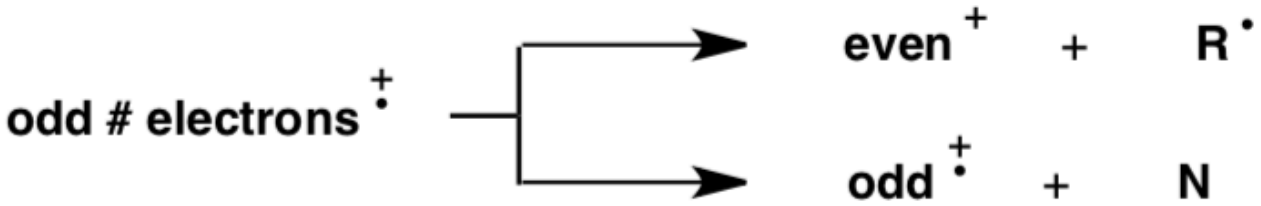


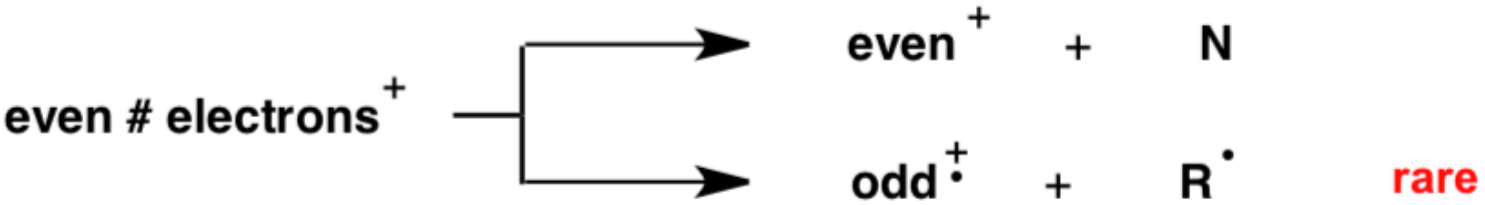
from a σ orbital



Generation of Molecular ion (M^+)

Electron Count & Fragmentation





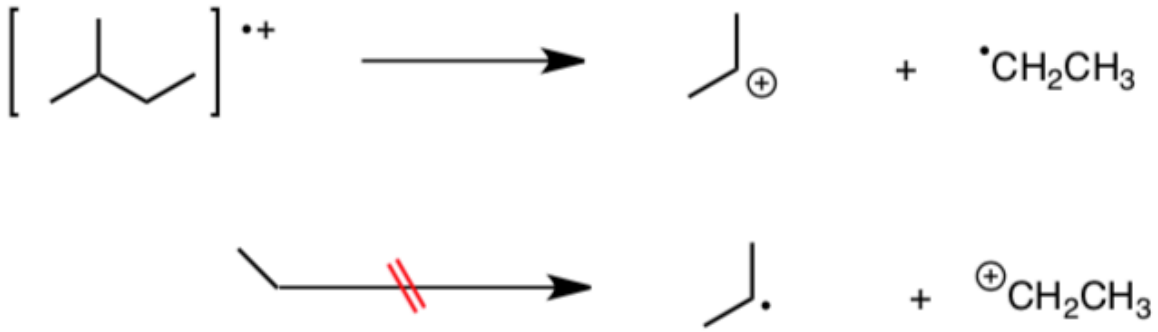
General Rules for Fragmentation

Stevenson's Rule

- The most probable fragmentation is the one that leaves the positive charge on the fragment with the lowest ionization energy
  - fragmentation processes that lead to the formation of more stable ions are favored over processes that lead to less stable ions

- Cleavages that lead to formation of more stable carbocations are favored
  - cation stability is more important than radical stability

- When loss of more than one radical is possible, the largest alkyl radical will be lost preferentially



General Rules for Fragmentation

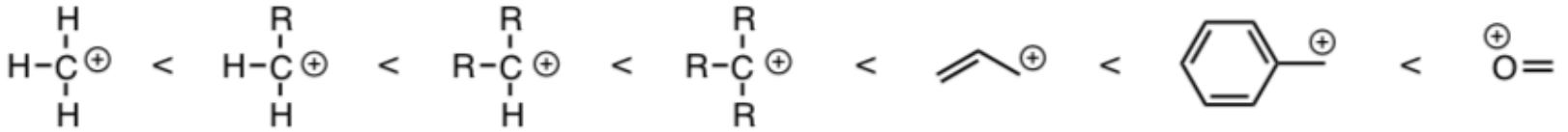
Factors that Impact Fragmentation

- **Energetic factors**

  - relative bond strengths (BDE)

bond:	C–Cl	C–Br	C–I	
BDE:	81	68	51	kcal mol^{-1}

  - stability of the resulting cations or radical ions



  - stability of the resulting radicals or neutrals

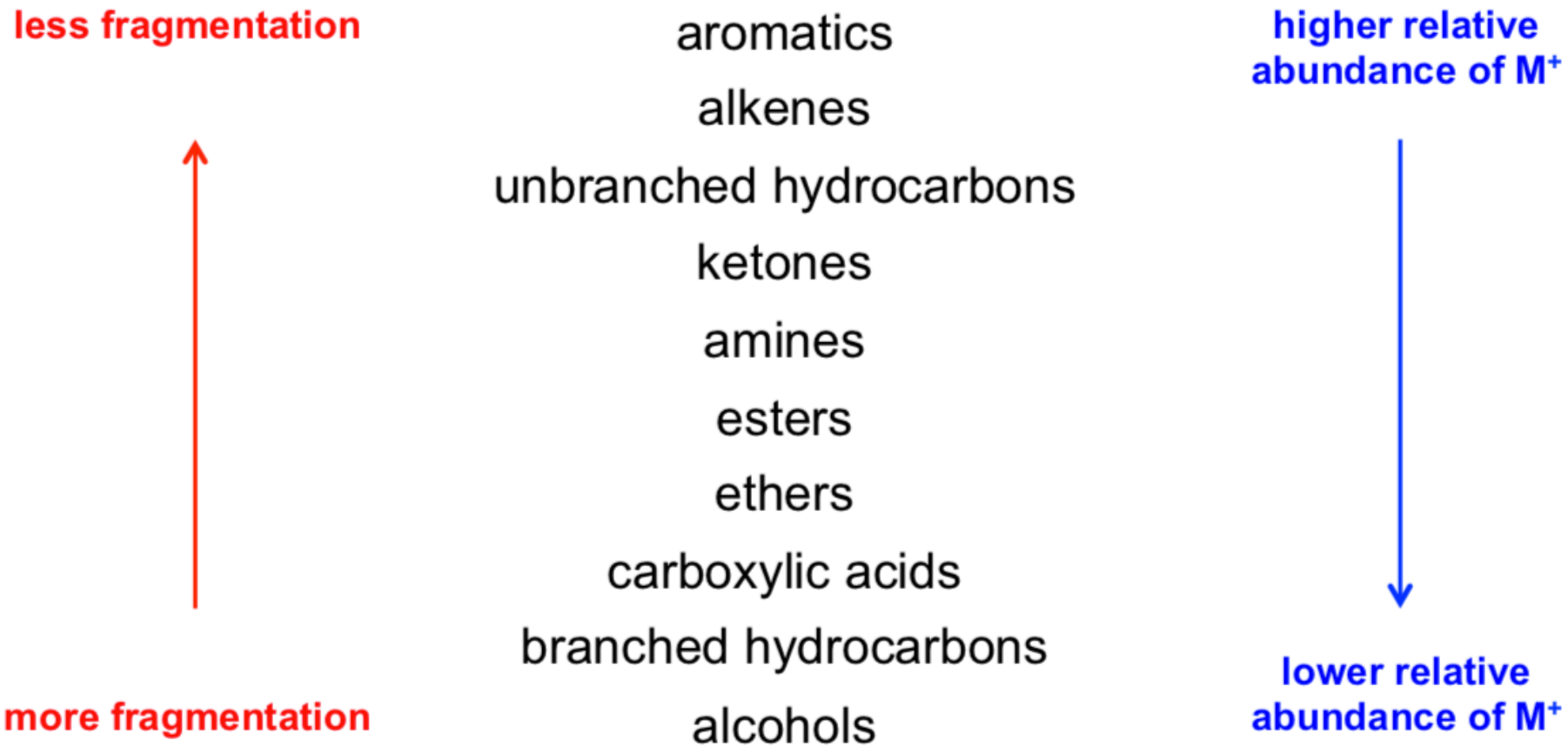
    radical stability as above

- **Kinetic factors**

  - availability of a favorable cyclic transition state

General Rules for Fragmentation

Ease of Fragmentation



Some common and reasonable losses from molecular ions (M^+)

M - 1	loss of hydrogen radical	M- $\cdot H$
M - 15	loss of methyl radical	M- $\cdot CH_3$
M - 29	loss of ethyl radical	M- $\cdot CH_2CH_3$
M - 31	loss of methoxyl radical	M- $\cdot OCH_3$
M - 43	loss of propyl radical	M- $\cdot CH_2CH_2CH_3$
M - 45	loss of ethoxyl radical	M- $\cdot OCH_2CH_3$
M - 57	loss of butyl radical	M- $\cdot CH_2CH_2CH_2CH_3$
M - 2	loss of hydrogen	M- H_2
M - 18	loss of water	M- H_2O
M - 28	loss of CO or ethylene	M- CO or M - CH_2H_4
M - 32	loss of methanol	M- CH_3OH
M - 44	loss of CO_2	M- CO_2
M - 60	loss of acetic acid	M- CH_3CO_2H
M - 90	loss of silanol: $HO\text{-}Si(CH_3)_3$	M- $HO - Si - (CH_3)_3$

Factors affecting Fragmentation

a) Energy of the molecular ion and the fragments formed from it

b) Stability of the bonds in the ions

c) For rearrangements: steric factors.

It is easier to move an H than a whole group

d) Stability of the formed ions or neutral particles

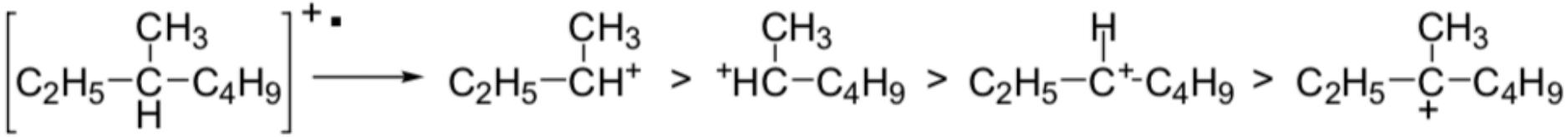
resonance stabilization such as in an acylium ion

Stevenson's Rule:

Upon dissociation of $AB^{+\cdot} \rightarrow A^+ + B^\cdot$ or $A^\cdot + B^+$

A^+ will be formed if it has the lower ionization energy

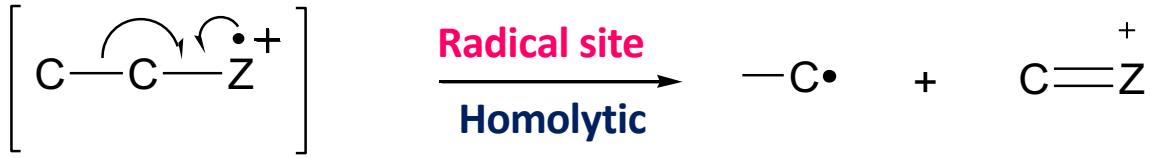
In branched radical cations, the largest group is preferentially lost

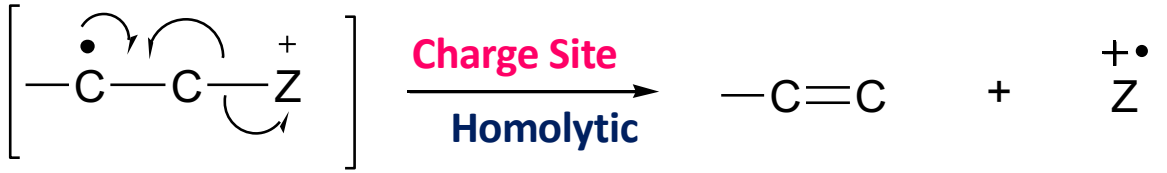


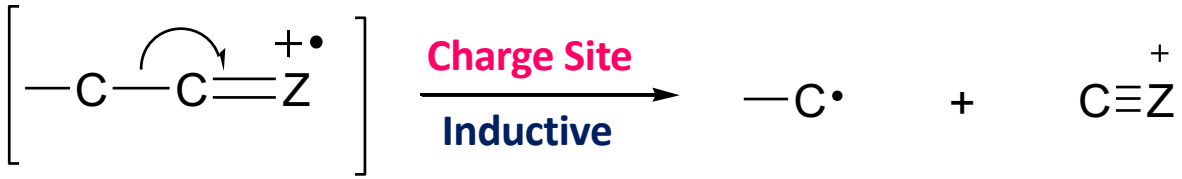
Modes of Fragmentation

1. One bond cleavages: (α-cleavage)

α-cleavage: Homolytic /Heterolytic (Inductive)
Radical site/Charge Site



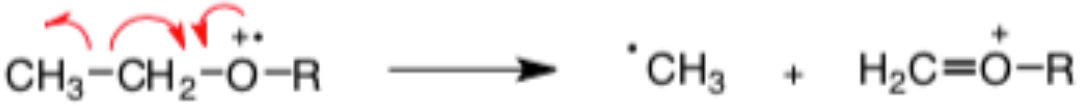




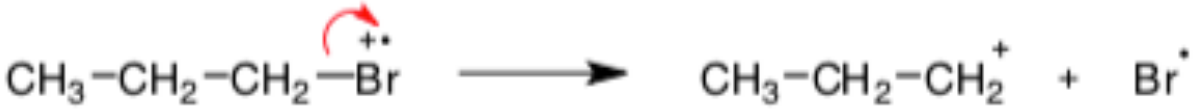
Modes of Fragmentation

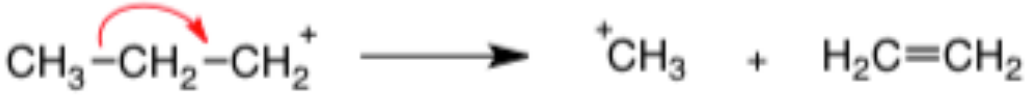
1. One bond cleavages: (α-cleavage)

homolytic cleavage



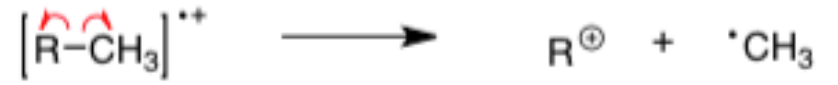
heterolytic cleavage

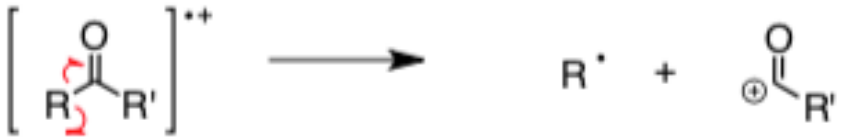




Modes of Fragmentation

1. One bond cleavages: (α-cleavage)



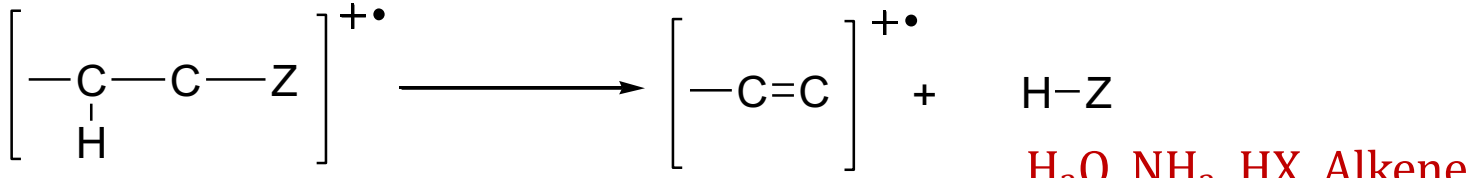


R, R' = H, alkyl, aryl
X = halogen, OR, SR, NR , etc.

Modes of Fragmentation

2. Two bond cleavages/rearrangements:

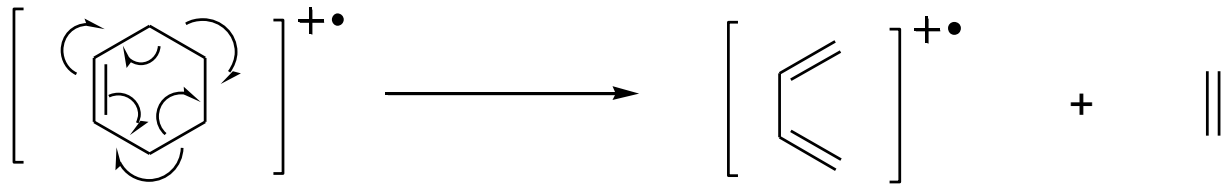
a. Elimination of a vicinal H and heteroatom:



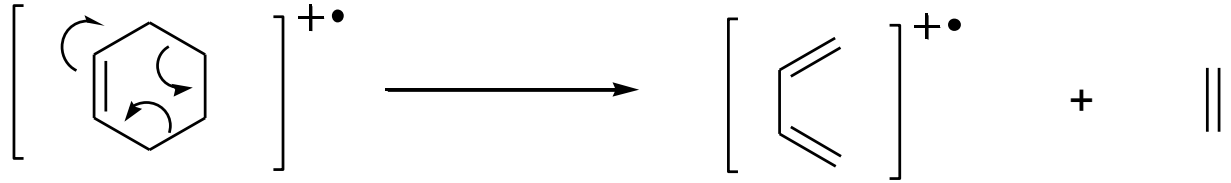
H_2O, NH_3, HX, Alkene

b. Retro-Diels-Alder reaction:

Full mechanism



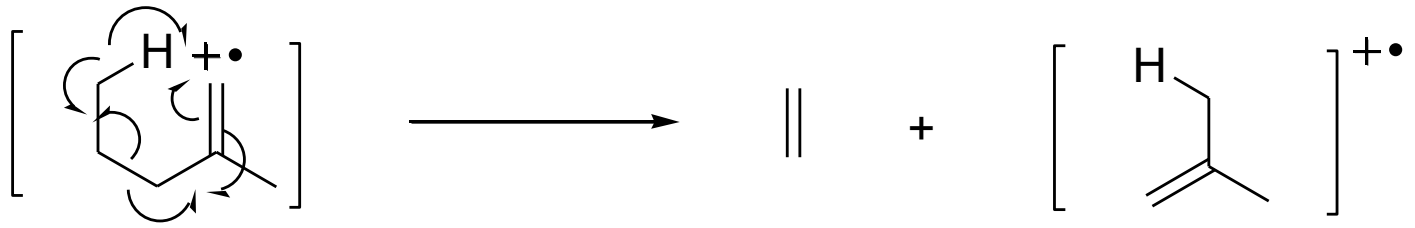
Abbreviated:



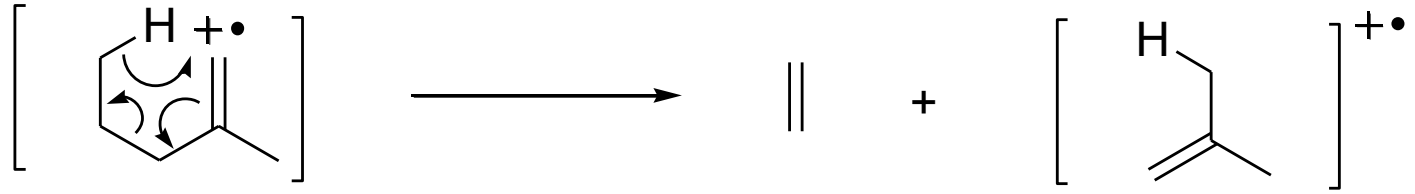
Modes of Fragmentation

c. McLafferty Rearrangement:

Full mechanism



Abbreviated:



Alkene, Aromatic, carbonyl, imine, Nitrile compounds

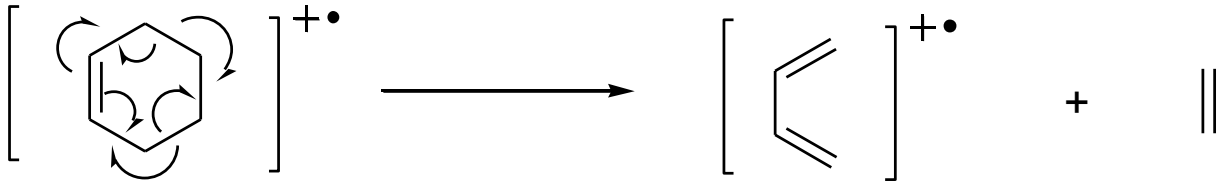
3. **Other types of fragmentation are less common, but in specific cases are dominant processes.**

   Include: a) Fragmentations from rearrangement,
   b) Migrations,
   c) Fragmentation of fragments.

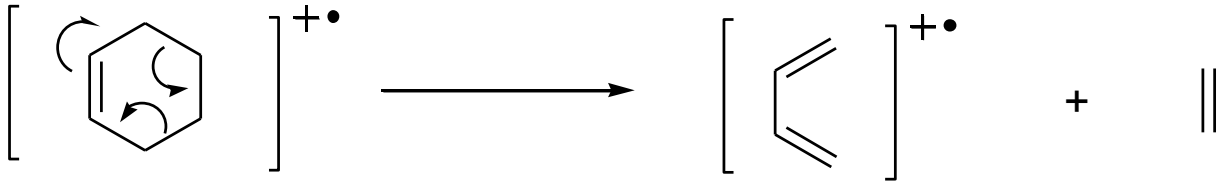
Retro-Diels-Alder reaction

The formation of conjugated-diolefinic **(diene)** and mono-olefinic **(ene)** fragments upon electron ionization (EI) of molecules containing a cyclohexene unit was recognized by Biemann (1) as formally analogous to the familiar retro-Diels–Alder (RDA) reaction (2). The term was recommended for use in the mass spectrometric nomenclature (3) and has been widely used. From the analytical point of view, RDA fragmentation appeared very promising for structure elucidation purposes, mainly for two reasons:

Full mechanism

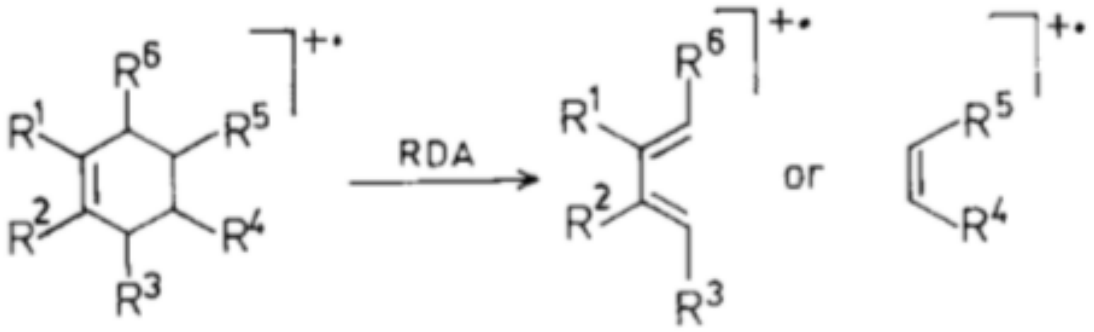


Abbreviated:

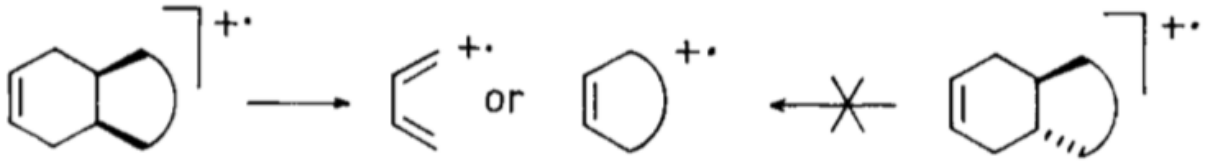


Retro-Diels-Alder reaction

(1) Specific formulation of a diene and an ene fragment ion from a substituted cyclohexene would enable reliable location of the double bond



(2) Provided orbital symmetry control operates, the presence or absence of an RDA reaction would distinguish between annulated isomers in bicyclic or polycyclic systems, for only cis isomers would undergo a symmetry-allowed, supra–supra fragmentation



Retro-Diels-Alder reaction

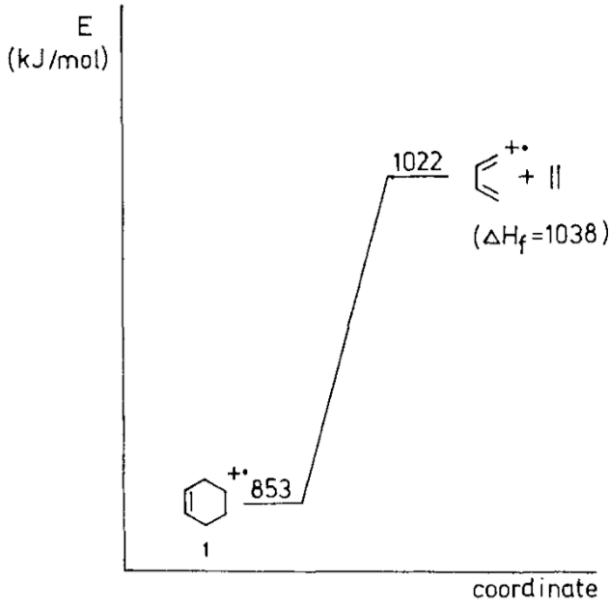
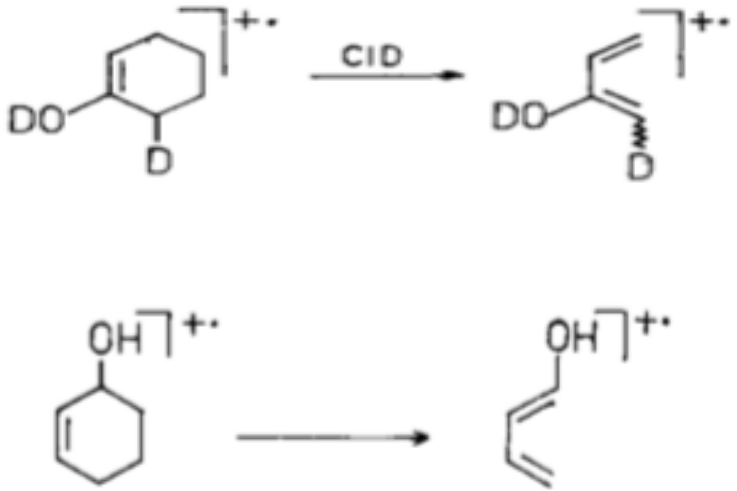
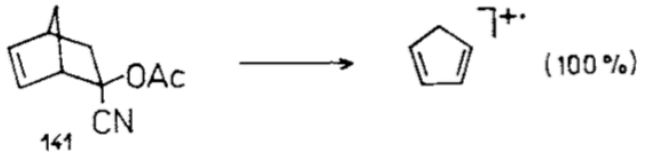
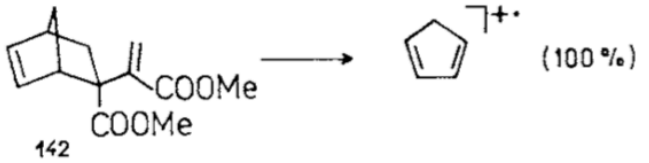
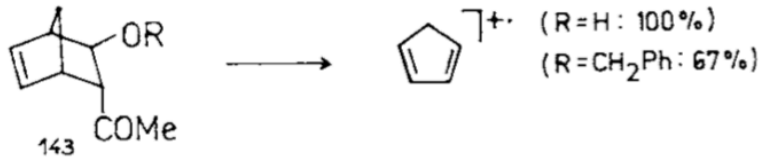


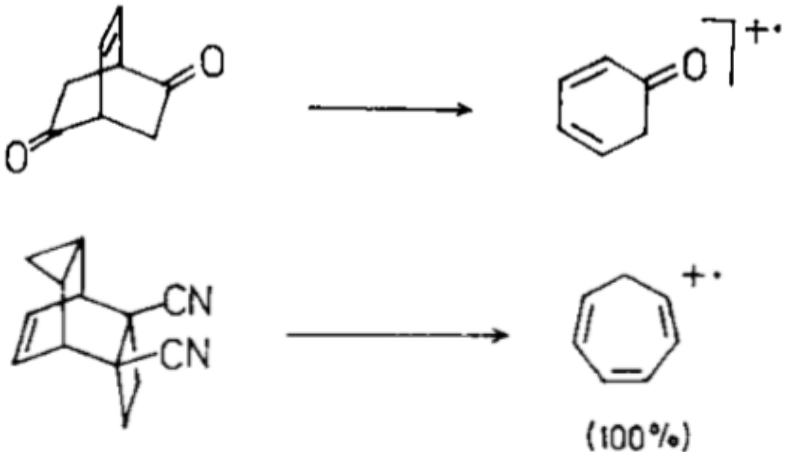
Figure 1. The schematic energy profile for the RDA reaction in ionized cyclohexene.







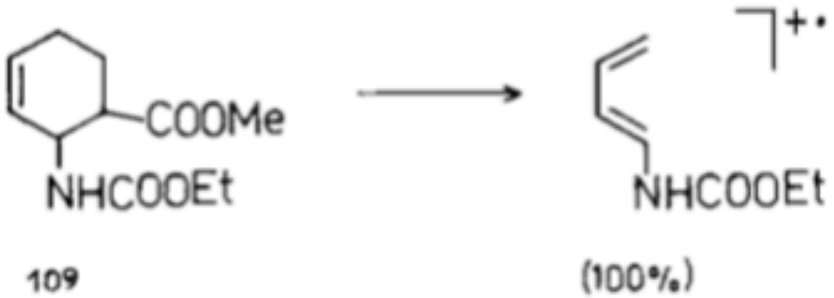




Retro-Diels-Alder reaction

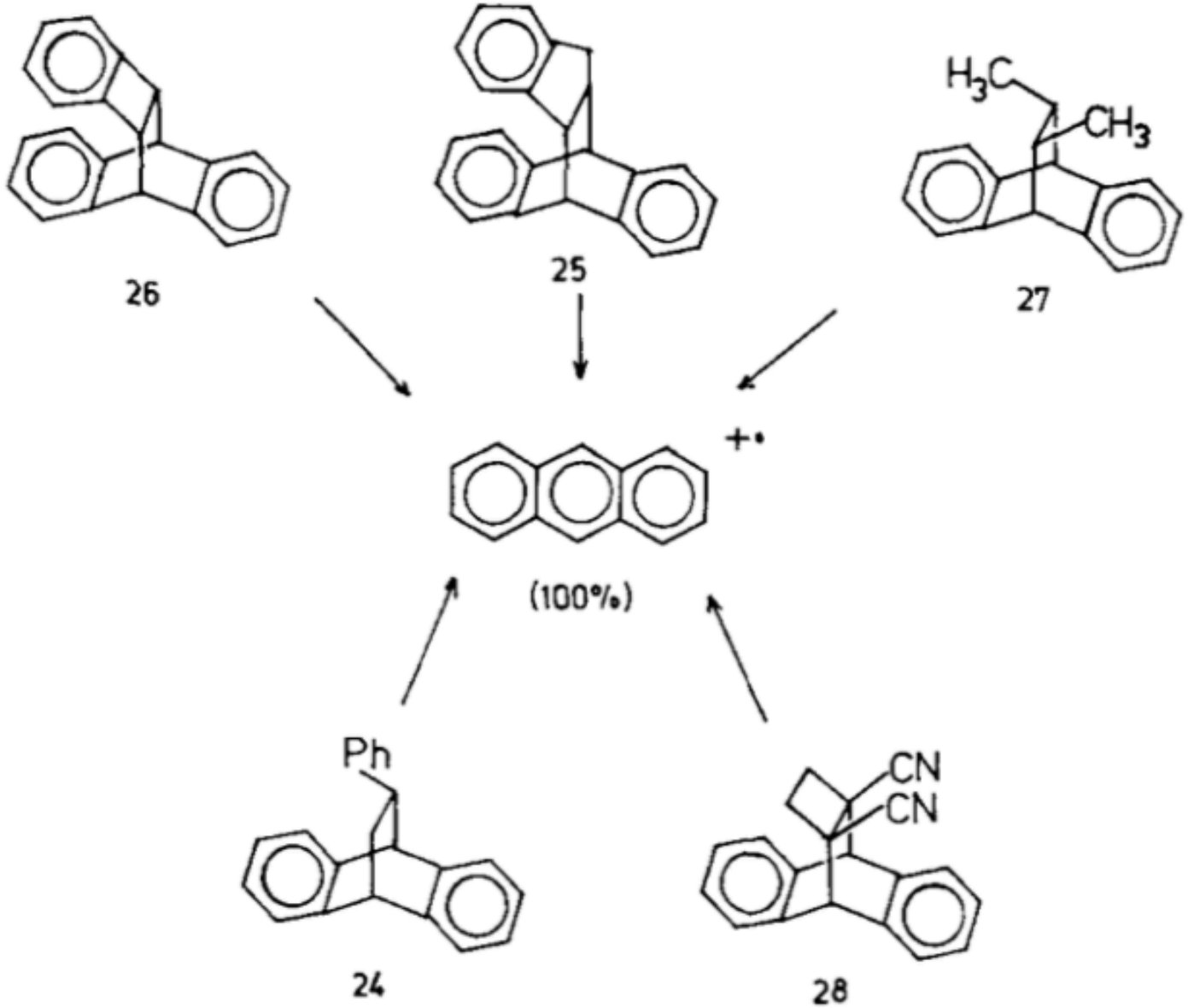
107

108

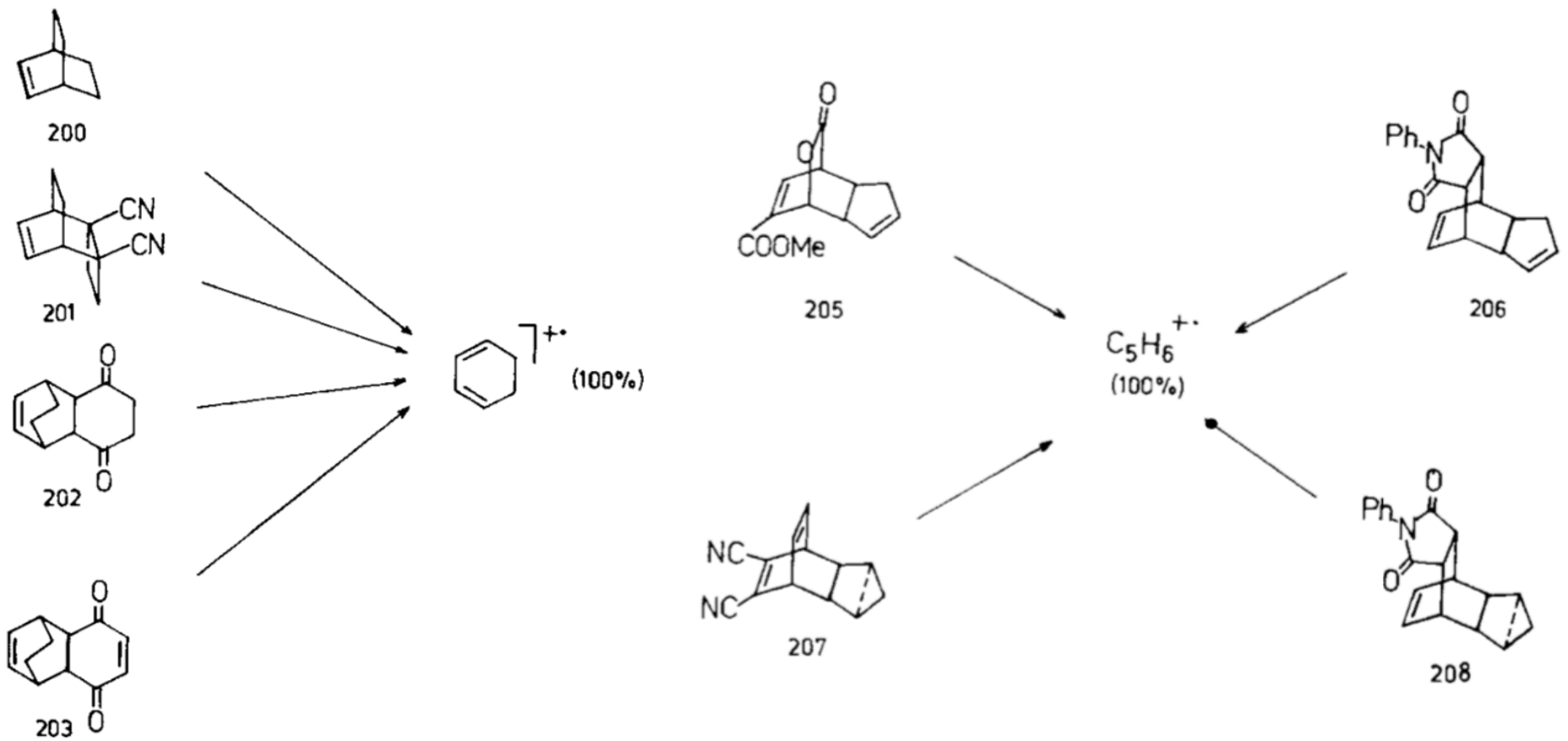


109

Retro-Diels-Alder reaction



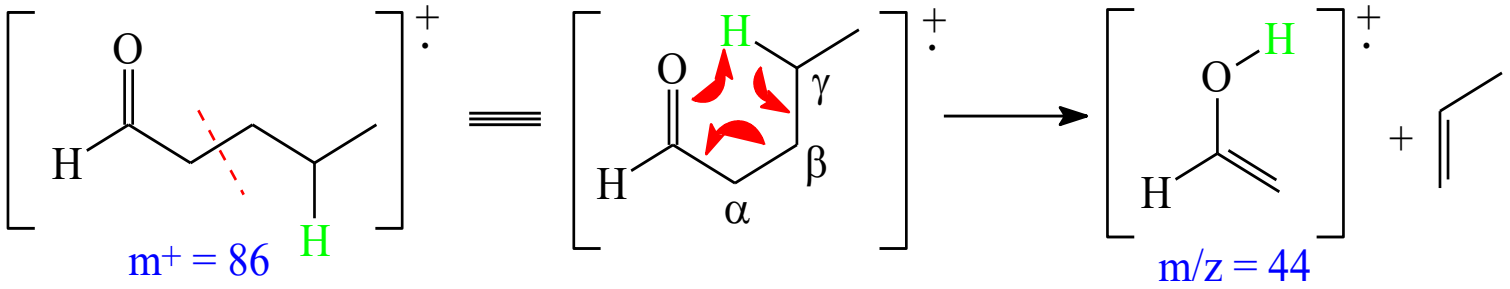
Retro-Diels-Alder reaction



McLafferty Rearrangement

Compounds containing hydrogen atom at position gamma to carbonyl group have been found to a relative intense peak. This is probably due to rearrangement and fragmentation is accompanied by the loss of neutral molecule. This rearrangement is known as McLafferty rearrangement.

First discovered by Fred McLafferty in 1956



The rearrangement results in the formation of charged enols and a neutral olefins.

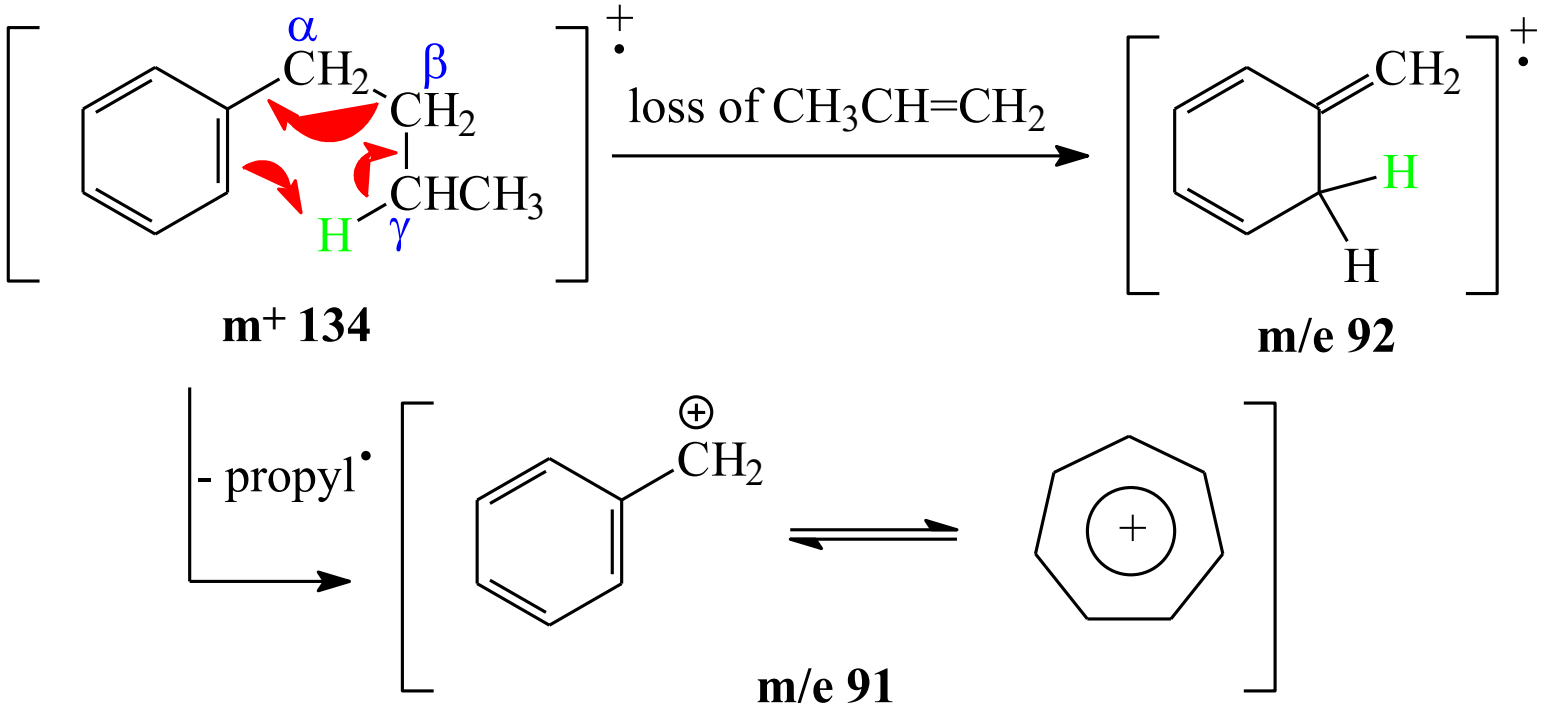
Such rearrangement involves the transfer of hydrogen from one part of the molecular ion to another via, preferably, a six-membered cyclic transition state. This process is favoured energetically because as many bonds are formed as are broken.

McLafferty Rearrangements in Alkyl Benzenes

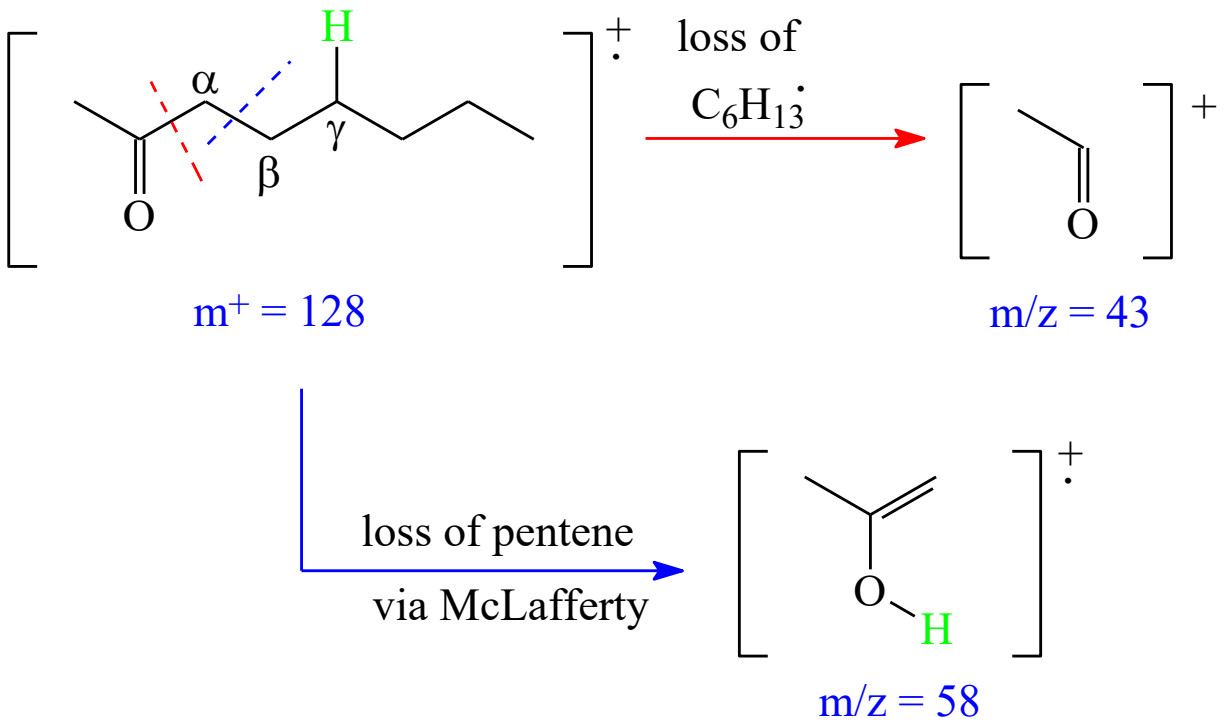
To undergo McLafferty rearrangement, a molecule must posses

a. An appropriately located heteroatom (ex.oxygen)
b. A double bond
c. An abstractable Hydrogen atom which is γ (gamma) to C=Osystem.

41



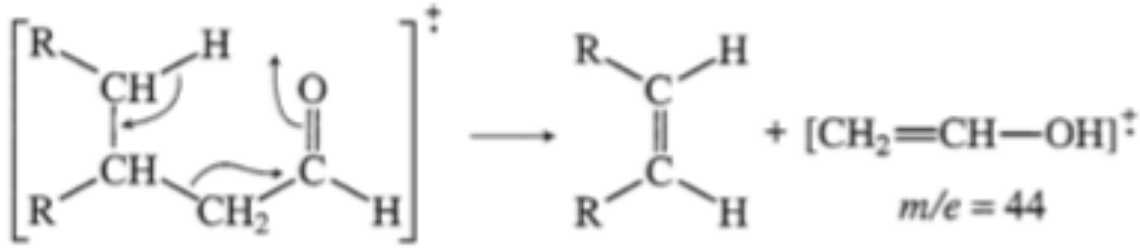
McLafferty Rearrangement



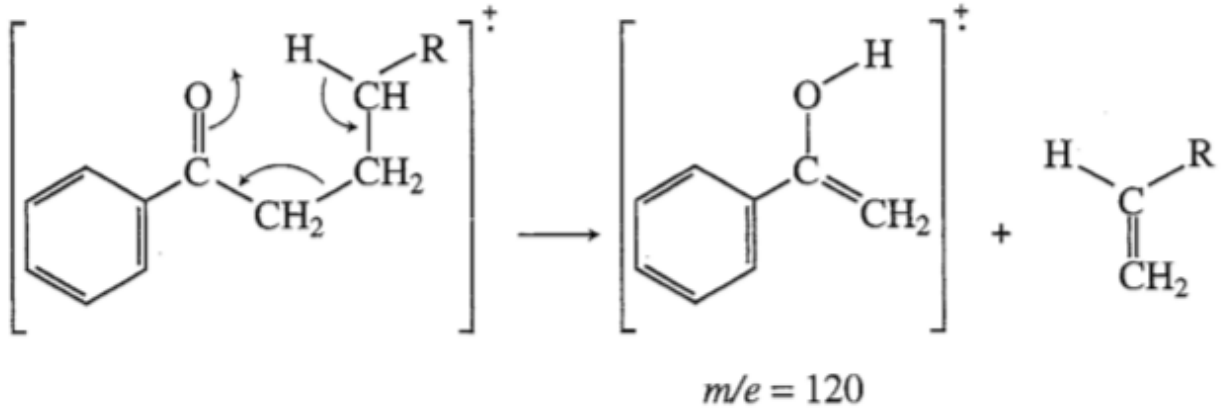
Fragmentation due to rearrangement of Molecular or Parent ion: Here cleavage of bonds in Molecular ion is due to the intramolecular atomic rearrangement. This leads to fragmentation whose origin cannot be described by simple cleavage of bonds. When fragments are accompanied by bond formation as well as bond for breaking, a rearrangement process is said to have occurred.

McLafferty Rearrangement

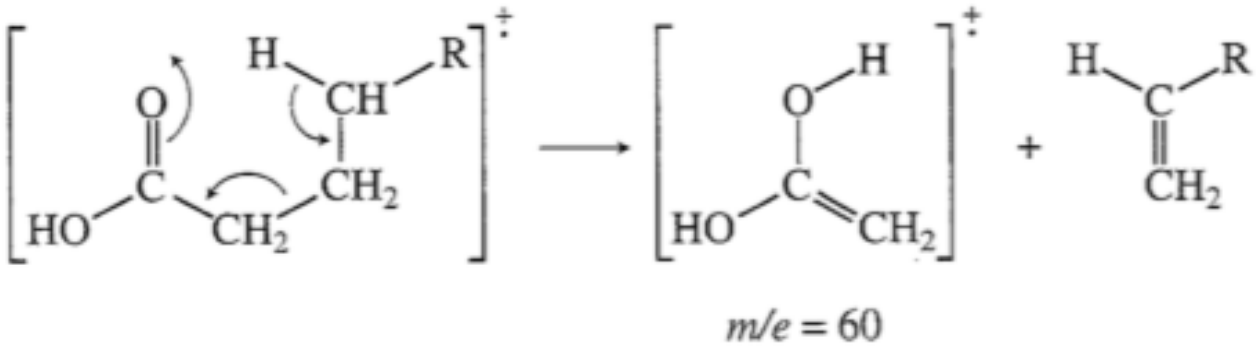
Aldehydes



Ketones

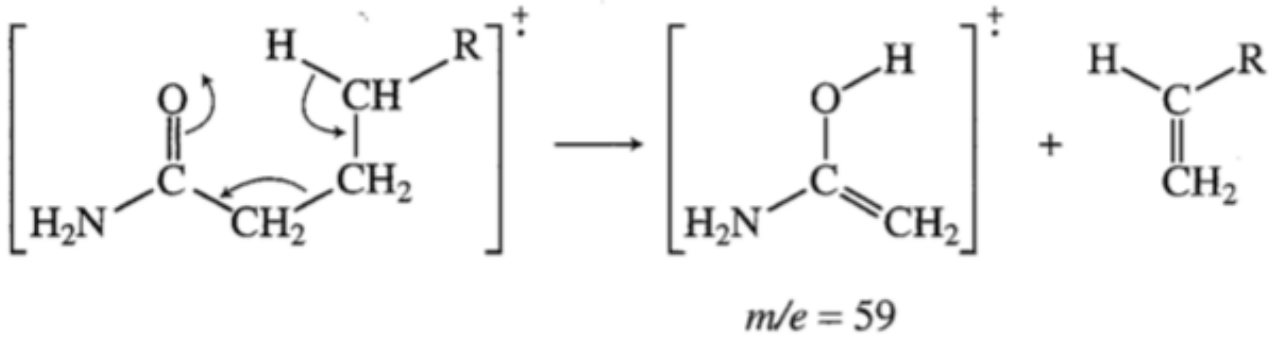


Acids

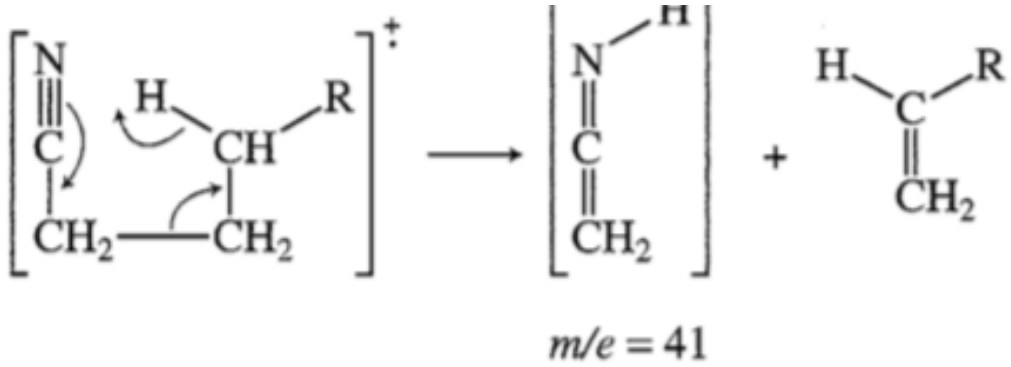


McLafferty Rearrangement

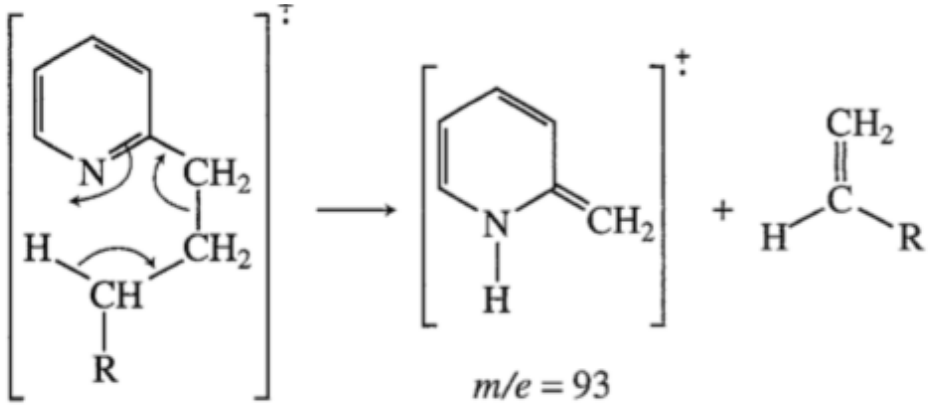
Amides



Nitriles

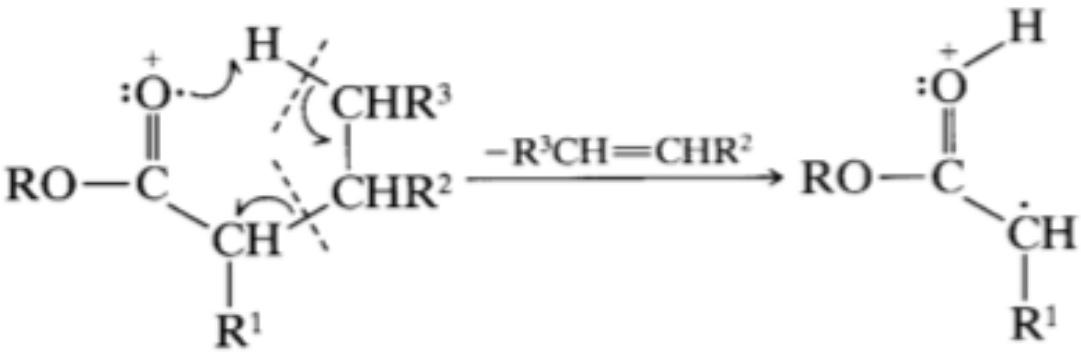


Pyridines

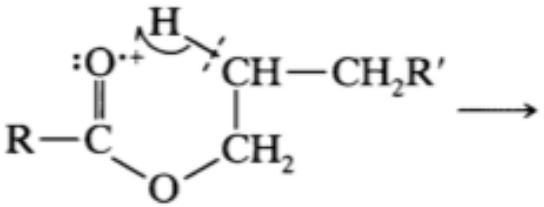


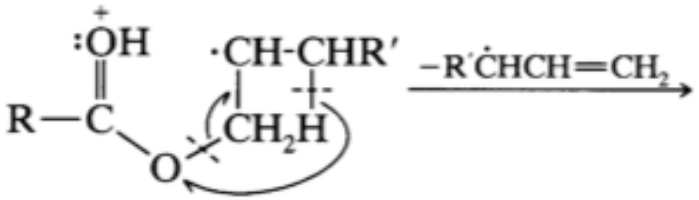
McLafferty Rearrangement

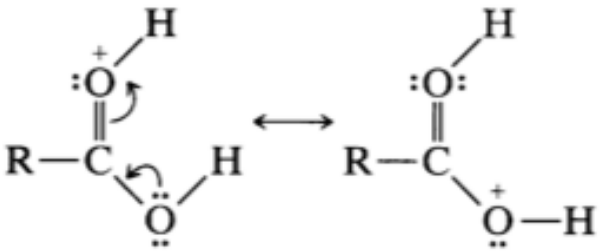
Esters



McLafferty rearrangement

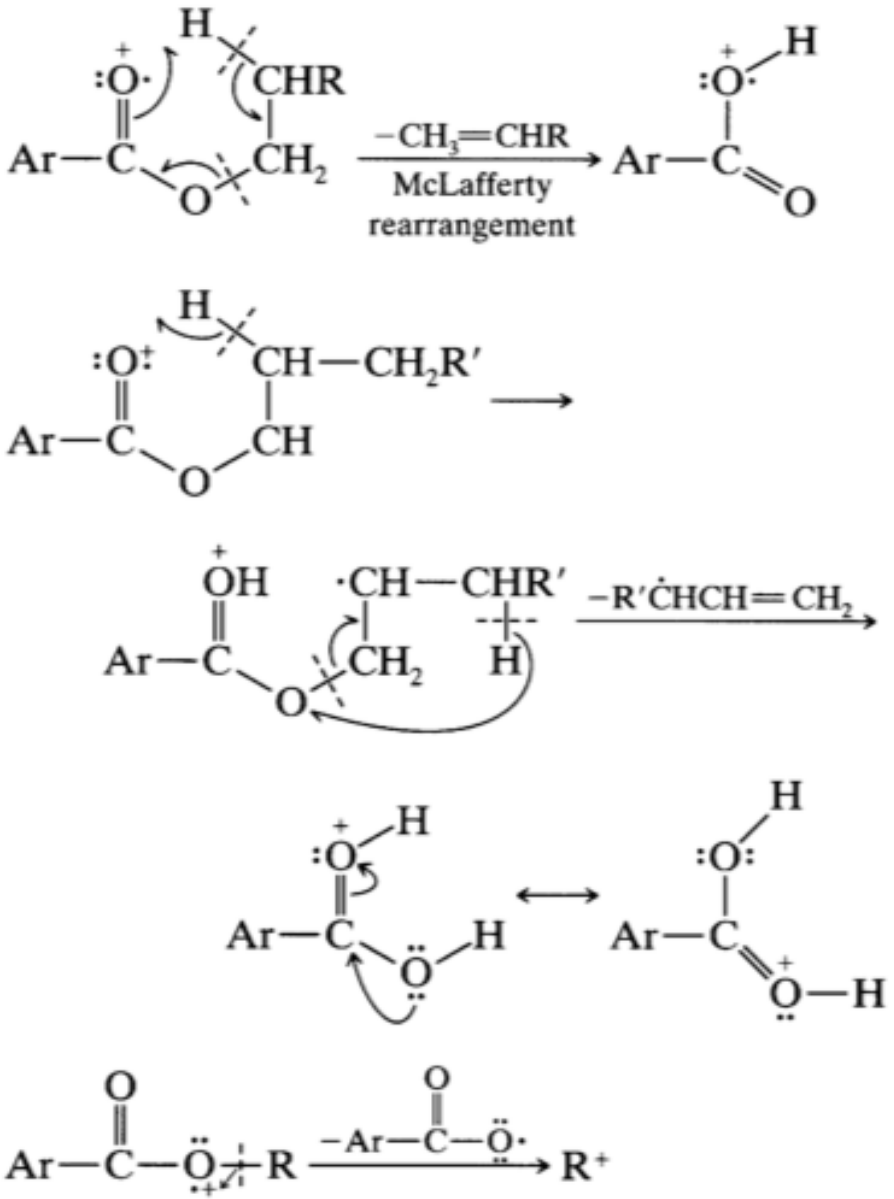




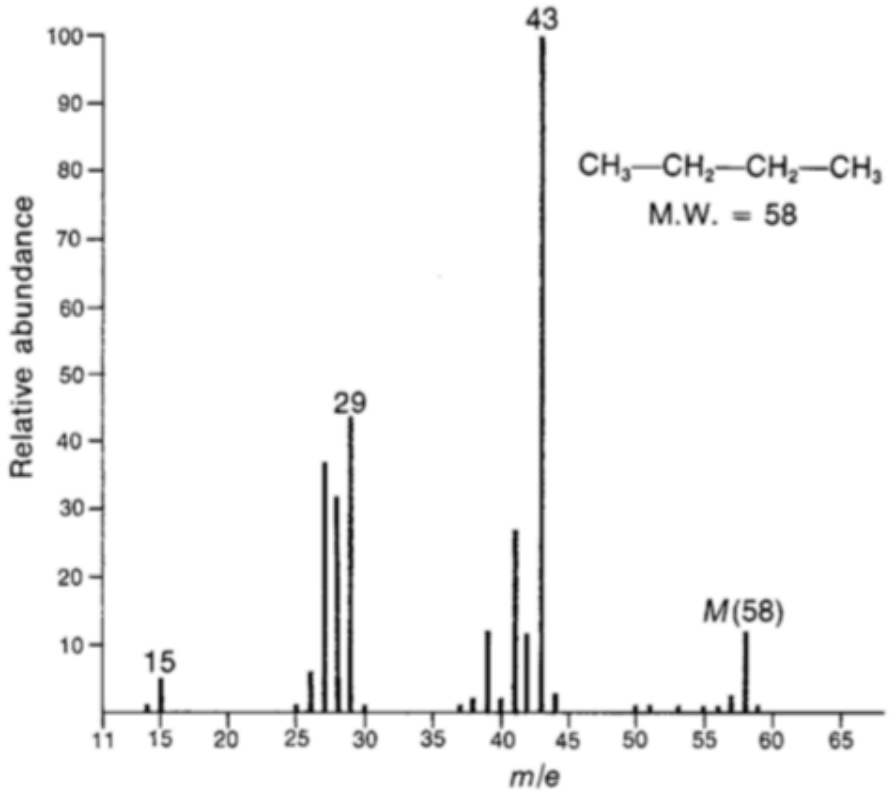


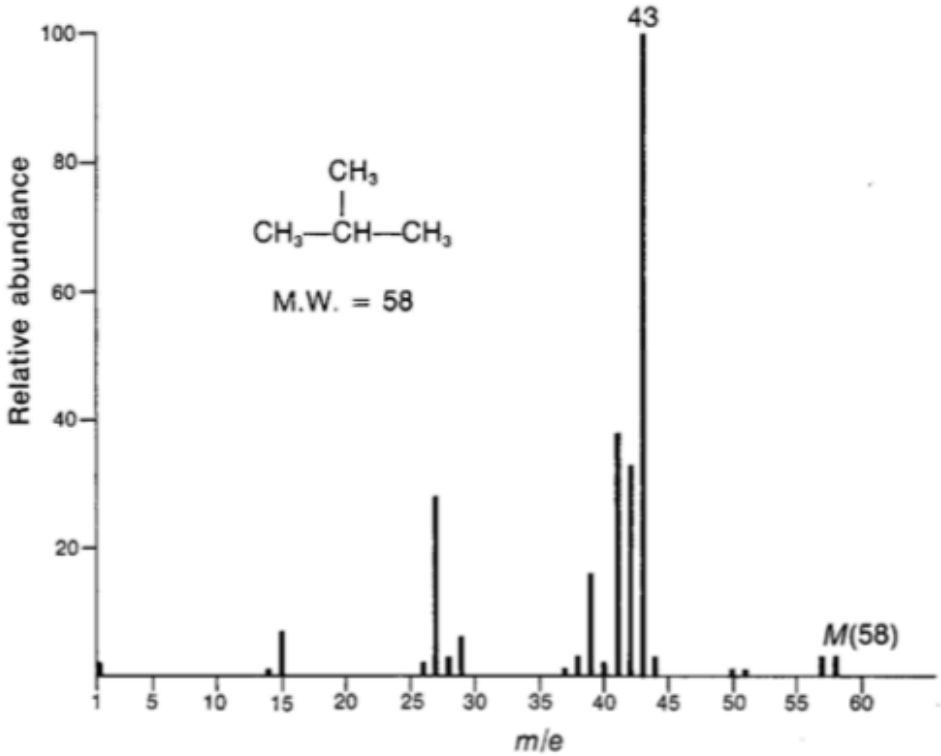
McLafferty Rearrangement

Esters

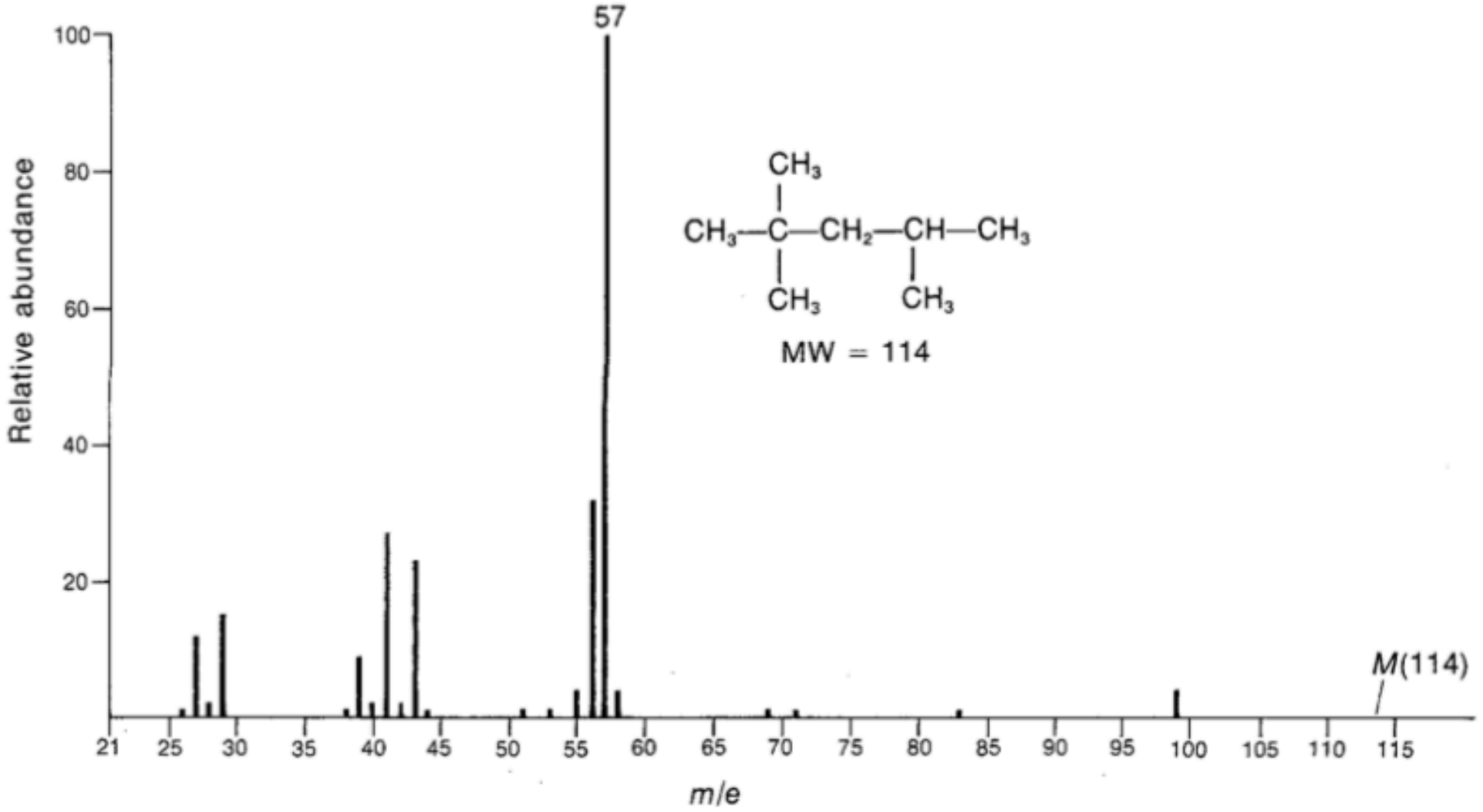


Alkane

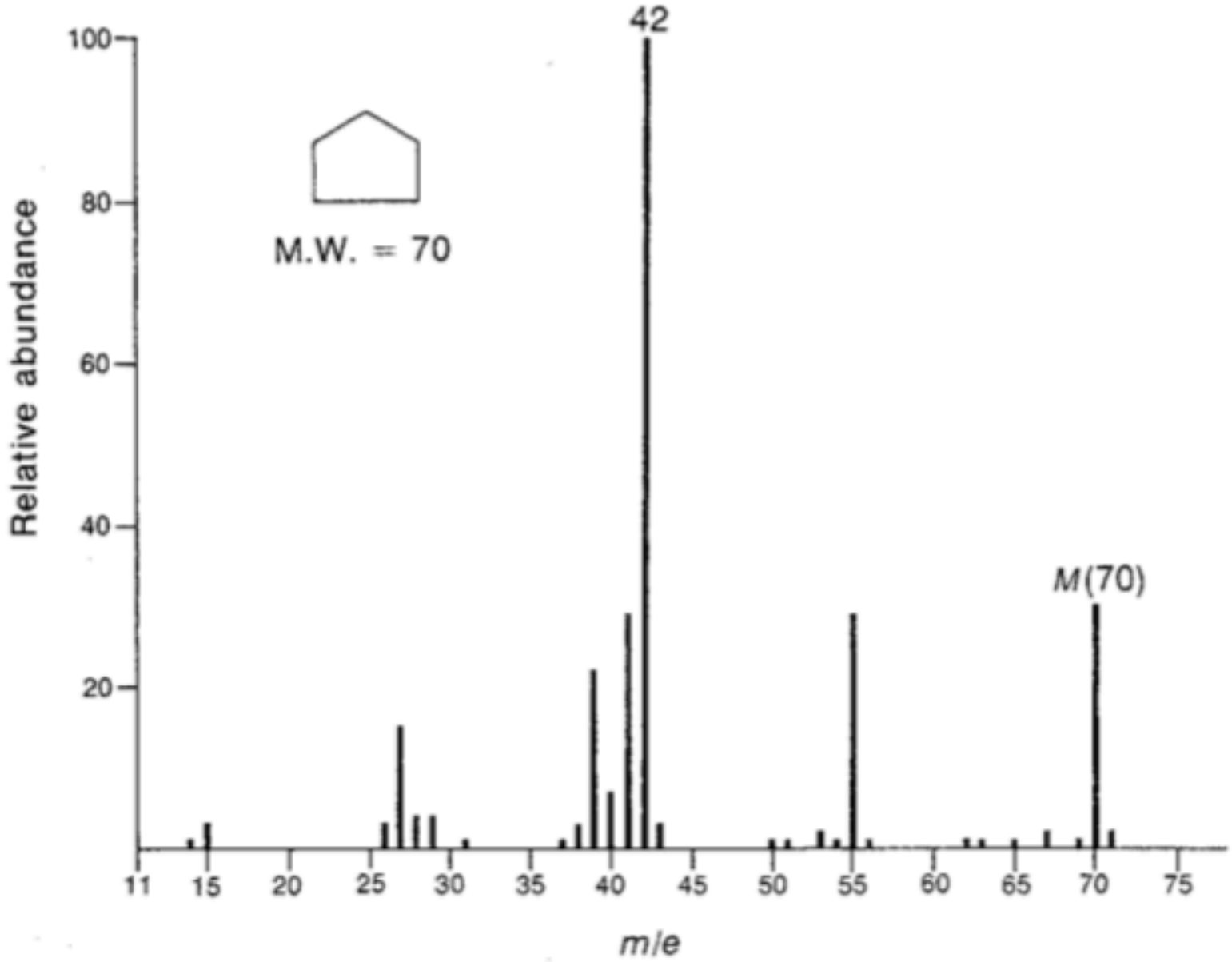

43
29
15
M(58)
CH3—CH2—CH2—CH3
M.W. = 58
Relative abundance
m/e


43
M(58)
CH3
CH3—CH—CH3
M.W. = 58
Relative abundance
m/e

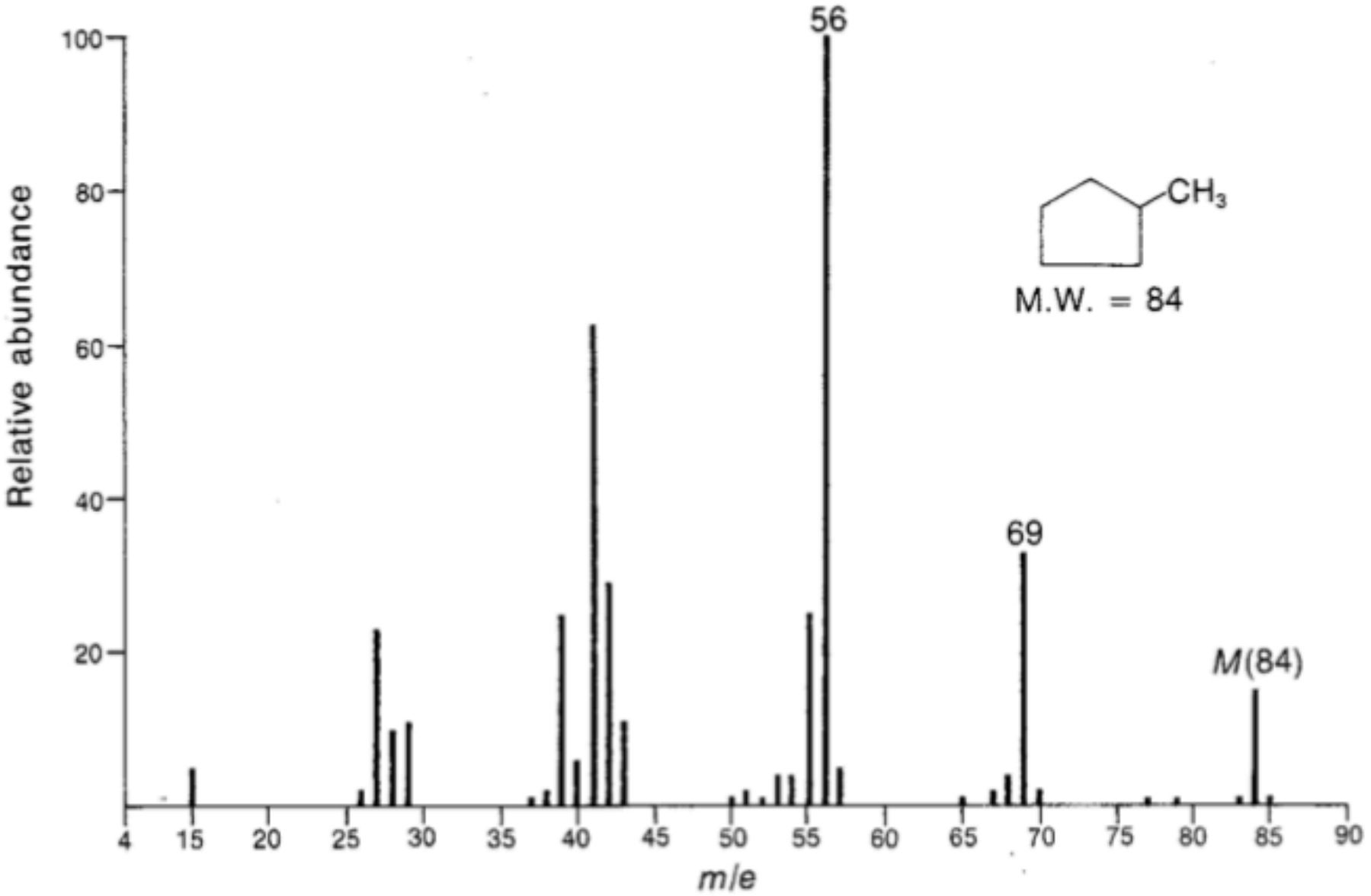
Alkane


57
100
80
60
40
20
Relative abundance
CH3
CH3—C—CH2—CH—CH3
CH3
CH3
MW = 114
M(114)
21 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115
m/e

Alkane

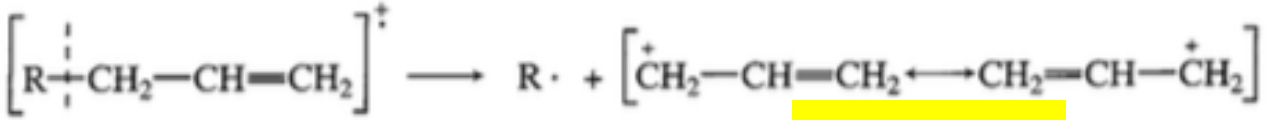

42
100
80
60
40
20
M.W. = 70
M(70)
Relative abundance
11
15
20
25
30
35
40
45
50
55
60
65
70
75
m/e

Alkane

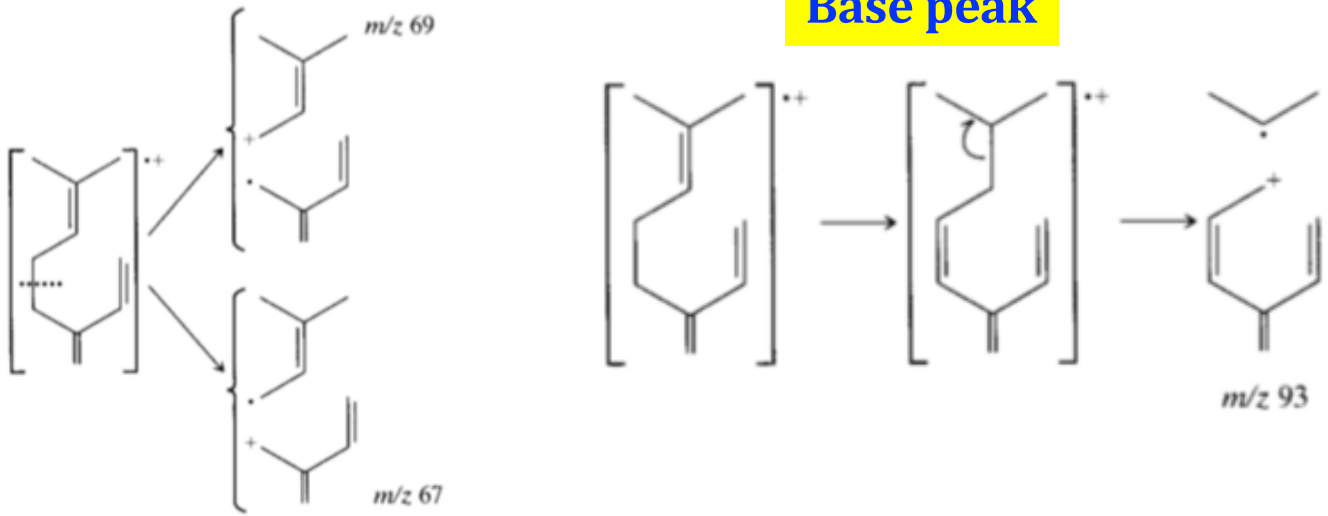

56
100
80
60
40
20
Relative abundance
CH3
M.W. = 84
69
M(84)
4
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
m/e

Alkene

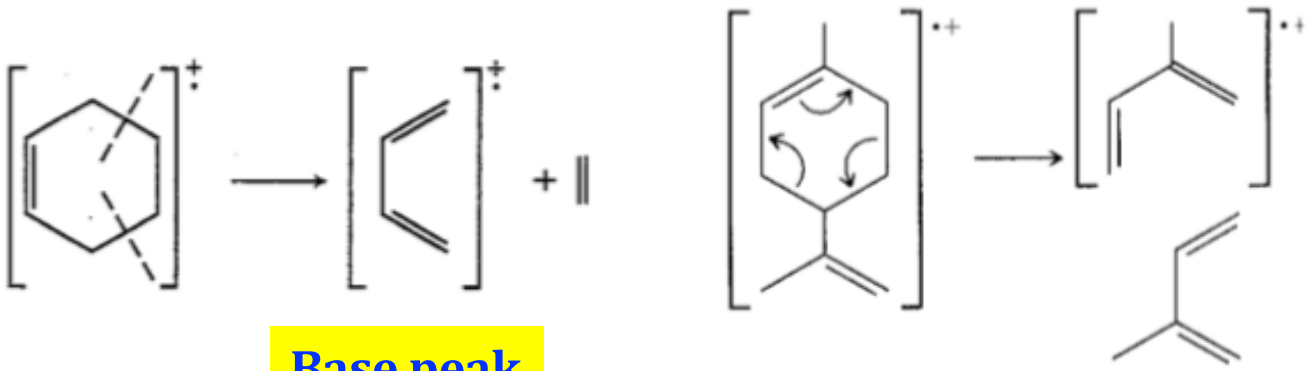
Allylic cleavage



Base peak

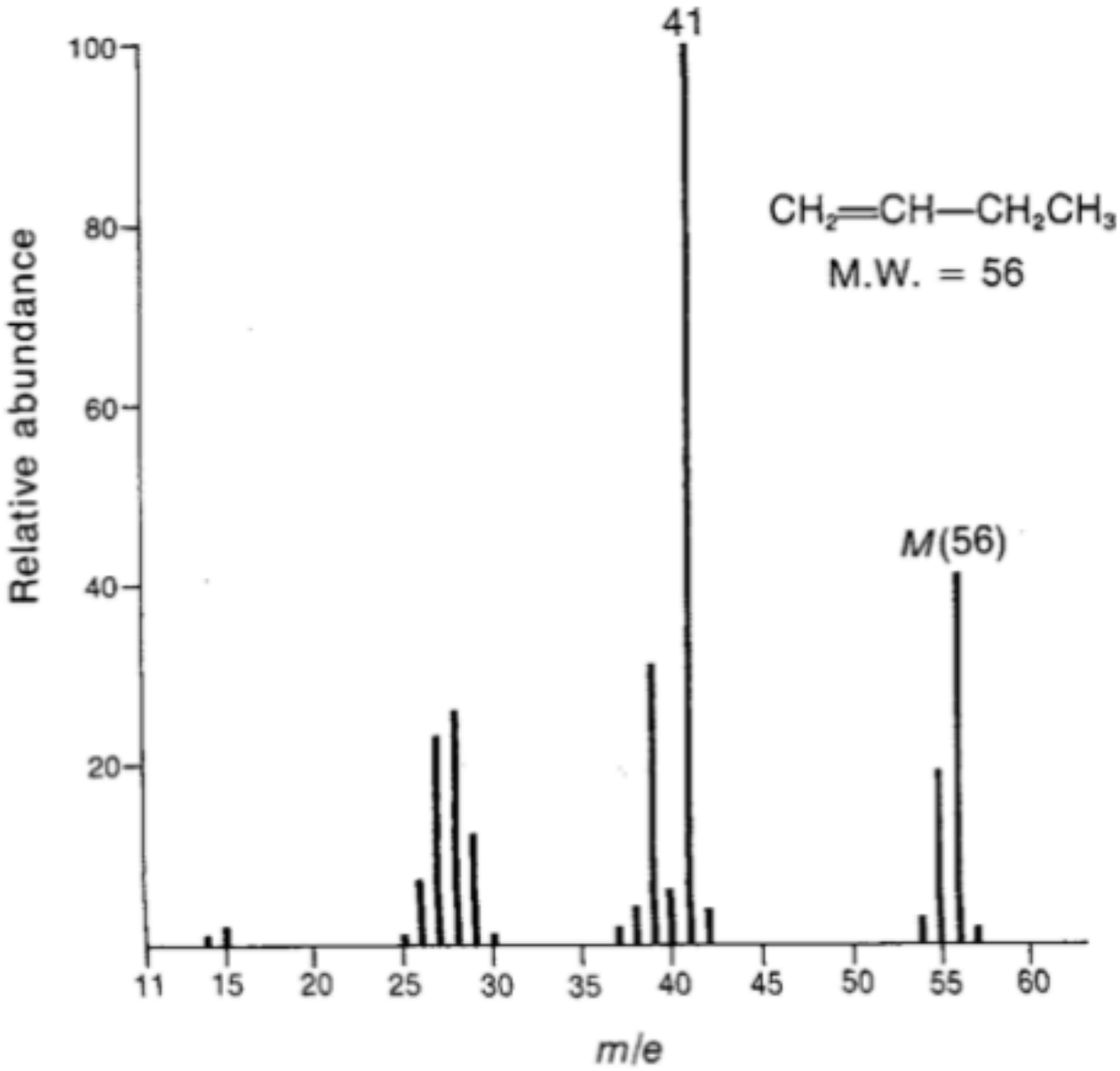


Retro Diels-Alder



Base peak

Alkene

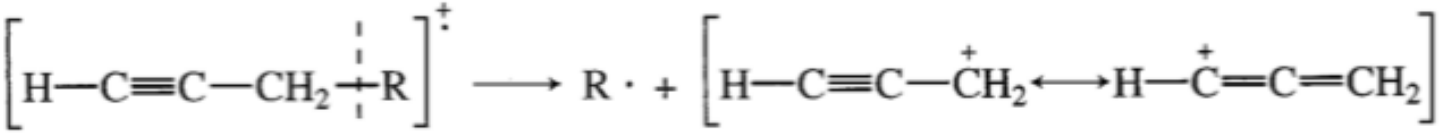

41
100
80
60
40
20
Relative abundance
$CH_2{=}CH{-}CH_2CH_3$
M.W. = 56
M(56)
11
15
20
25
30
35
40
45
50
55
60
m/e

Alkyne

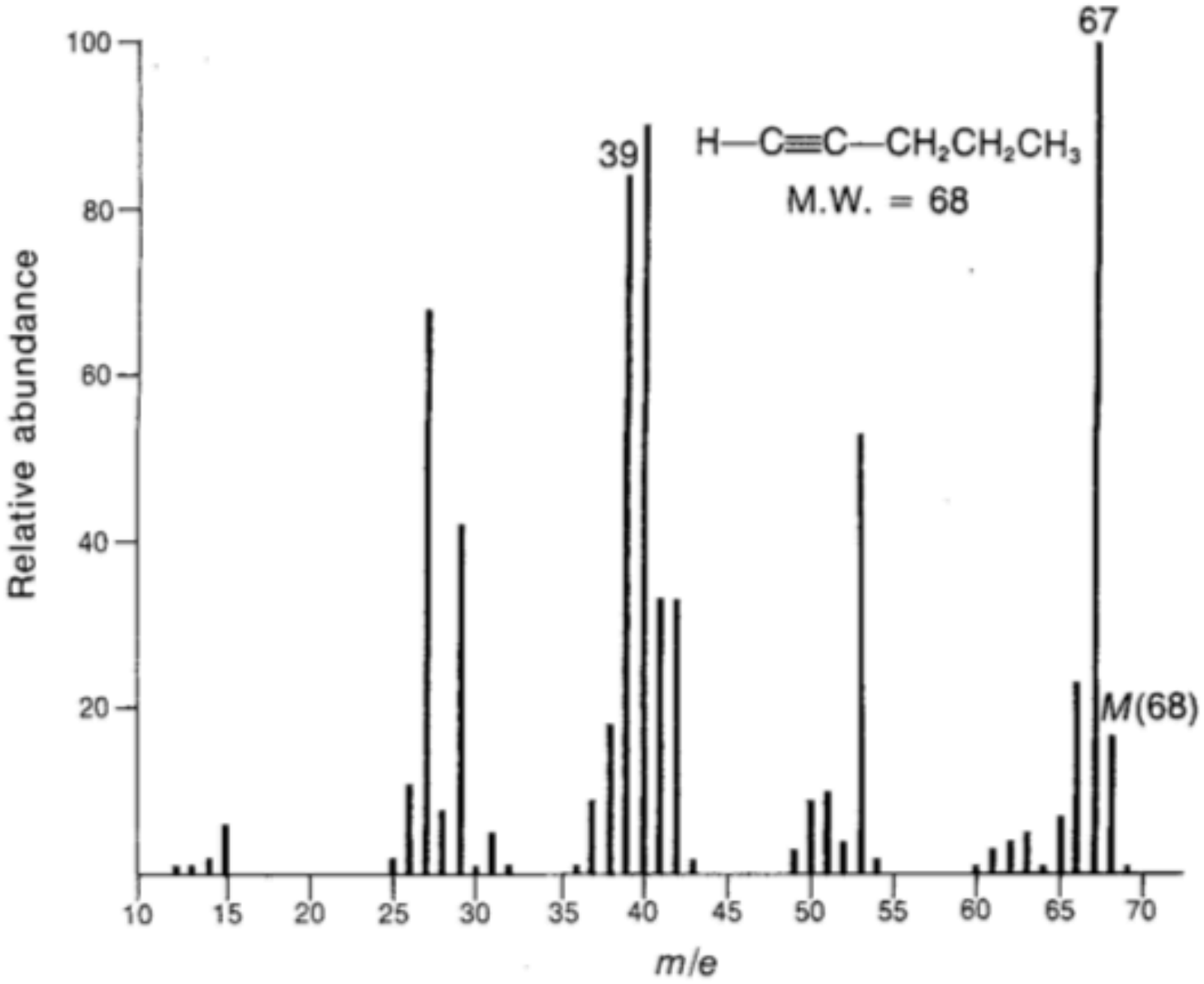
Loss of -H

Base peak

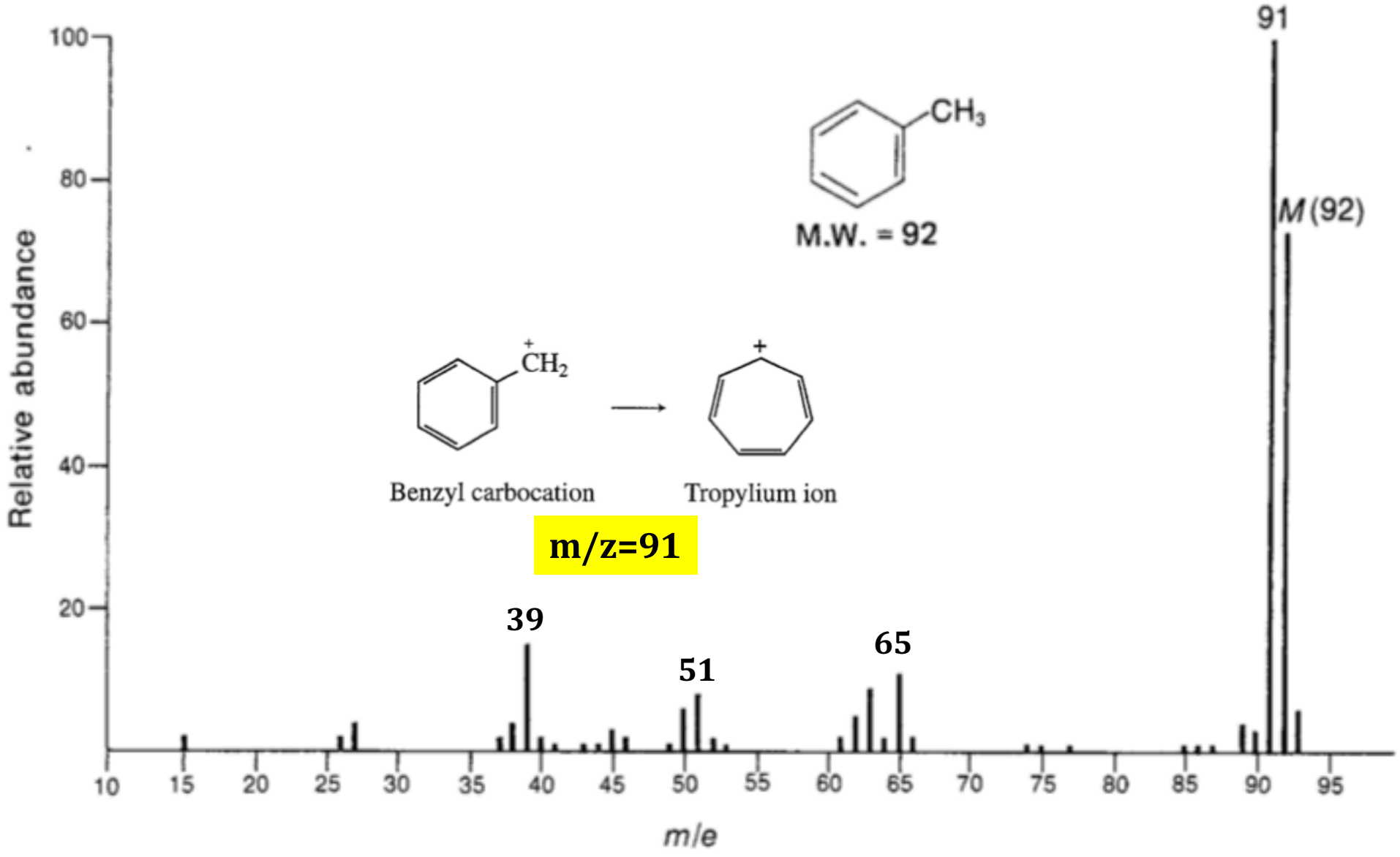
Allylic cleavage



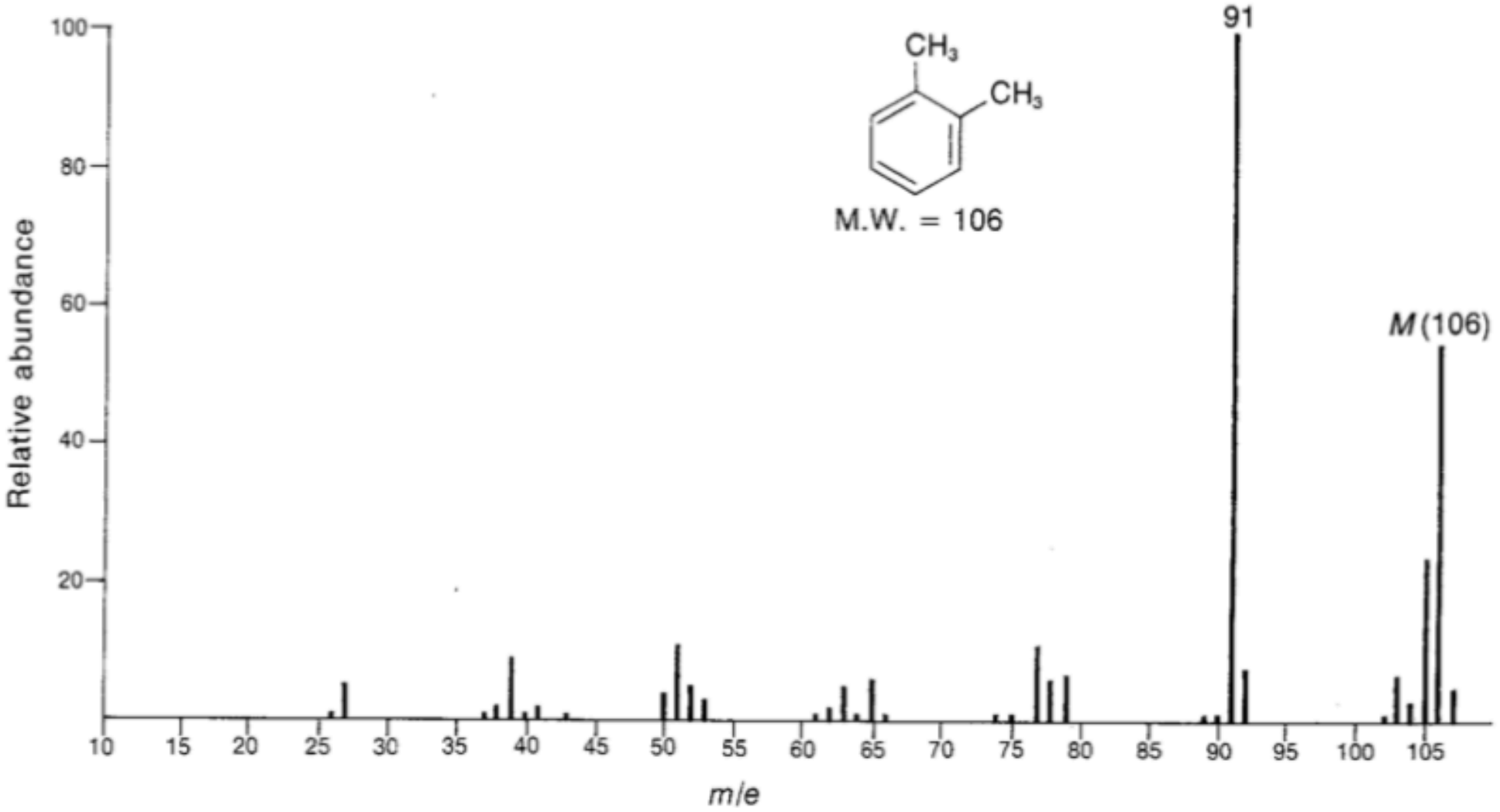
Alkyne


67
39
H—C≡C—CH2CH2CH3
M.W. = 68
M(68)
Relative abundance
100
80
60
40
20
10
15
20
25
30
35
40
45
50
55
60
65
70
m/e

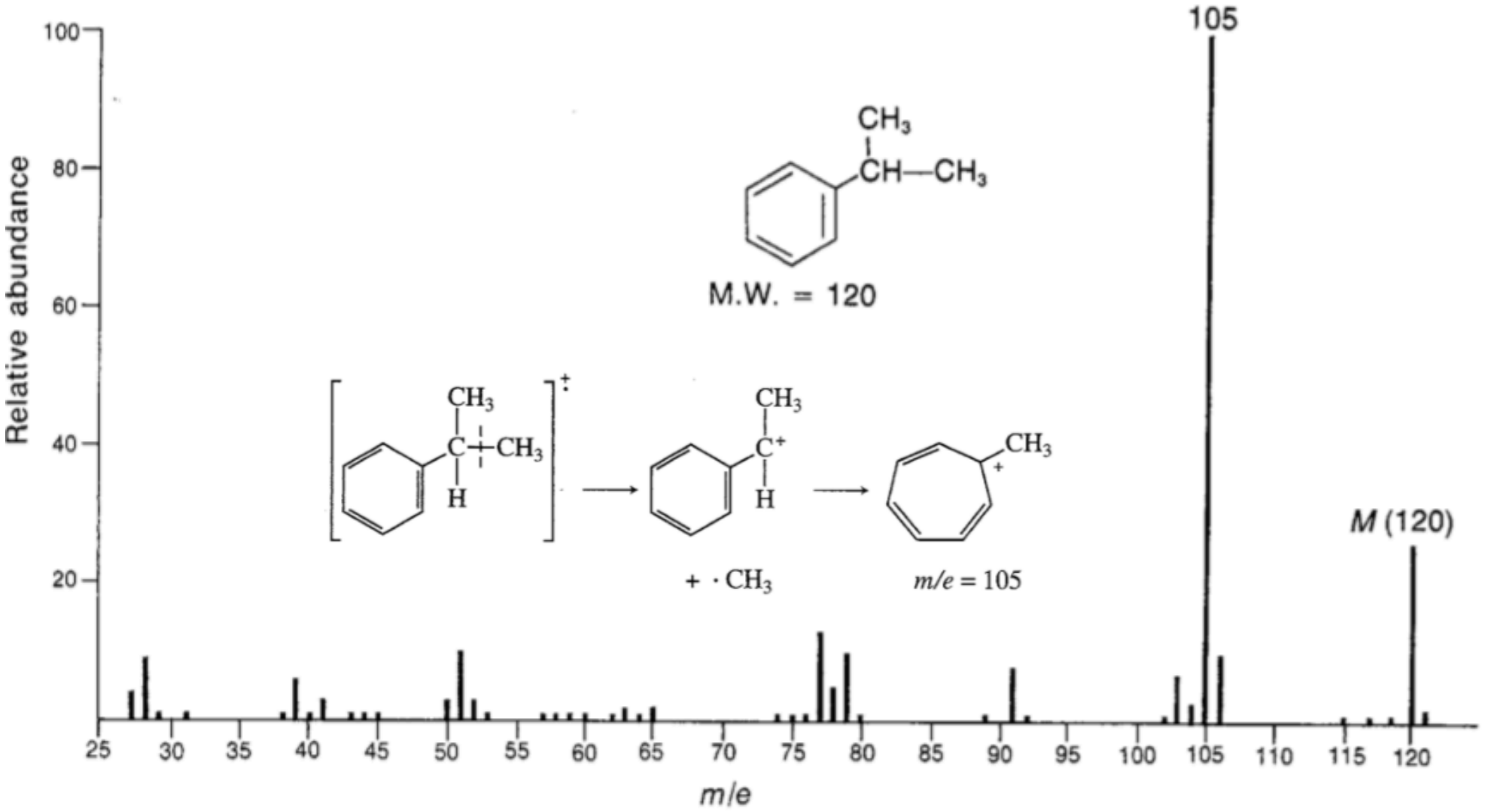
Aromatic compounds



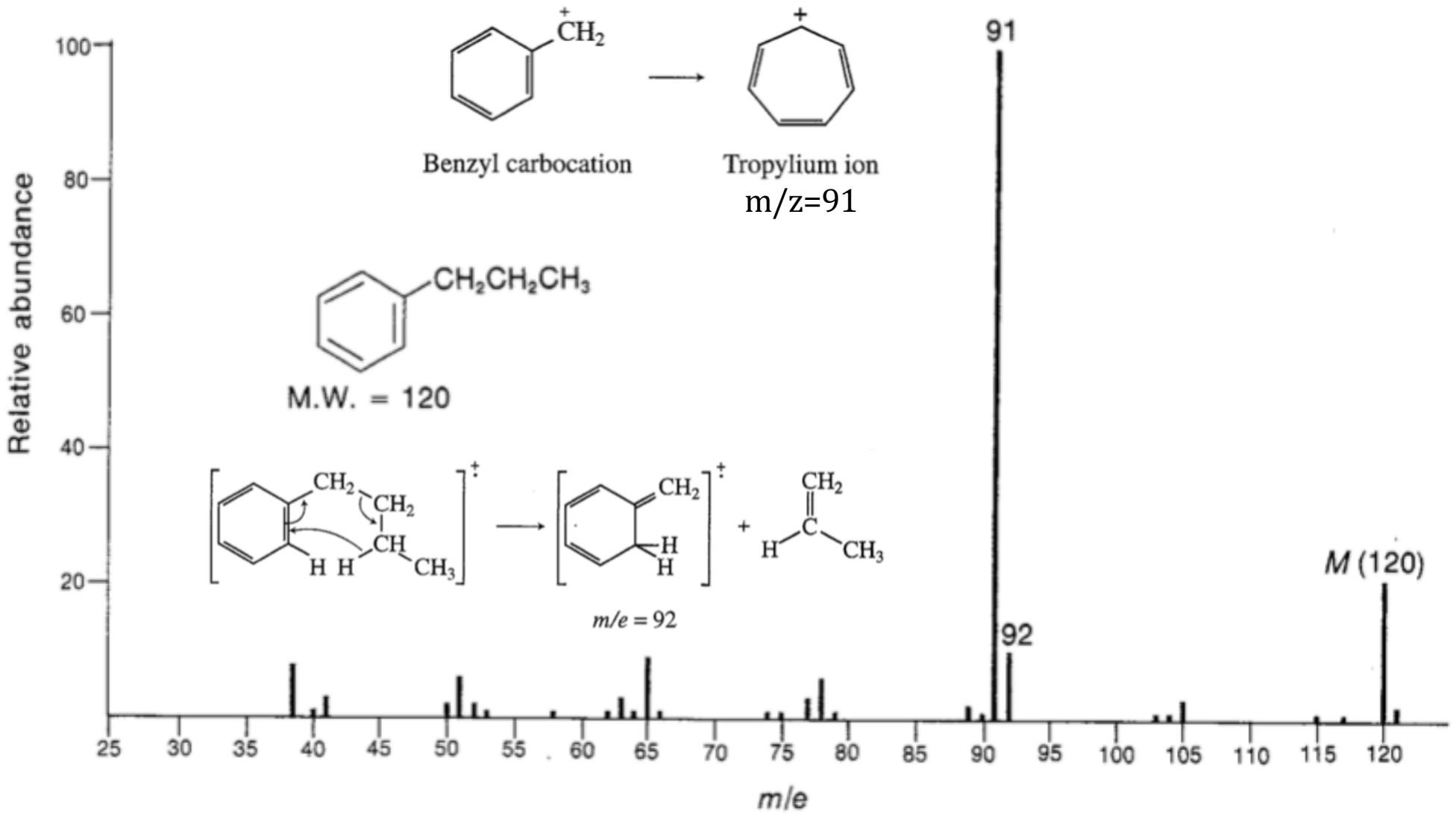
Aromatic compounds



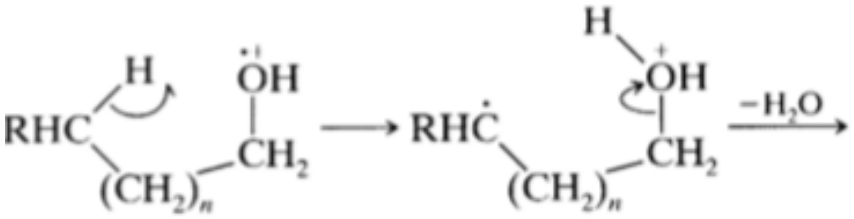
Aromatic compounds

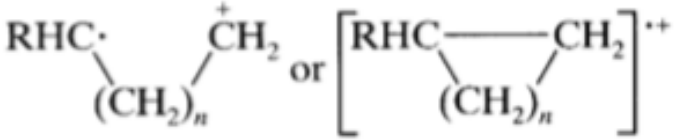


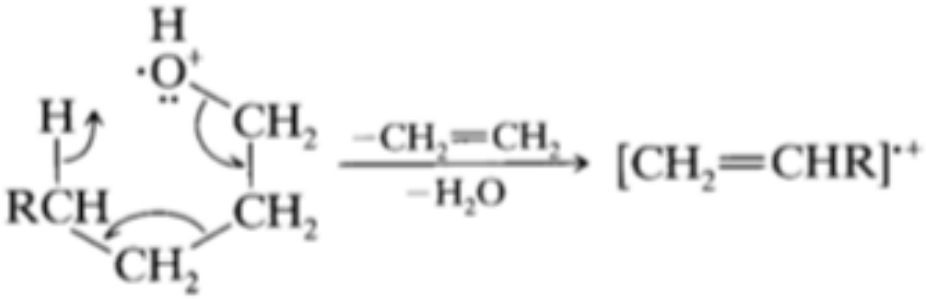
Aromatic compounds



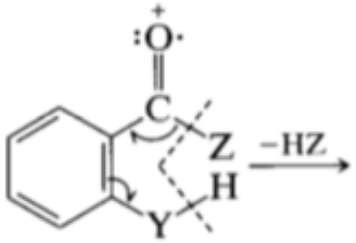
Loss of Water

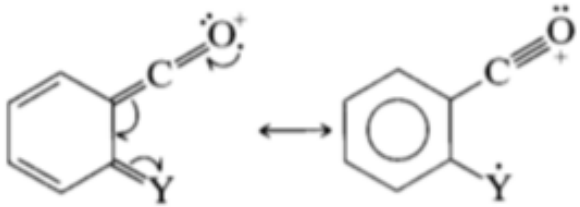




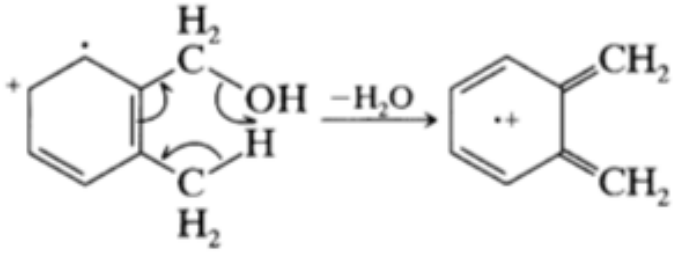


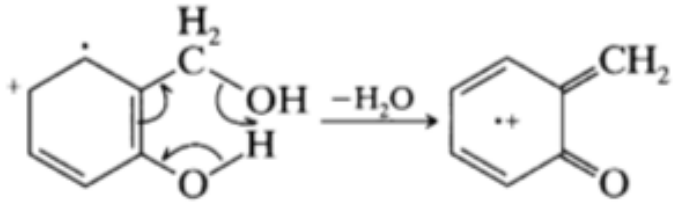
M − (alkene + H_2O)

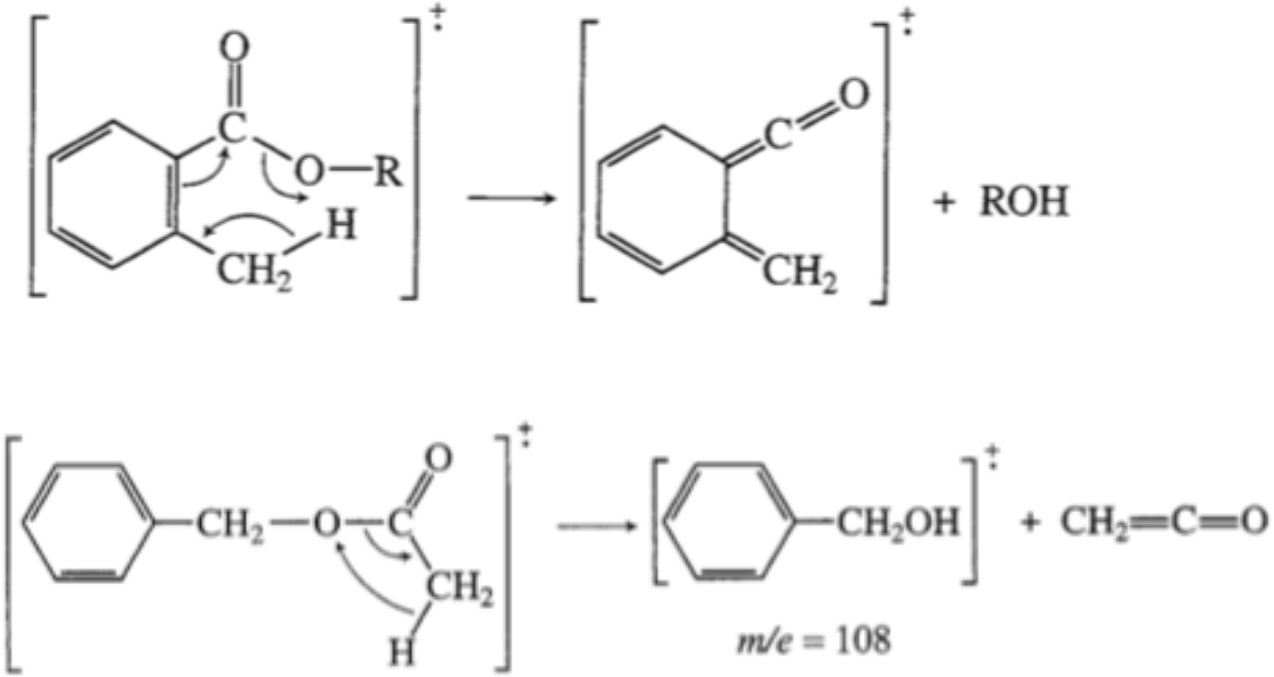


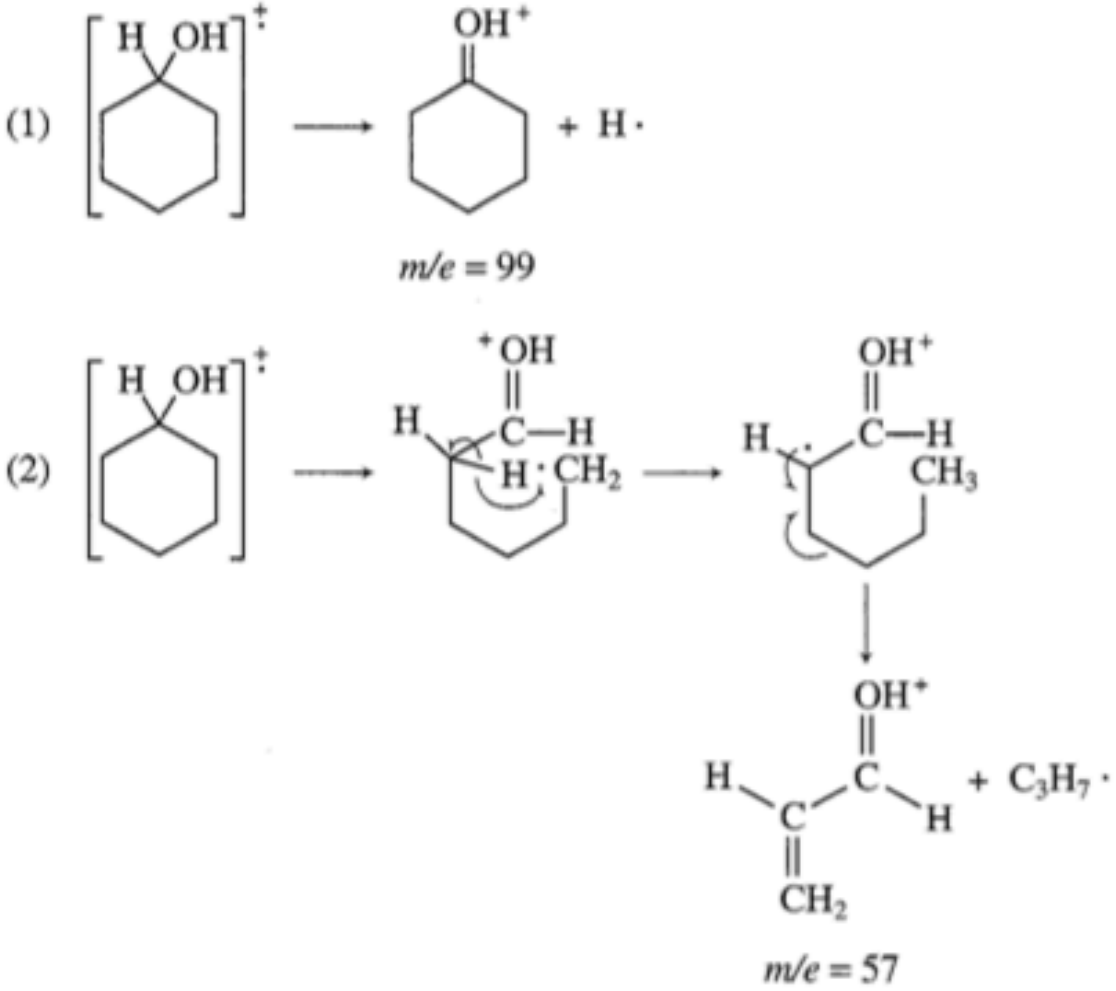


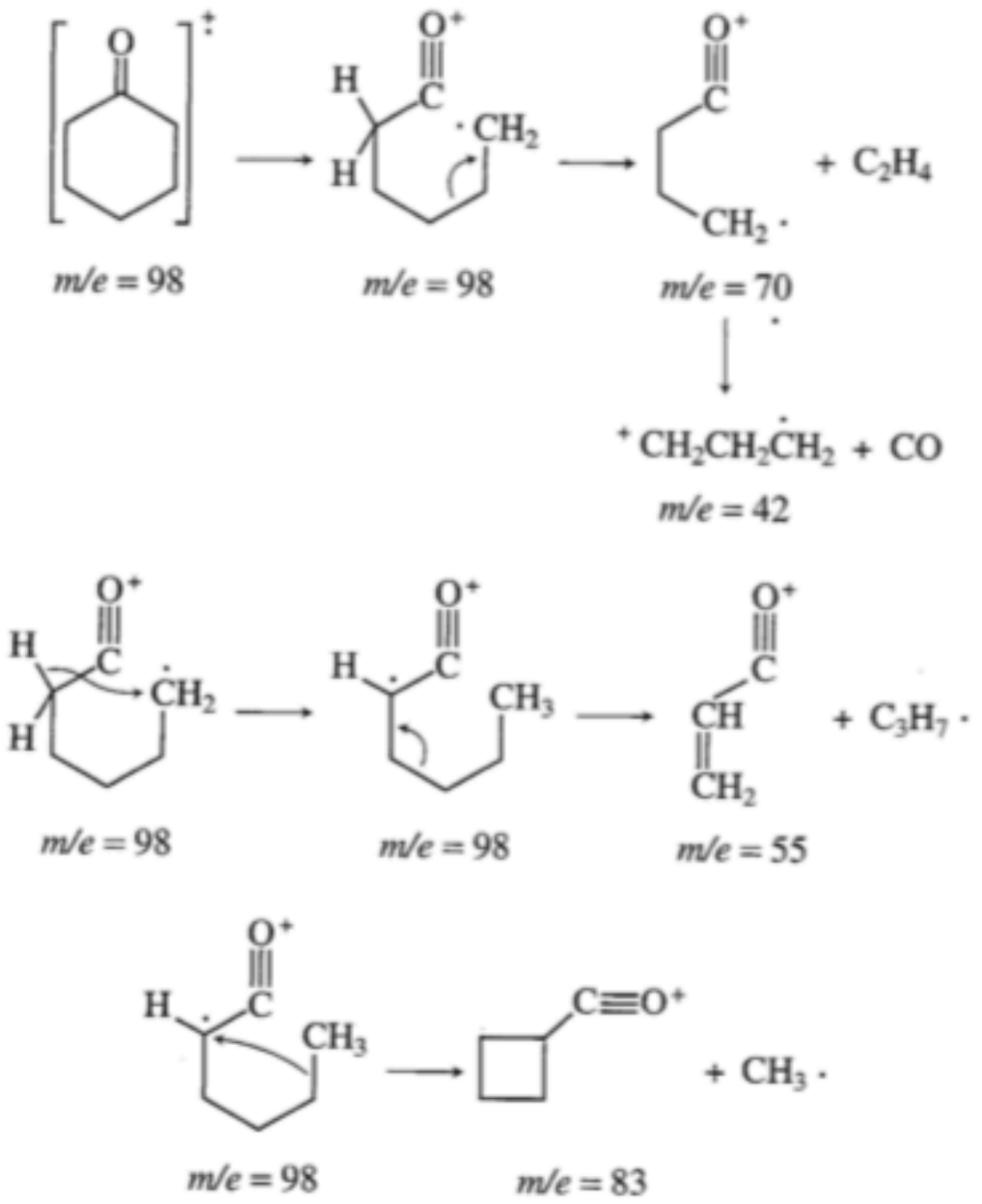
where Z = OH, OR, NH_2; Y = CH_2, O, NH.



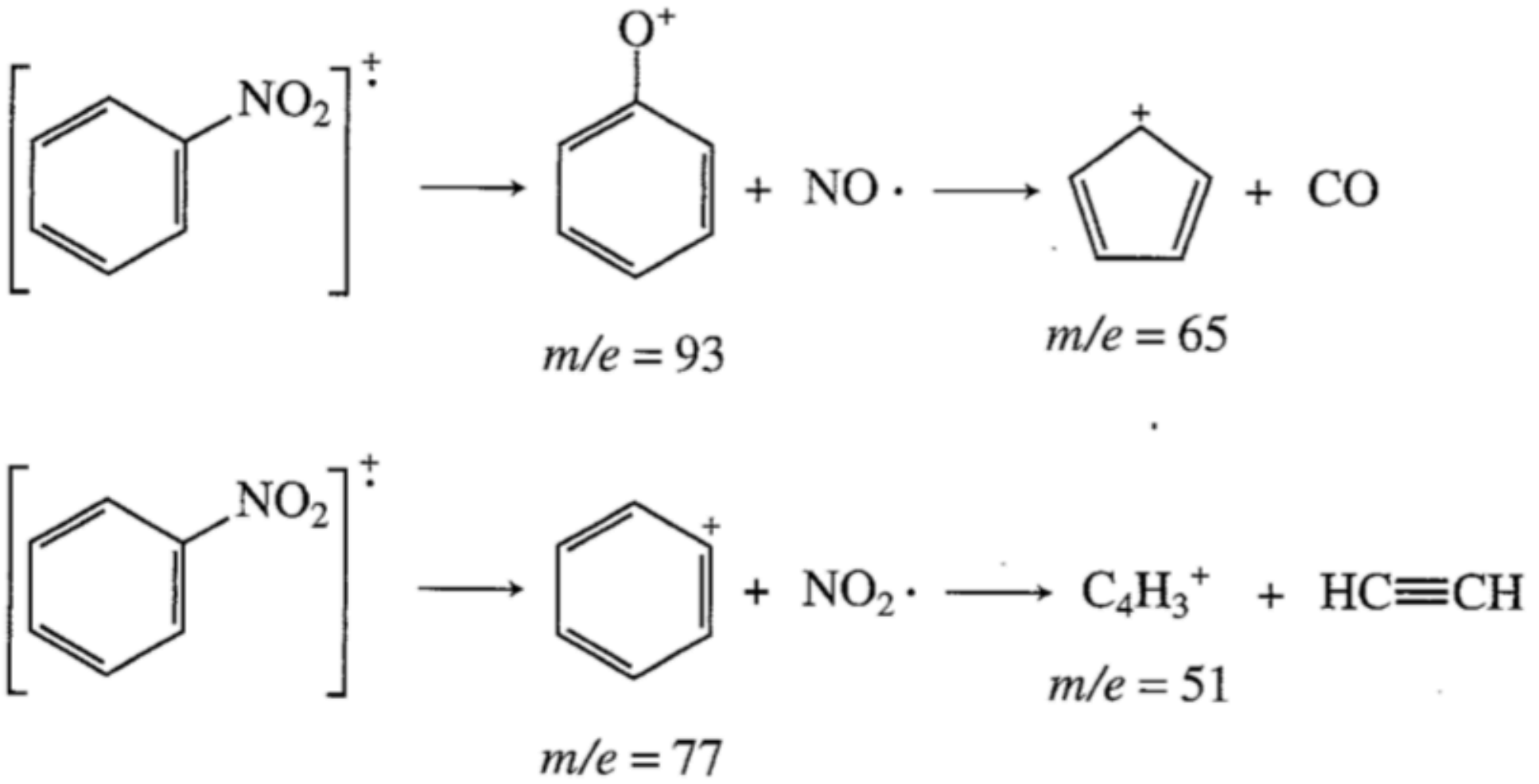



O
C
O—R
H
CH2
+ ROH
CH2—O—C
O
CH2
H
CH2OH
+ CH2=C=O
m/e = 108

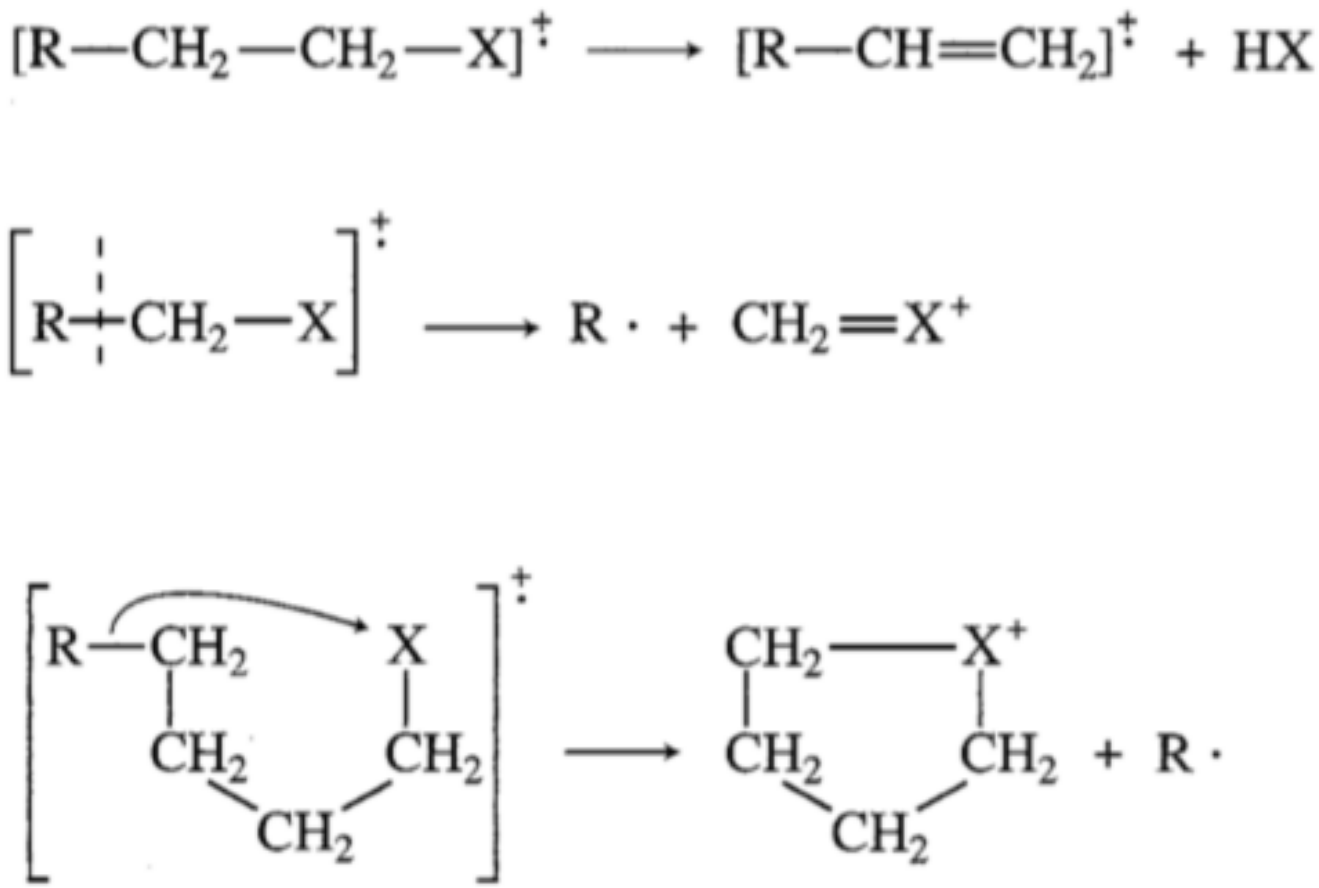

(1)
H OH
OH+
+ H·
m/e = 99
(2)
H OH
+OH
H
C—H
H CH2
OH+
H
C—H
CH3
OH+
H
C
C
H
CH2
+ C3H7·
m/e = 57
(3)
H OH
+
+ H2O
m/e = 82


m/e = 98
m/e = 98
m/e = 70
+ C2H4
+ CH2CH2CH2 + CO
m/e = 42
m/e = 98
m/e = 98
m/e = 55
+ C3H7 ·
m/e = 98
m/e = 83
+ CH3 ·

Nitrobenzne

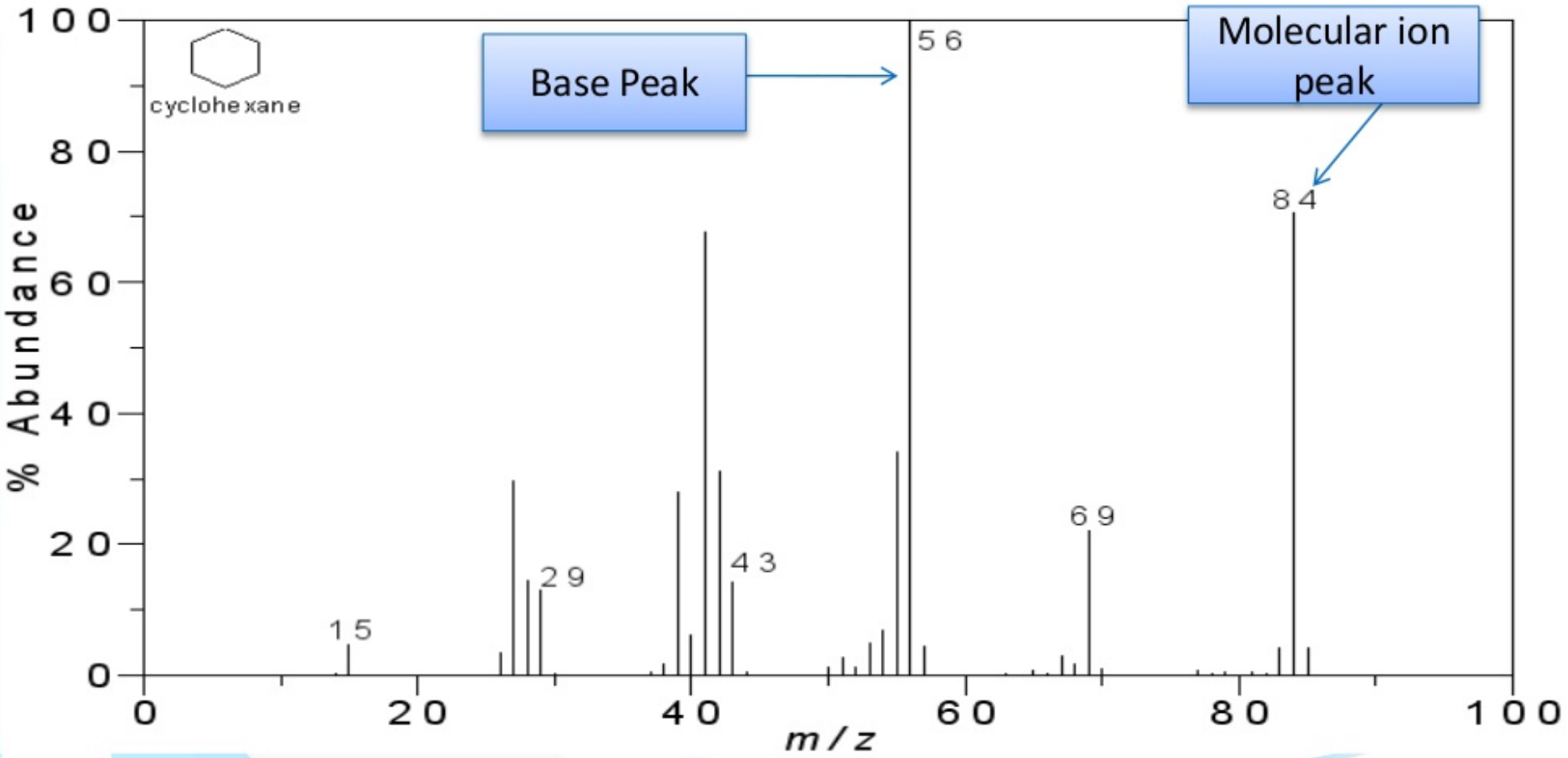


Halides



Mass Spectrum of compounds:-

Alkane:-



Alcohol

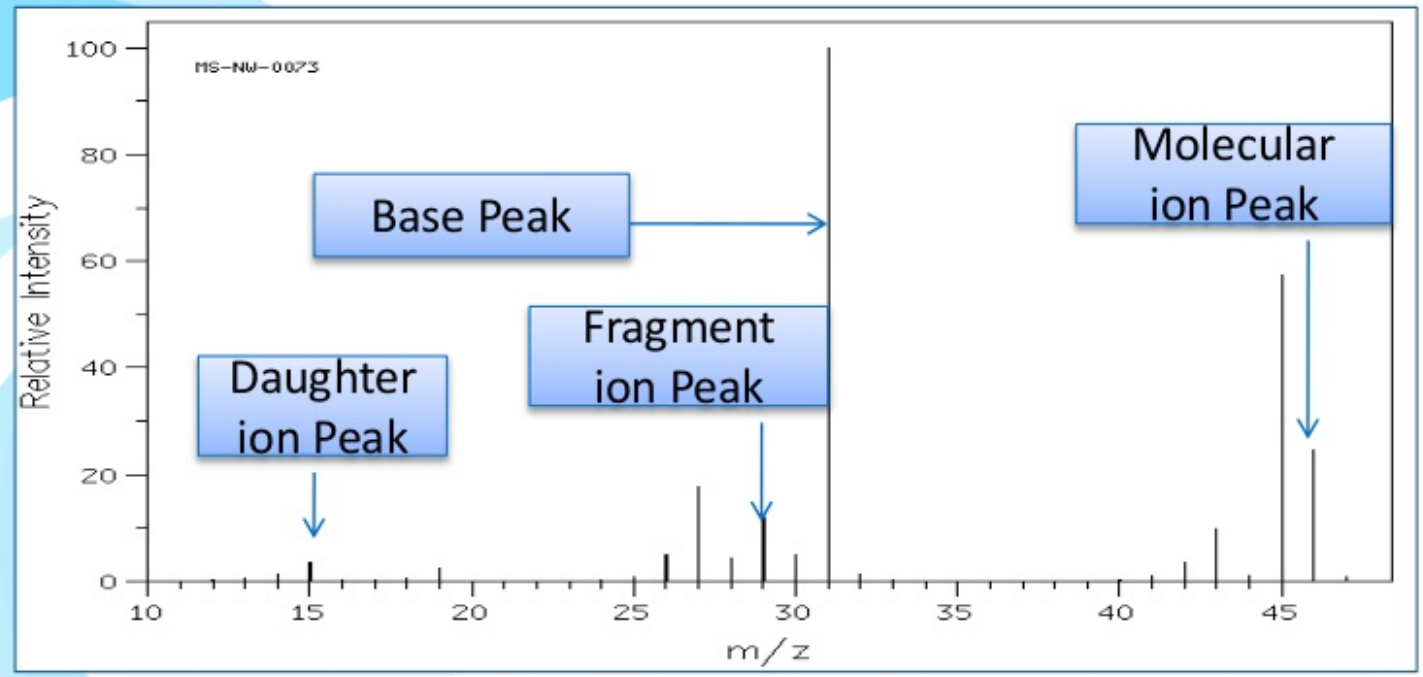
Possible Fragmentations are:- Mol.wt-46

$C_2H_5OH^+$ =46 (Molecular ion peak)

CH_3O^+ = 31 (Base Peak)

CHO^+ = 27 (Fragment ion Peak)

CH_3^+ = 15 (Daughter ion Peak)



Aldehyde:-

Molecular formula:-$C_6H_{12}O$

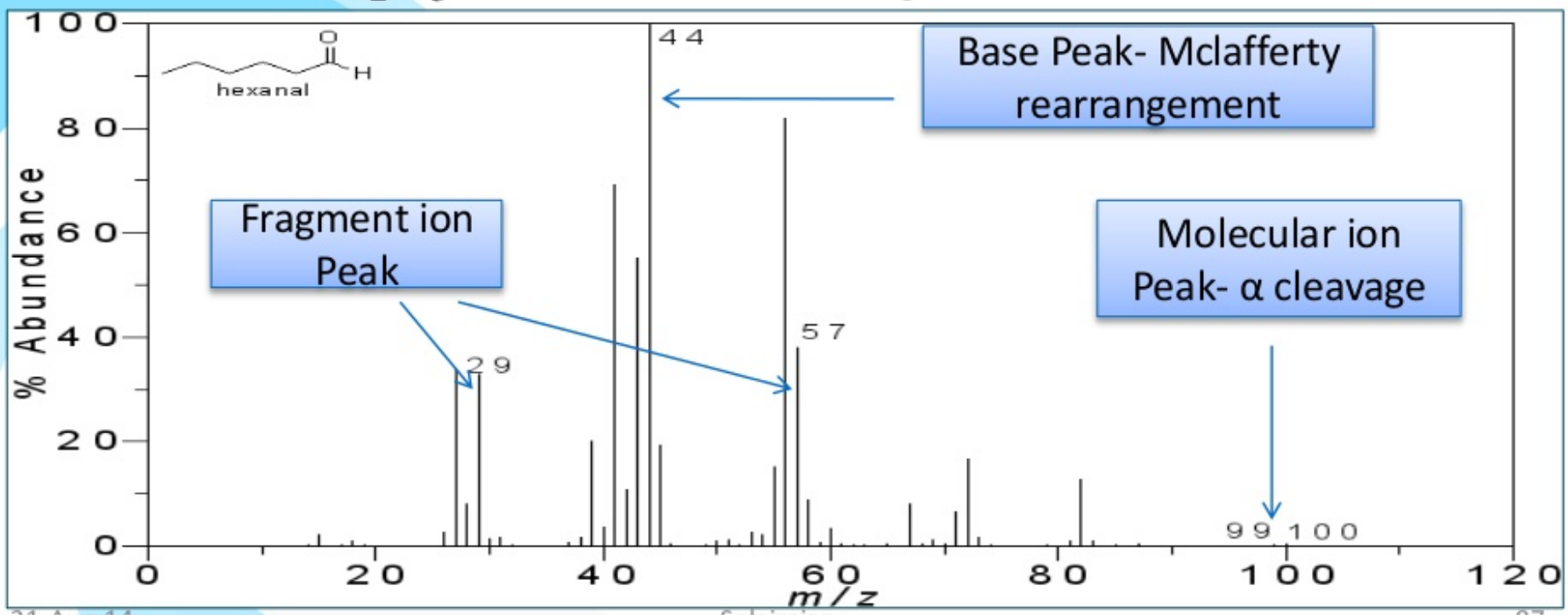
Molecular Weight:-100

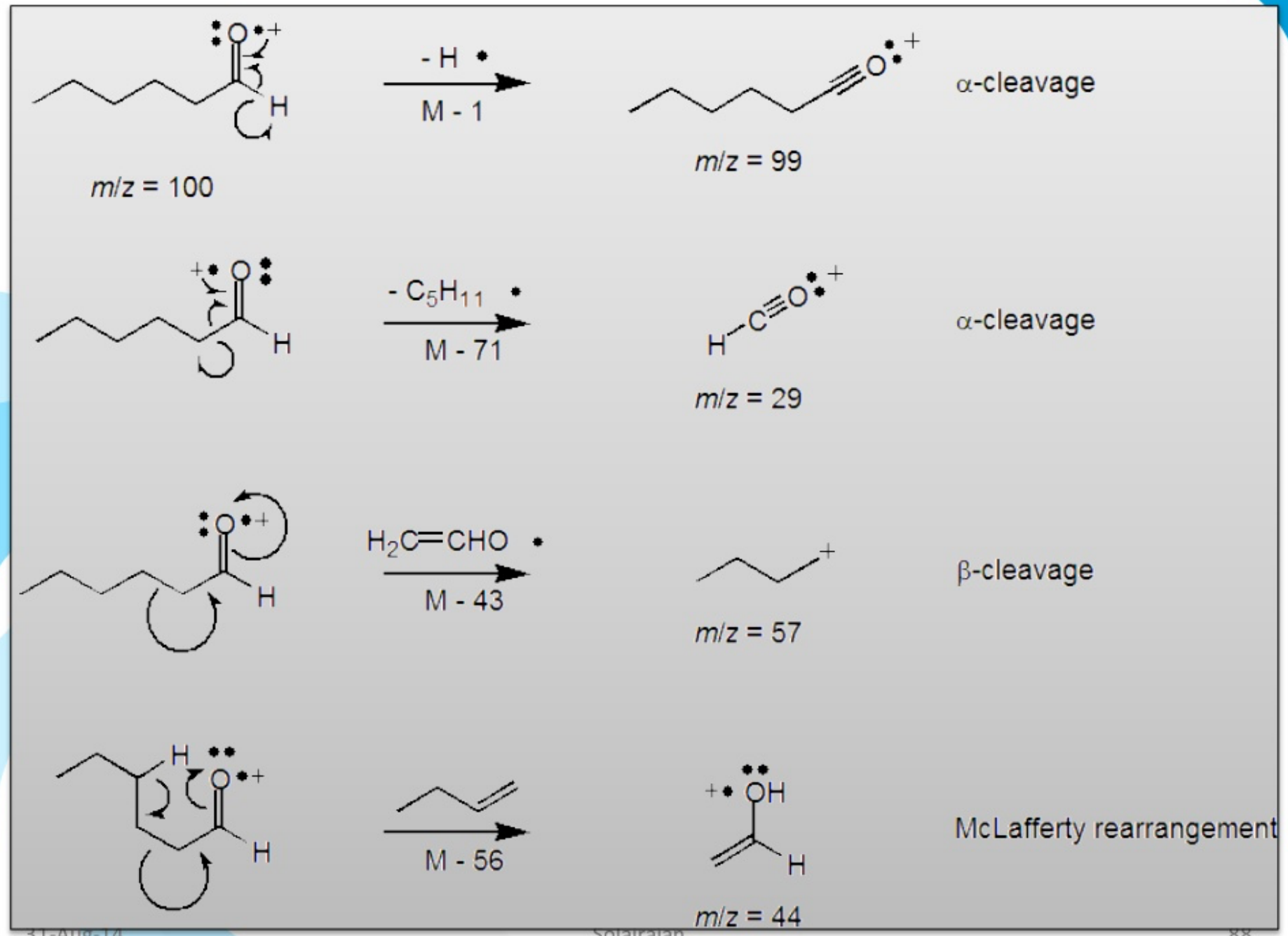
$C_6H_{12}O^+$ = 99 (Molecular ion Peak)

$C_3H_8^+$ = 44 (Base Peak)

$C_4H_9^+$ = 57 (Fragment ion Peak)

$C_2H_5^+$ = 29 (Fragment ion Peak)




- H •
M - 1
m/z = 100
m/z = 99
α-cleavage
- C5H11 •
M - 71
m/z = 29
α-cleavage
H2C=CHO •
M - 43
m/z = 57
β-cleavage
M - 56
m/z = 44
McLafferty rearrangement

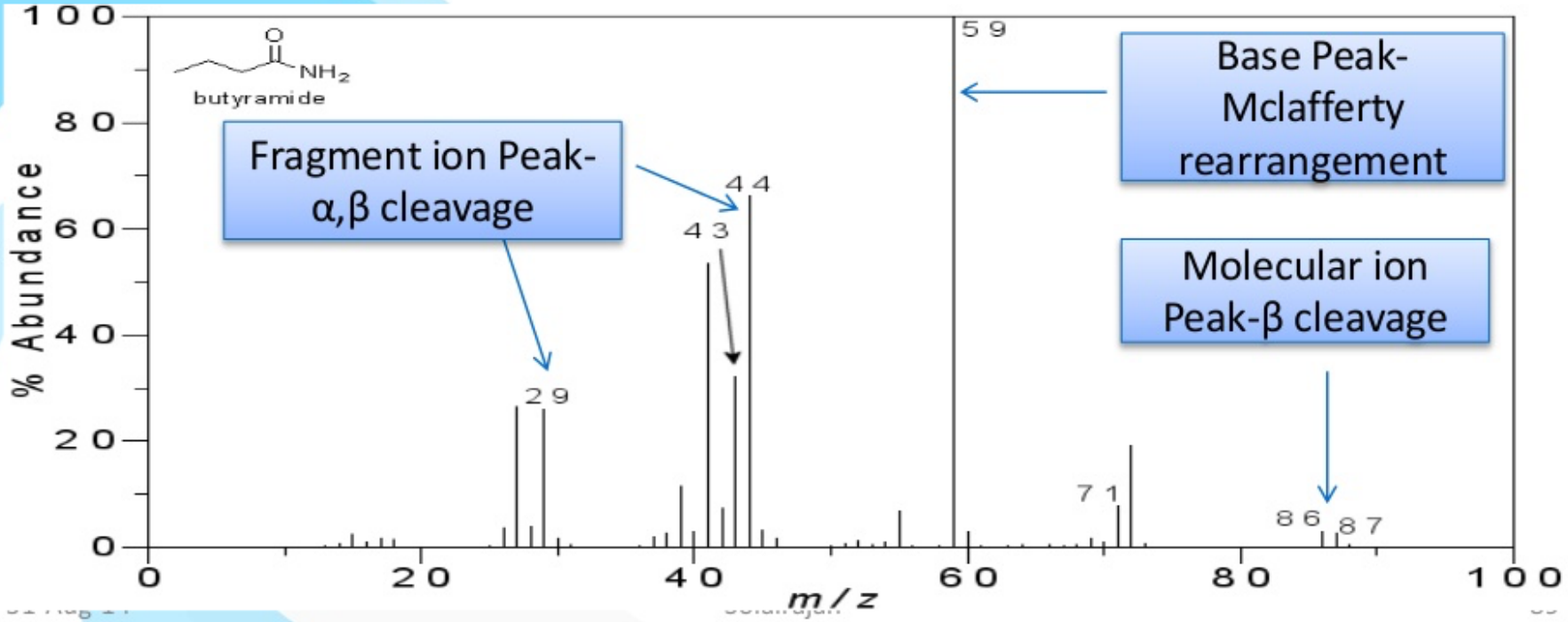
Amide:-

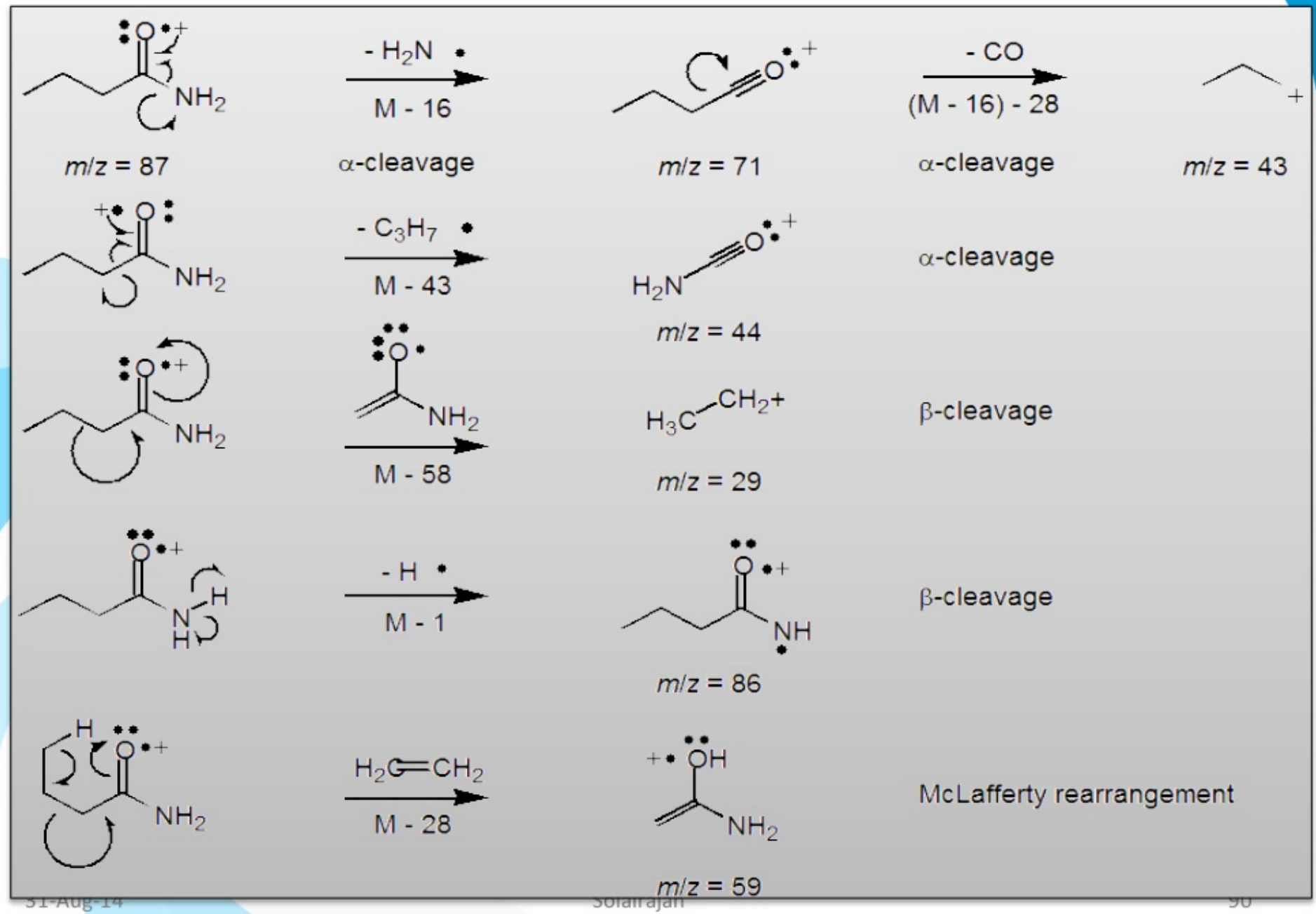
Molecular wt :- 87,

Molecular formula :- C_4H_9NO

$C_4H_9NO^+$ = 87 (Molecular ion Peak),

$C_2H_5NO^+$ = 59 (Base Peak)

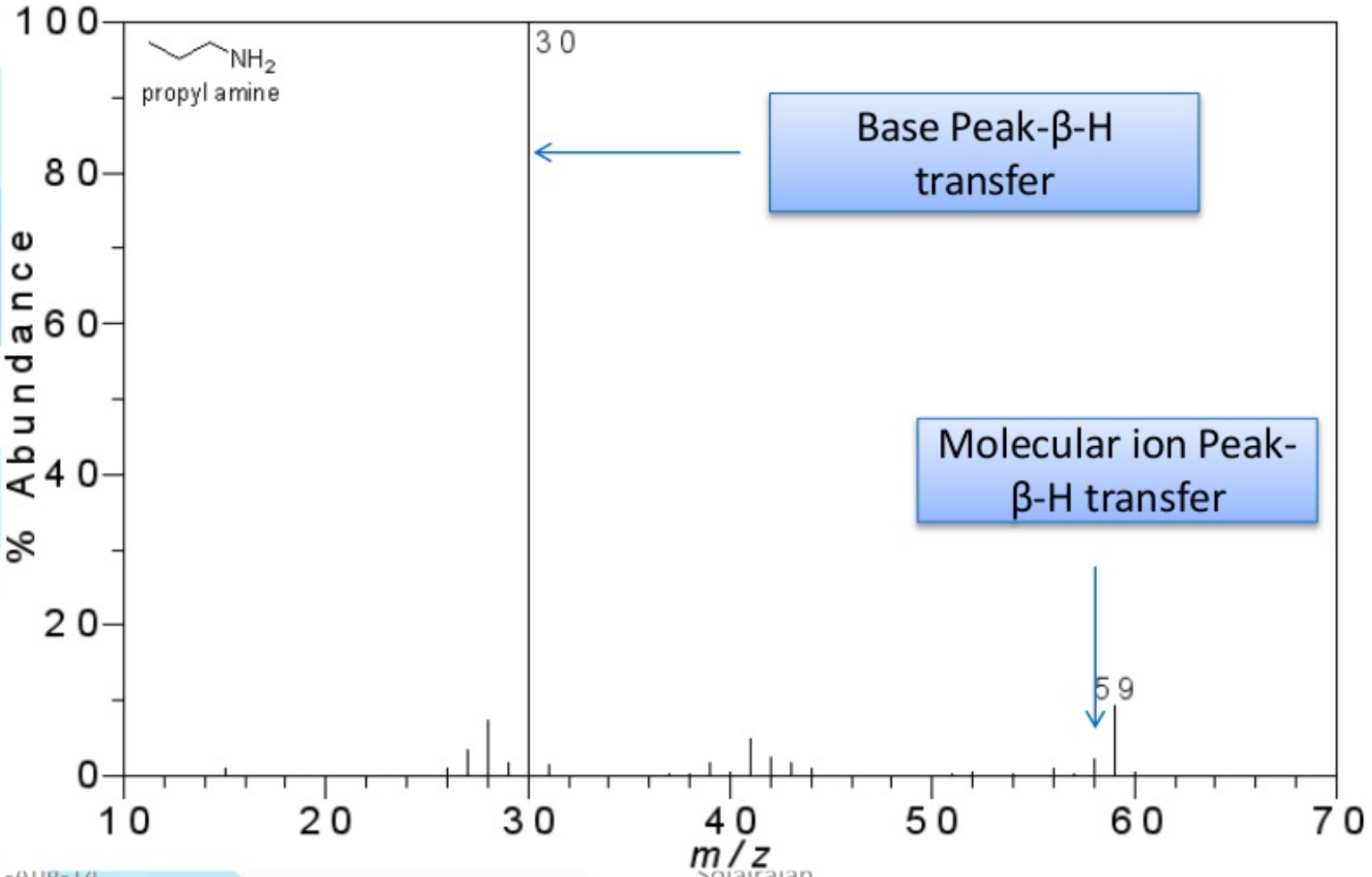


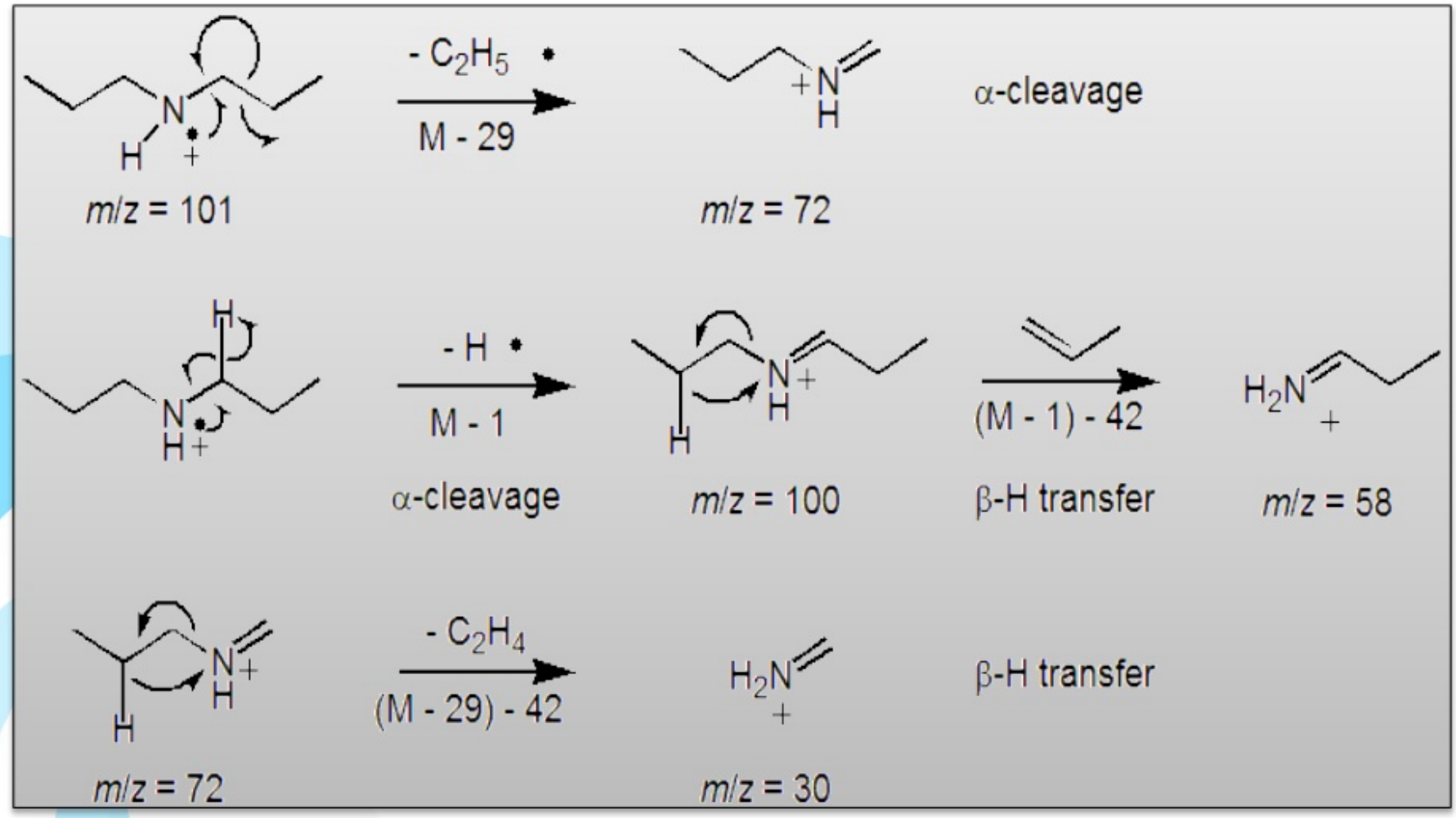

- H_2N •
M - 16
α-cleavage
m/z = 87
m/z = 71
- CO
(M - 16) - 28
α-cleavage
m/z = 43
- C_3H_7 •
M - 43
α-cleavage
H_2N
m/z = 44
M - 58
H_3C
CH_2+
β-cleavage
m/z = 29
- H •
M - 1
β-cleavage
m/z = 86
H_2C=CH_2
M - 28
McLafferty rearrangement
m/z = 59

Amine:-

Molecular wt:-59

Mol.formula :-C_3H_9N

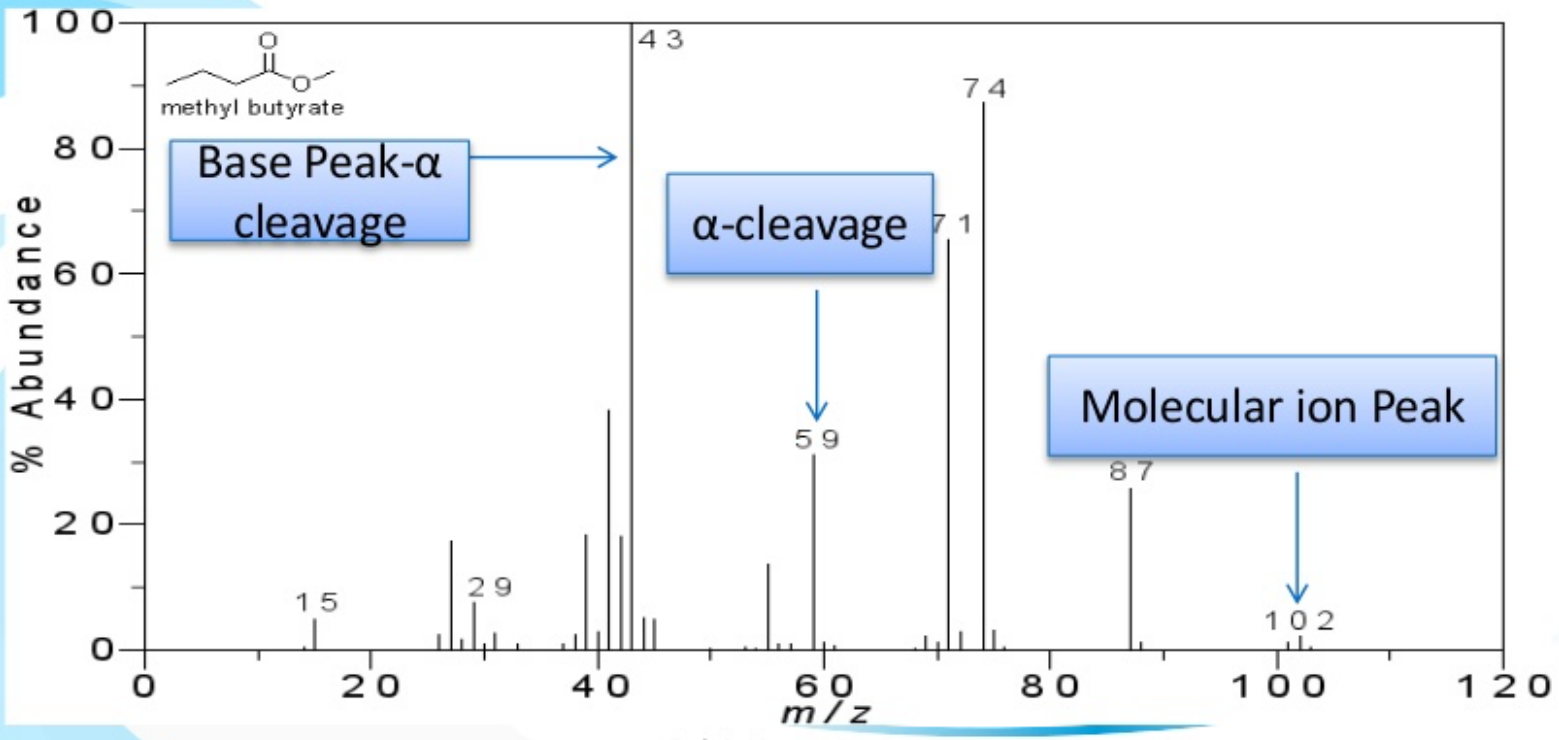


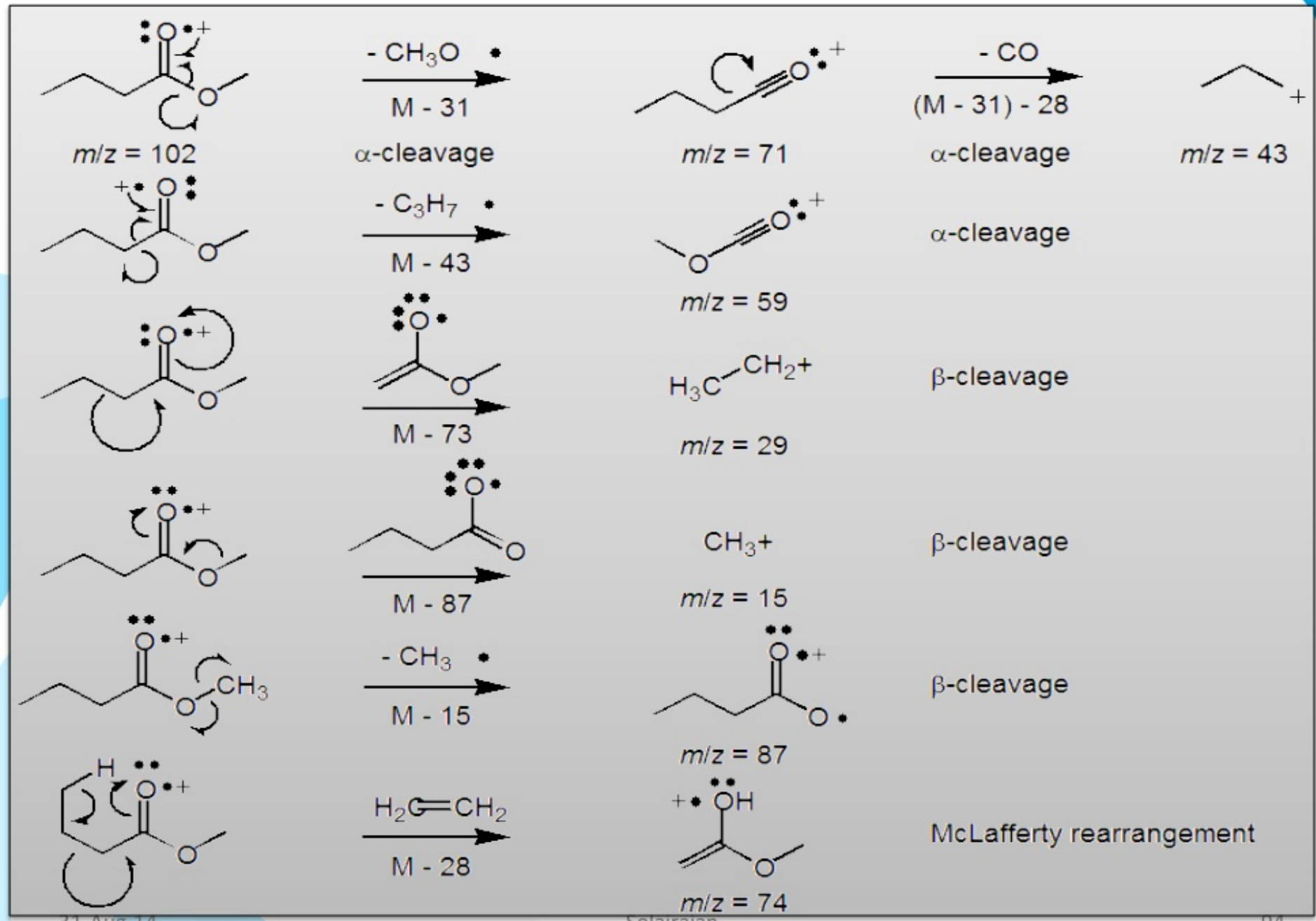

- C_2H_5 •
M - 29
α-cleavage
m/z = 101
m/z = 72
- H •
M - 1
α-cleavage
m/z = 100
(M - 1) - 42
β-H transfer
m/z = 58
- C_2H_4
(M - 29) - 42
β-H transfer
m/z = 72
m/z = 30

Ester

Mol.wt:-102,

Mol.formula:-$C_5H_{10}O_2$

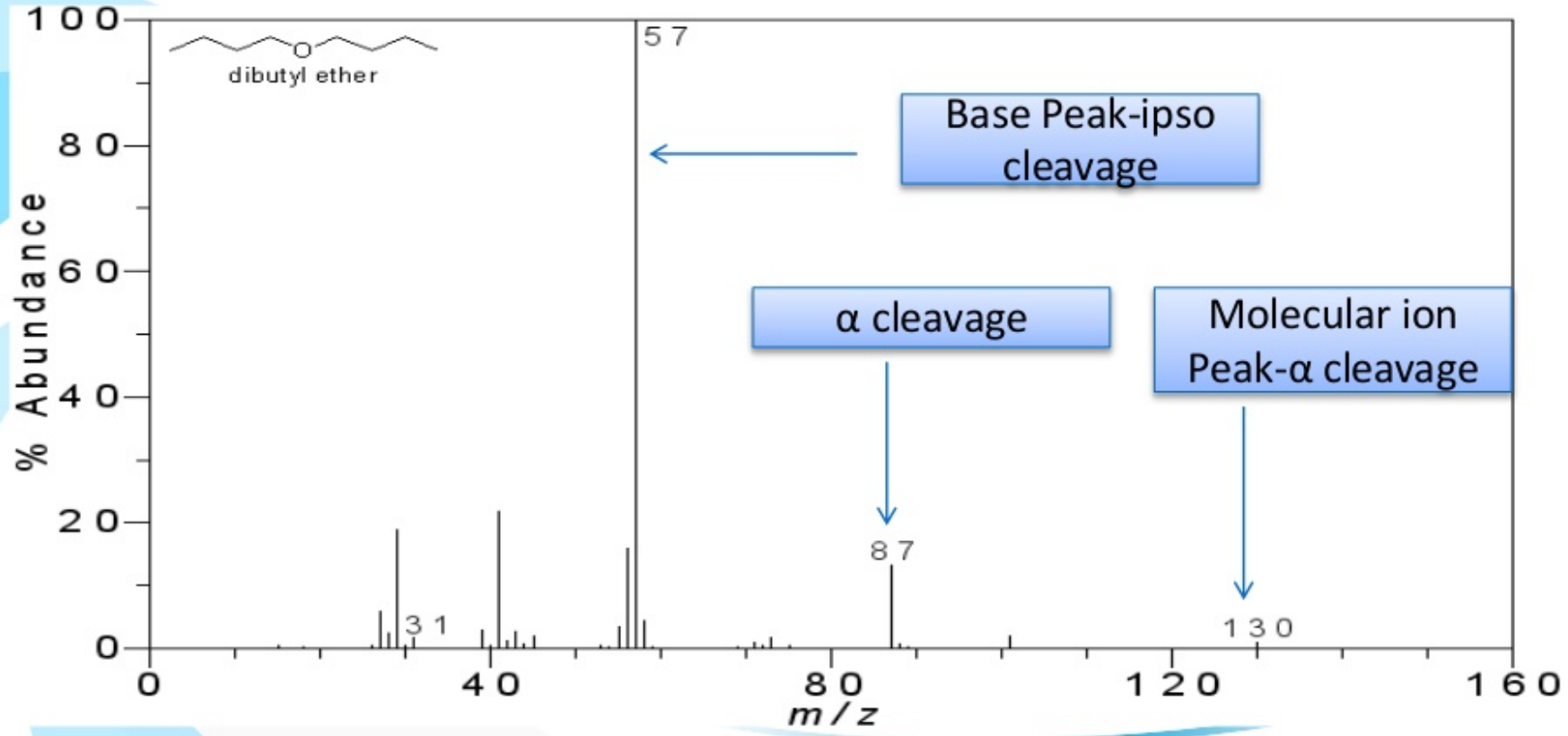


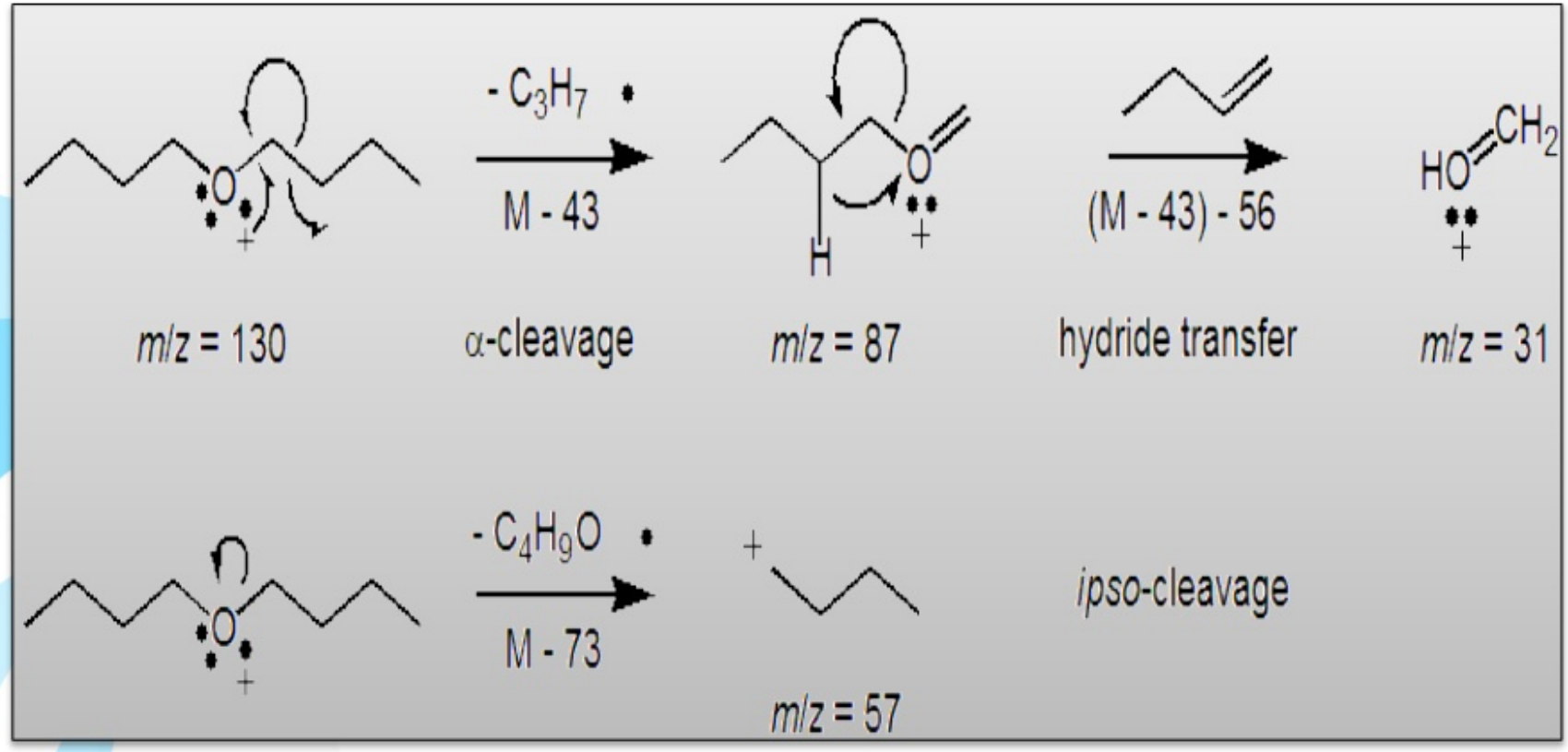

- CH_3O
M - 31
α-cleavage
m/z = 102
m/z = 71
- CO
(M - 31) - 28
α-cleavage
m/z = 43
- C_3H_7
M - 43
α-cleavage
m/z = 59
M - 73
H_3C CH_2+
β-cleavage
m/z = 29
M - 87
CH_3+
β-cleavage
m/z = 15
- CH_3
M - 15
β-cleavage
m/z = 87
H_2C=CH_2
M - 28
McLafferty rearrangement
m/z = 74

Ether

Mol.wt:-130

Mol.formula:-$C_8H_{18}O$




- C_3H_7 •
M - 43
m/z = 130
α-cleavage
m/z = 87
(M - 43) - 56
hydride transfer
m/z = 31
- C_4H_9O •
M - 73
m/z = 57
ipso-cleavage