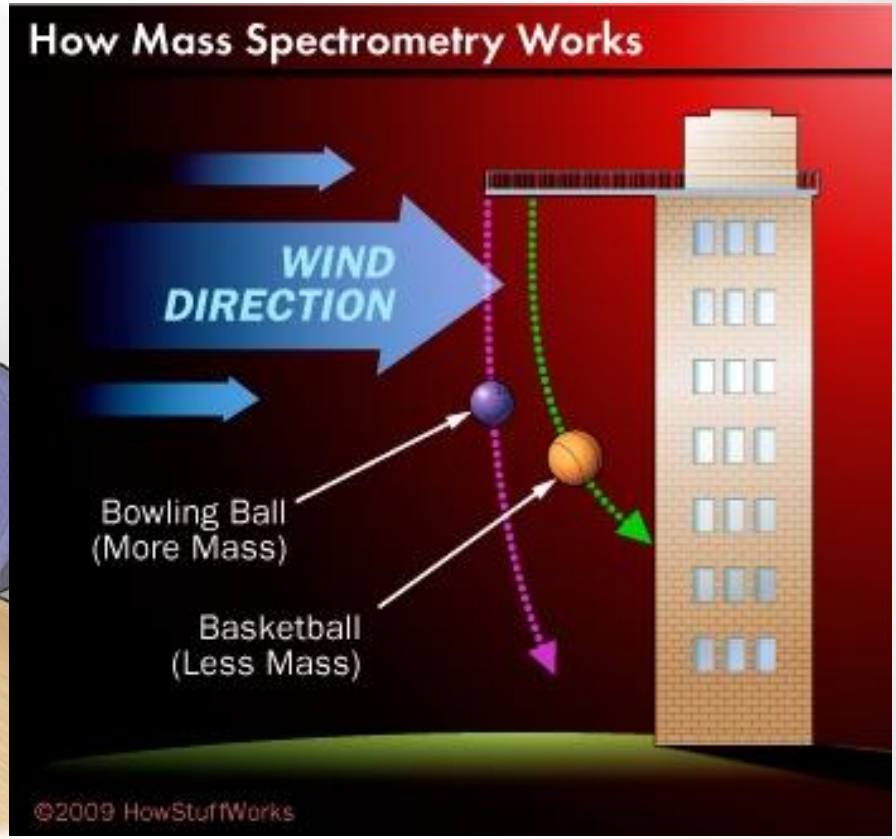
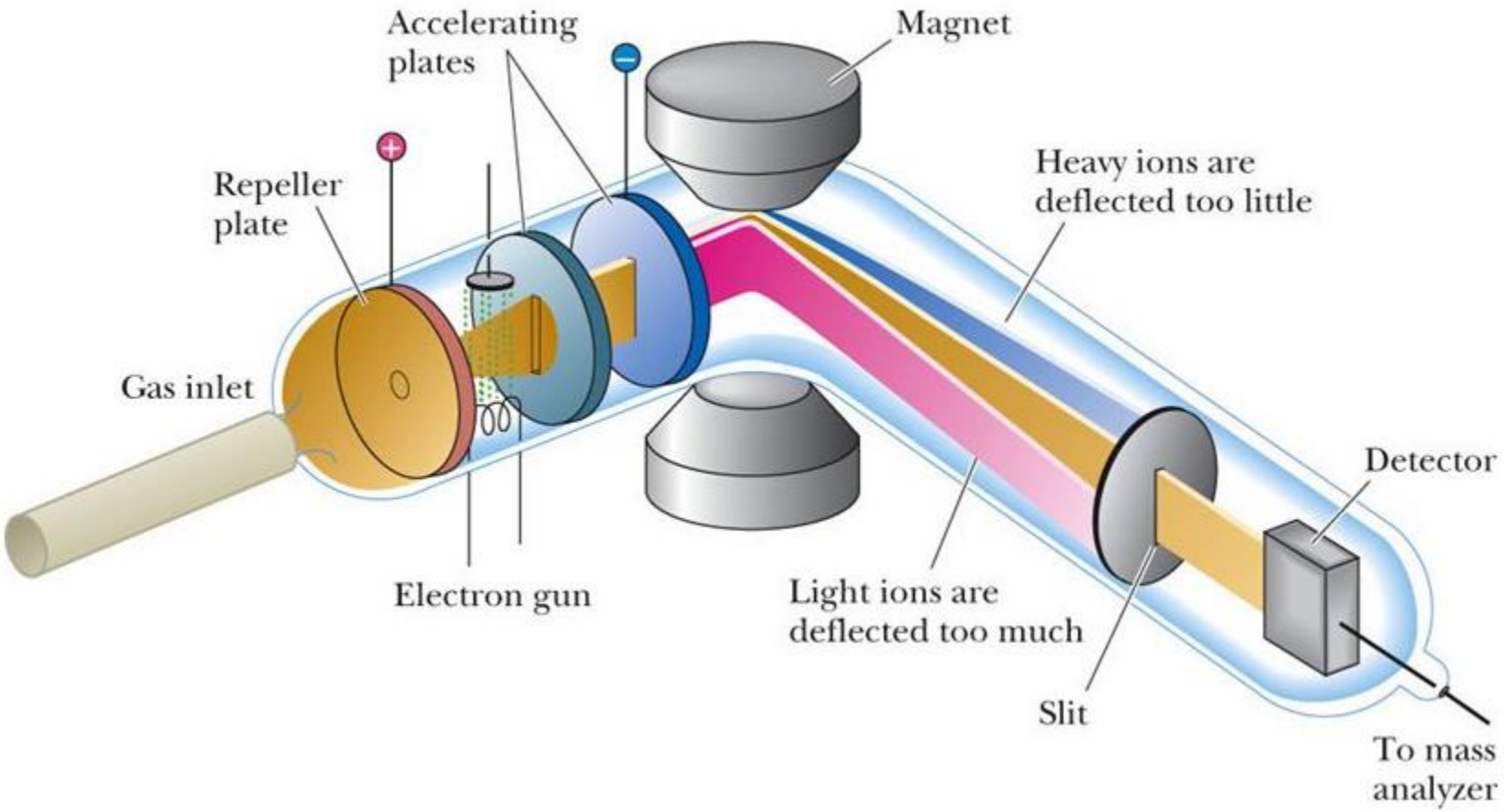
Mass – Spectrometry

"Okay—who put my lunch through the mass spectrometer..?"

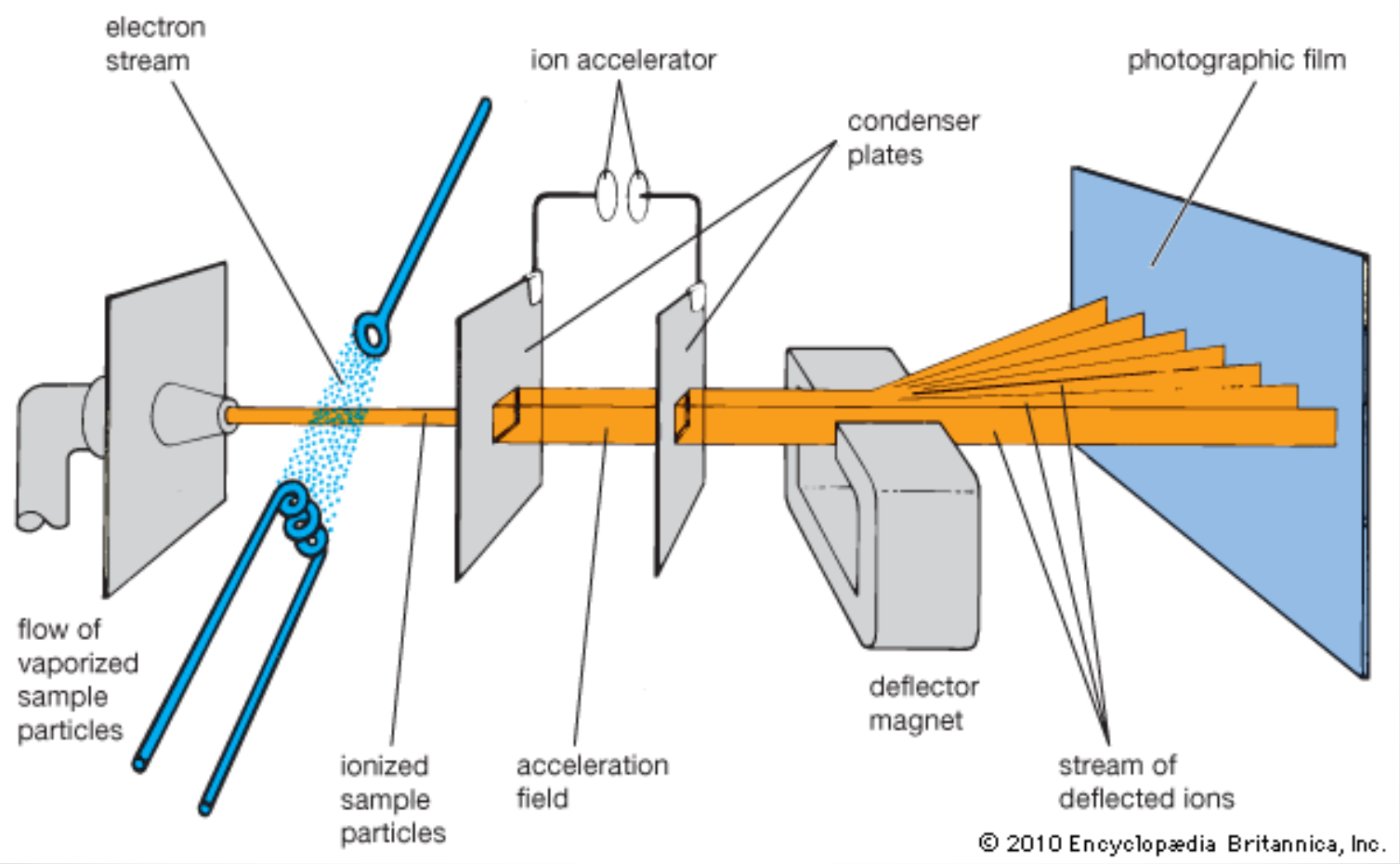


Department of Organic Chemistry
Dr hab. Sławomir Makowiec prof. PG

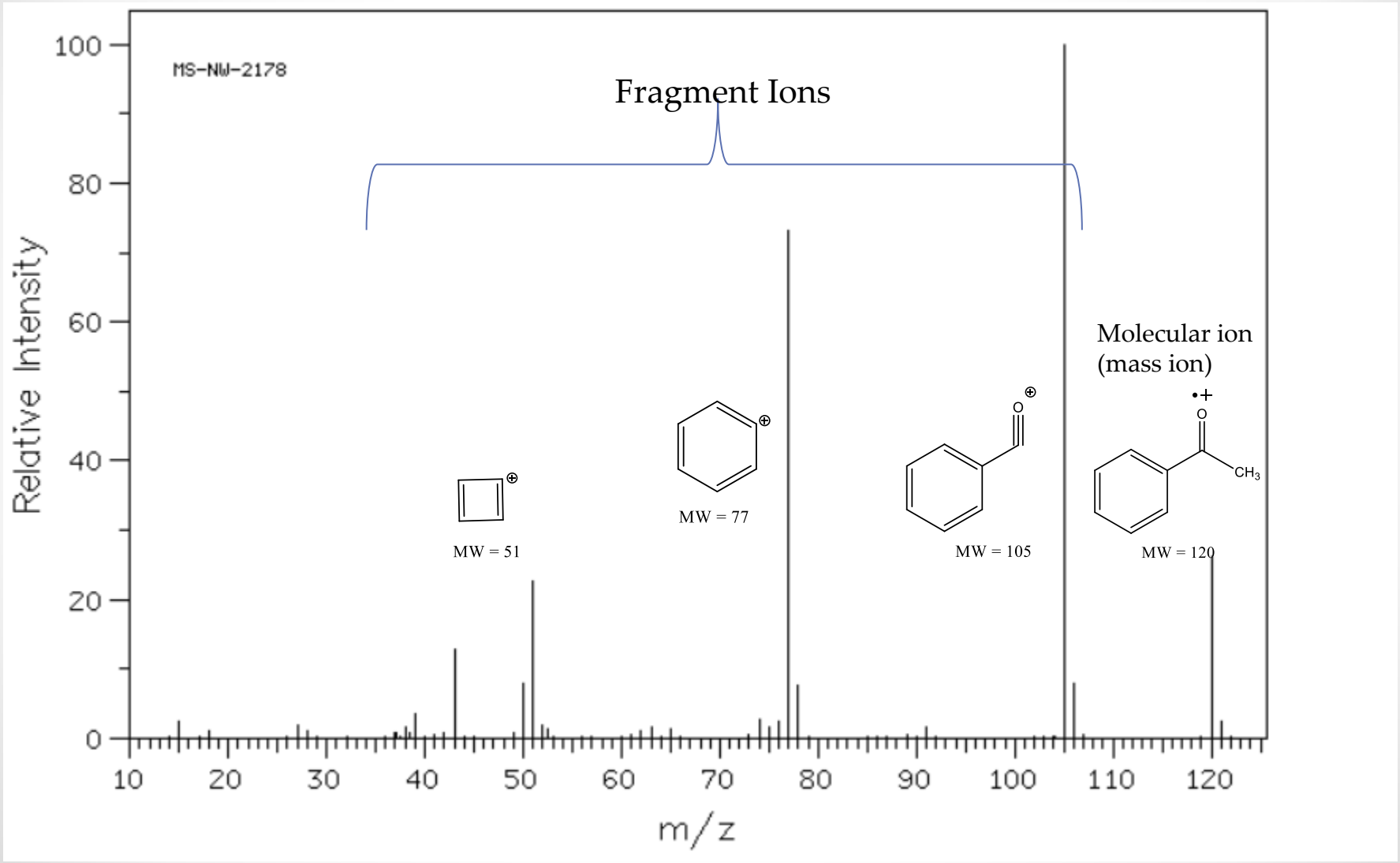
Mass spectrometry : Apparatus



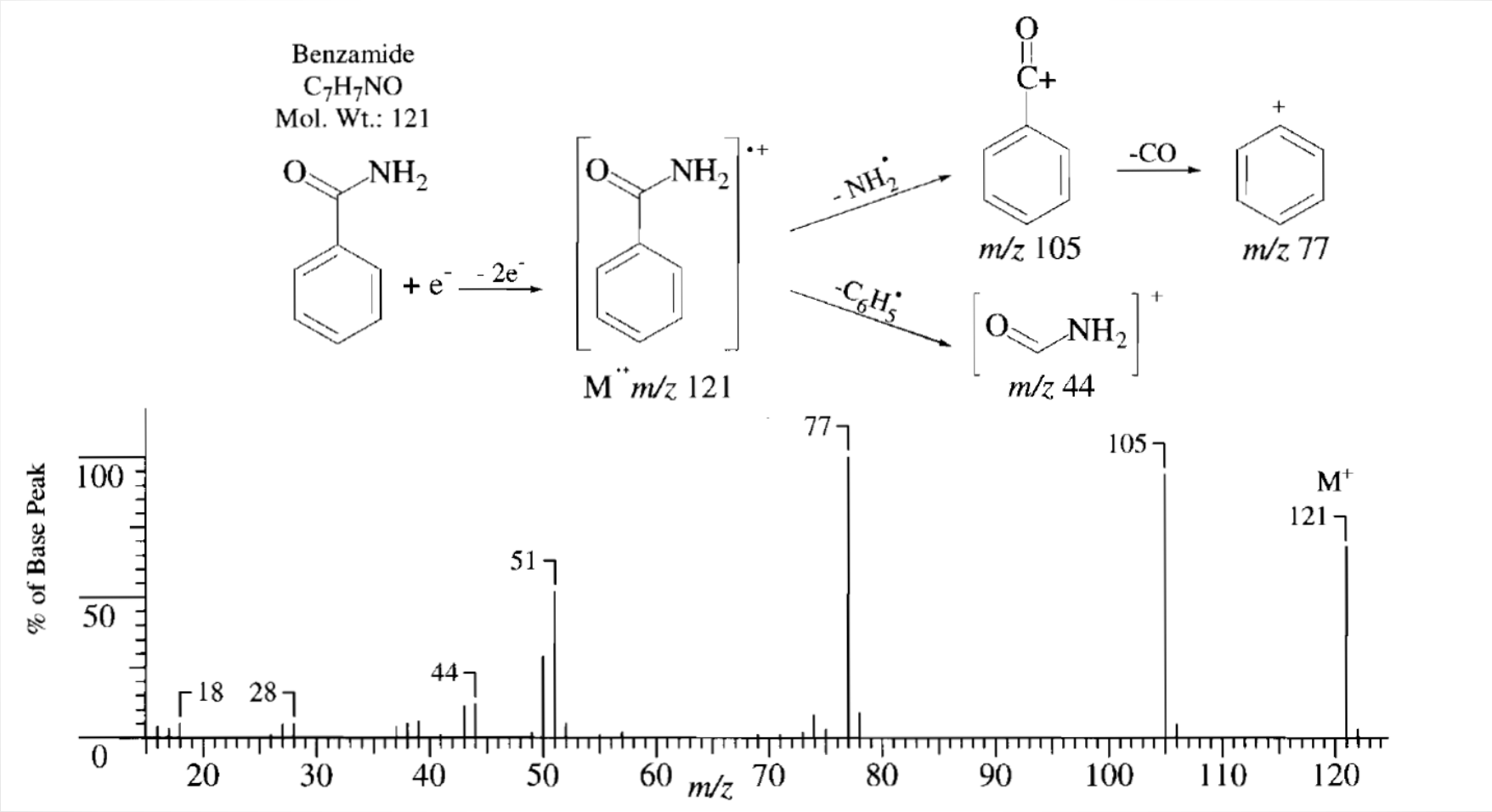
Mass spectrometry :Aparatus



MASS Spectrum of acetophenone

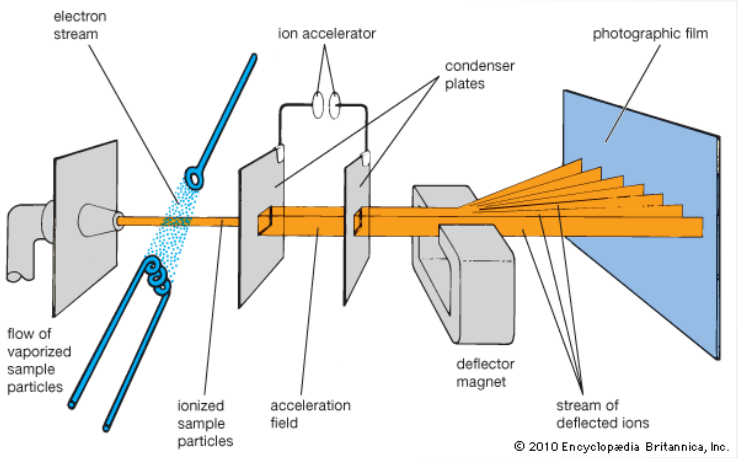


MASS Spectrum of Benzamide



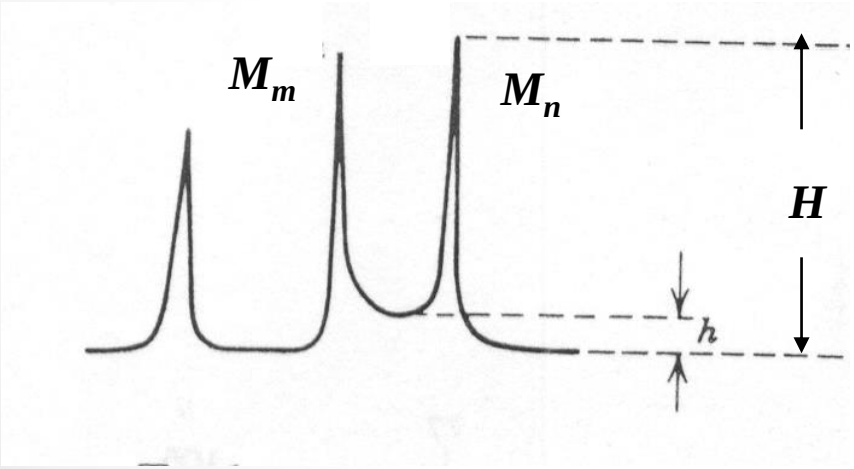
Mass spectrometry:
Processing steps of the sample

1. Ionization of molecules
2. Fragmentation of ionized molecules
3. Acceleration of ions
4. Analysys of the ions



Resolution of mass spectrometer

$$R = \frac{M}{\Delta M} = \frac{M_n}{M_n - M_m} = \frac{10001}{10001 - 10000} = 10000$$

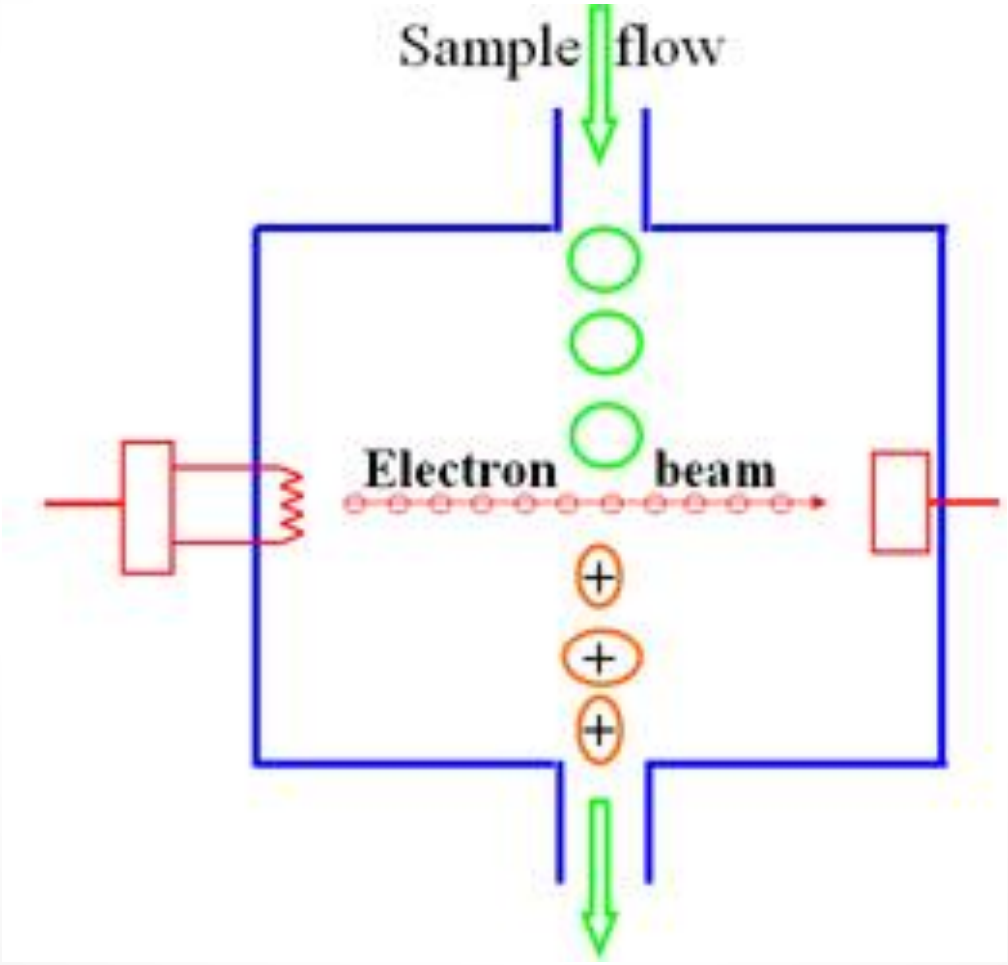


$$\frac{h}{H} * 100 \leq 10\ \%$$

Ion sources

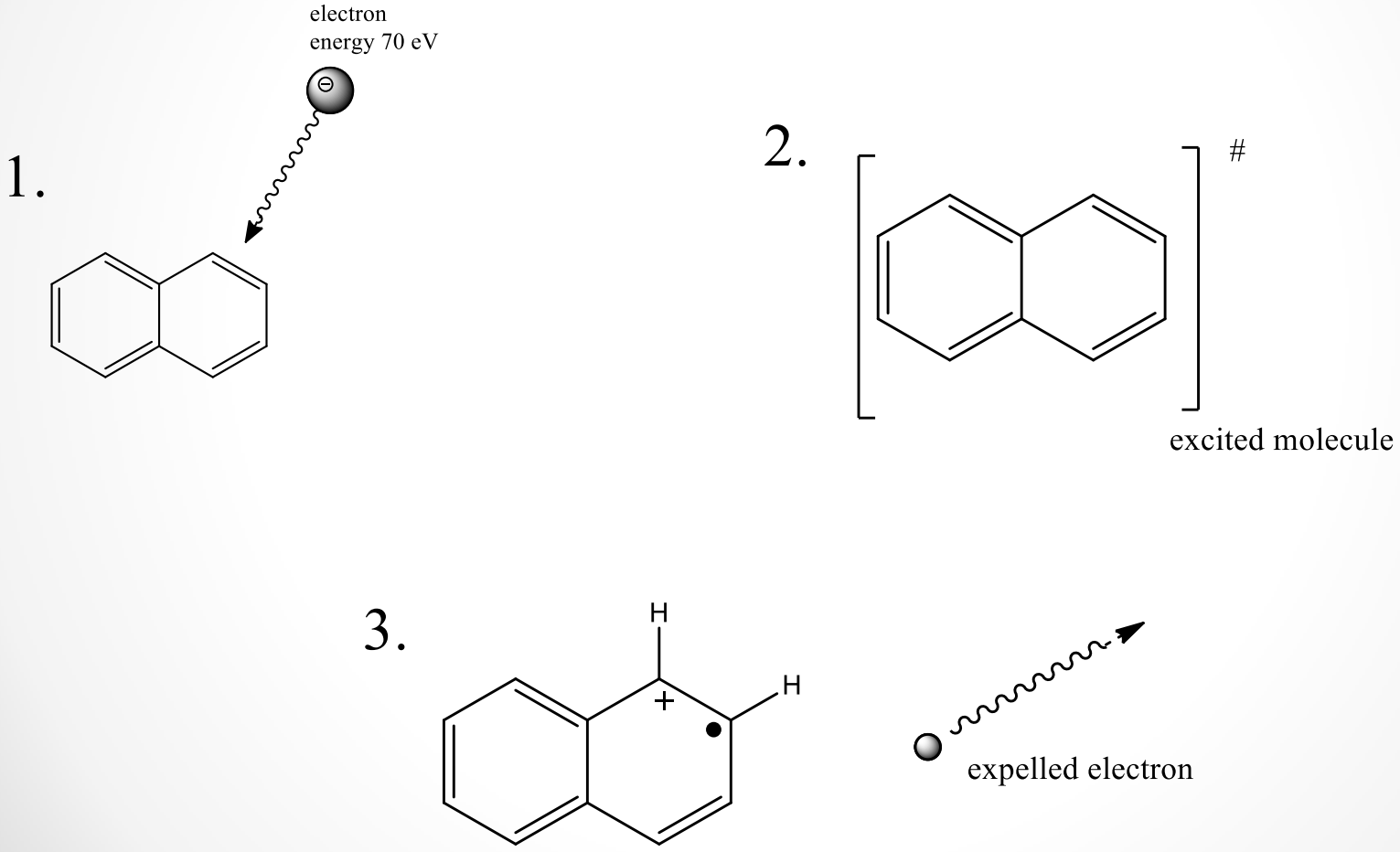
- 1. Electron ionization (EI) (Electron Impact)
- 2. Chemical Ionization (CI)
- 3. Fast Atom Bombardment (FAB)
- 4. Laser Desorption (LD)
- 5. Matrix-Assisted Laser Desorption Ionization (MALDI)
- 6. ElectroSpray ionization (ESI)

Electron Ionization (EI) – Ionization Chamber



Electron Ionization (EI)

What is going on physically?

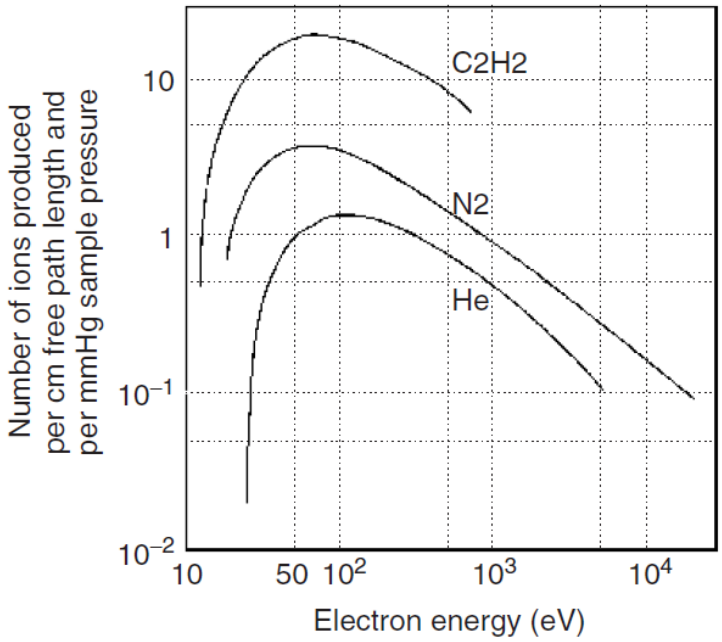


Electron Ionization - Energy of electrons

Each electron is associated to a wave whose wavelength λ is given by

$$\lambda = \frac{h}{m\,\upsilon}$$

where m is its mass, v its velocity and h Planck's constant.
Wavelength is 2.7Å for a kinetic energy of 20 eV and **1.4 Å for 70 eV.**



Number of ions produced as a function of the electron energy.

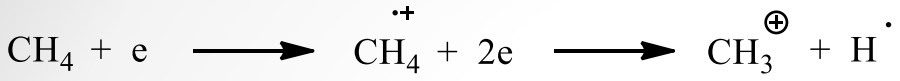
Advantages of EI

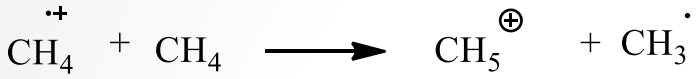
1. Reproducible method
2. High Ionization Efficiency
3. All vaporized molecules can be ionized (non polar and insoluble)
4. Molecular structural information (fragmentation)

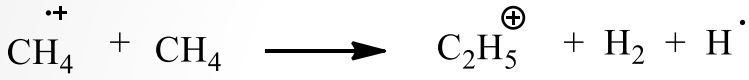
Disadvantages of EI

1. Only +ve ions are formed
2. Sample has to be volatile
3. Limits to 600Da or less MW
4. Extensive fragmentation

Chemical Ionization (CI)



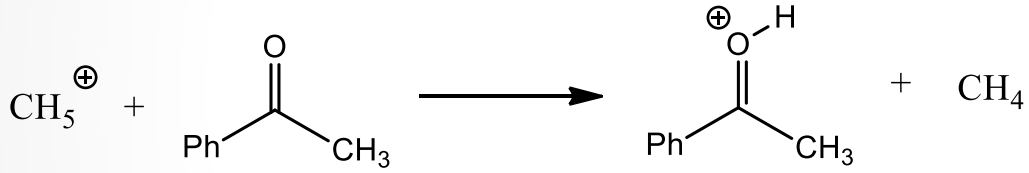


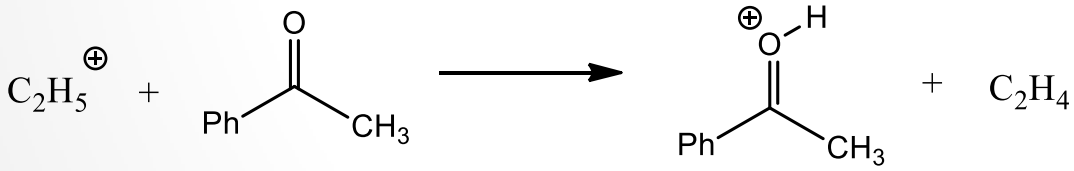


1. Sample is injected in atmosfere of gas (methane, izobutane, ammonia).

2. Gas is ionised by EI method.

3. During the collisions of methane ions with molecules of sample, energy is transfered, as well as protons are transfered.

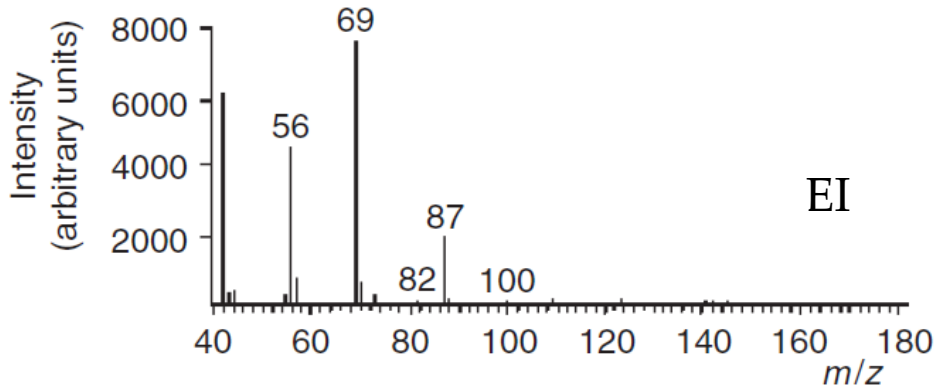




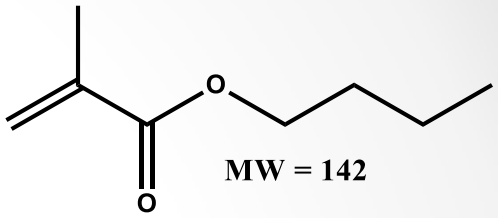
When methane is a ionizing gas: the relevant peak observed are $[M+H]^+$, $[M+CH_5]^+$, and $[M+C_2H_5]^+$

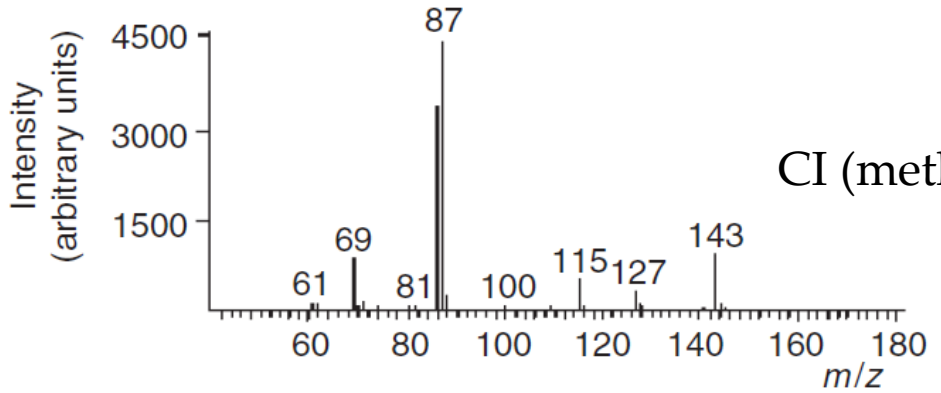
Chemical ionization (CI) is a technique that produces ions with little excess energy - less fragmentation - higher molecular ion

Comparison of EI and CI

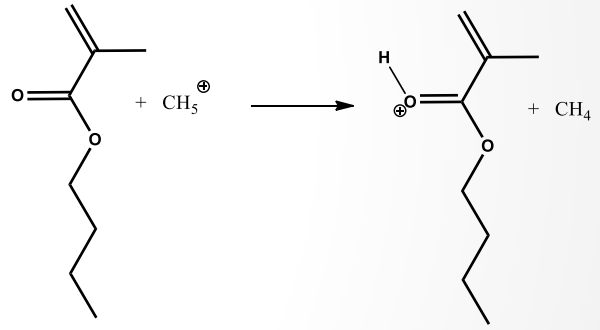


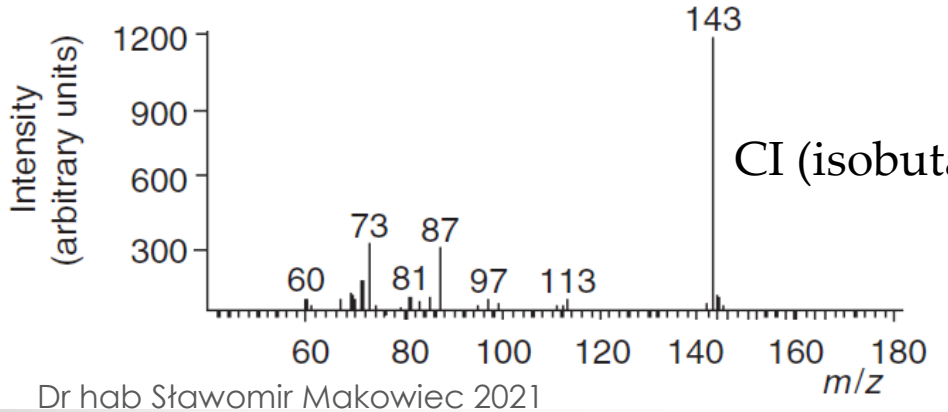
EI



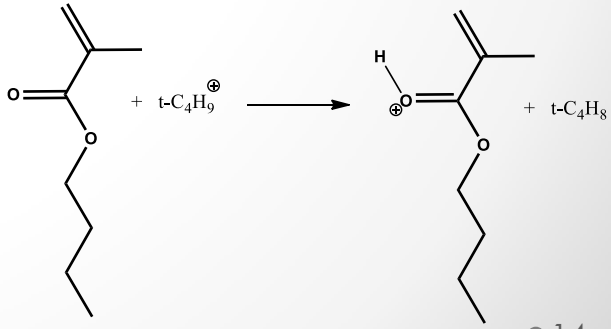


CI (methane)

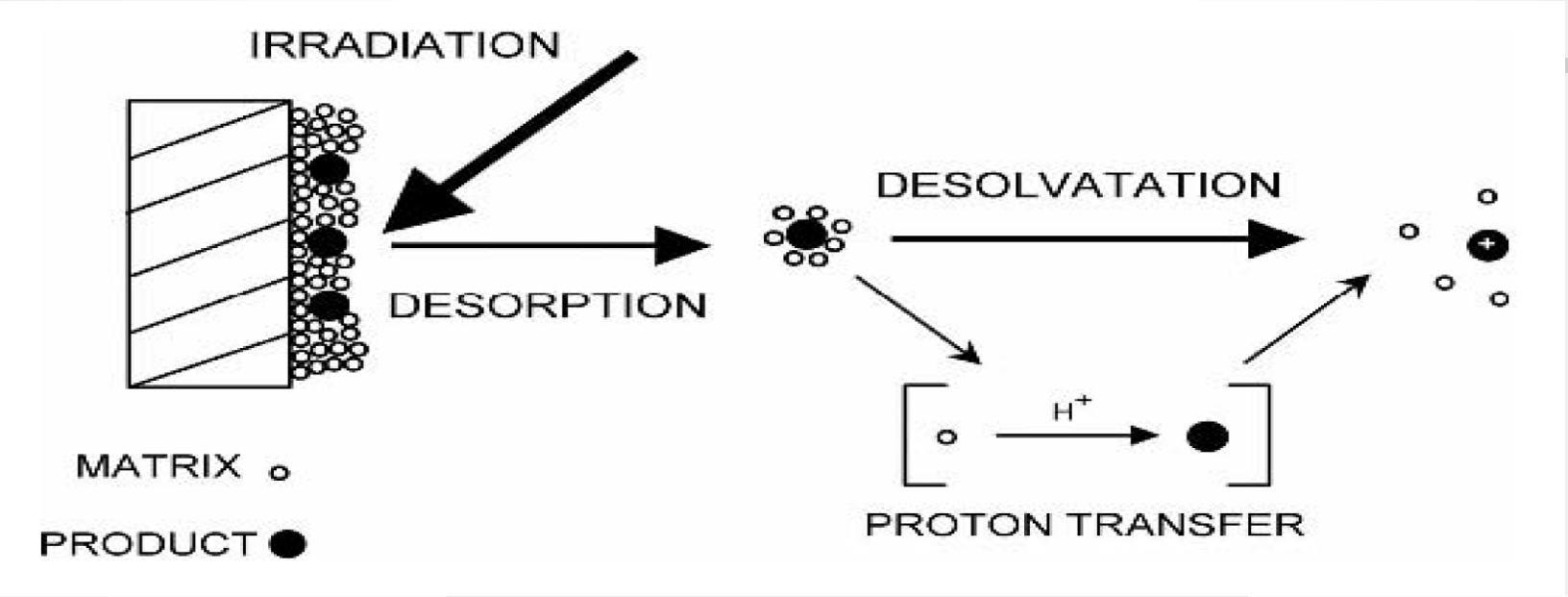




CI (isobutane)



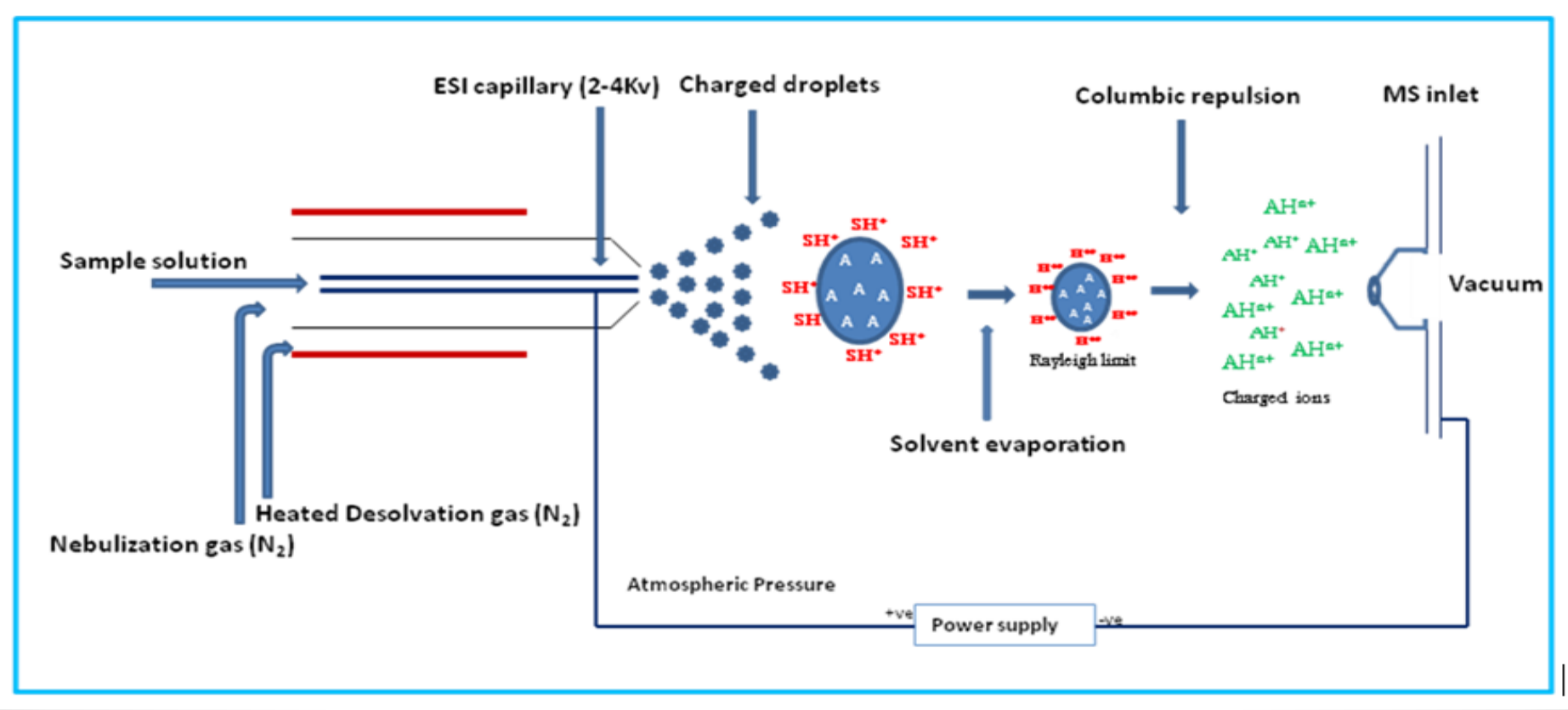
MALDI Matrix-Assisted Laser Desorption Ionization



The matrix minimizes sample damage from the laser pulse by absorbing most of the incident energy and increases the efficiency of energy transfer from the laser to the analyte.

MALDI allows the desorption and ionization of analytes with very high molecular mass , **up to 300 000 Da**.

Electrospray ionization ESI



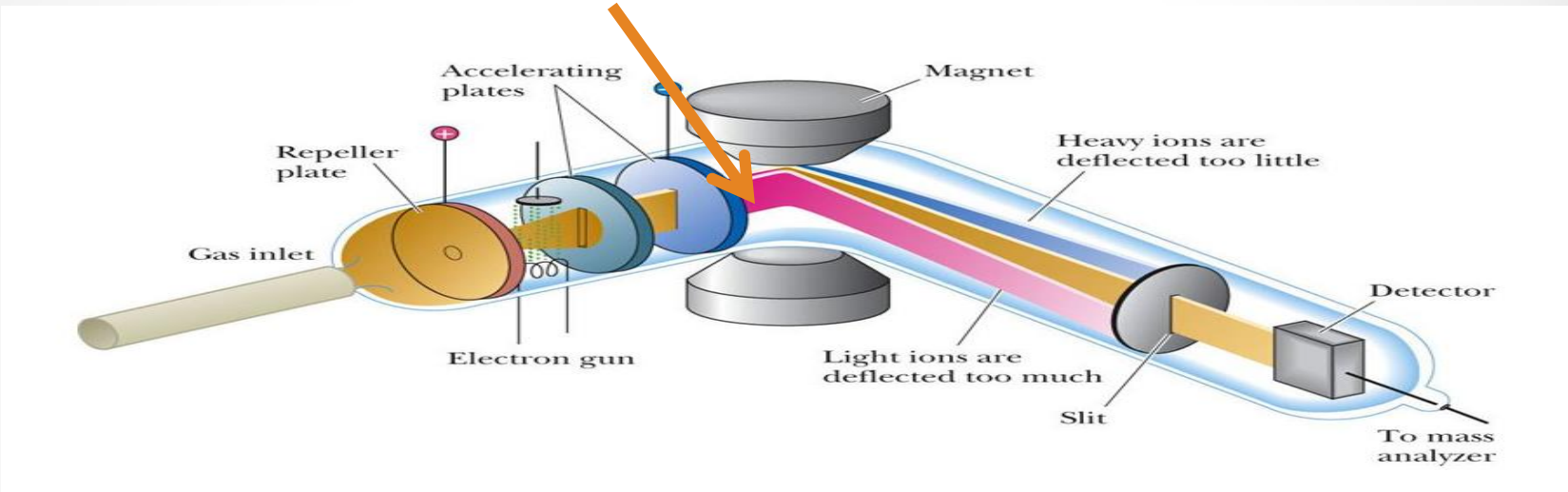
1. The electric field is obtained by applying a potential difference of 2–4 kV
2. This field induces a charge accumulation at the liquid surface located at the end of the capillary, which will break to form highly charged droplets.
3. Solvent is evaporated by the stream of heated inert gas.
4. When the electric field on their surface becomes large enough, desorption of ions from the surface occurs.

Ions in Mass Spectrometry

- ***Molecular ion*** - An unfragmented (parent) *ion* that results from the loss of an electron by a *molecule* following ionization, has the same mass as the molecule (sample).
- ***Main ion*** – An ion that corresponds to the highest intensity peak.
- ***Metastable ion*** - Ion formed with internal energy higher than the threshold for dissociation but with a lifetime great enough to allow it to exit the ion source and enter the mass analyzer region where it dissociates before detection.

Metastable Ions in Mass Spectrometry

- ***Metastable ion*** **[AB]⁺** - dissociates before detection but after acceleration.



$$[AB]^{\oplus} \longrightarrow A^{\oplus} + B$$

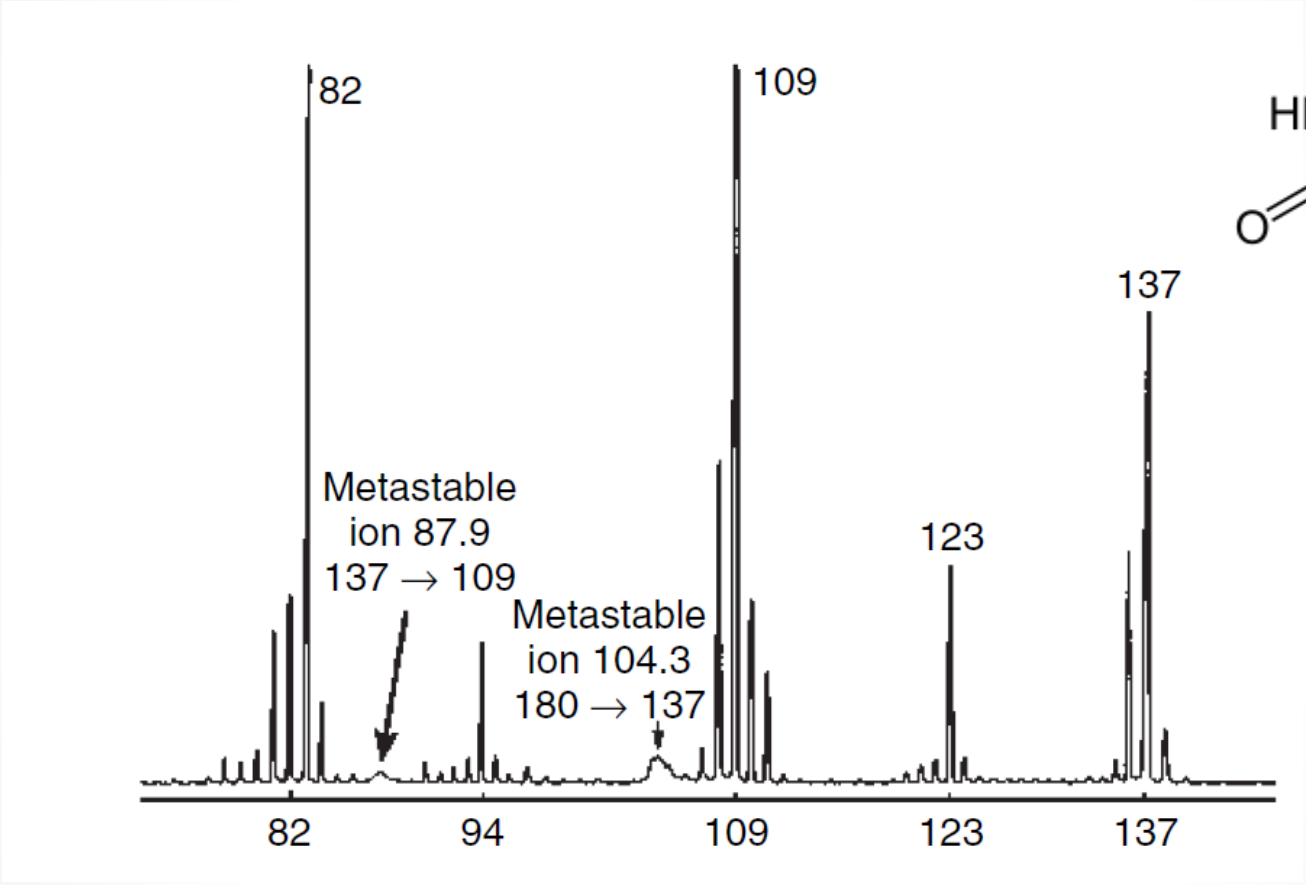
$$m_1 \qquad m_2 \quad (m_1 - m_2)$$

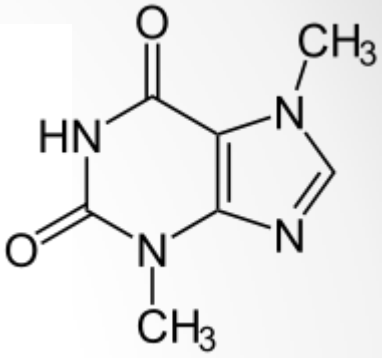
Fragment „B" takes part of kinetic energy.
Ion A^+ (metastable) has lower kinetic energy that „ordinary" Ion A^+

Metastable ion A^+ is recorded as ion with an apparent mass m*

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

Metastable ions





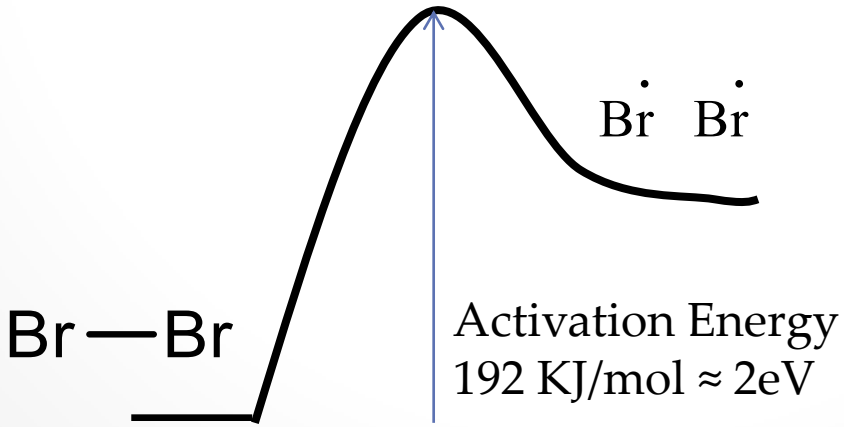
Theobromine
MW = 180

The signal detected at an apparent *m/z* 87.9 comes from the metastable fragmentation of the ion at 137 Da, which loses 28 Da and yields the 109 Da fragment.

Similarly, the signal at apparent *m/z* 104.3 comes from the metastable fragmentation 180→137 Da.

Fragmentation Reactions

- During EI ionization we transfer 10-20 eV energy to molecule, it is enough to exceed activation energy for bond breaking
- 1eV ≈ 96 KJ/mol, typical activation energy is in range **50-250kJ·mol^{-1}.**



Fragmentation Rules

- **Molecules fragment to (the most) stable particles.**
- **The most stable particles has lowest energy.**

- **Stablization factors:**

a) **High heat of formation - stable compounds - cleavage is often associated with elimination of small stable molecule:**

H_2O, N_2, $C{\equiv}O$, CO_2, $HC{\equiv}N$, H_2S

also other compounds with multiple bond :

$R_2C{=}O$, $R\text{-}C{\equiv}N$, $R_2C{=}CR_2$,

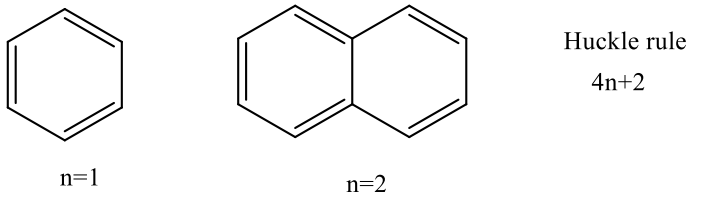
and compounds with strong single bonds:

C-F, C-O **They don't undergo fragmentaion**

Fragmentation Rules

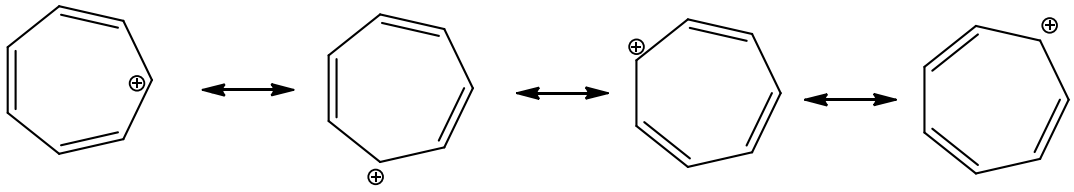
- Stablization factors:

b) Aromaticity: phenyl, naphtyl, cyclopentadienyl cation



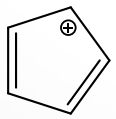
Huckle rule

4n+2



4n+2

n=1

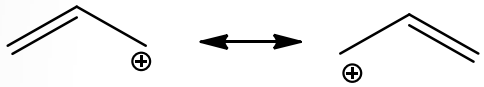


Is it aromatic?

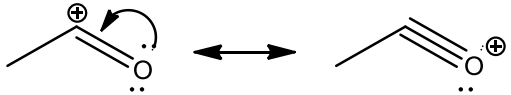
Fragmentation Rules

- Stablization factors:

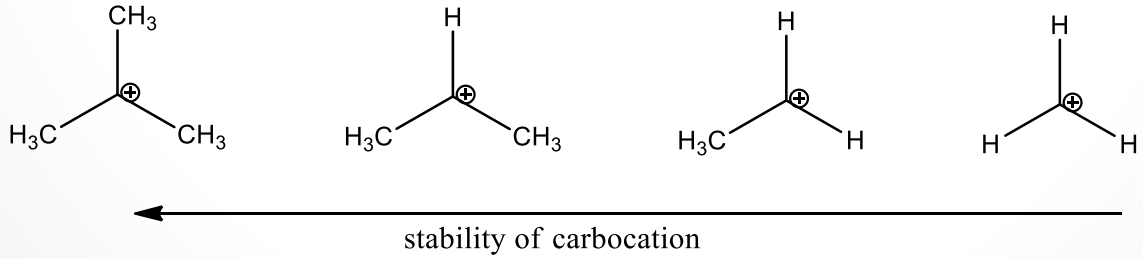
c) Mezomeric effect: allilic cation



acylium cation

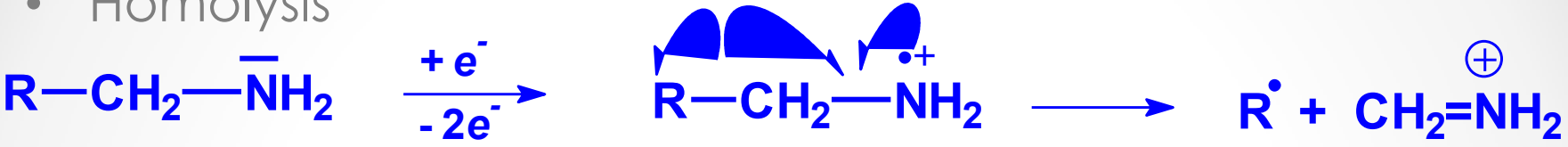


d) Hyperconjugation : tertiary carbocation more stable than secondary

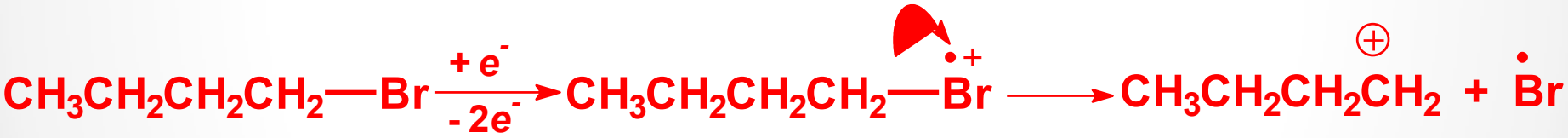


Fragmentation pathways

- Homolysis



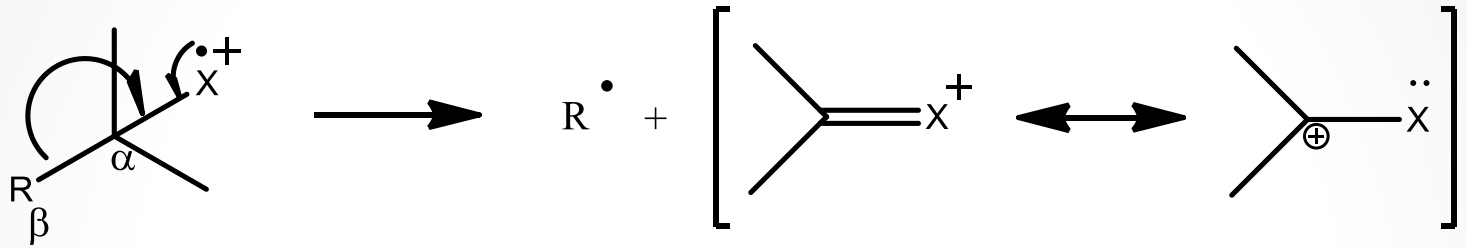
- Heterolysis



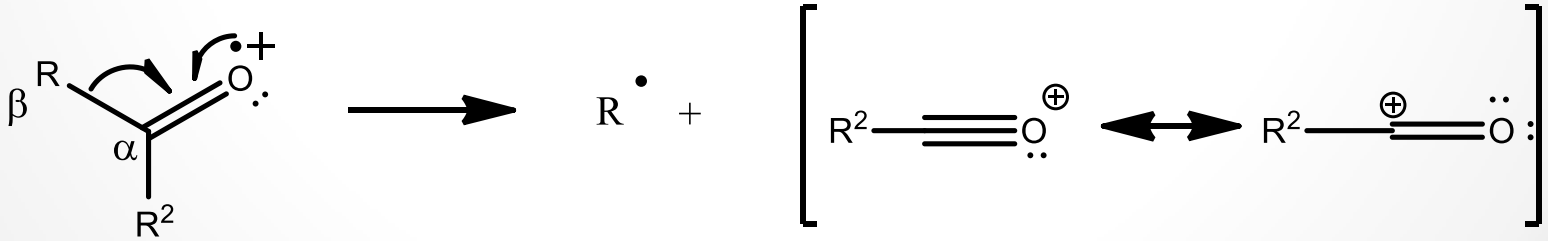
- Fragmentation starting points:
- lone pair of electrons on heteroatoms,
- π- bonds of non-conjugated systems

Fragmentation pathways

- α – Cleavage - homolysis between α and β atoms



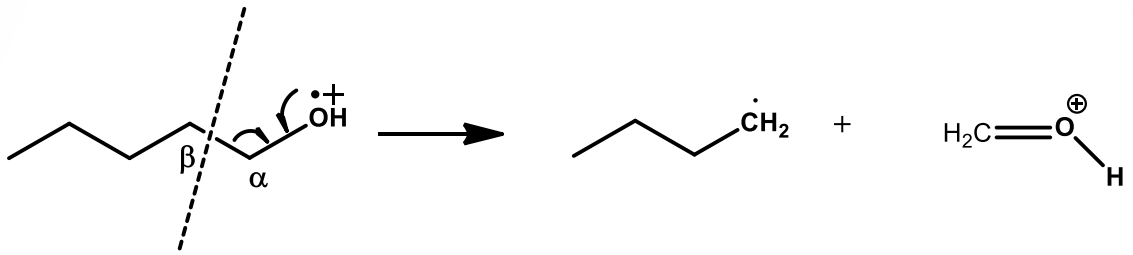
X = NR_2, OH, OR, SH, SR

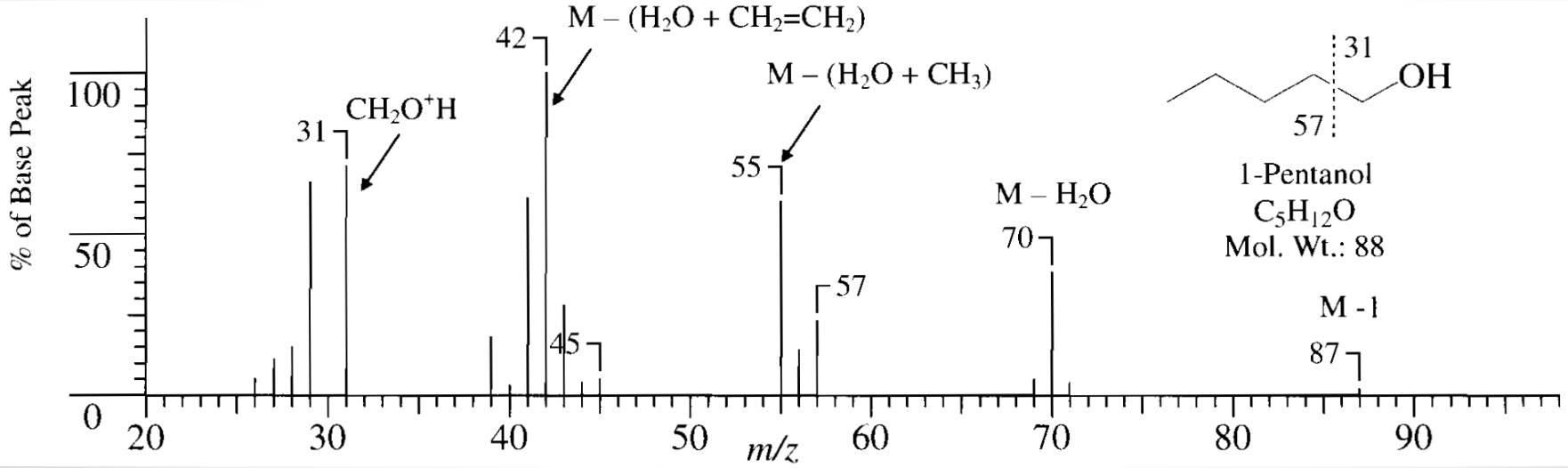


R^2 = H, Alk, Ar, NR_2, OH, OR

Fragmentation pathways

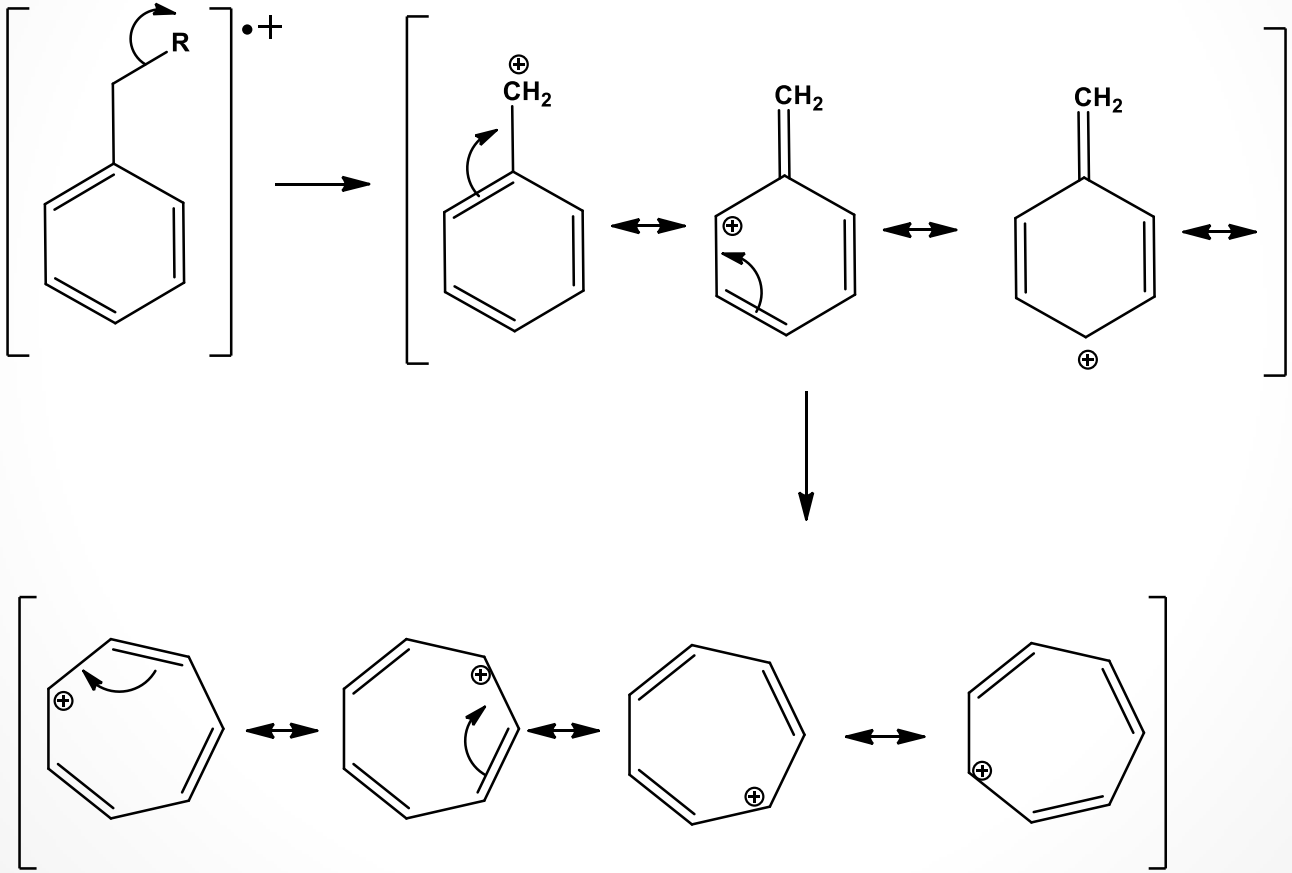
- α – Cleavage of pentanol, peak at 31 m/z





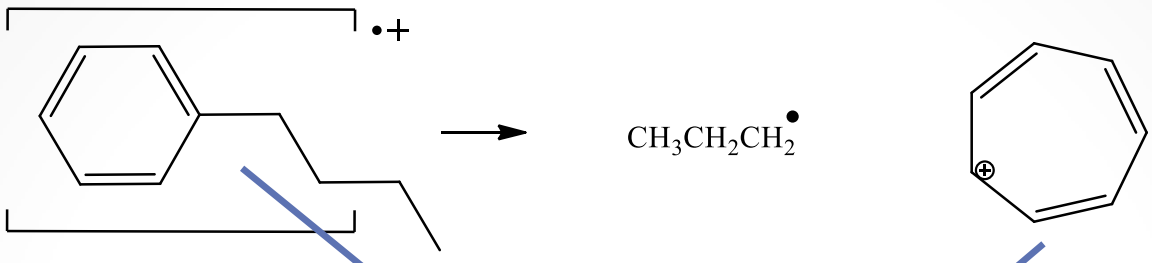
Fragmentation pathways

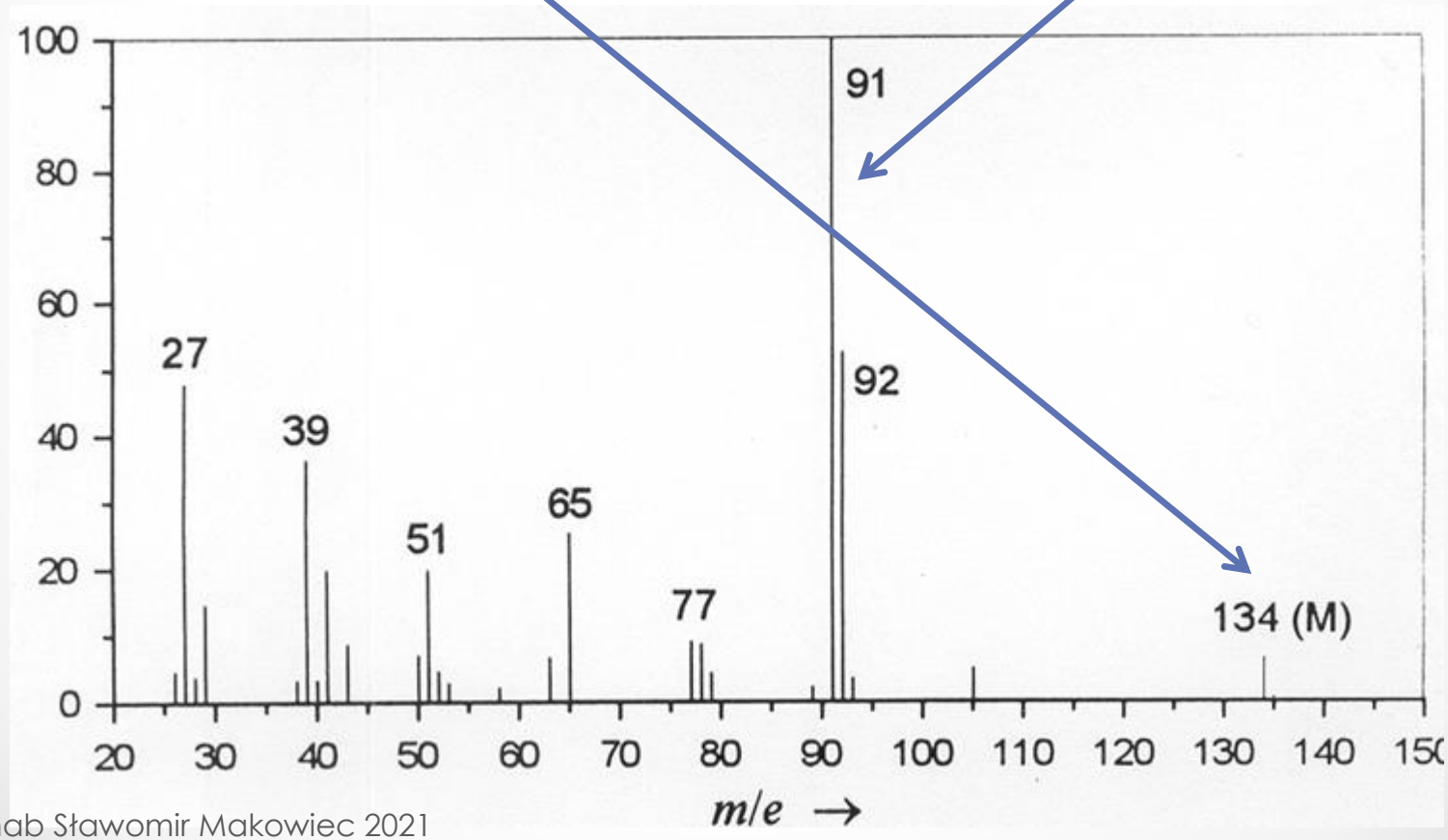
- Homolytic cleavage of alkyl aromatic derivatives, β – bond to aromatic ring



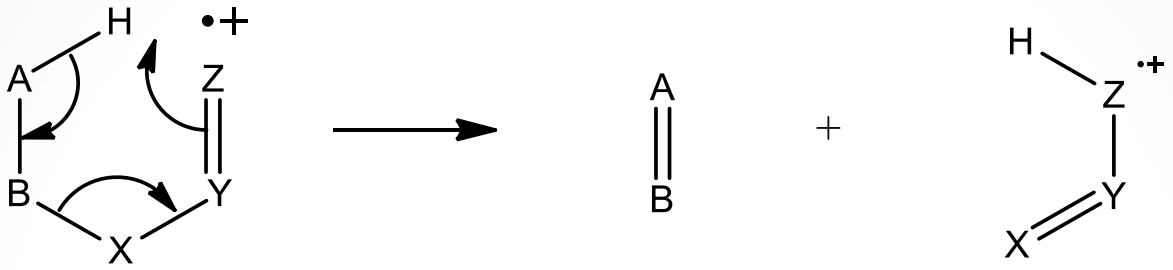
Tropylium cation m/z = 91

Cleavage of alkyl aromatic derivatives



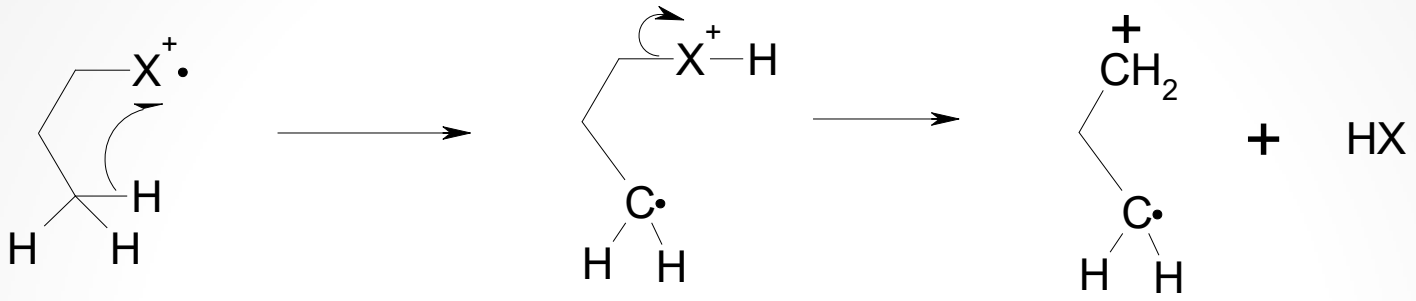


Mc Lafferty Rearrangement



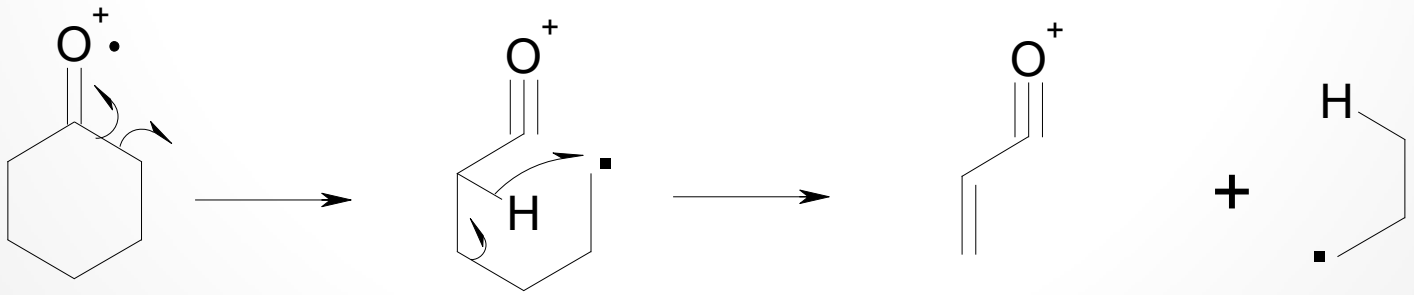
Type of Compounds	A	B	X	Y	Z
Alkenes	C	C	C	C	C
Aldehydes & ketones	C	C	C	C	O
Carboxyilc acids	C	C	C	C	O
Esters	C	C	C	C	O
Nitriles	C	C	C	C	N
Amides	C	C	C	C	O
Sulfoxides	C	C	C	S	O

Heterolytic cleavage carbon- heteroatom bond with hydrogen shift and elimination of stable molecule (H_2O, HCl)

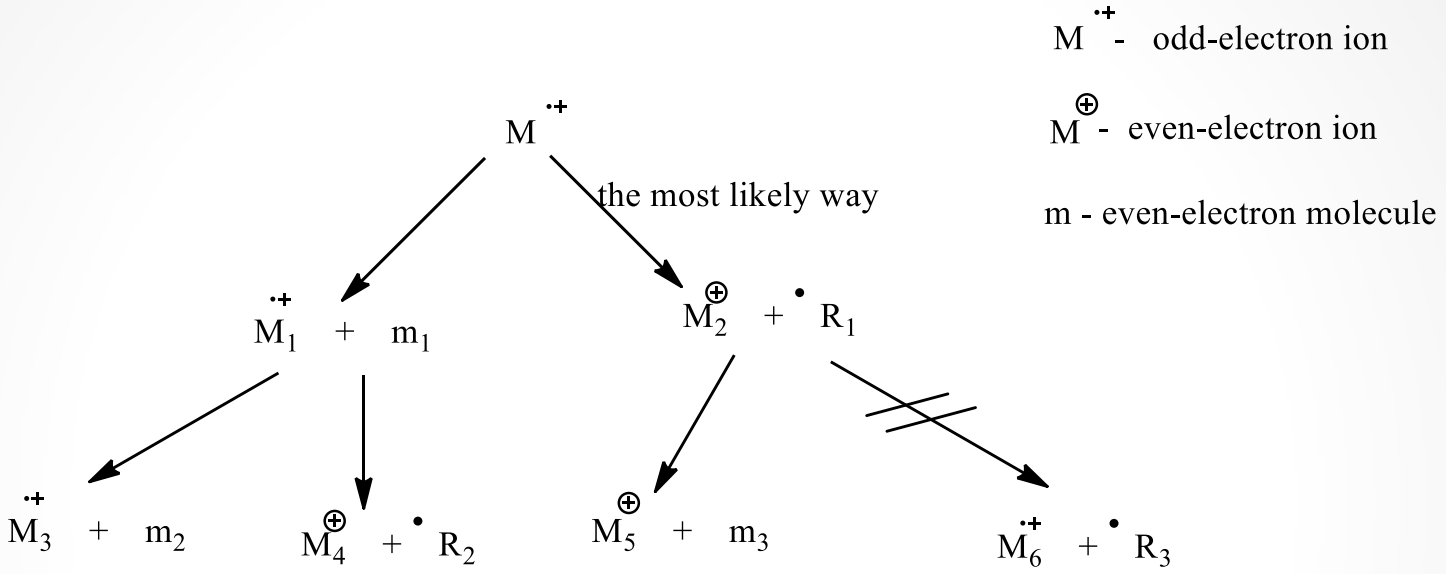


X = halogen, OH, SH

Cycloalkanes rearrangement with α-cleavage



Even and odd-electron cations



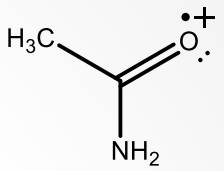
Even-electron ions are more stable than odd-electron ions

Nitrogen rule

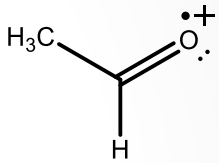
For odd-electron ions:

Odd- Molecular weight = odd number of nitrogen atoms

Even- Molecular weight = even number of nitrogen atoms



m /z = 59

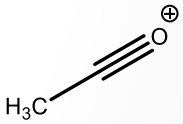


m /z = 44

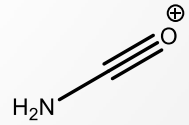
For even-electron ions:

Odd- Molecular weight = even number of nitrogen atoms

Even- Molecular weight = odd number of nitrogen atoms

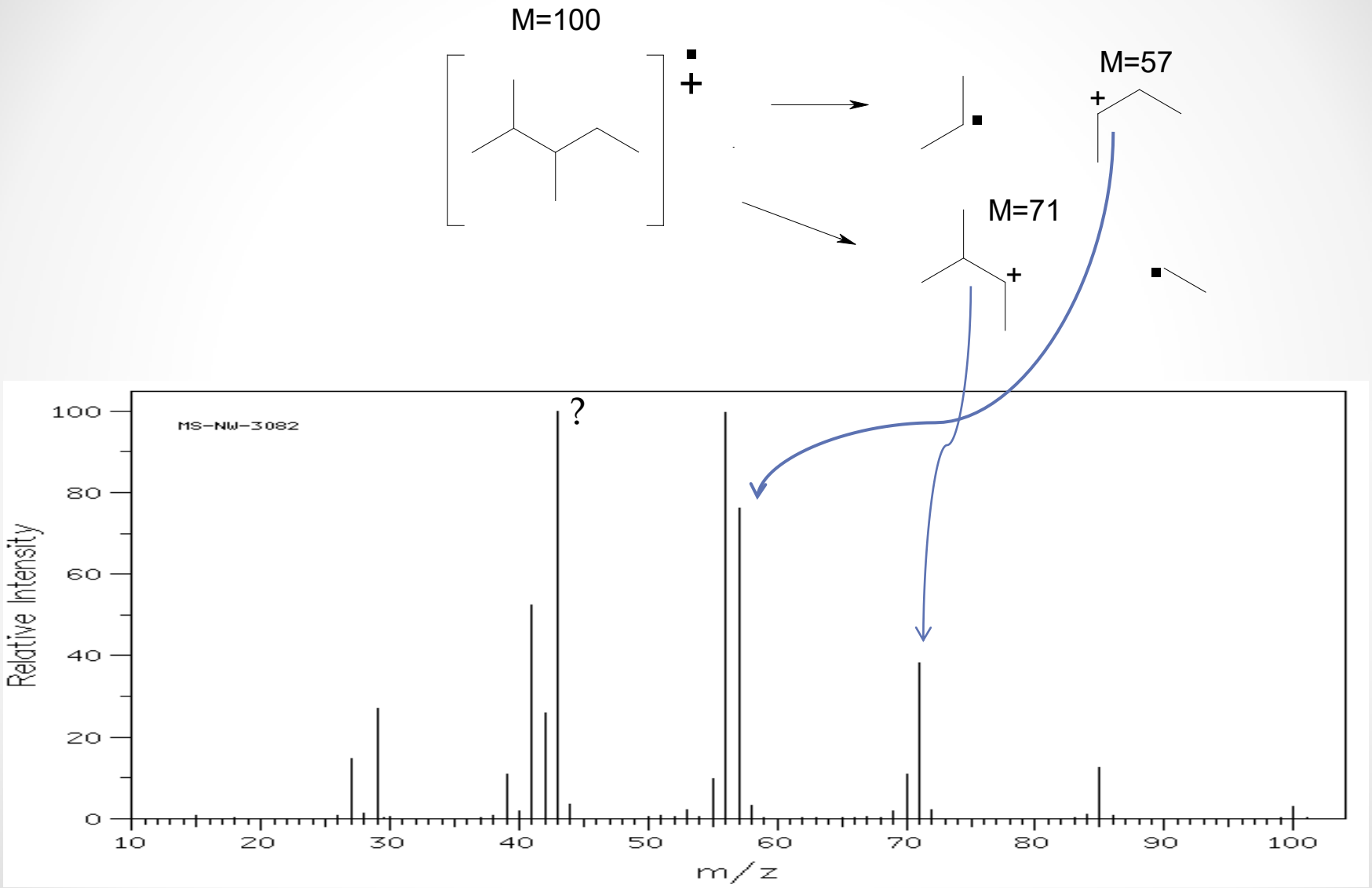


m/z = 43

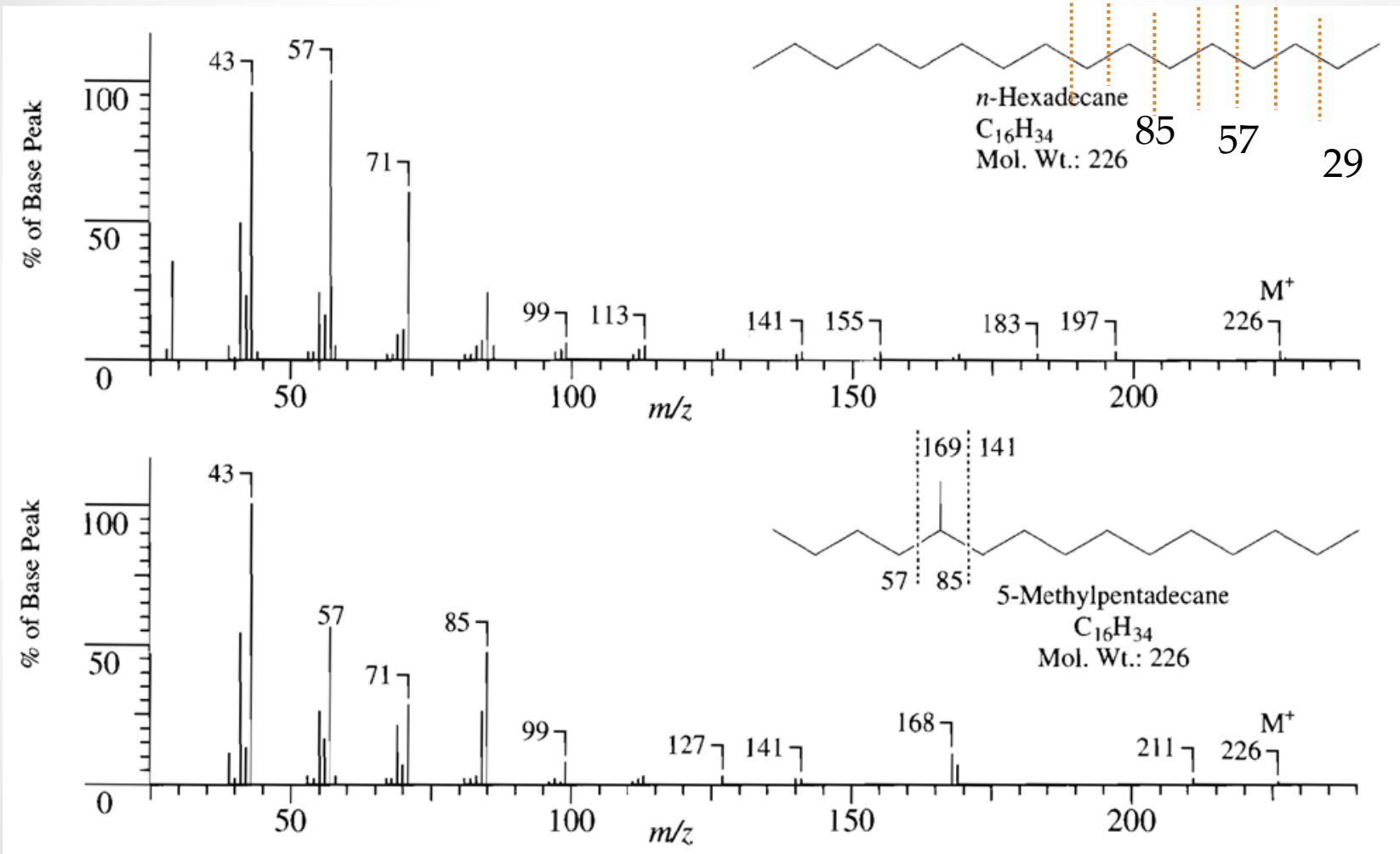


m/z = 44

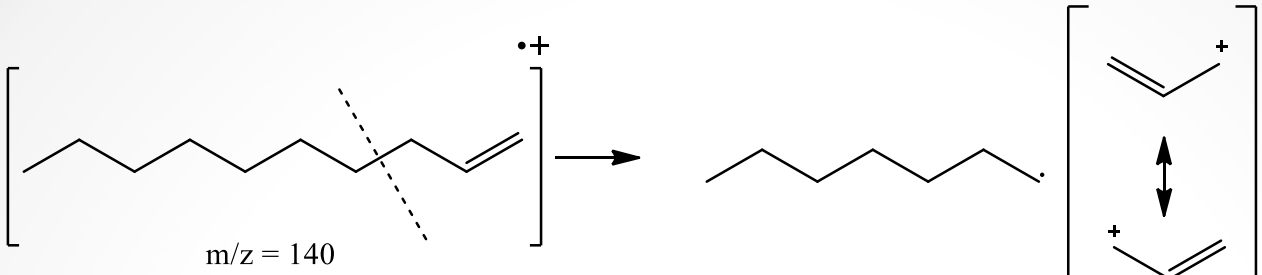
Alkanes


M=100
M=57
M=71
MS-NW-3082
?
Relative Intensity
m/z
0
20
40
60
80
100
10
20
30
40
50
60
70
80
90
100

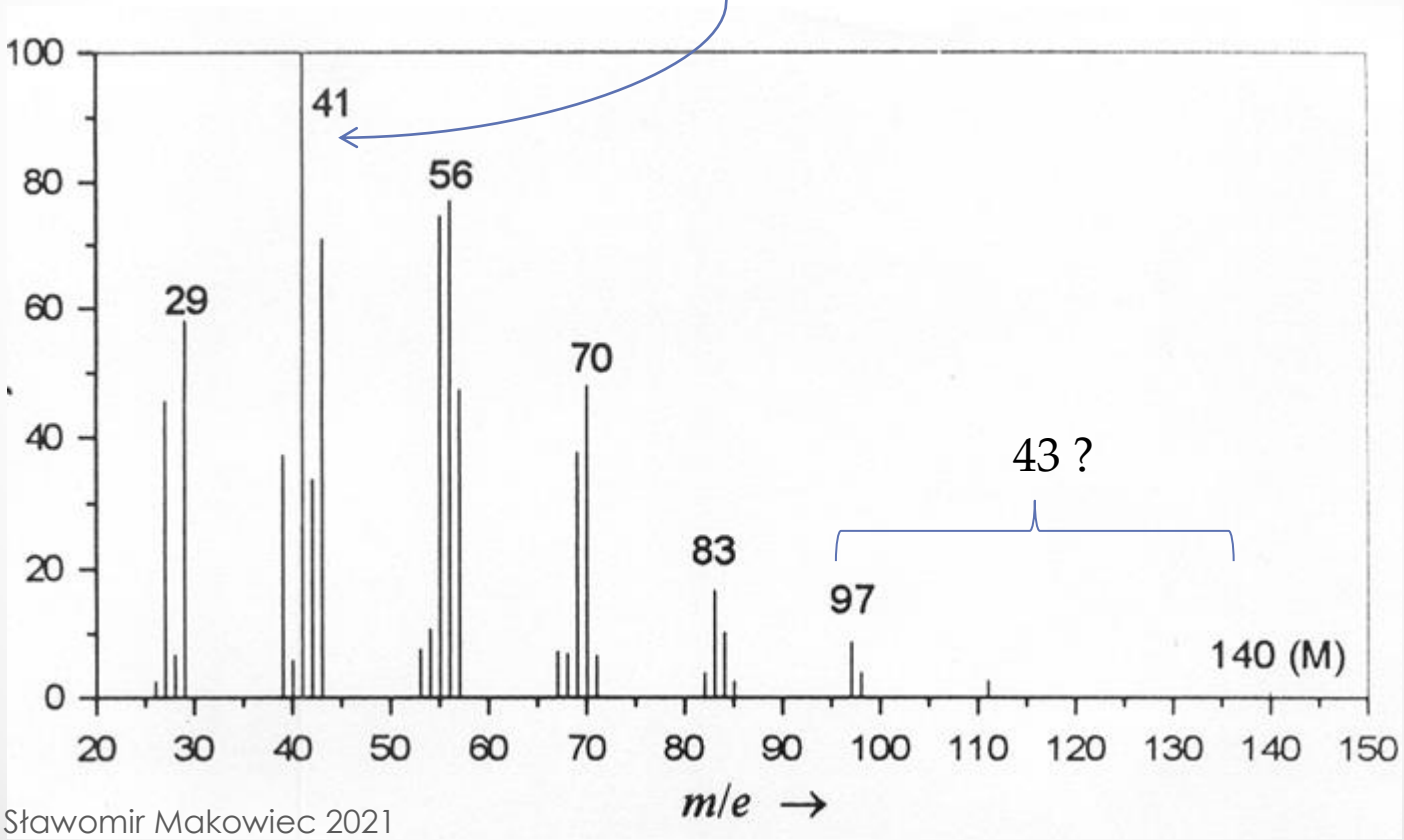
Alkanes


113 99 71 43
n-Hexadecane
C16H34
Mol. Wt.: 226
85 57 29
% of Base Peak
100
50
0
43
57
71
99
113
141
155
183
197
226
M+
50
100
150
200
m/z
169 141
57 85
5-Methylpentadecane
C16H34
Mol. Wt.: 226
% of Base Peak
100
50
0
43
57
85
71
99
127
141
168
211
226
M+
50
100
150
200
m/z

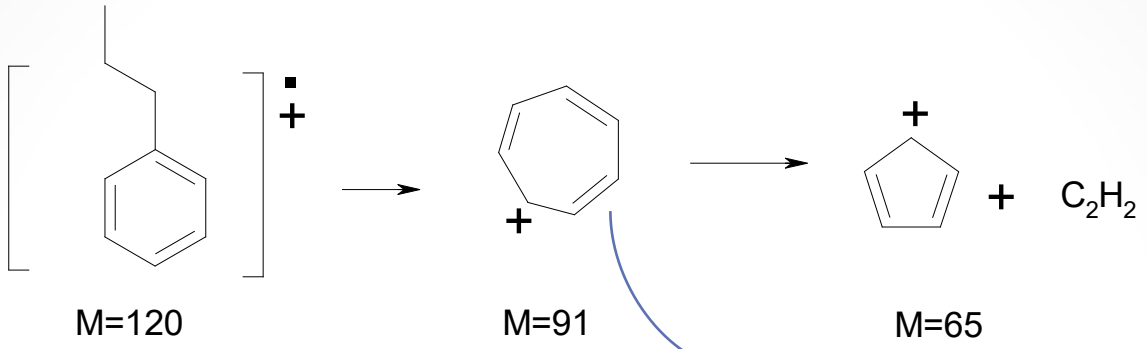
Alkenes

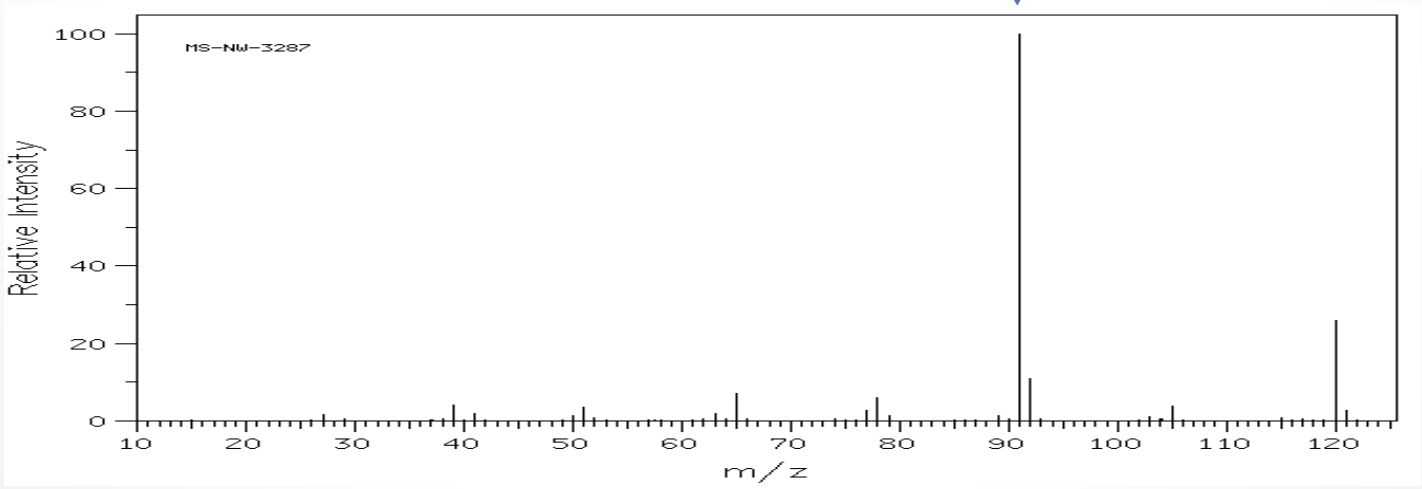


m/z = 41

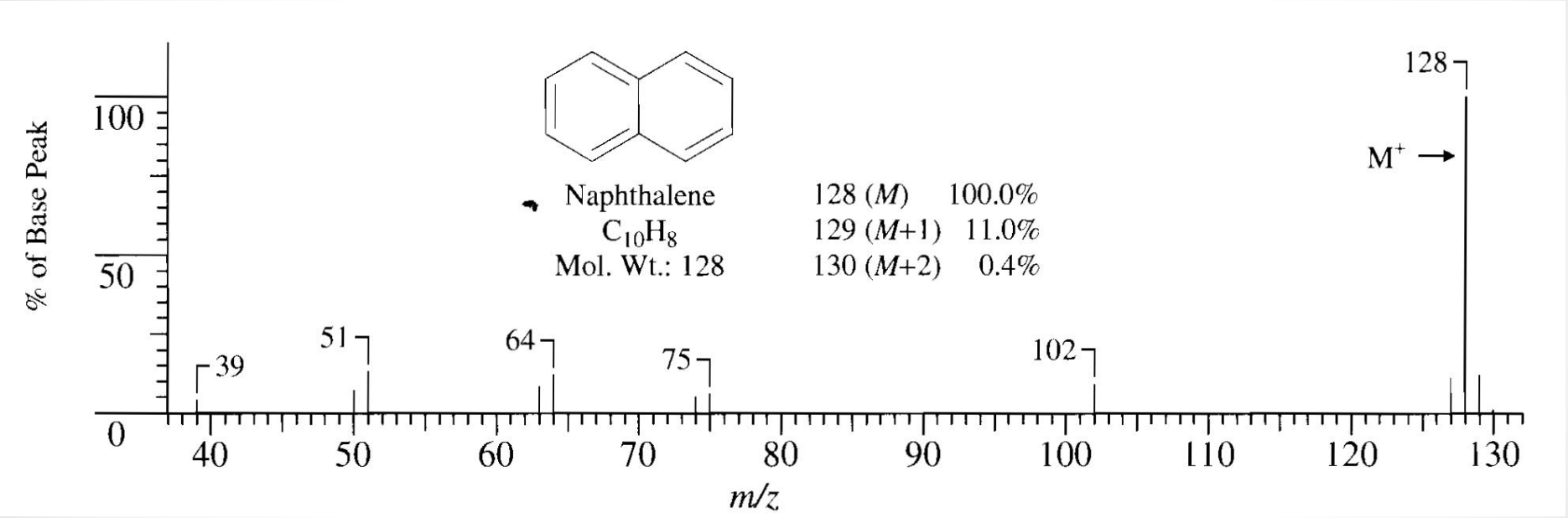


Aromatic hydrocarbons

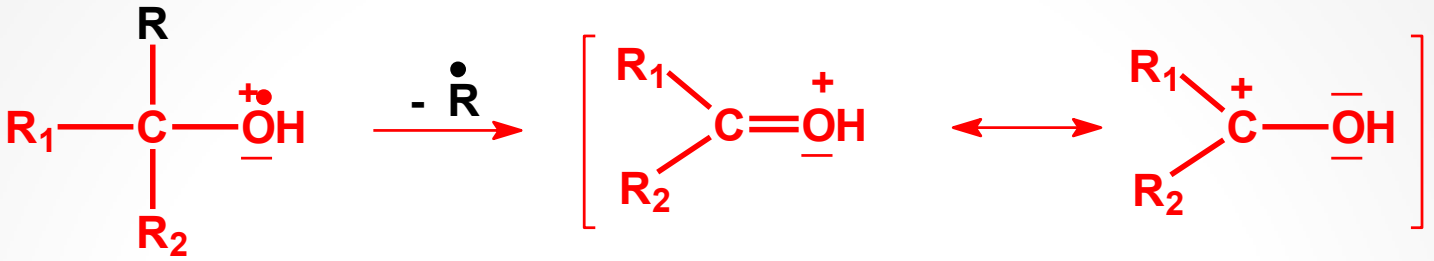




Aromatic hydrocarbons



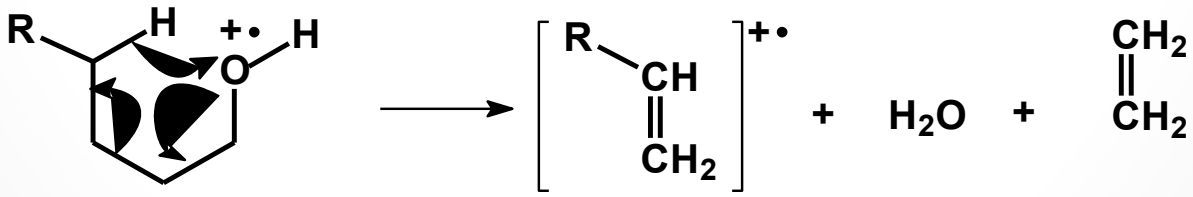
Alkohols



α-Cleavage

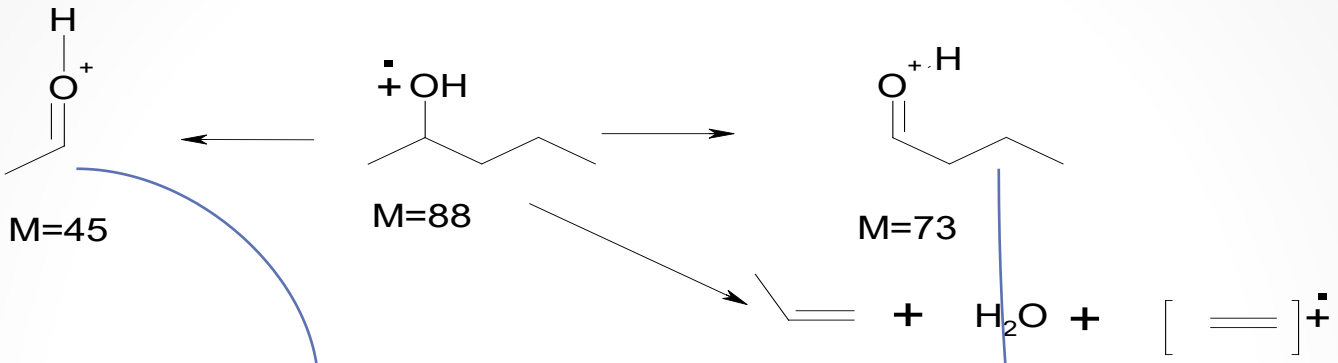
m/z for	$R_1 = R_2 = H$	31
	$R_1 = CH_3$	45
	$R_1 = CH_3CH_2$	59
	$R_1 = CH_3CH_2CH_2$	73

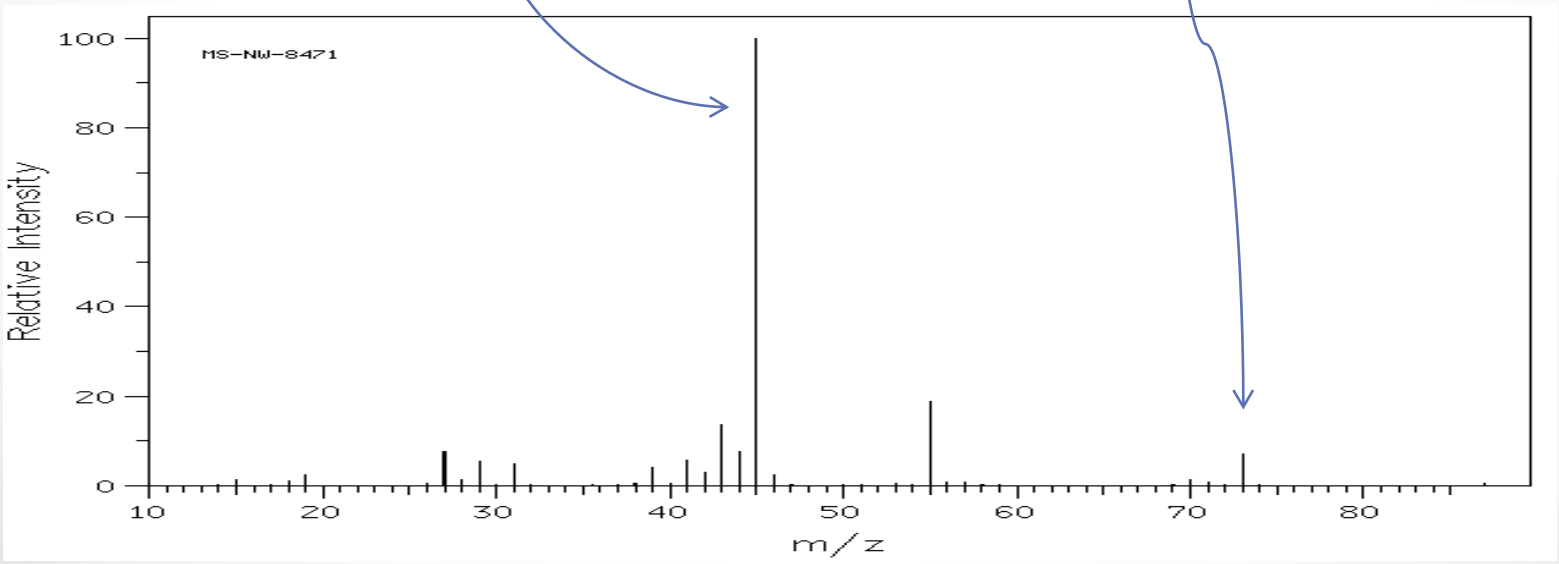
Alkohols with chain lenght at least four carbons .



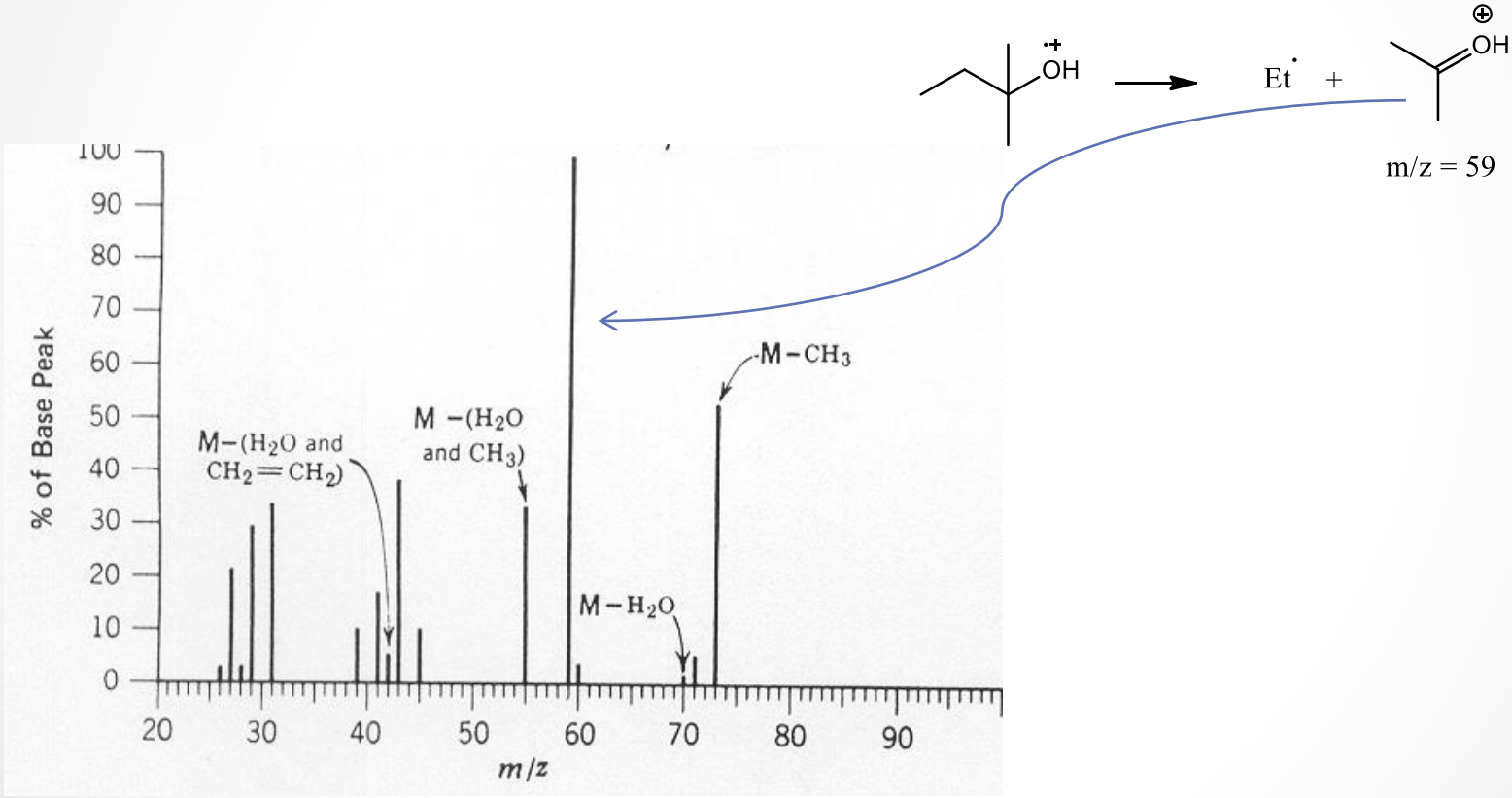
m /z 28 for n-butanol
m /z 42 for n-pentanol
etc….

Alkohols

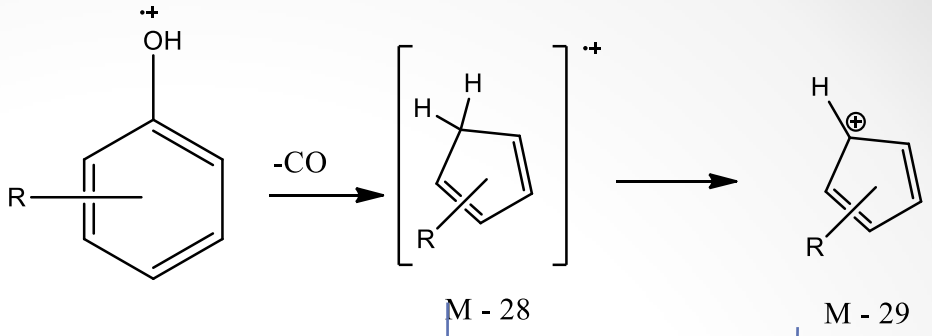

M=45
M=88
M=73
+ H2O +


MS-NW-8471
Relative Intensity
100
80
60
40
20
0
10
20
30
40
50
60
70
80
m/z

Alkohols: tert-pentanol

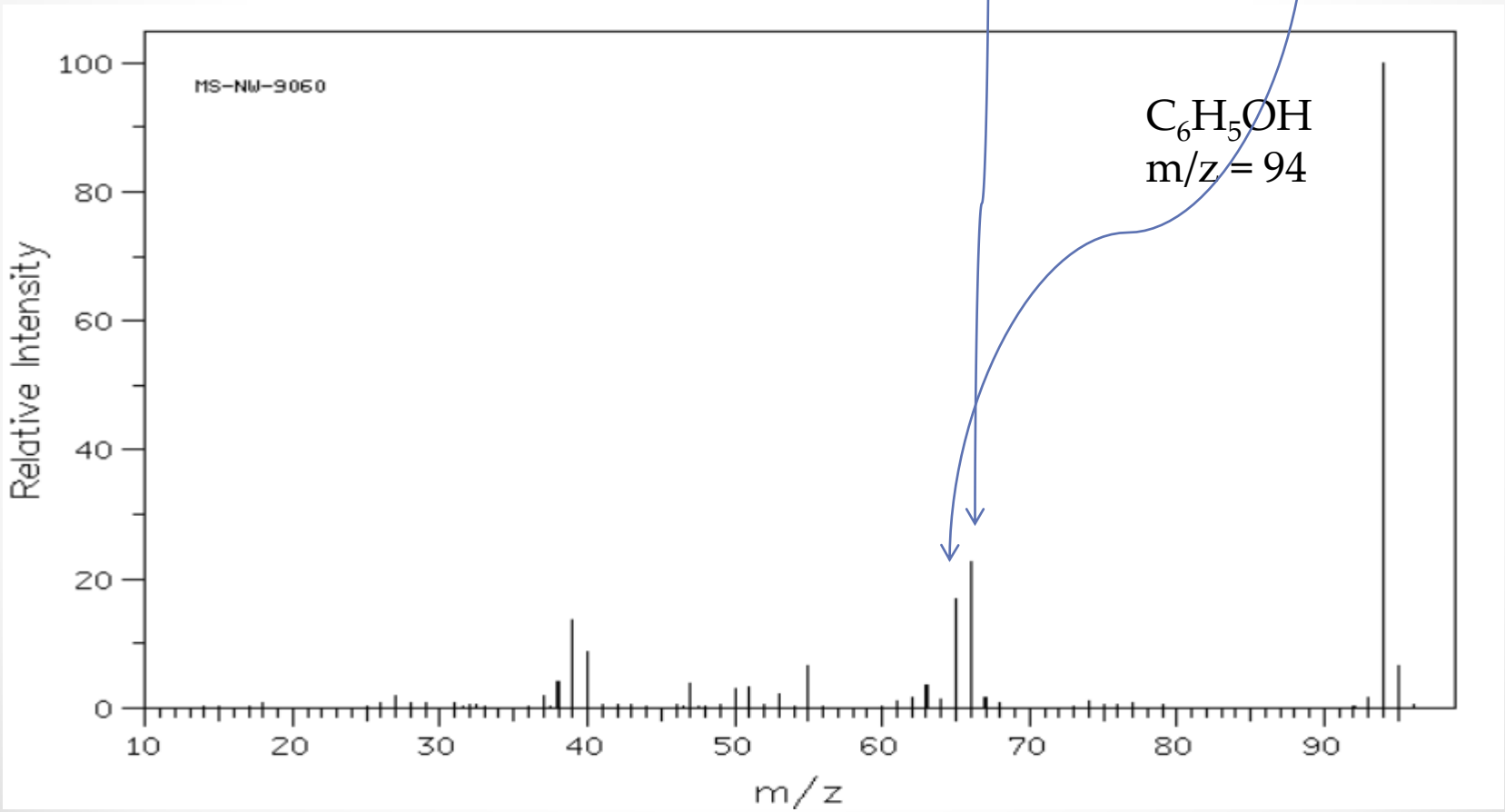


Phenols

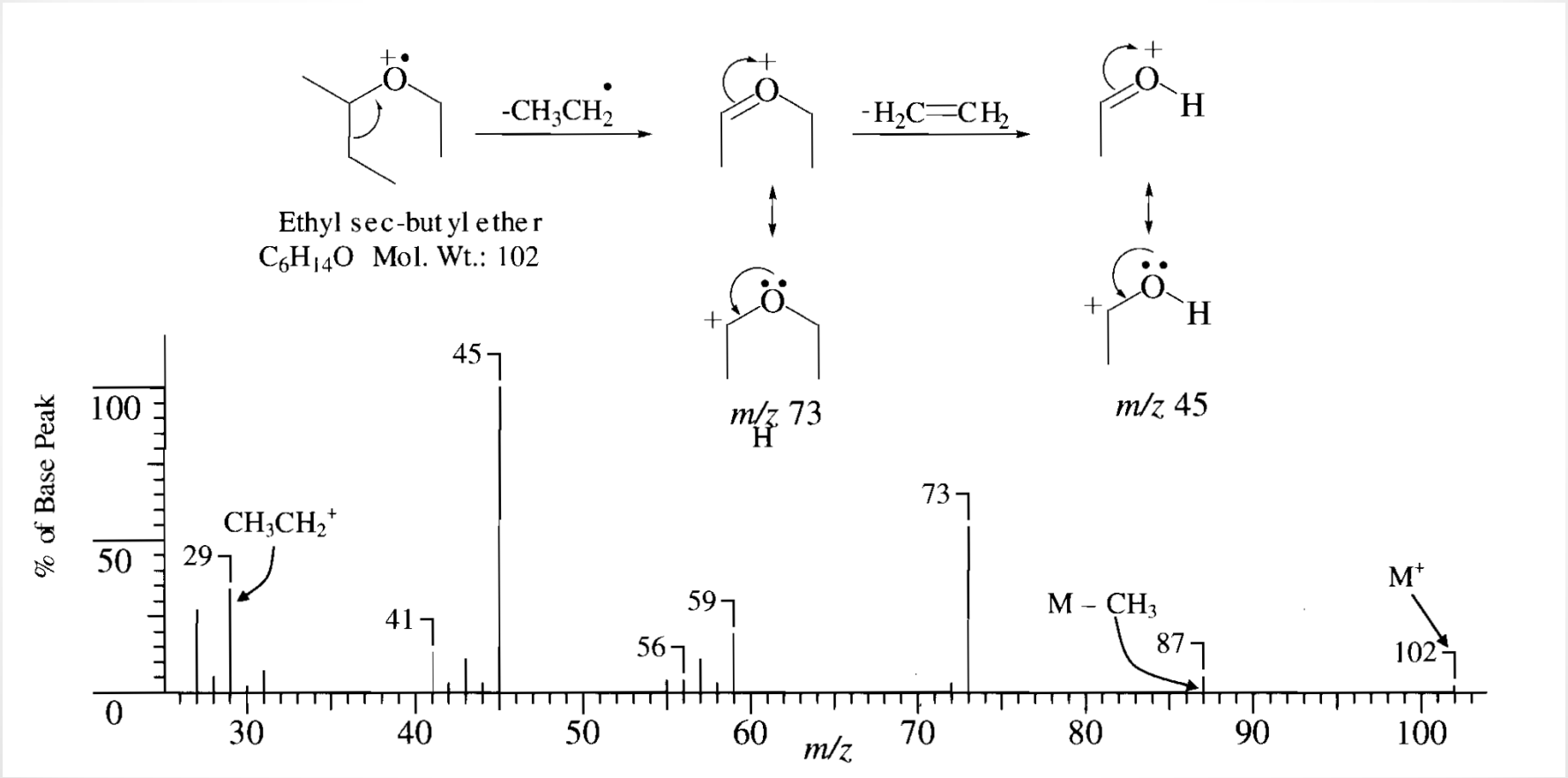


M - 28

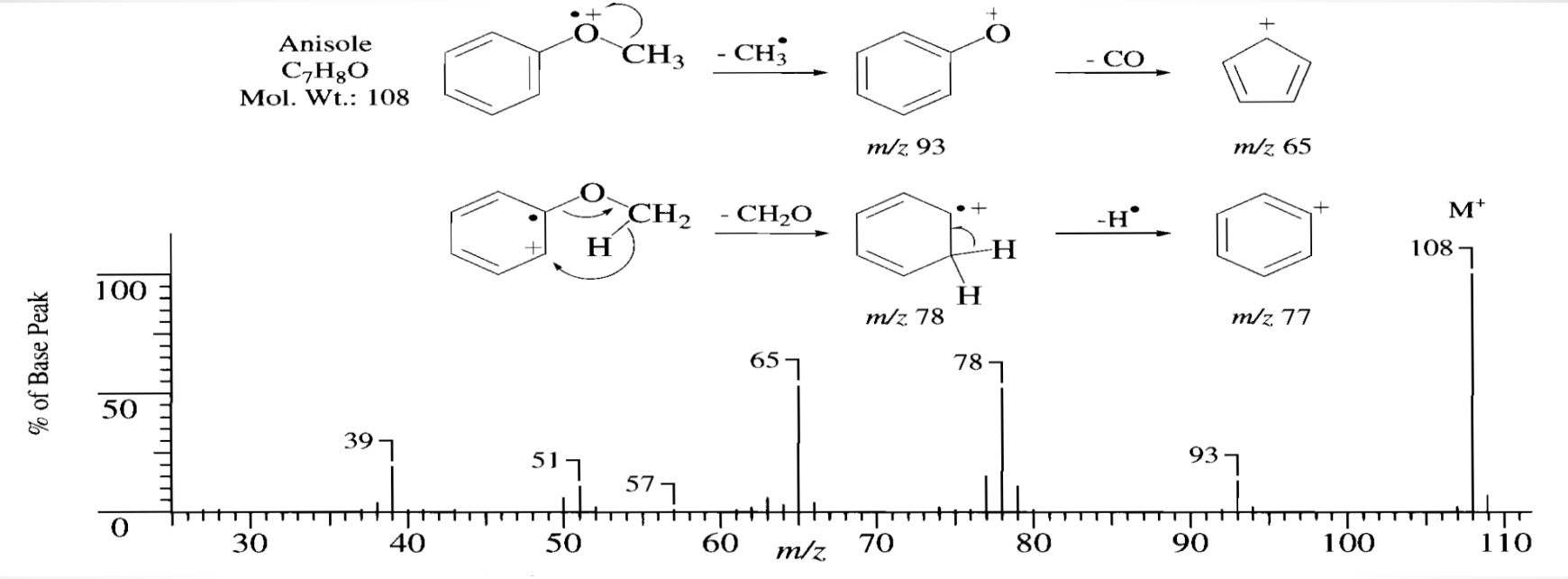
M - 29



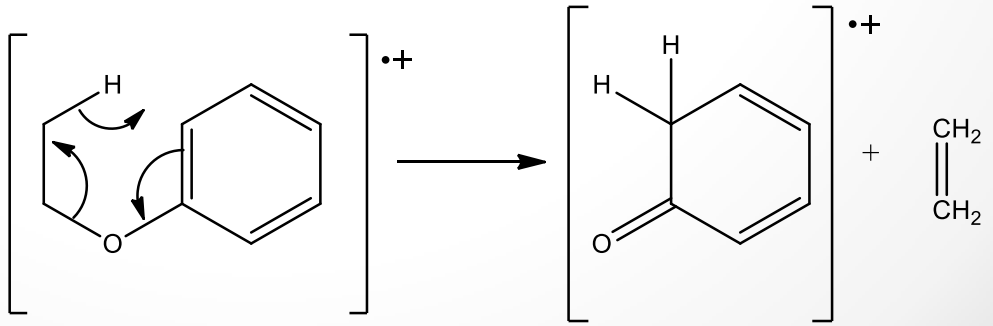
Ethers α-cleavage



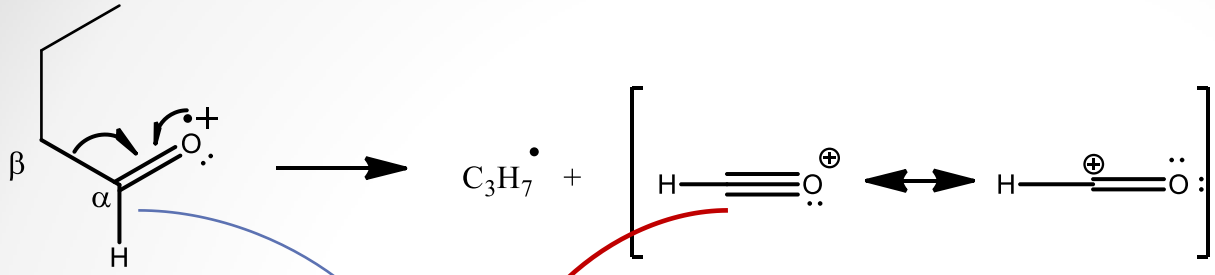
Alkyl-aryl ethers

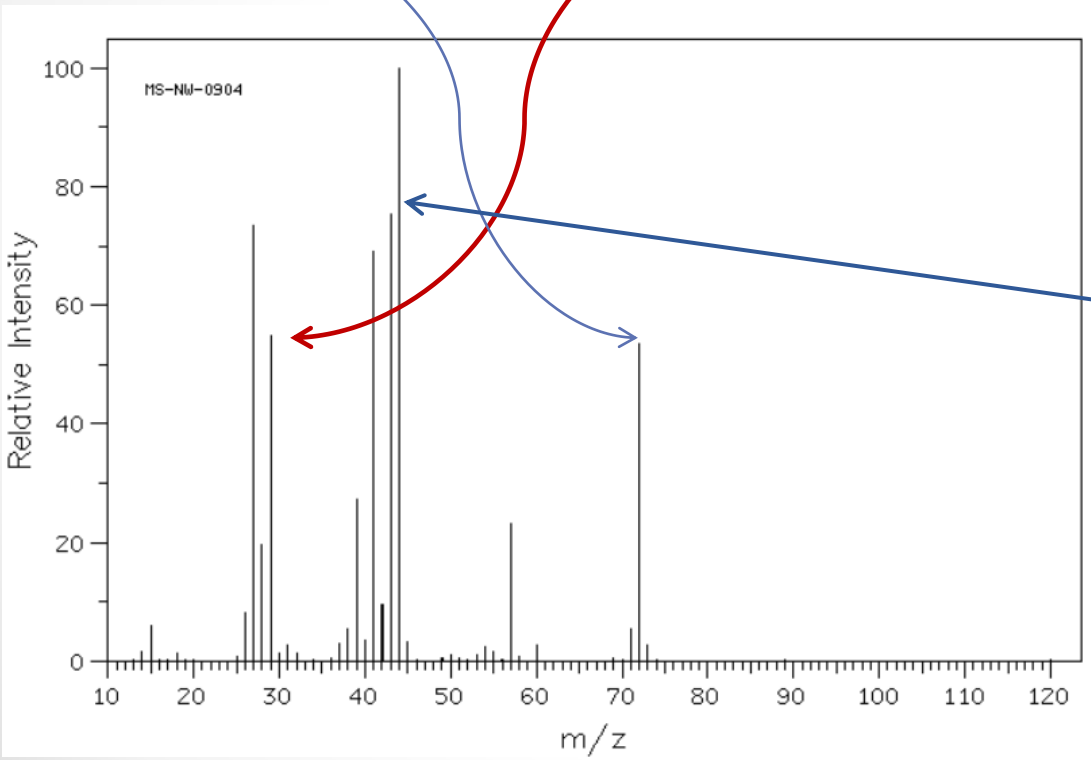


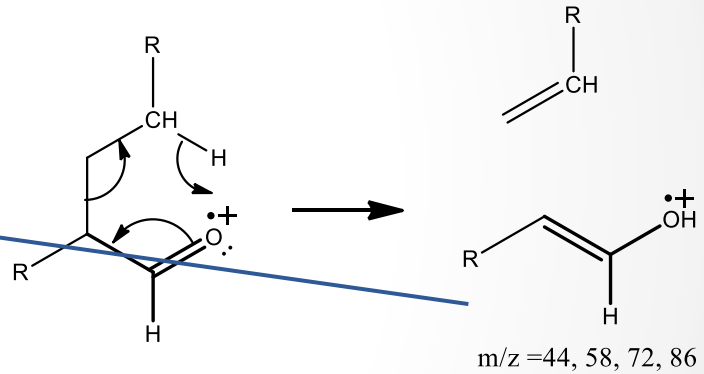
Alkyl-aryl ethers with side chain longer than C_2 undergoes Mc Lafferty Rearrangement



Aldehydes and ketones



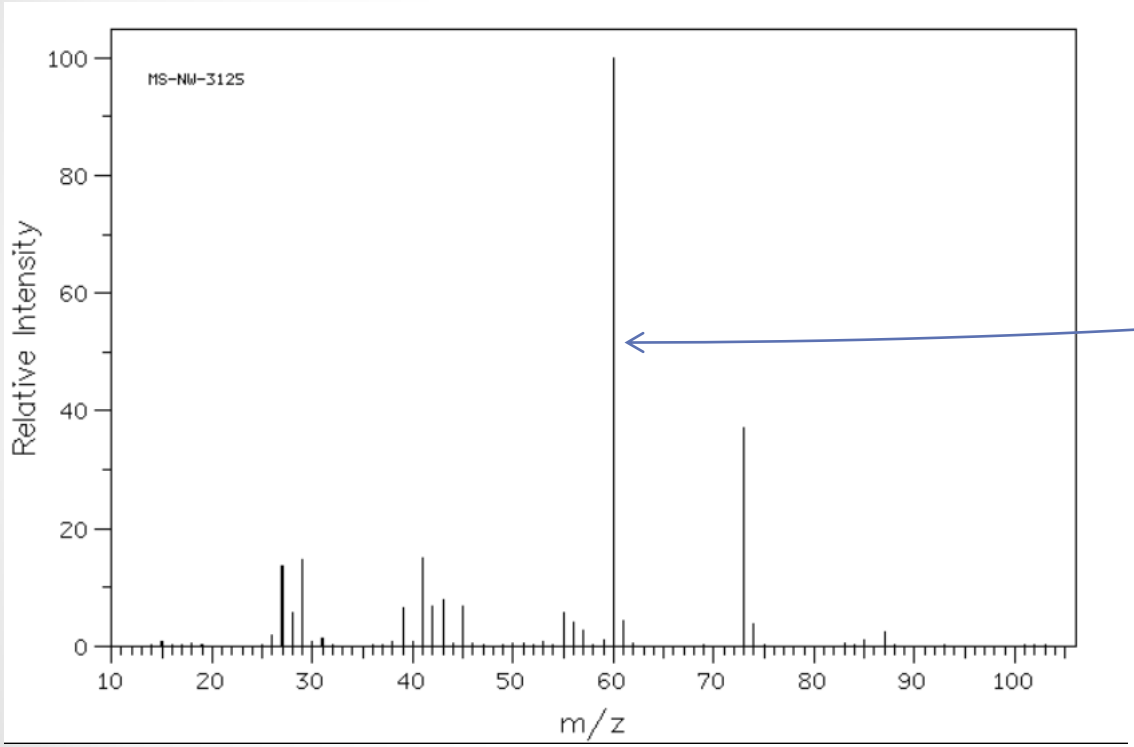


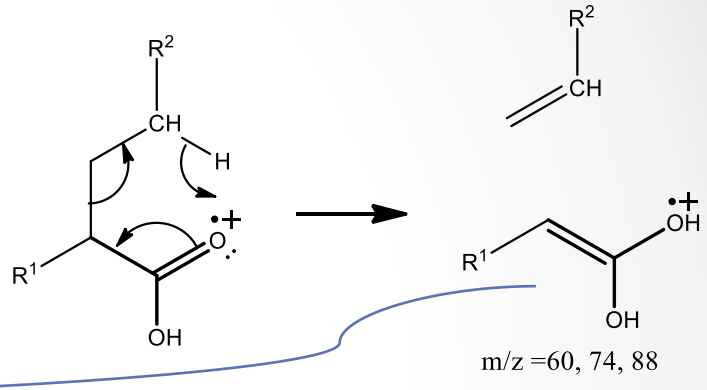


Aldehydes and Ketones with chain longer than C_3 undergoes McLafferty rearrangement

Butyric aldehyde

Carboxylic acids



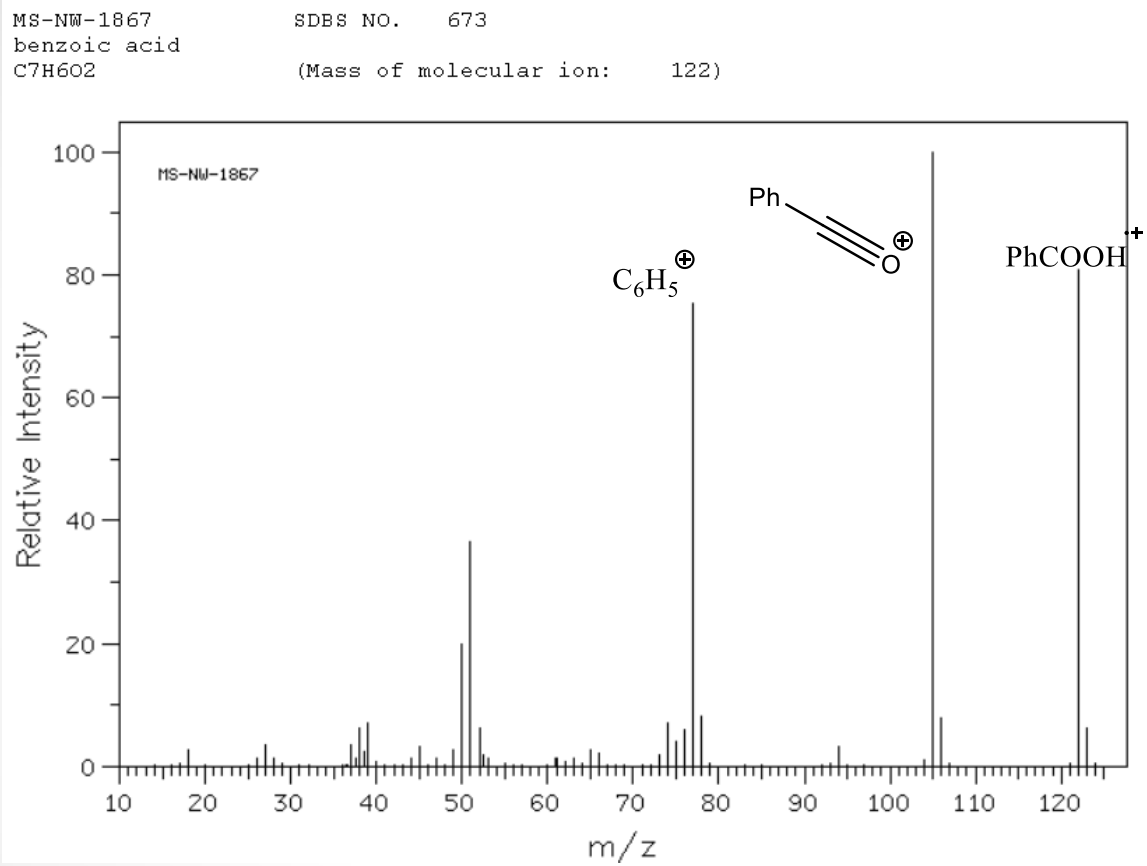


m/z =60, 74, 88

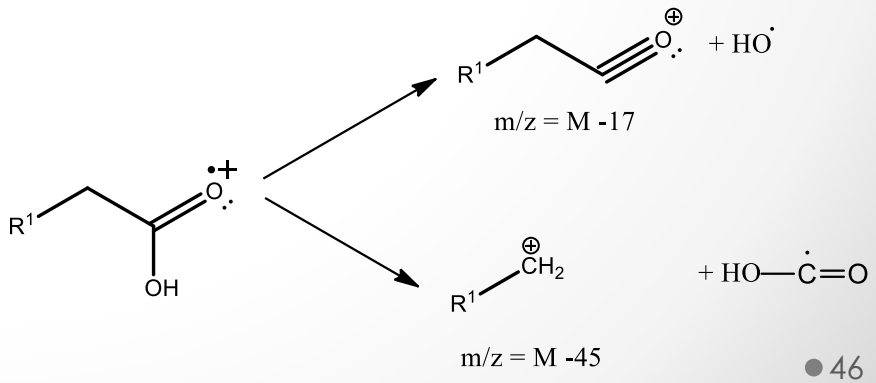
McLafferty rearrangement

Valeric acid (pentanoic acid)

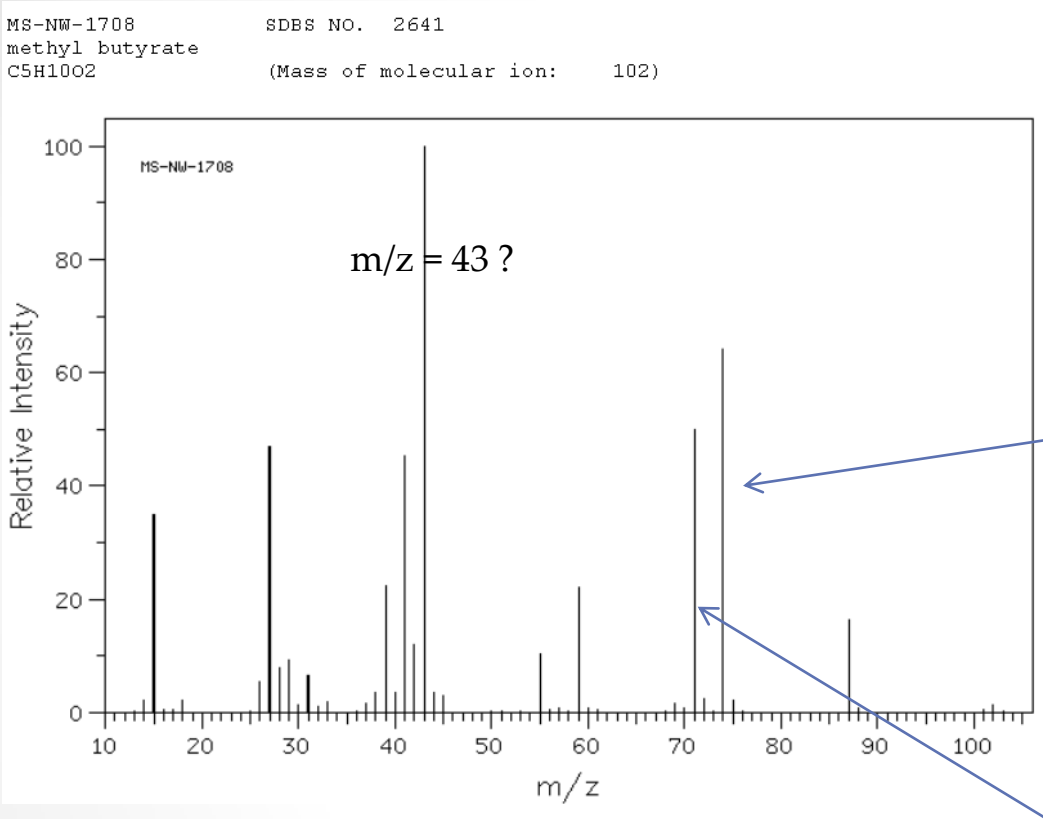
Carboxylic acids - aromatic and aliphatic with short chain



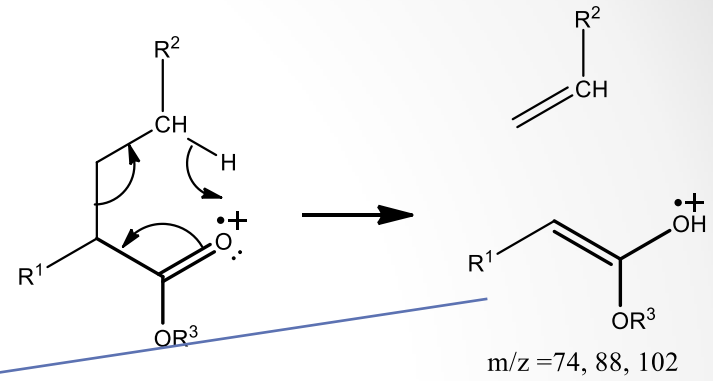
Benzoic acid



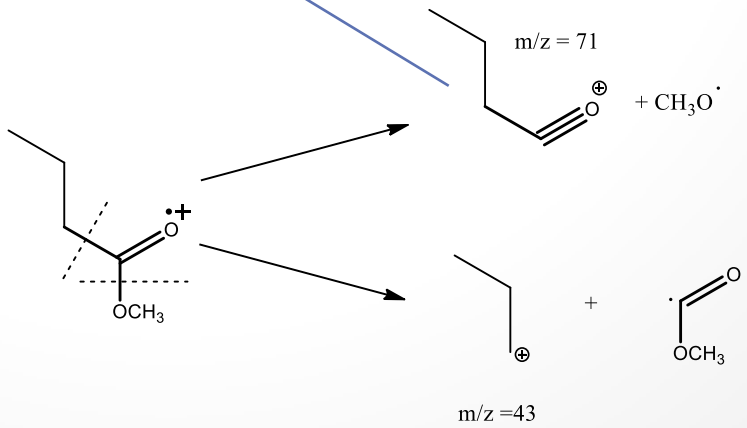
Carboxylic esters



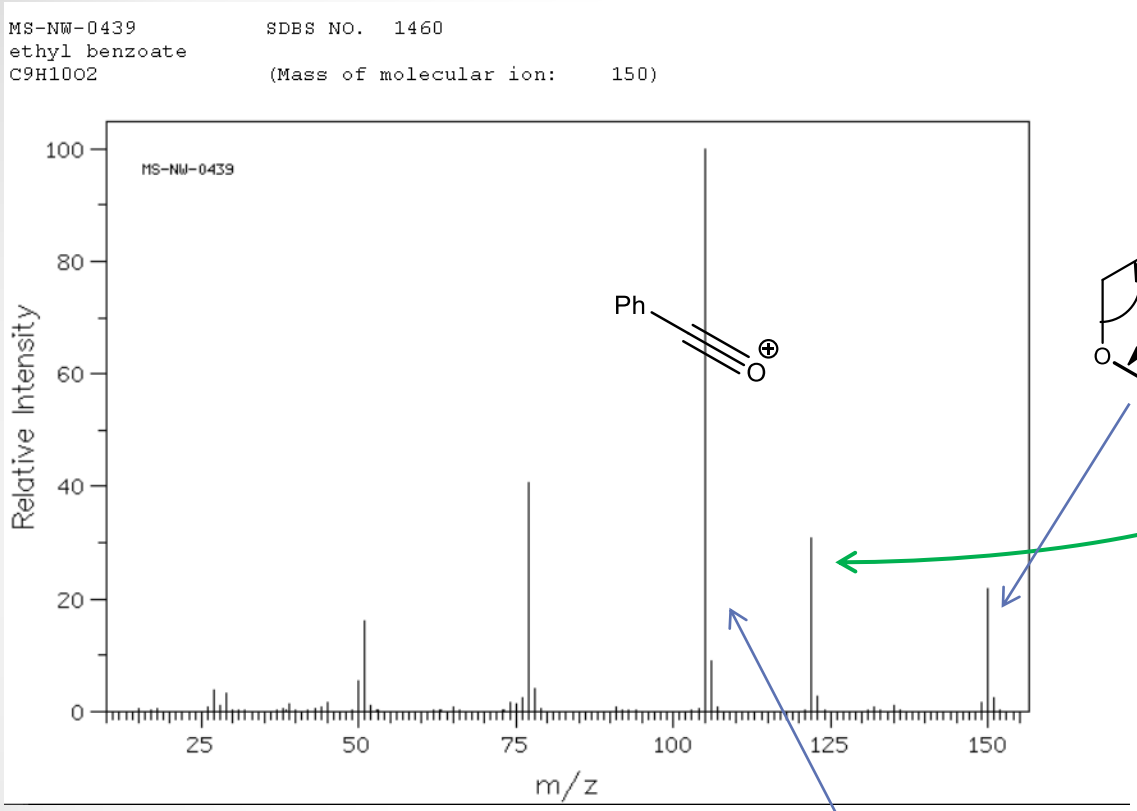
Methyl butyrate

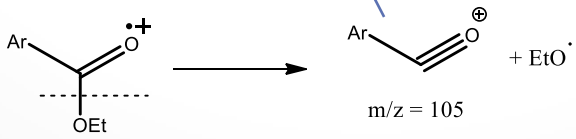


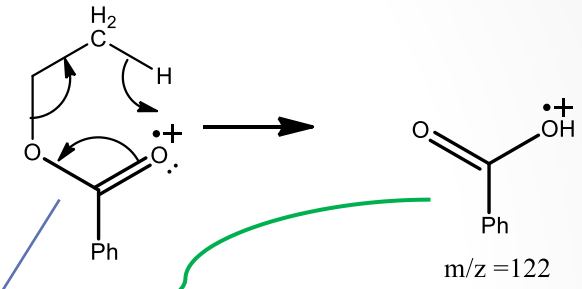
McLafferty rearrangement



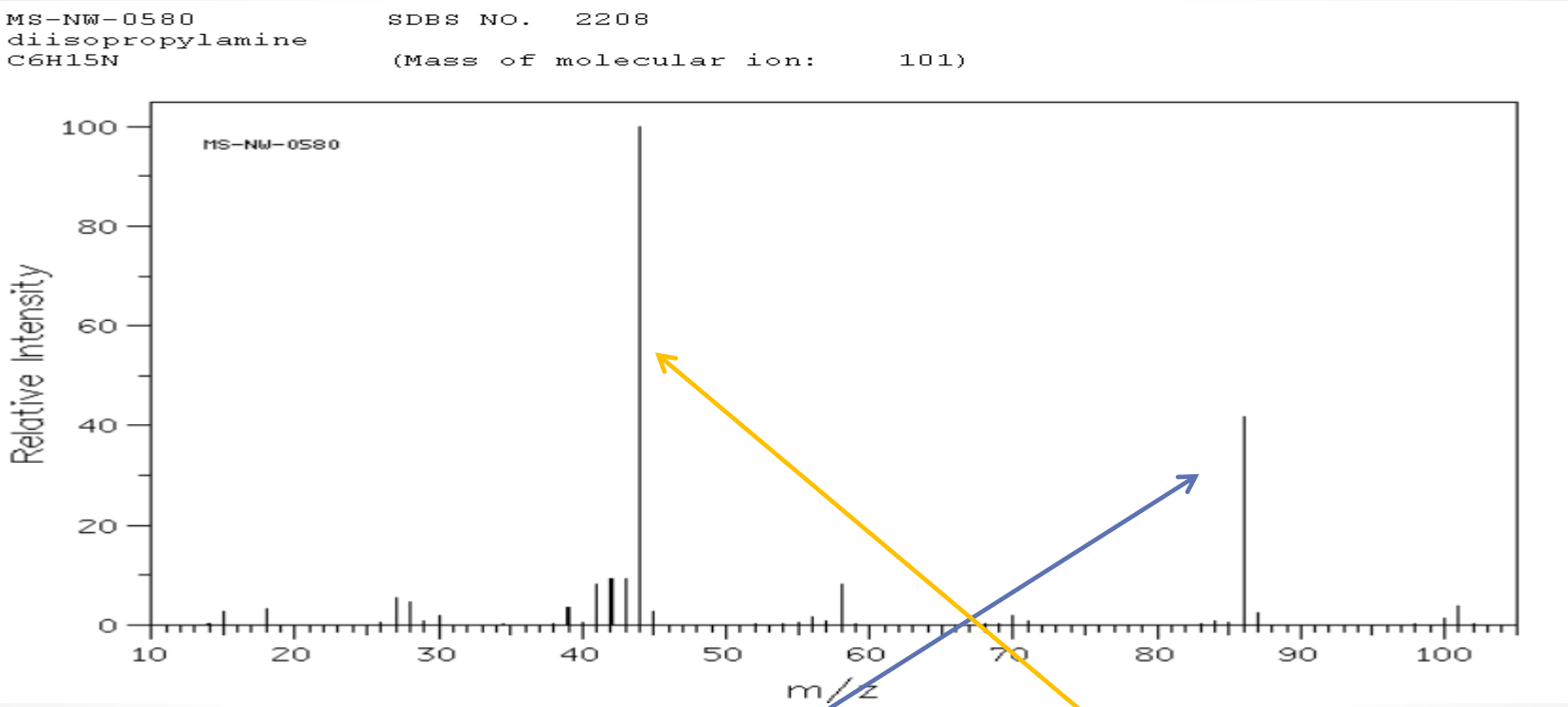
Carboxylic esters - aromatic and aliphatic with short chain

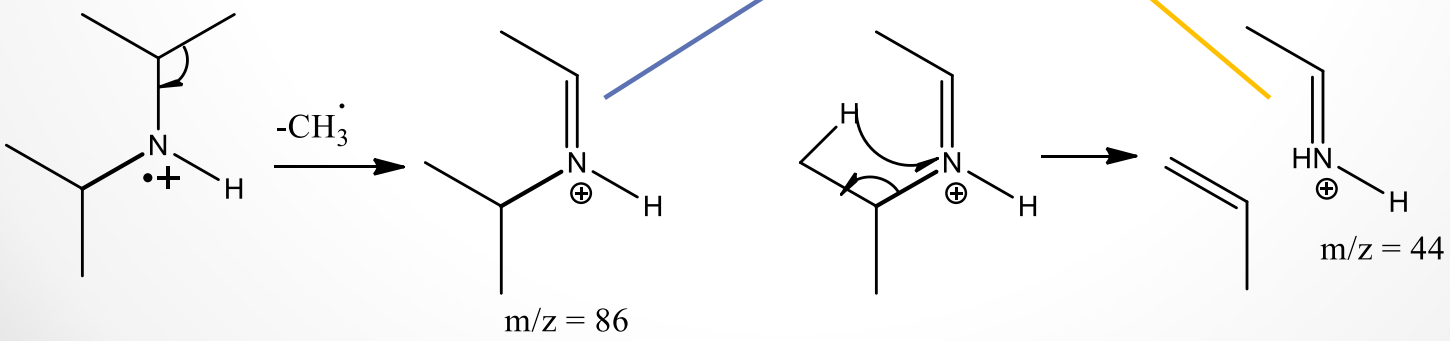






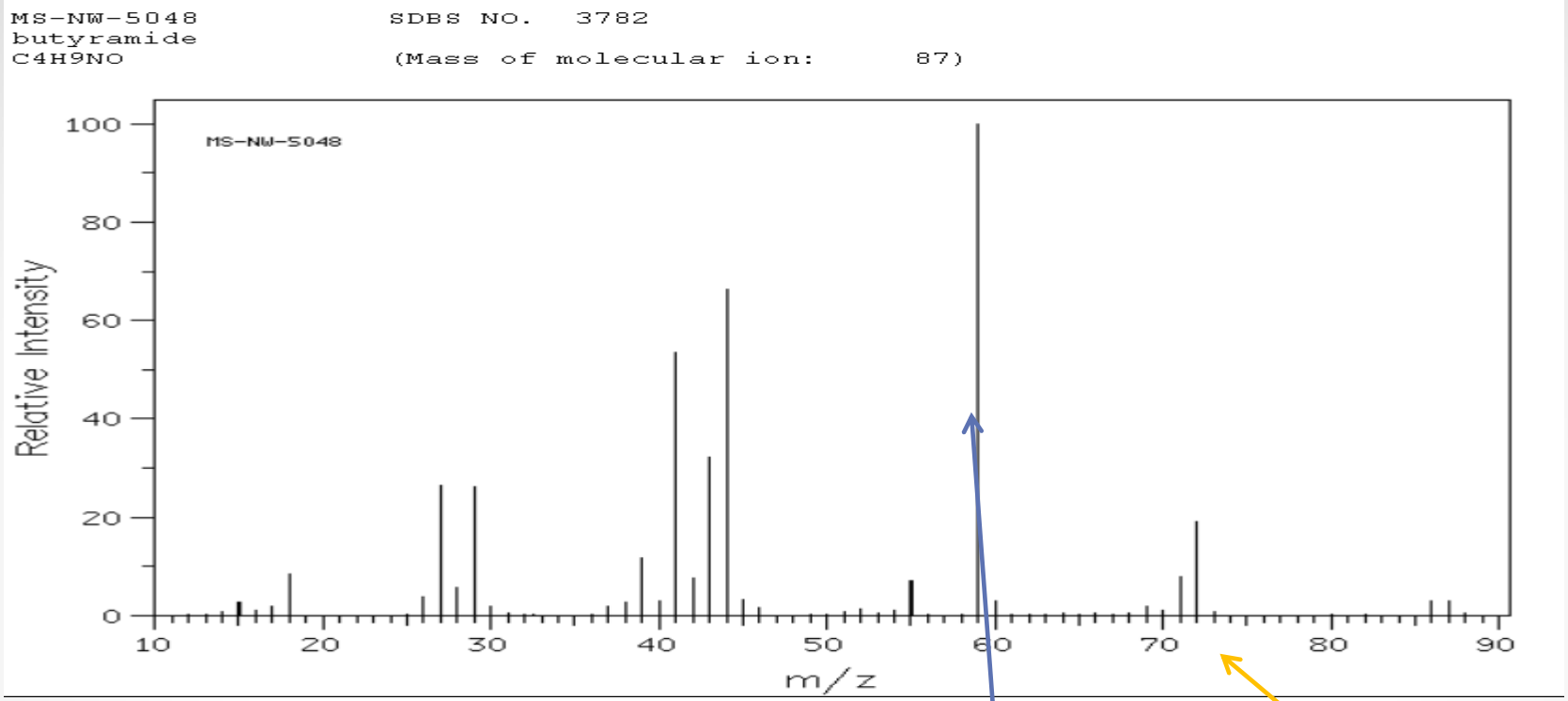
Amines

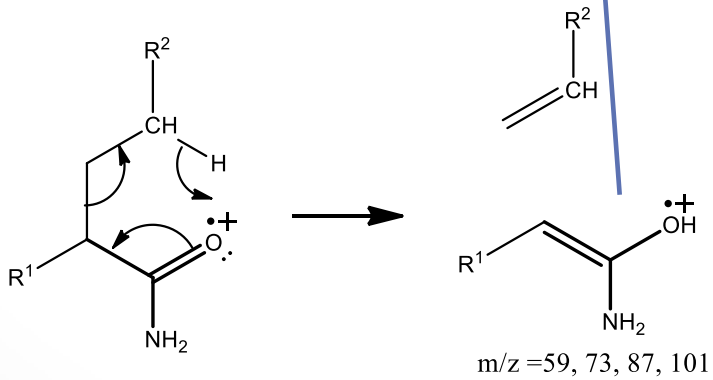




α – Cleavage

Amides

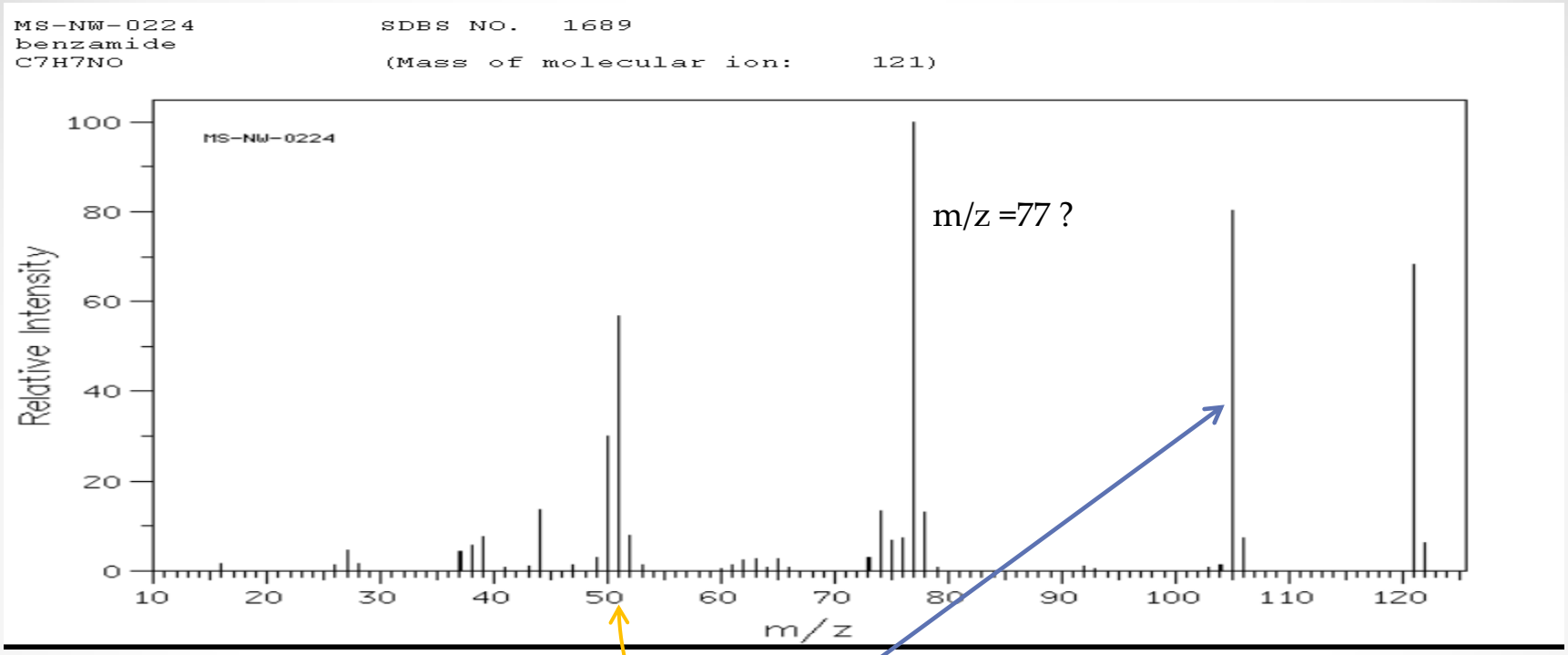


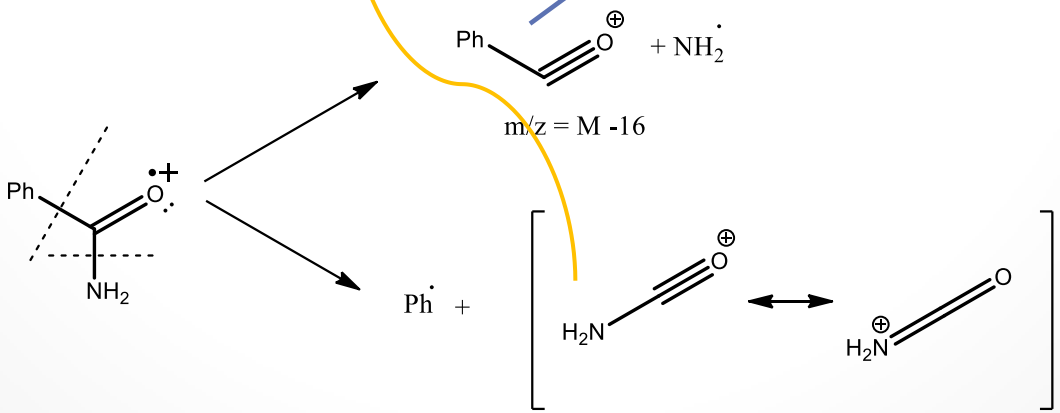


m/z =59, 73, 87, 101

McLafferty rearrangement

Amides – aromatic and with short chain





α – Cleavage

Determination of molecular formula

Relative Isotope Abundances of Common Elements.

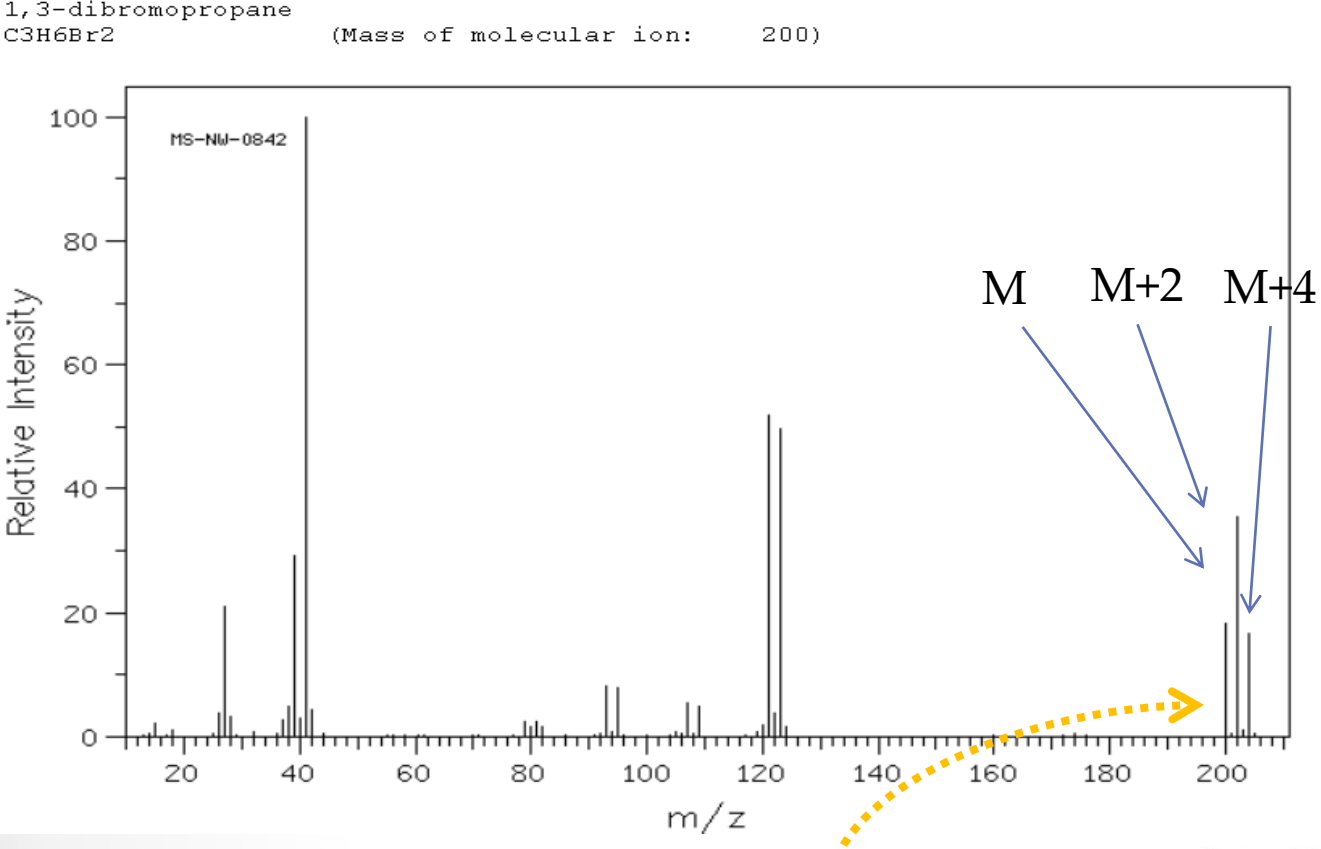
Elements	Isotope	Relative Abundance	Isotope	Relative Abundance	Isotope	Relative Abundance
Carbon	^{12}C	100	^{13}C	1.11		
Hydrogen	^{1}H	100	^{2}H	0.016		
Nitrogen	^{14}N	100	^{15}N	0.38		
Oxygen	^{16}O	100	^{17}O	0.04	^{18}O	0.2
Fluorine	^{19}F	100				
Silicon	^{28}Si	100	^{29}Si	5.1	^{30}Si	3.35
Phosphorus	^{31}P	100				
Sulfur	^{32}S	100	^{33}S	0.78	^{34}S	4.4
Chlorine	^{35}Cl	100			^{37}Cl	32.5
Bromine	^{79}Br	100			^{81}Br	98
Iodine	^{127}I	100				

$CH_3 Br^{79}$ **$CH_3 Br^{81}$**

m/z = 94 **m/z = 96**

Two molecular ions M and M+2

Determination of molecular formula



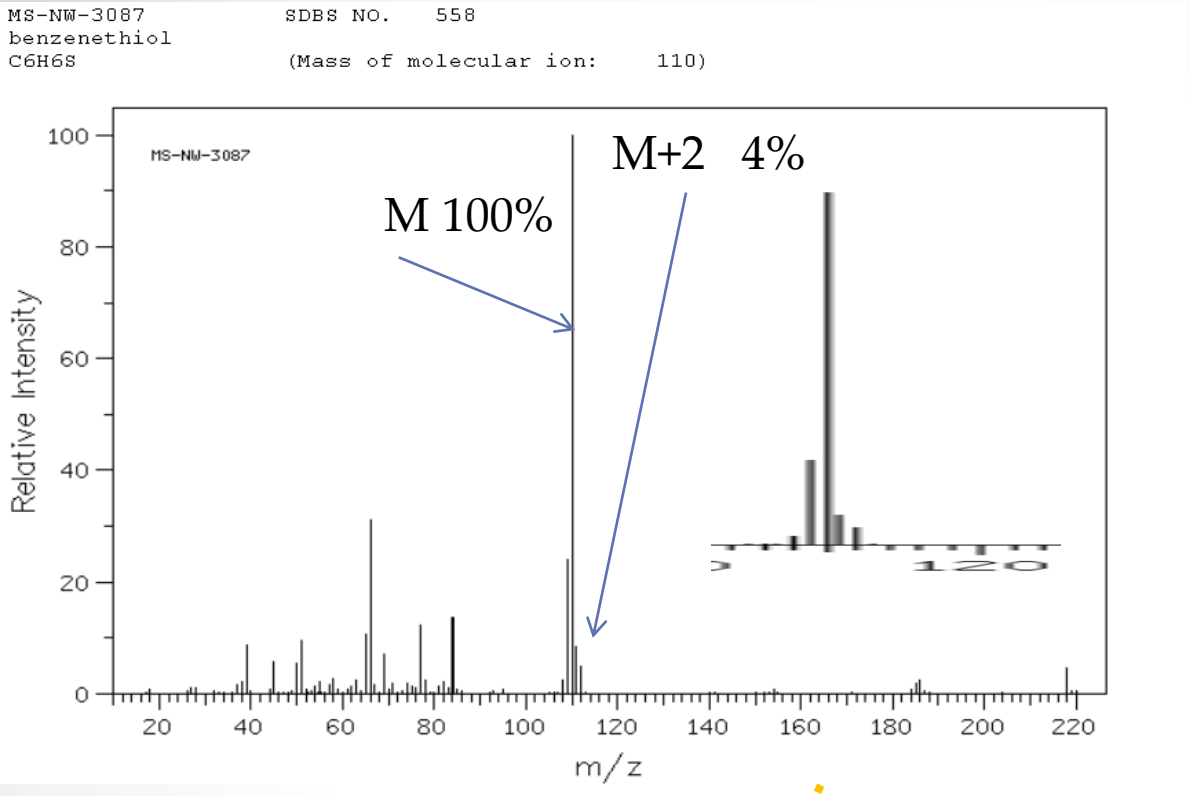
If intensity of peaks- M: M+2: M+4 are as ≈ 1:2:1

Molecule contain two bromine atoms

Intensities of Isotope Peaks (Relative to the Molecular Ion) for Combination of Chlorine and Bromine.

Halogen Present	% M+2	% M+4	% M+6	% M+8	% M+10	% M+12
Cl	32.6					
Cl_2	65.3	10.6				
Cl_3	97.8	31.9	3.5			
Cl_4	131.0	63.9	14.0	1.2		
Cl_5	163.0	106.0	34.7	5.7	0.4	
Cl_6	196.0	161.0	69.4	17.0	2.2	0.1
Br	97.9					
Br_2	195.0	95.5				
Br_3	293.0	286.0	93.4			
BrCl	130.0	31.9				
$BrCl_2$	163.0	74.4	10.4			
Br_2Cl	228.0	159.0	31.2			

Determination of molecular formula

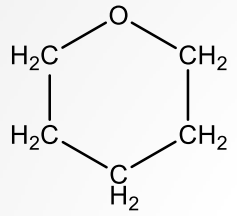


If intensity of peaks, M: M+2: are as ≈ 100:4

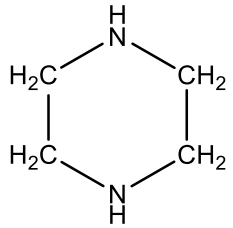
Molecule contain one sulfur atom

Elements	Isotope	Relative Abundance	Isotope	Relative Abundance	Isotope	Relative Abundance
Fluorine	^{19}F	100				
Silicon	^{28}Si	100	^{29}Si	5.1	^{30}Si	3.35
Phosphorus	^{31}P	100				
Sulfur	^{32}S	100	^{33}S	0.78	^{34}S	4.4

High resolution Molecular ion



Chemical Formula: $C_5H_{10}O$
Exact Mass: 86.07
Molecular Weight: 86.13



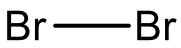
Chemical Formula: $C_4H_{10}N_2$
Exact Mass: 86.08
Molecular Weight: 86.14

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

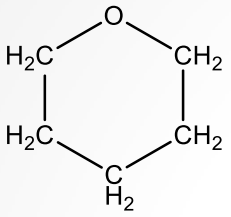
Exact mass: mass of moleule calculated
with exact masses of most most abundant isotopes.

Molecular weight: mass of moleule calculated
with atomic weight (weighted average for all isotopes).

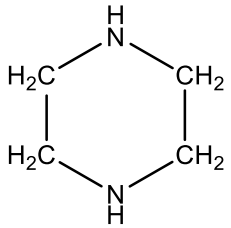


Exact Mass: 157.84
Molecular Weight:
159.81

High resolution Molecular ion



Chemical Formula: $C_5H_{10}O$
Exact Mass: 86.07
Molecular Weight: 86.13



Chemical Formula: $C_4H_{10}N_2$
Exact Mass: 86.08
Molecular Weight: 86.14

Exact Masses of Isotopes.

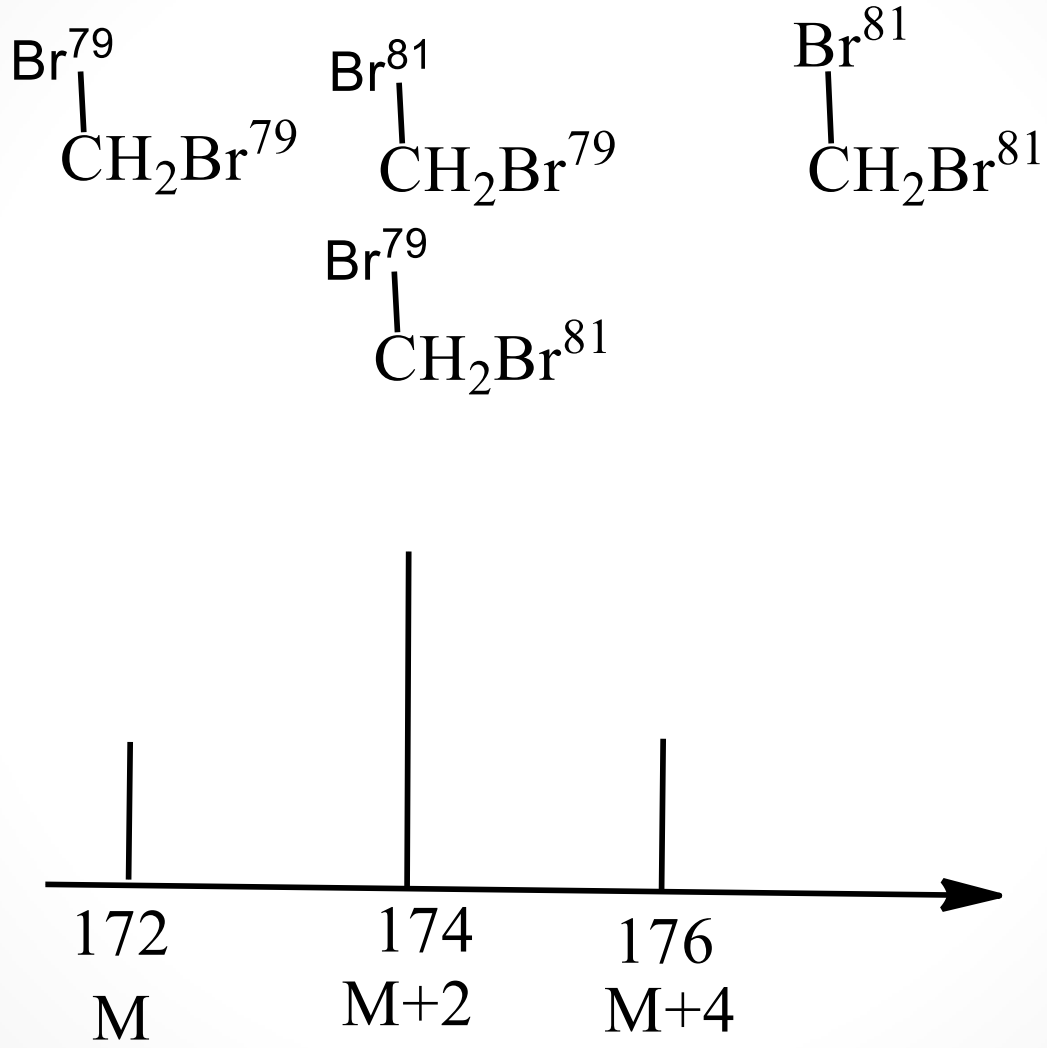
Element	Atomic Weight	Nuclide	Mass
Hydrogen	1.00794	^{1}H	1.00783
		D(^{2}H)	2.01410
Carbon	12.01115	^{12}C	12.00000 (std)
		^{13}C	13.00336
Nitrogen	14.0067	^{14}N	14.0031
		^{15}N	15.0001
Oxygen	15.9994	^{16}O	15.9949
		^{17}O	16.9991
		^{18}O	17.9992
Fluorine	18.9984	^{19}F	18.9984
Silicon	28.0855	^{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

High resolution exact mass:
for $C_5H_{10}O$ is 86.073165

for $C_4H_{10}N_2$ is 86.084398

With the resolution power of spectrometer = 10000.
We can distinguish peak 86.000 and 86.008
The tolerane of measurment (TOF) is 5 ppm - means
We measure mass with accuracy 86.000 +/- 0.004 D.

This approach is usually used instead of elemental analysis.


Br79
CH2Br79
Br81
CH2Br79
Br81
CH2Br81
Br79
CH2Br81
172
M
174
M+2
176
M+4