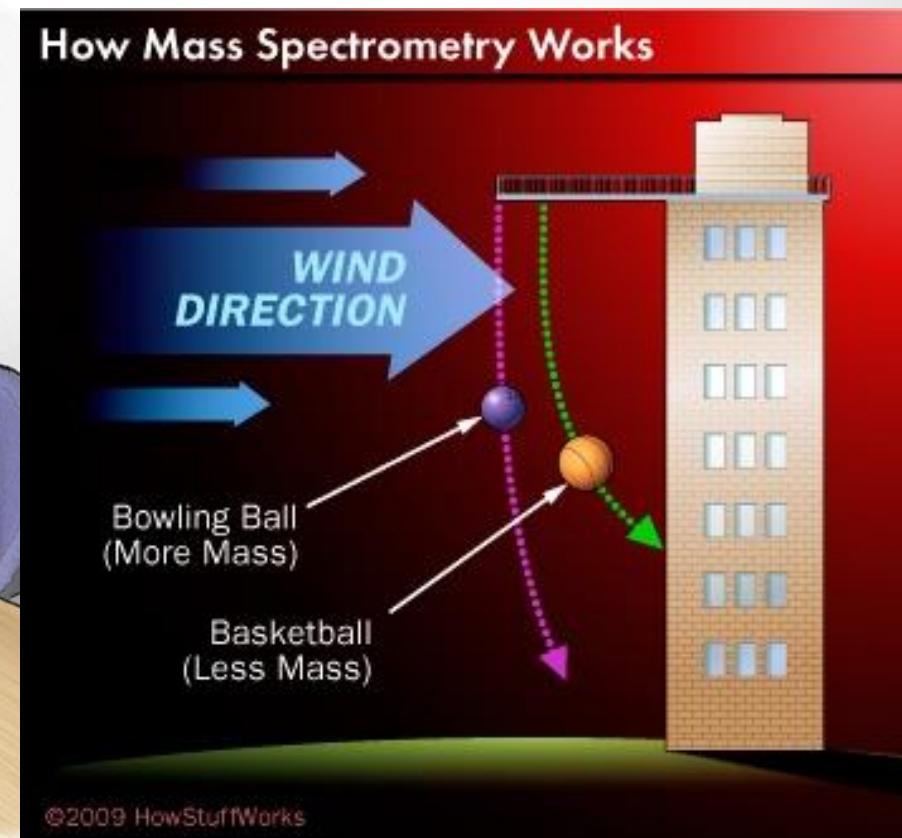
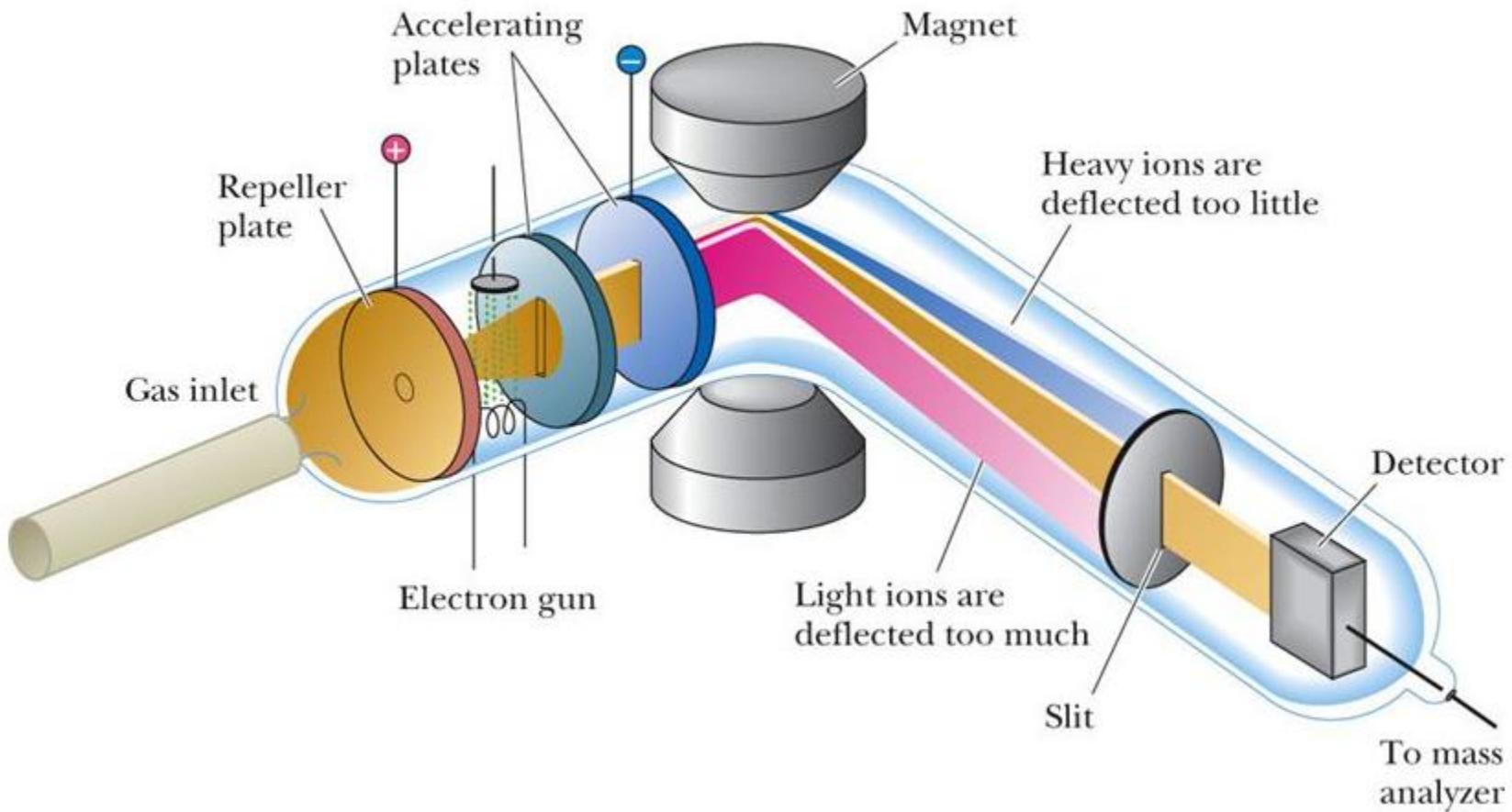


# Mass – Spectrometry

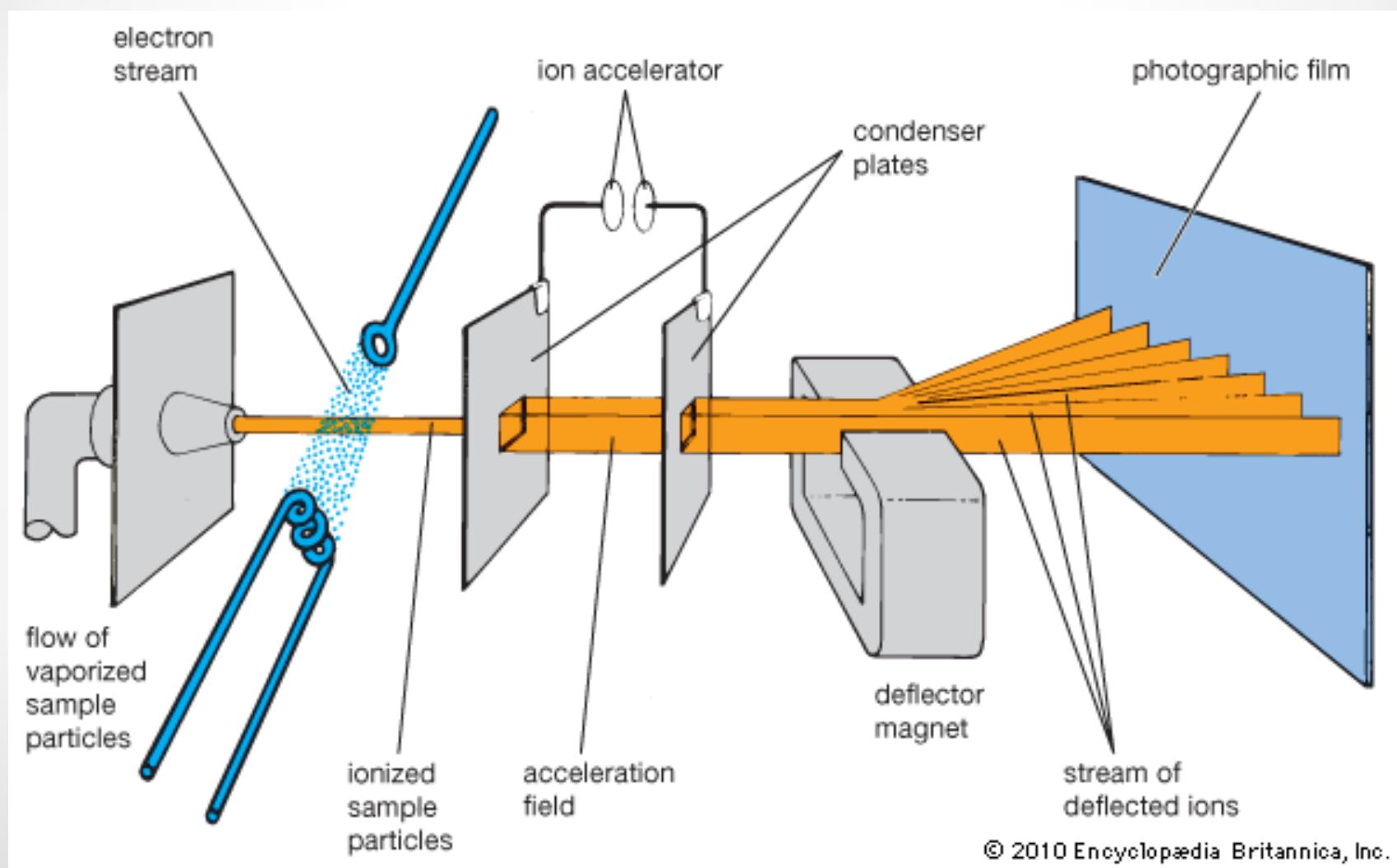


"Okay—who put my lunch through the mass spectrometer..?"

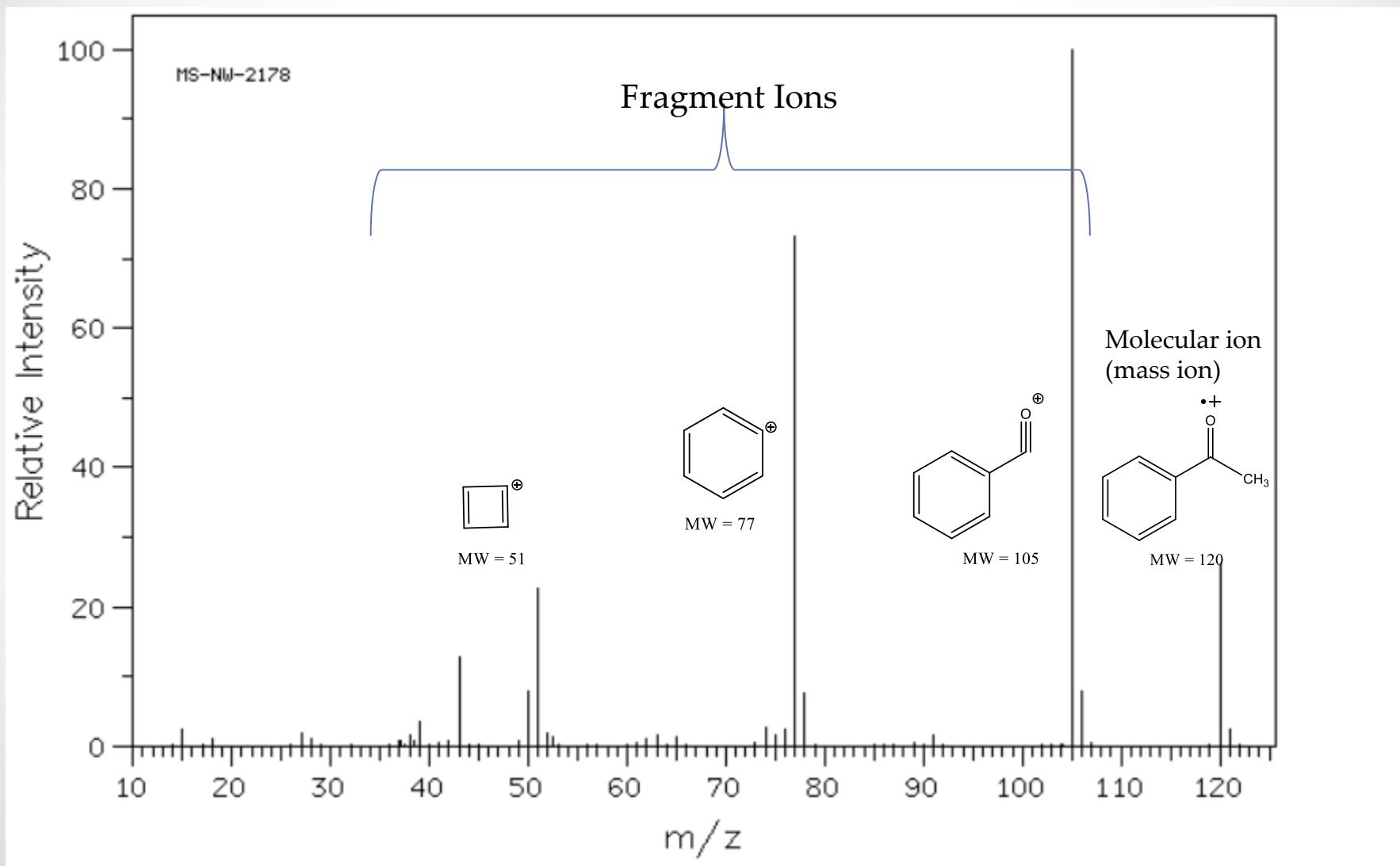
# Mass spectrometry : Apparatus



# Mass spectrometry :Aparatus

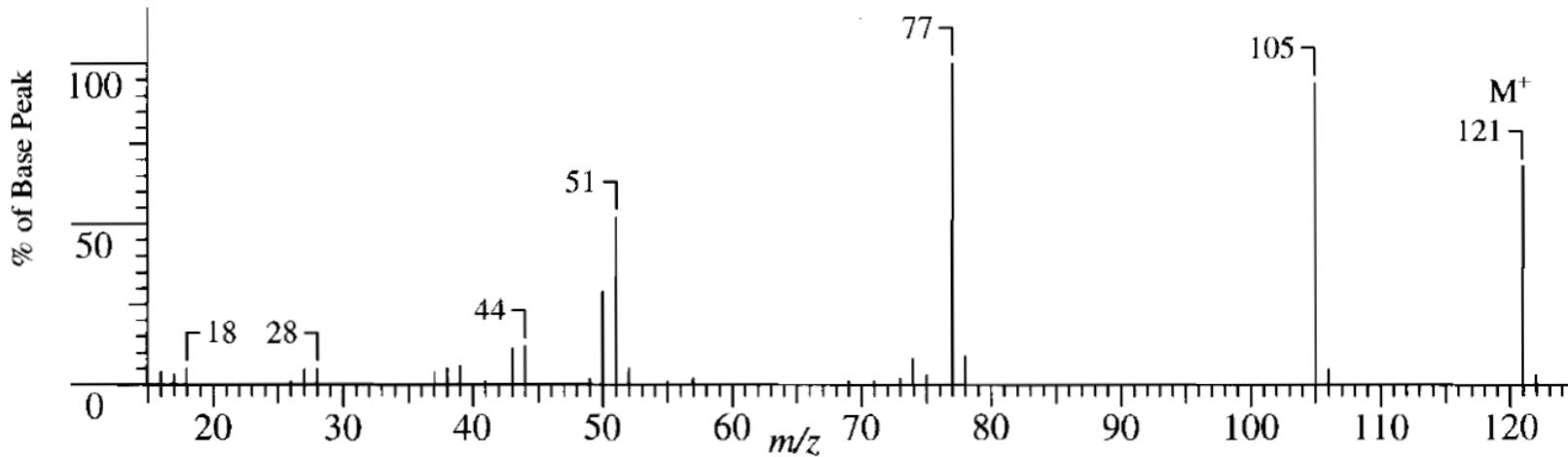
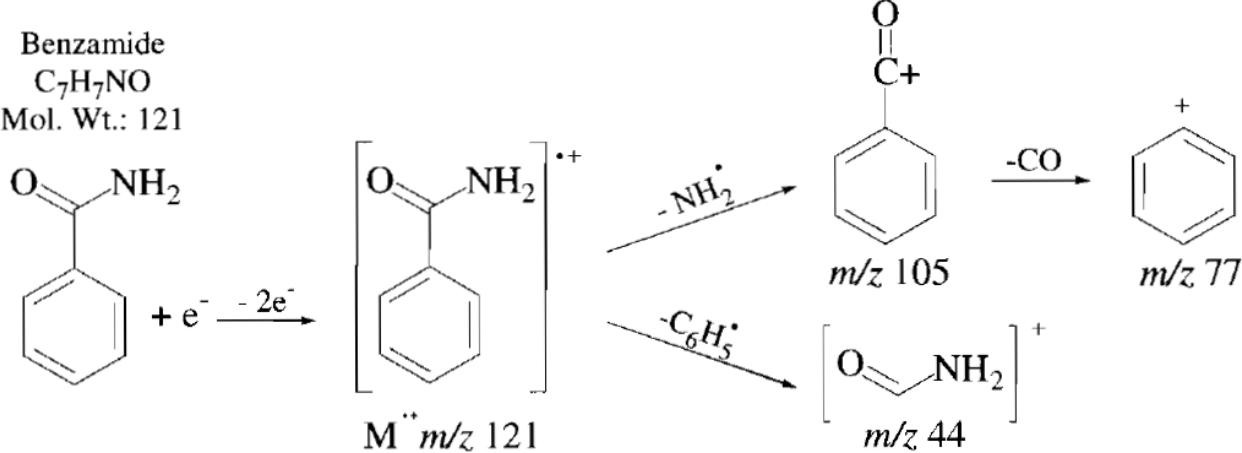


# MASS Spectrum of acetophenone



# MASS Spectrum of Benzamide

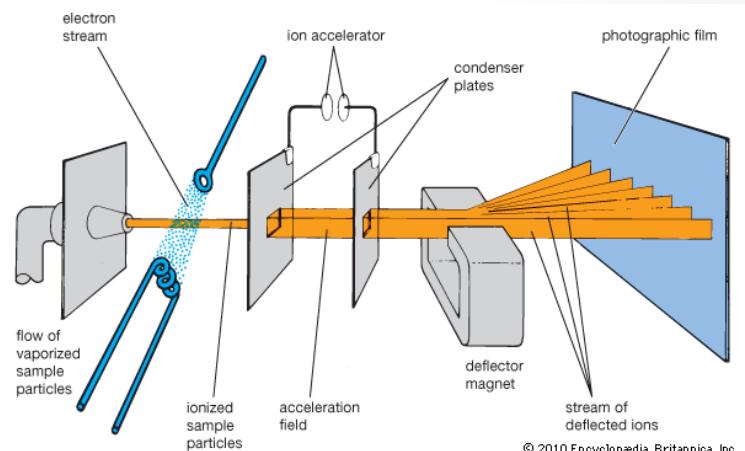
Benzamide  
 $C_7H_7NO$   
Mol. Wt.: 121



# Mass spectrometry:

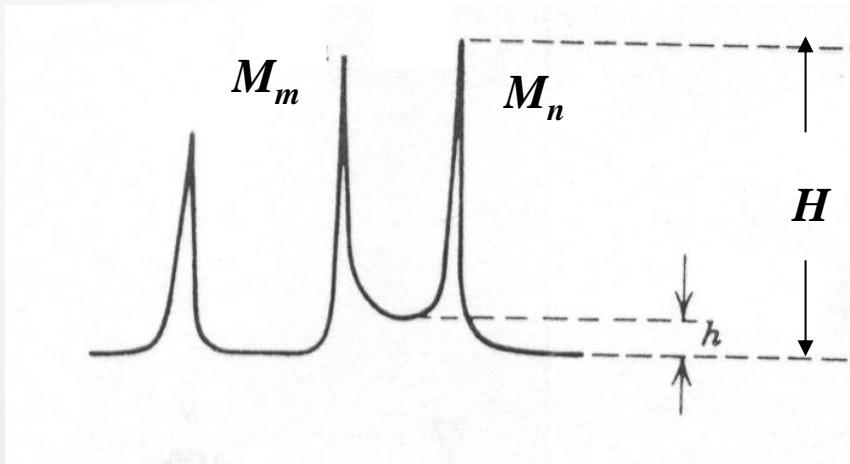
## Processing steps of the sample

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



# Resolution of mass spectrometer

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

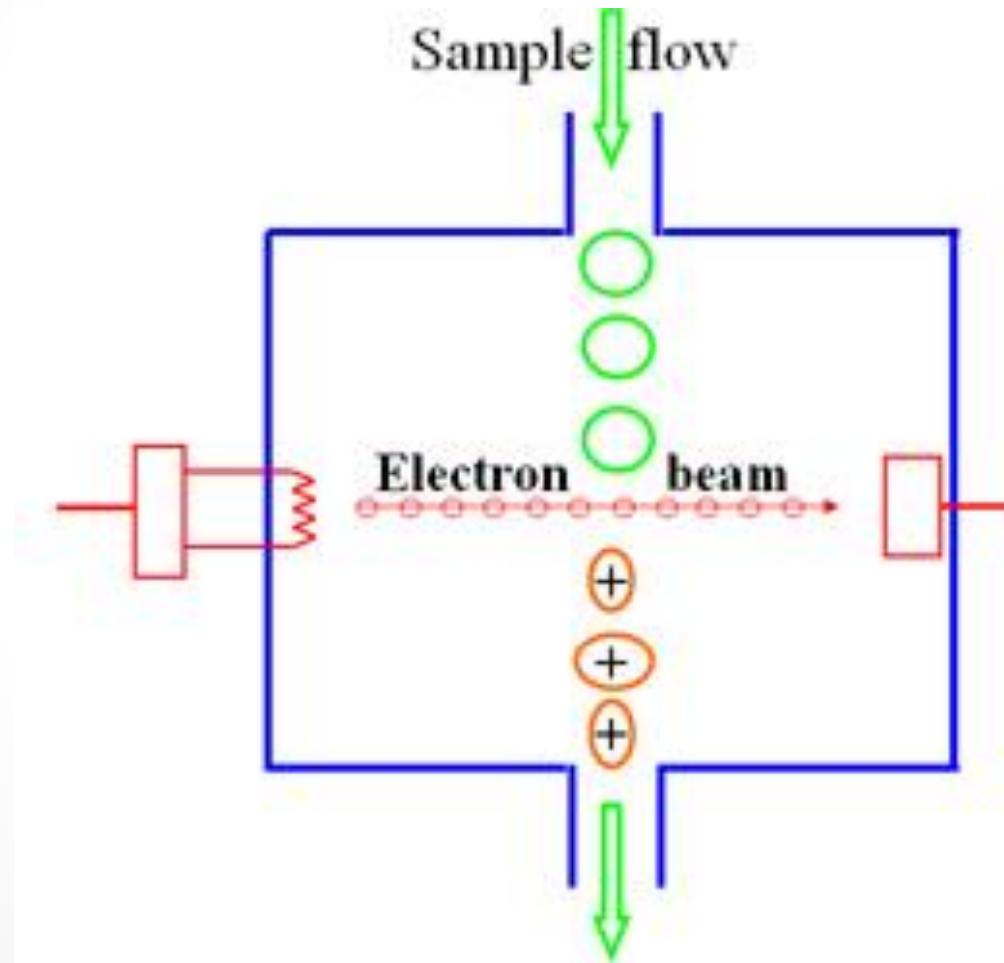


$$\frac{h}{H} * 100 \leq 10 \%$$

# Ion sources

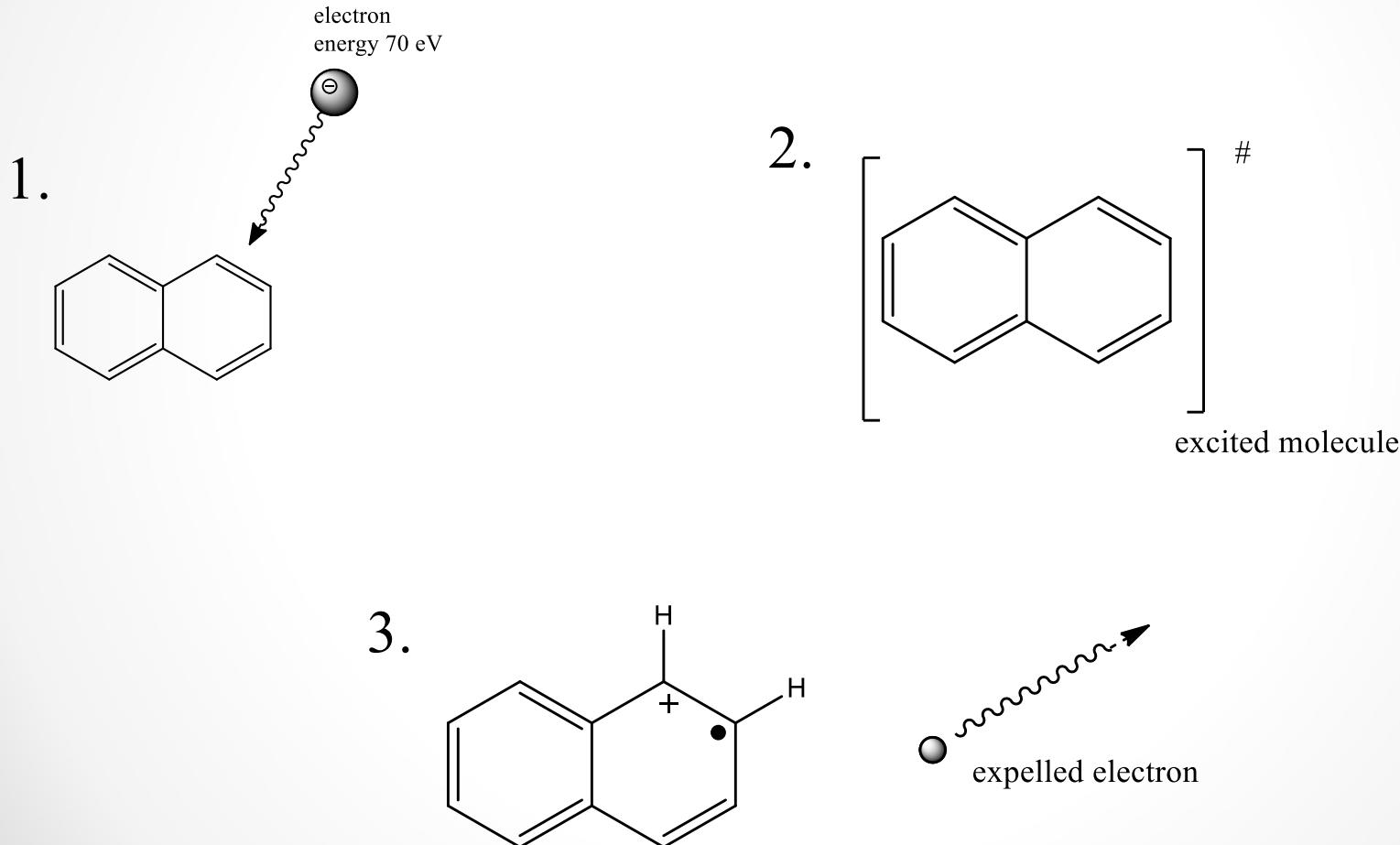
- 1. Electron ionization (EI) (Electron Impact)
- 2. Chemical Ionization (CI)
- 3. Fast Atom Bombardment (FAB)
- 4. Laser Desorption (LD)
- 5. Matrix-Assisted Laser Desorption Ionization (MALDI)
- 6. ElectroSpray ionization (ESI)

# Electron Ionization (EI) – Ionization Chamber



# Electron Ionization (EI)

## What is going on physically?



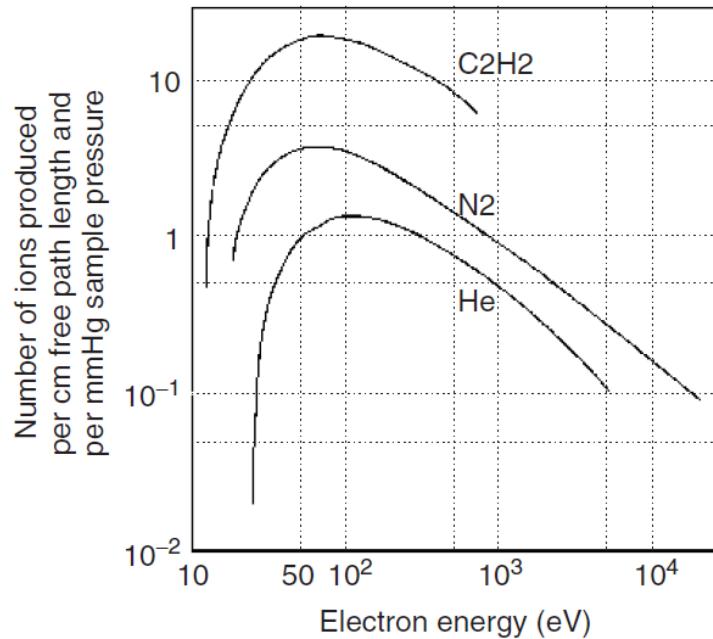
# Electron Ionization - Energy of electrons

Each electron is associated to a wave whose wavelength  $\lambda$  is given by

$$\lambda = \frac{h}{m v}$$

where  $m$  is its mass,  $v$  its velocity and  $h$  Planck's constant.

Wavelength is 2.7 Å 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.

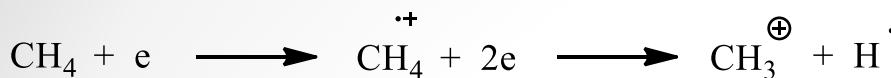
## Advantages of EI

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

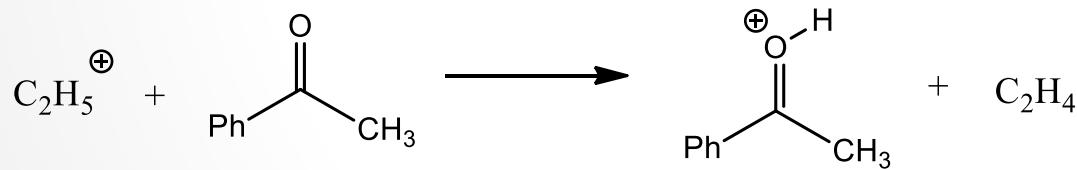
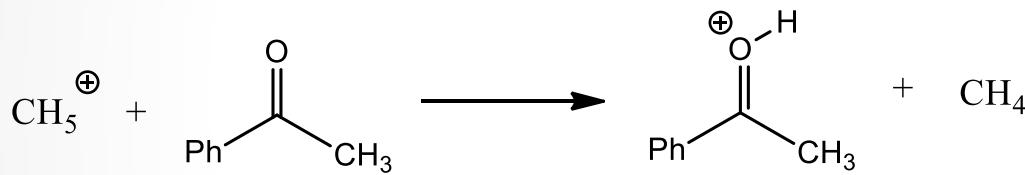
# Chemical Ionization (CI)



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

2. Gas is ionised by EI method.

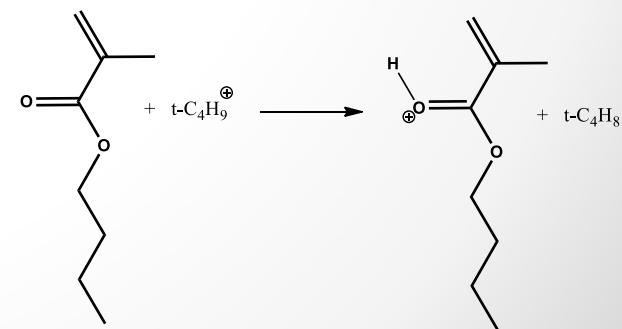
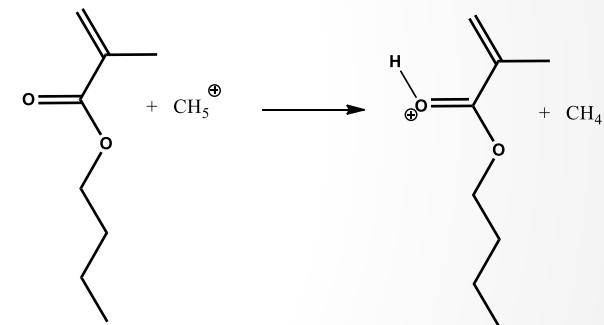
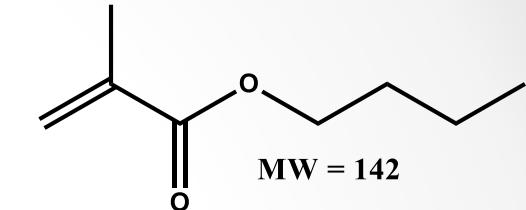
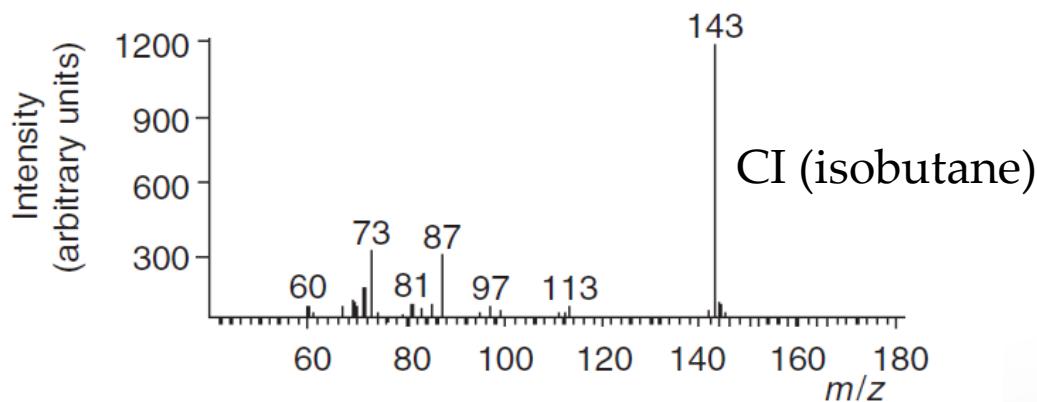
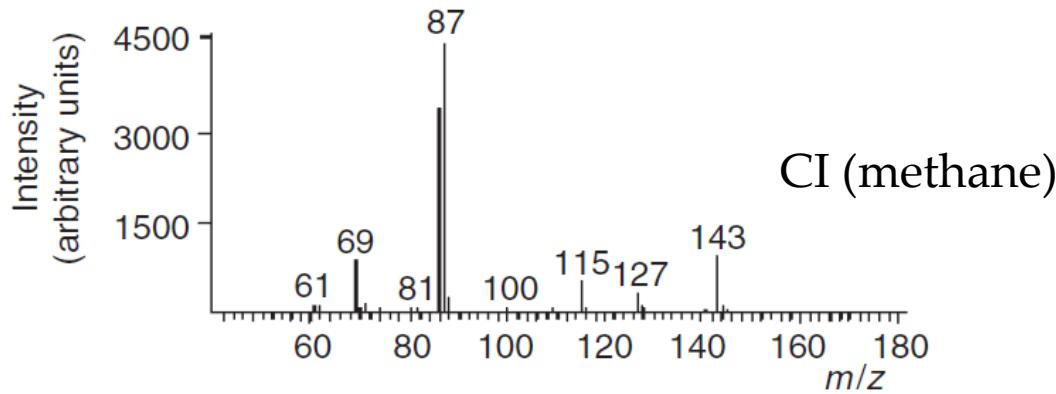
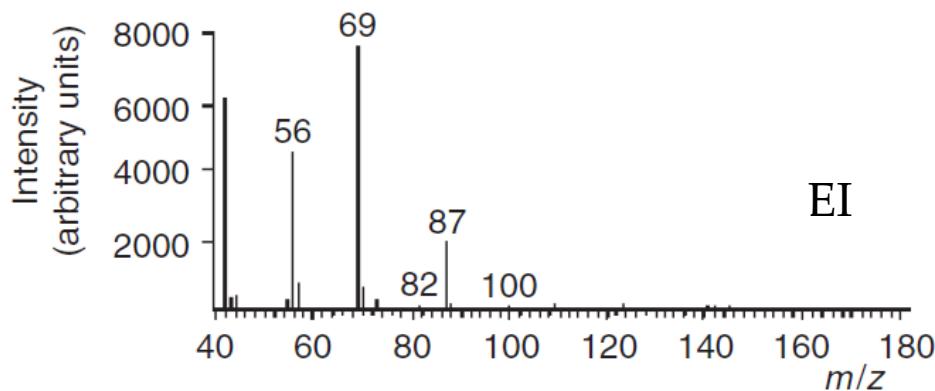
3. During the collisions of methane ions with molecules of sample, energy is transferred, as well as protons are transferred.



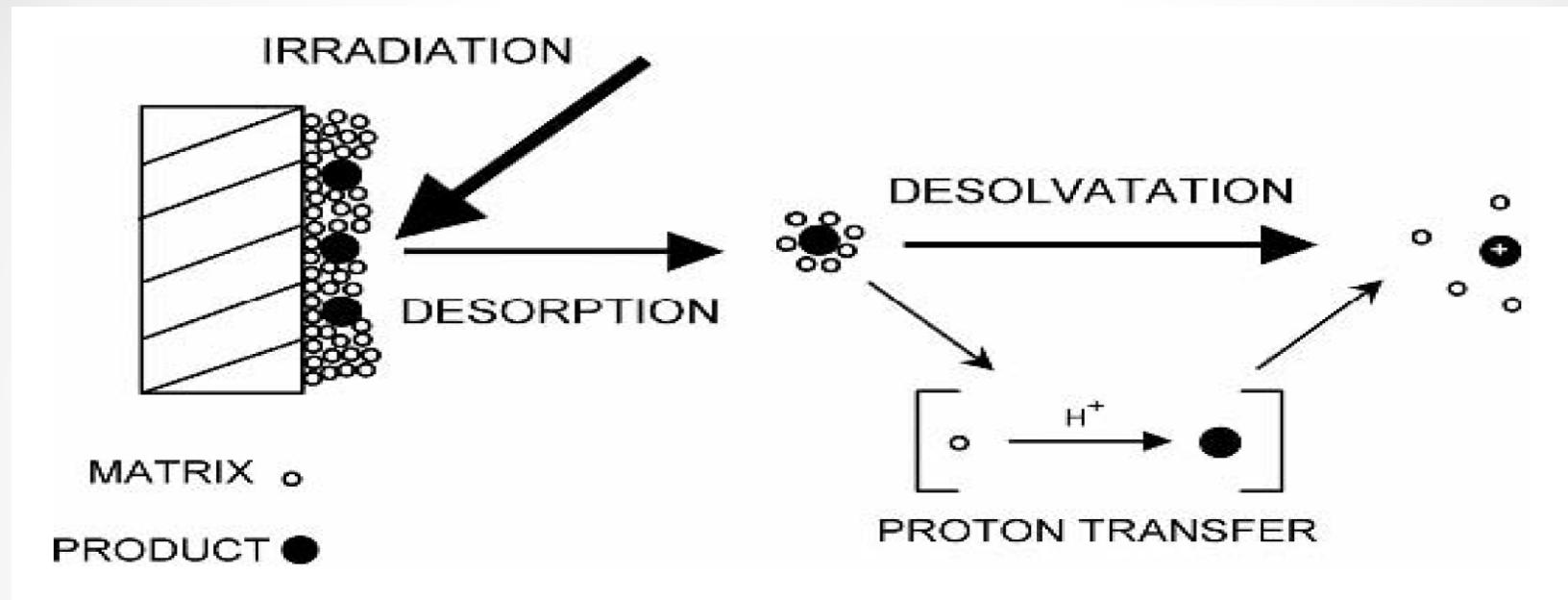
When methane is a ionizing gas: the relevant peak observed are  $[\text{M}+\text{H}]^+$ ,  $[\text{M}+\text{CH}_5]^+$ , and  $[\text{M}+\text{C}_2\text{H}_5]^+$

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

# Comparison of EI and CI



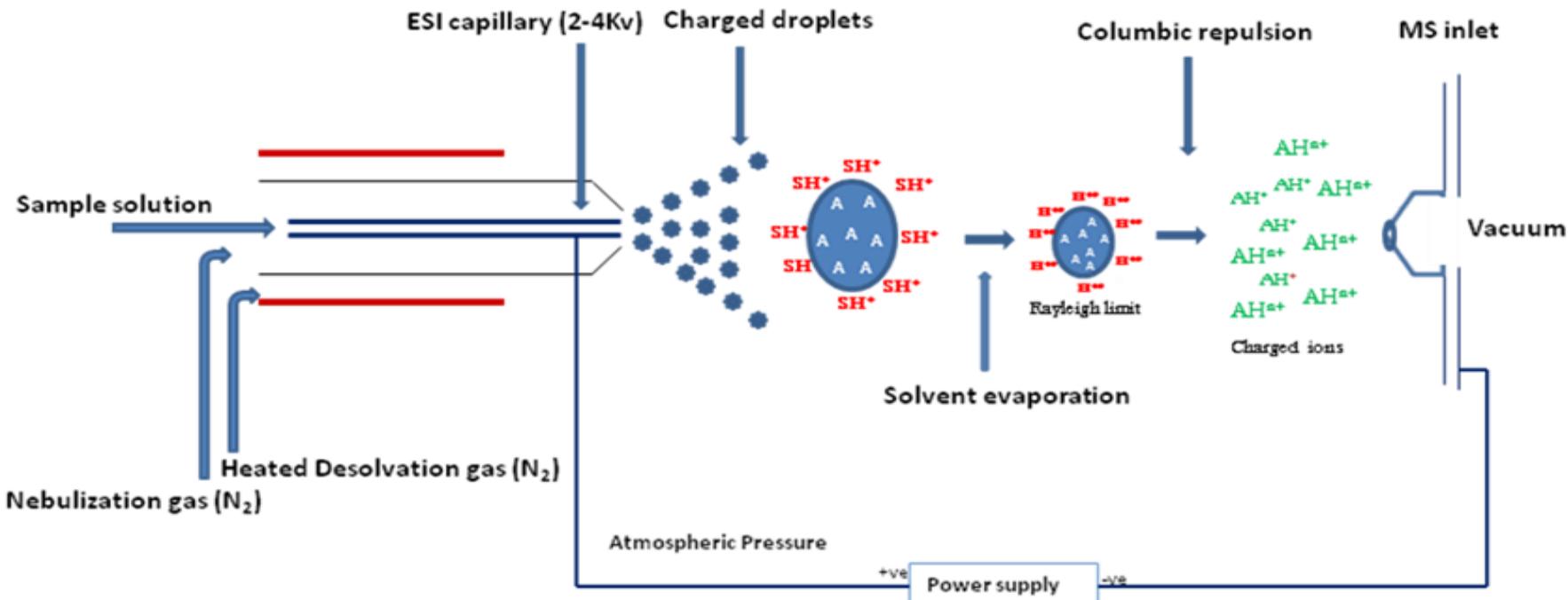
# MALDI Matrix-Assisted Laser Desorption Ionization



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



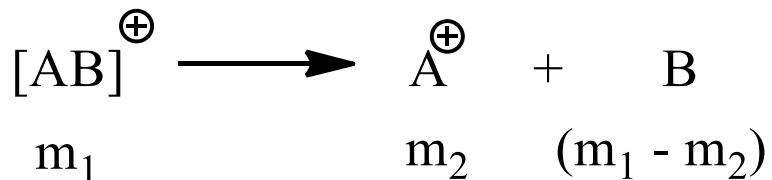
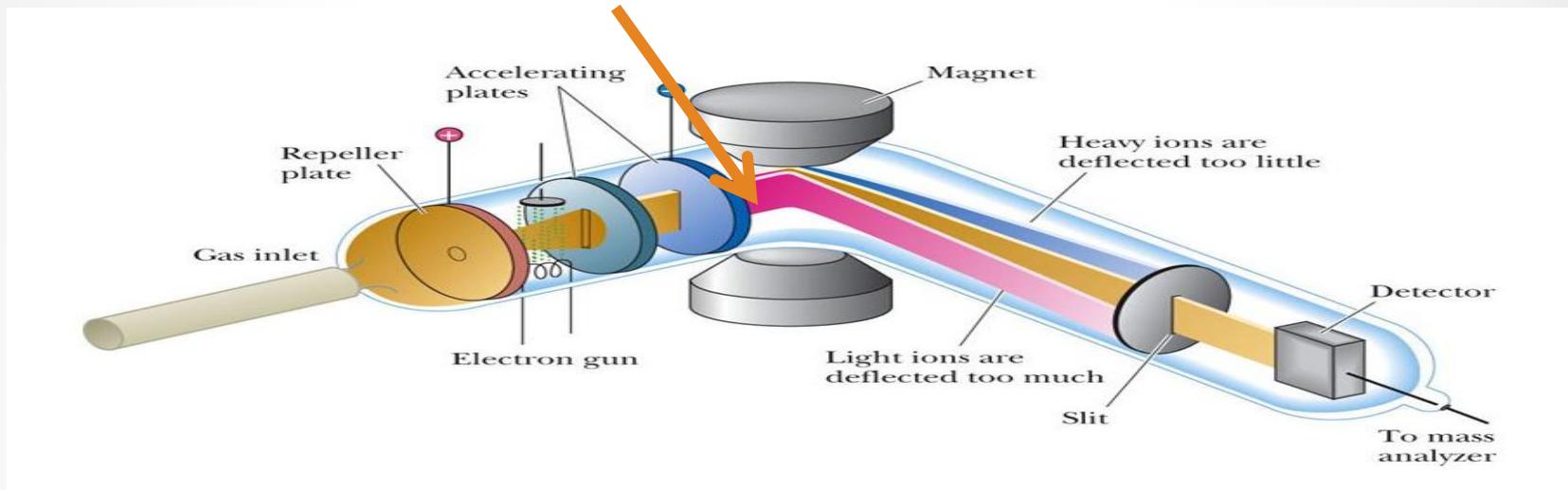
1. The electric field is obtained by applying a potential difference of 2–4 kV
2. This field induces a charge accumulation at the liquid surface located at the end of the capillary, which will break to form highly charged droplets.
3. Solvent is evaporated by the stream of heated inert gas.
4. When the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



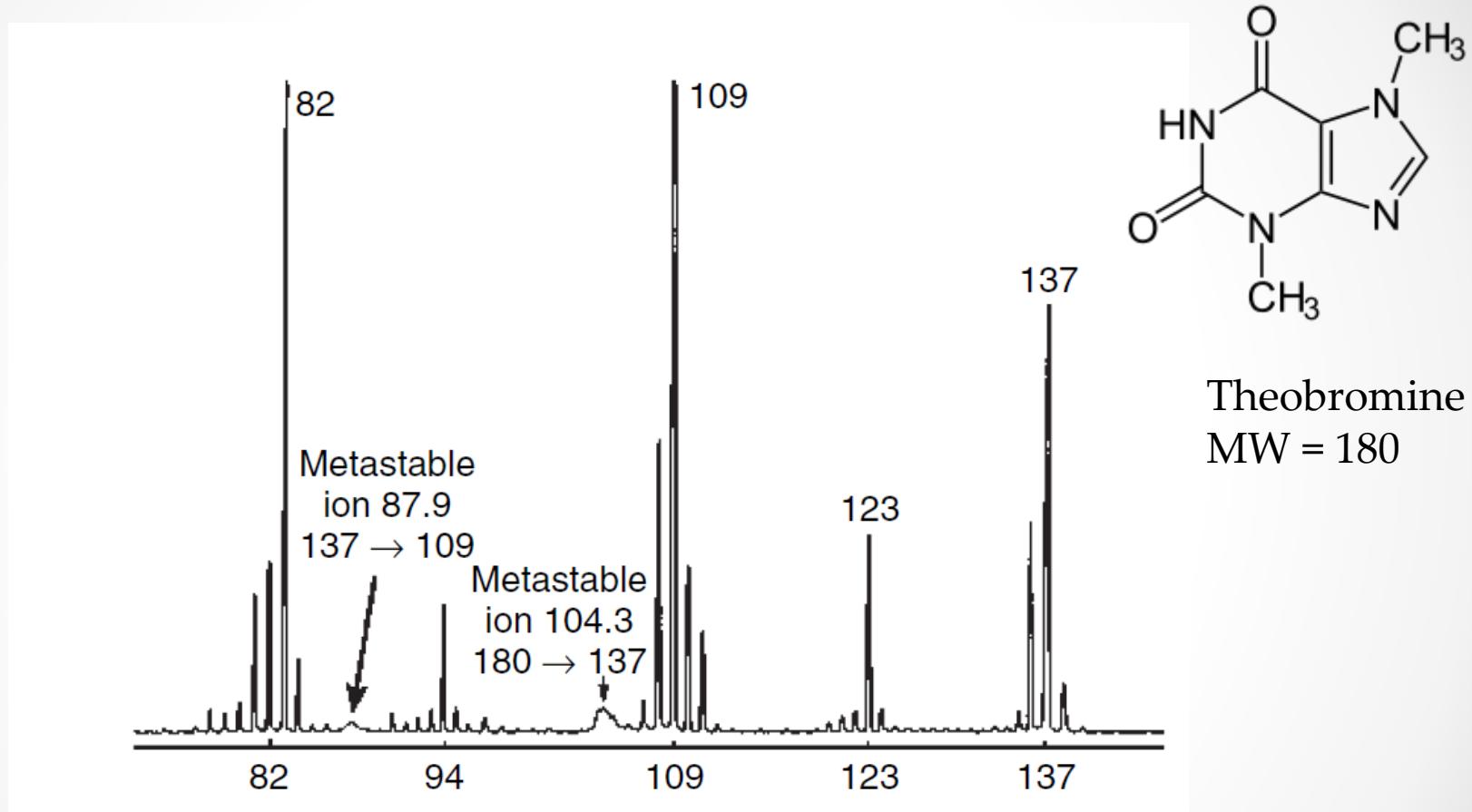
Fragment „B” takes part of kinetic energy.

Ion  $A^+$  (metastable) has lower kinetic energy than „ordinary” Ion  $A^+$

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

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

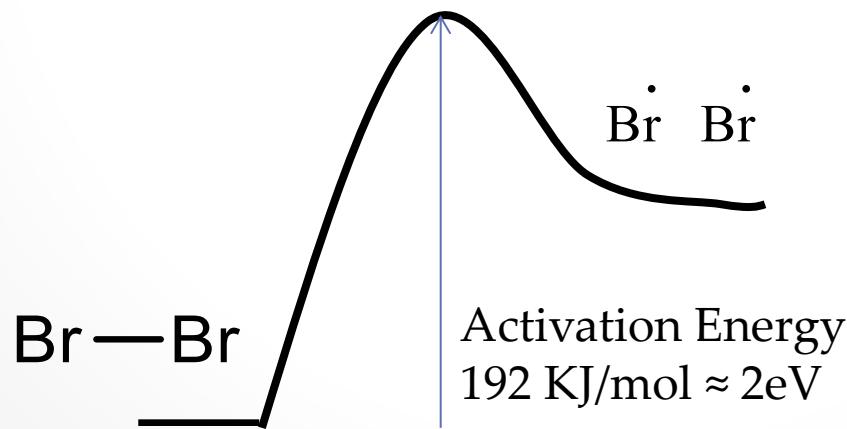
# Metastable ions



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  $180 \rightarrow 137$  Da.

# Fragmentation Reactions

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



# Fragmentation Rules

- Molecules fragment to (the most) stable particles.
  - The most stable particles has lowest energy.
- Stabilization factors:
- a) High heat of formation - stable compounds - cleavage is often associated with elimination of small stable molecule:



also other compounds with multiple bond :



and compounds with strong single bonds:

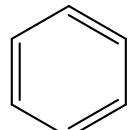


They don't undergoes fragmentation

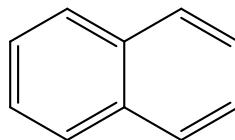
# Fragmentation Rules

- Stabilization factors:

b) Aromaticity: phenyl, naphtyl, cyclopentadienyl cation



$n=1$

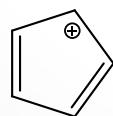
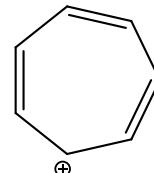


$n=2$

Hückel rule  
 $4n+2$



$4n+2$   
 $n=1$

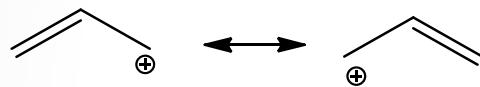


Is it aromatic?

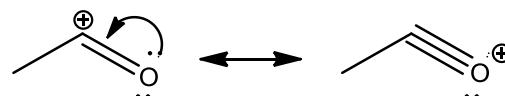
# Fragmentation Rules

- Stabilization factors:

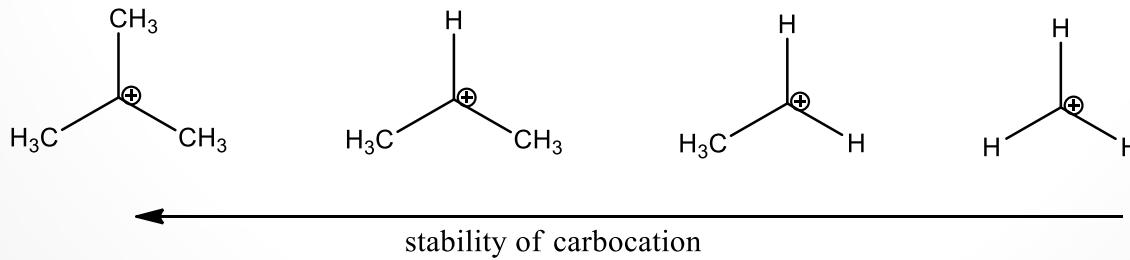
c) Mezomeric effect: allylic cation



acylium cation



d) Hyperconjugation : tertiary carbocation more stable than secondary

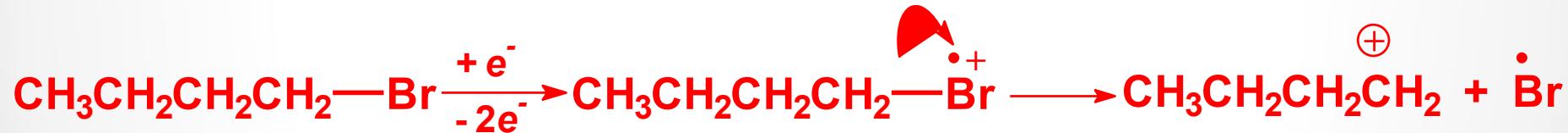


# Fragmentation pathways

- Homolysis



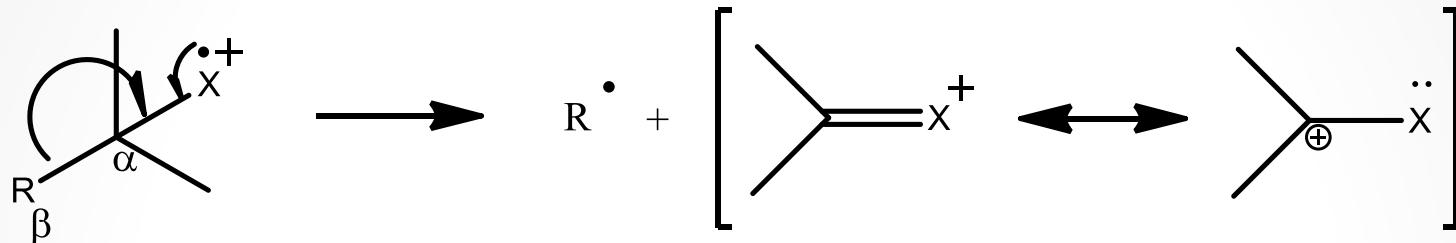
- Heterolysis



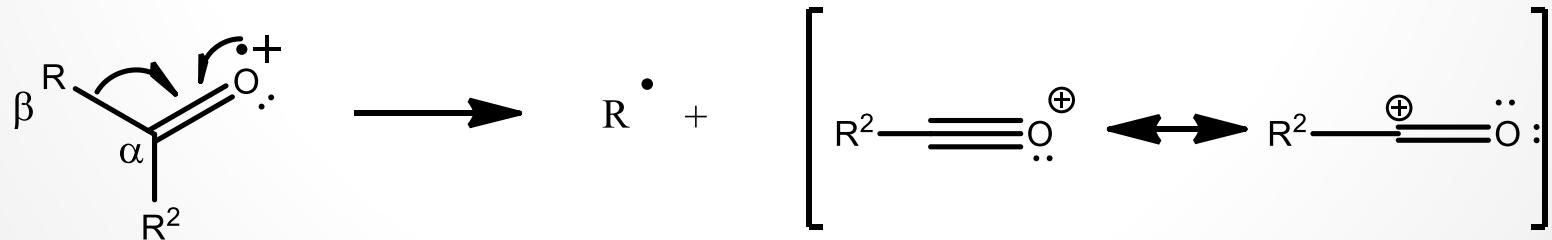
- Fragmentation starting points:
  - lone pair of electrons on heteroatoms,
  - $\pi$ - bonds of non-conjugated systems

# Fragmentation pathways

- $\alpha$  – Cleavage - homolysis between  $\alpha$  and  $\beta$  atoms



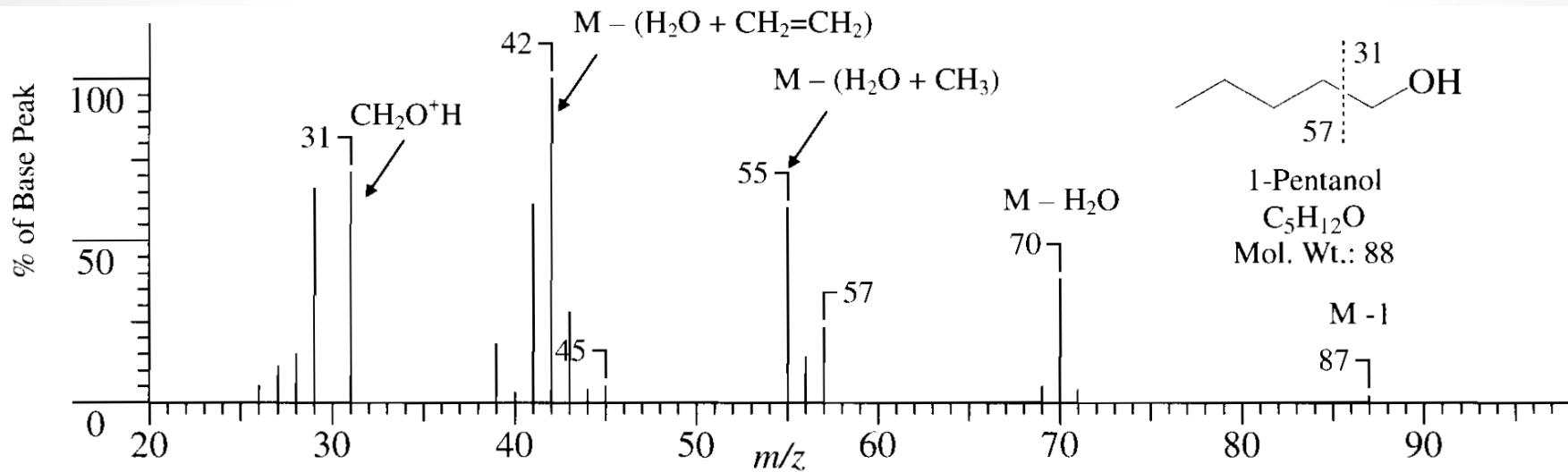
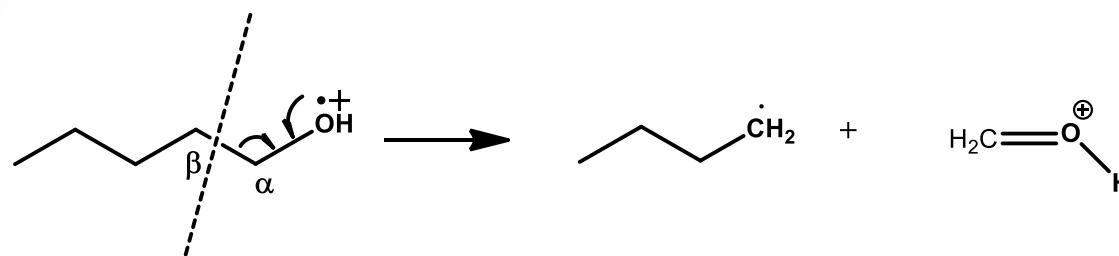
$X = NR_2, OH, OR, SH, SR$



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

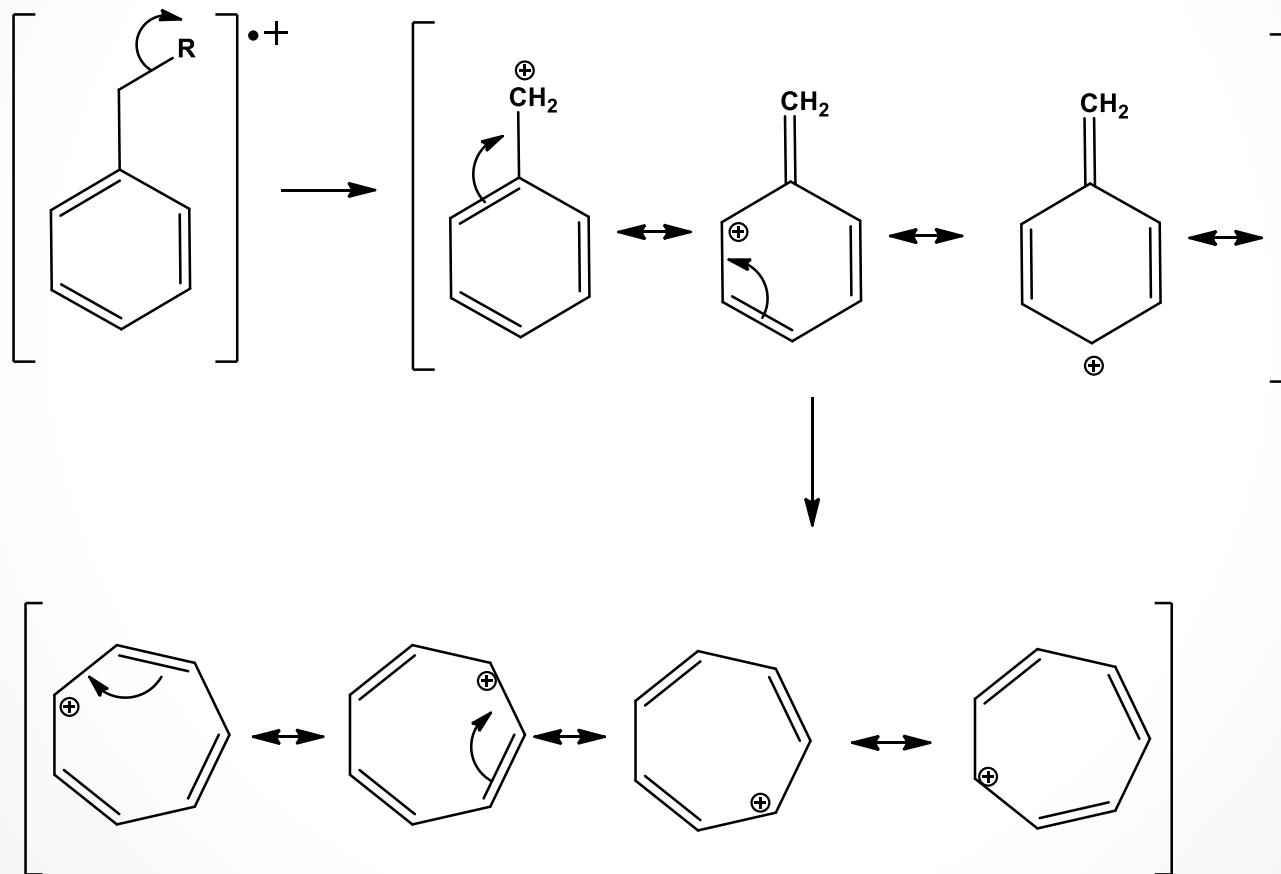
# Fragmentation pathways

- $\alpha$  – Cleavage of pentanol, peak at 31 m/z



# Fragmentation pathways

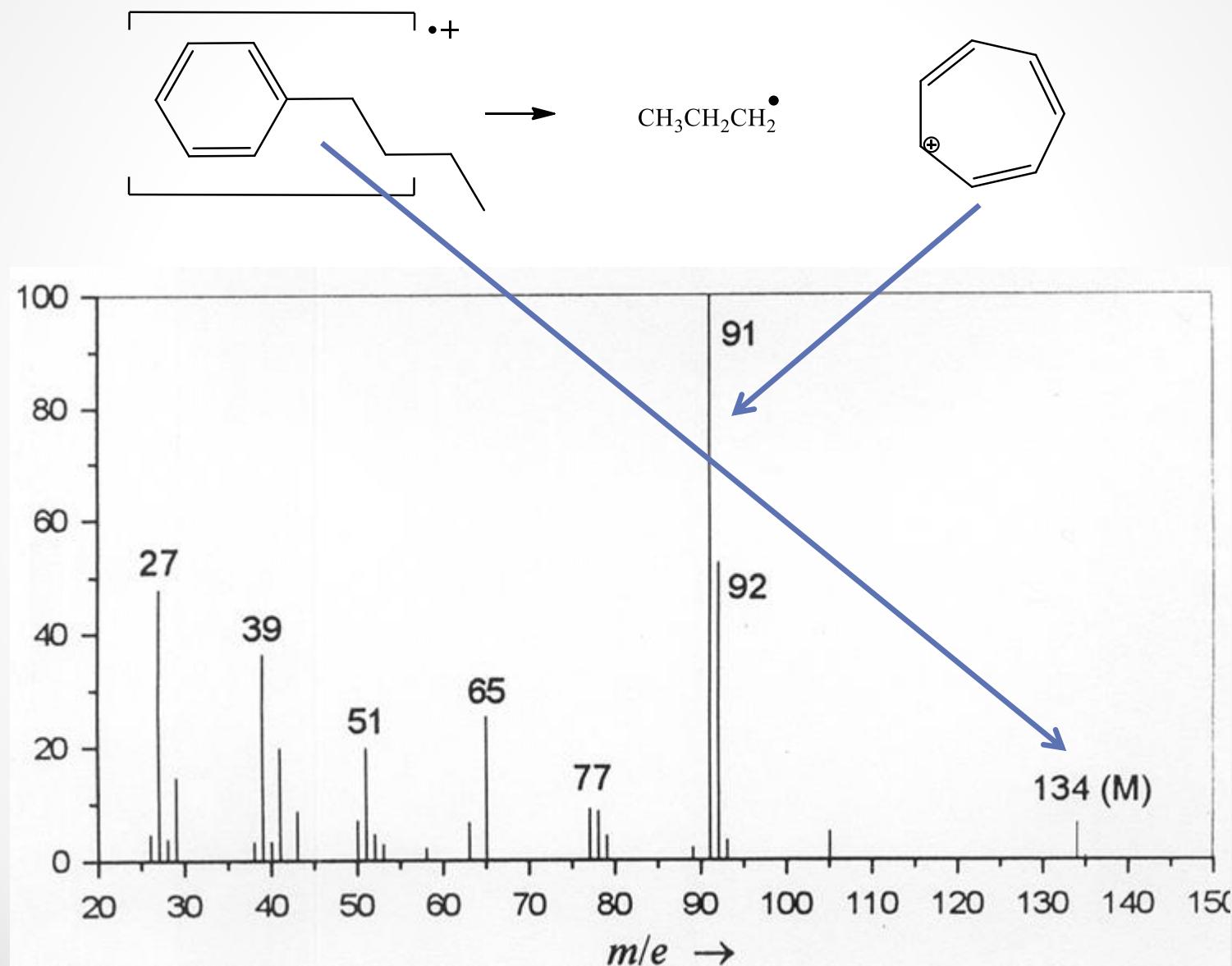
- Homolytic cleavage of alkyl aromatic derivatives,  $\beta$  – bond to aromatic ring



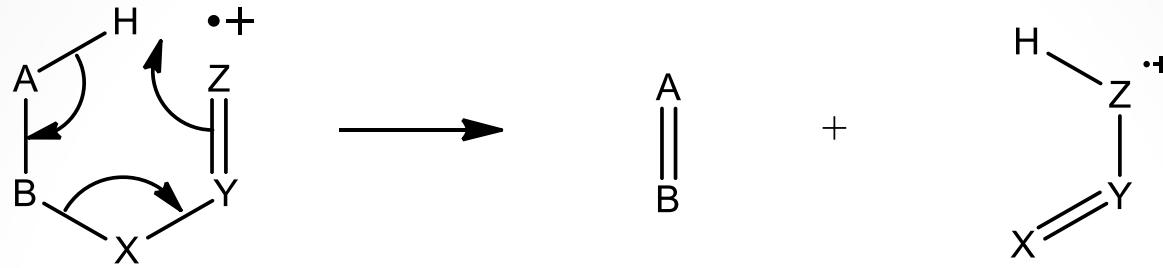
Tropylium cation

$m/z = 91$

# Cleavage of alkyl aromatic derivatives

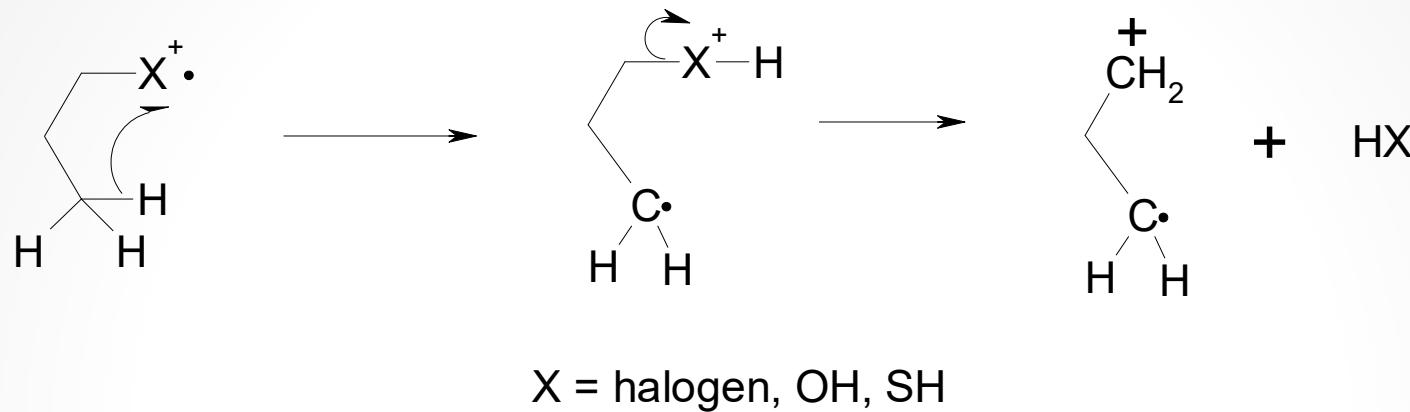


# Mc Lafferty Rearrangement

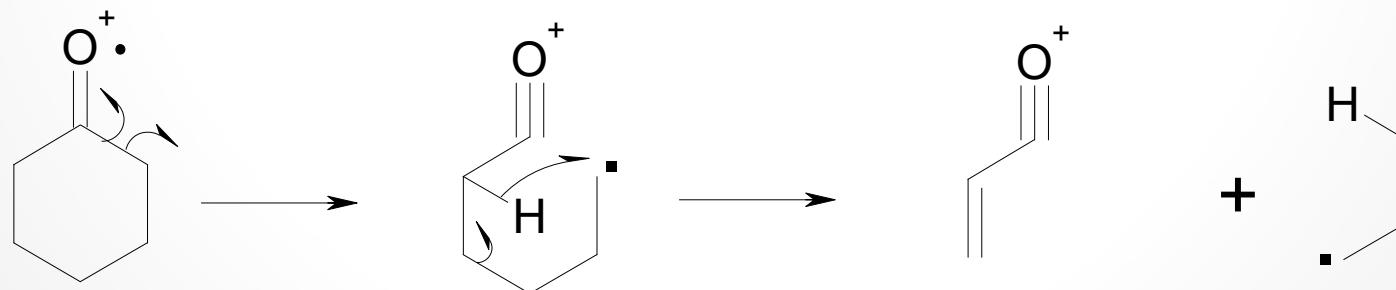


Type of Compounds	A	B	X	Y	Z
Alkenes	C	C	C	C	C
Aldehydes & ketones	C	C	C	C	O
Carboxylic acids	C	C	C	C	O
Esters	C	C	C	C	O
Nitriles	C	C	C	C	N
Amides	C	C	C	C	O
Sulfoxides	C	C	C	S	O

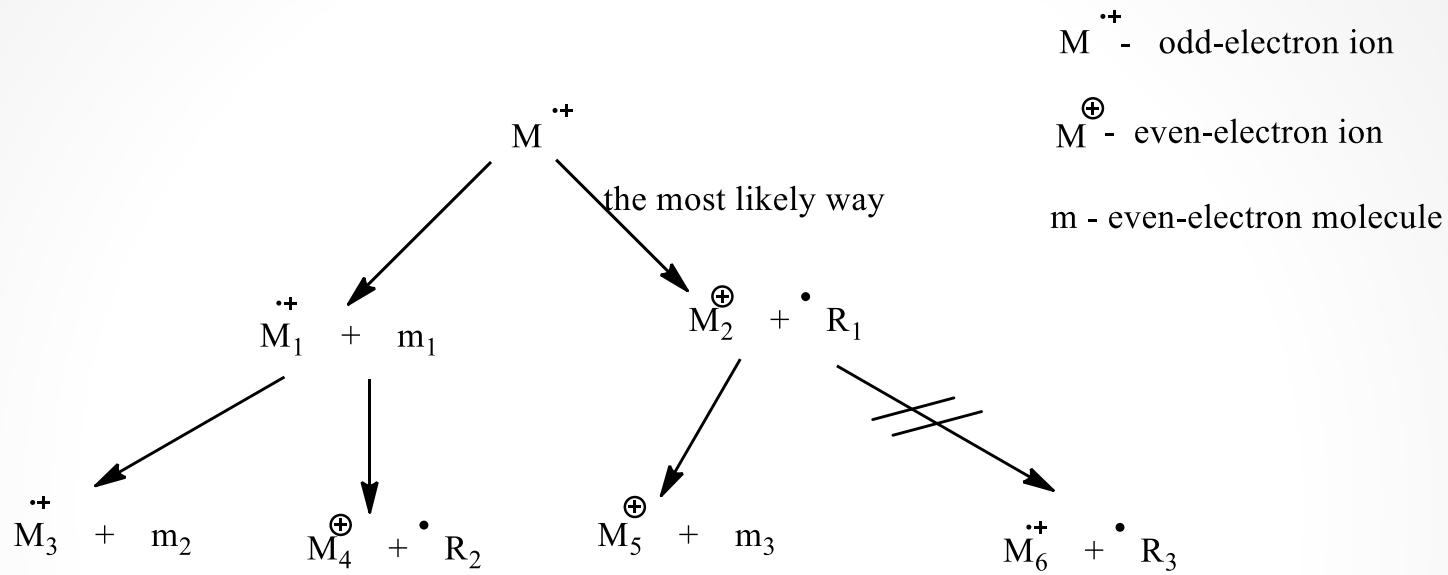
Heterolytic cleavage carbon- heteroatom bond with hydrogen shift and elimination of stable molecule ( $\text{H}_2\text{O}$ ,  $\text{HCl}$ )



Cycloalkanes rearrangement with  $\alpha$ -cleavage



# Even and odd-electron cations

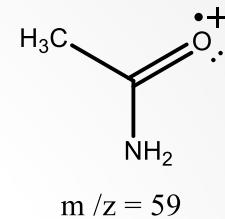


Even-electron ions are more stable than odd-electron ions

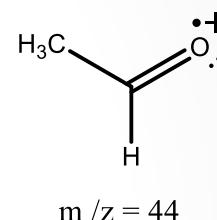
# Nitrogen rule

For odd-electron ions:

Odd- Molecular weight = odd number of nitrogen atoms

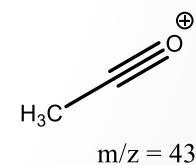


Even- Molecular weight = even number of nitrogen atoms

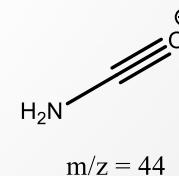


For even-electron ions:

Odd- Molecular weight = even number of nitrogen atoms

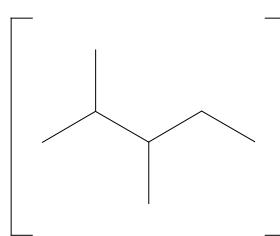


Even- Molecular weight = odd number of nitrogen atoms



# Alkanes

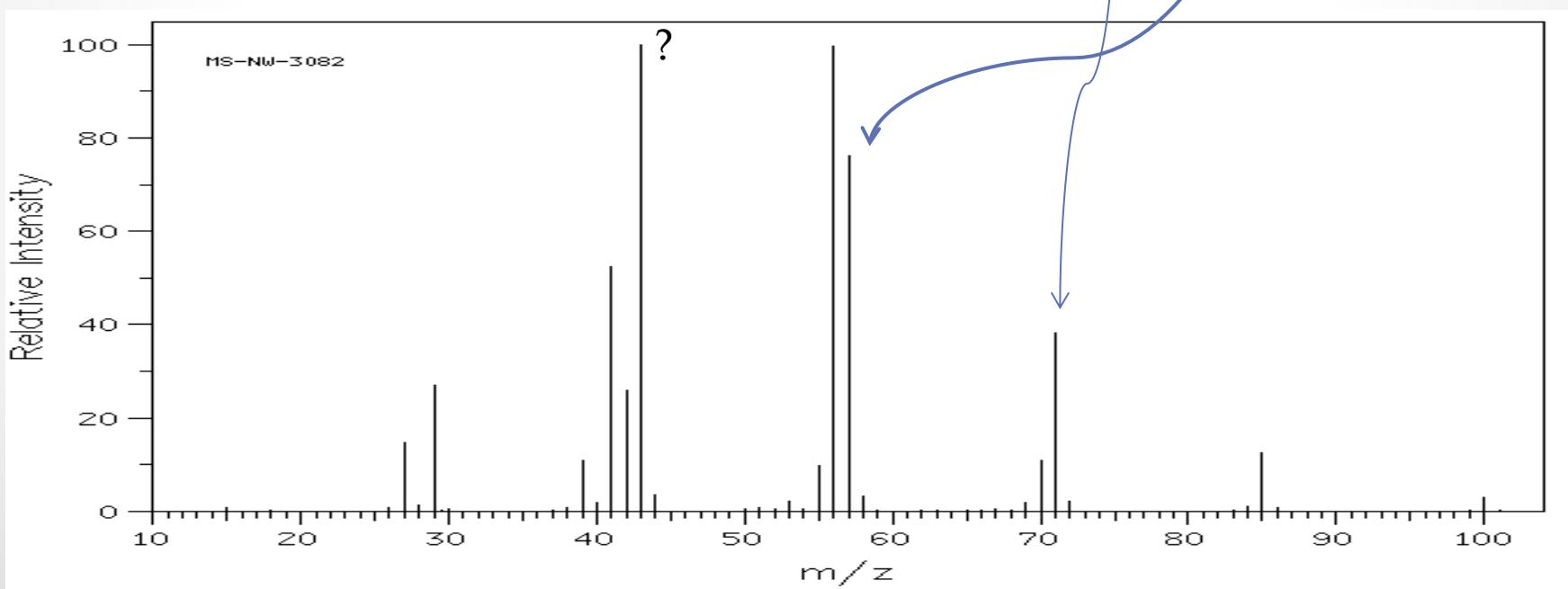
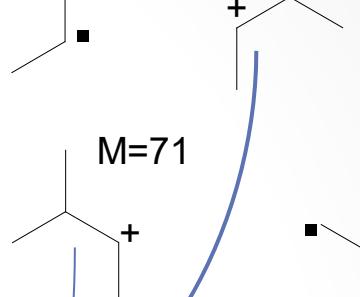
M=100



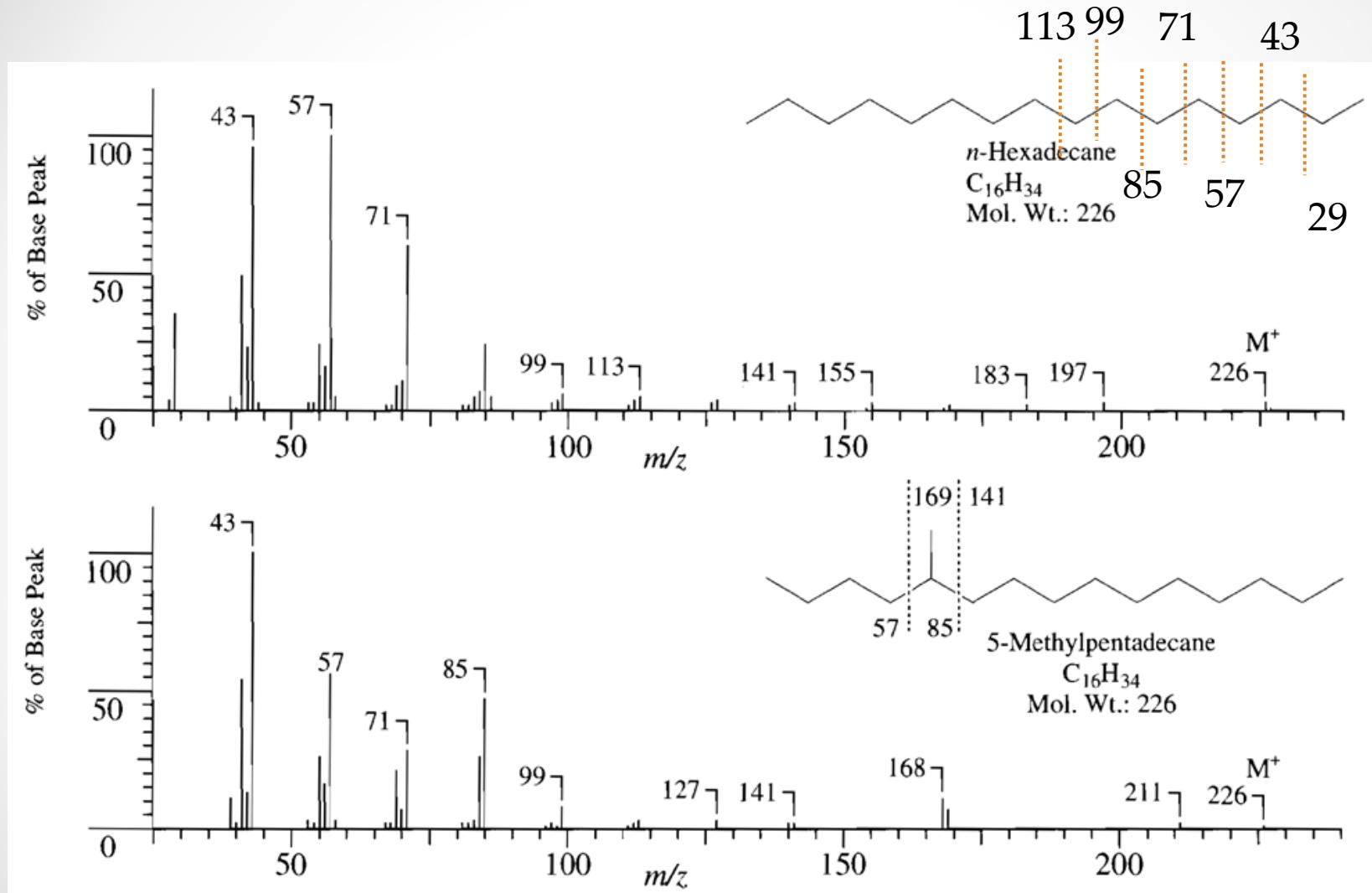
+



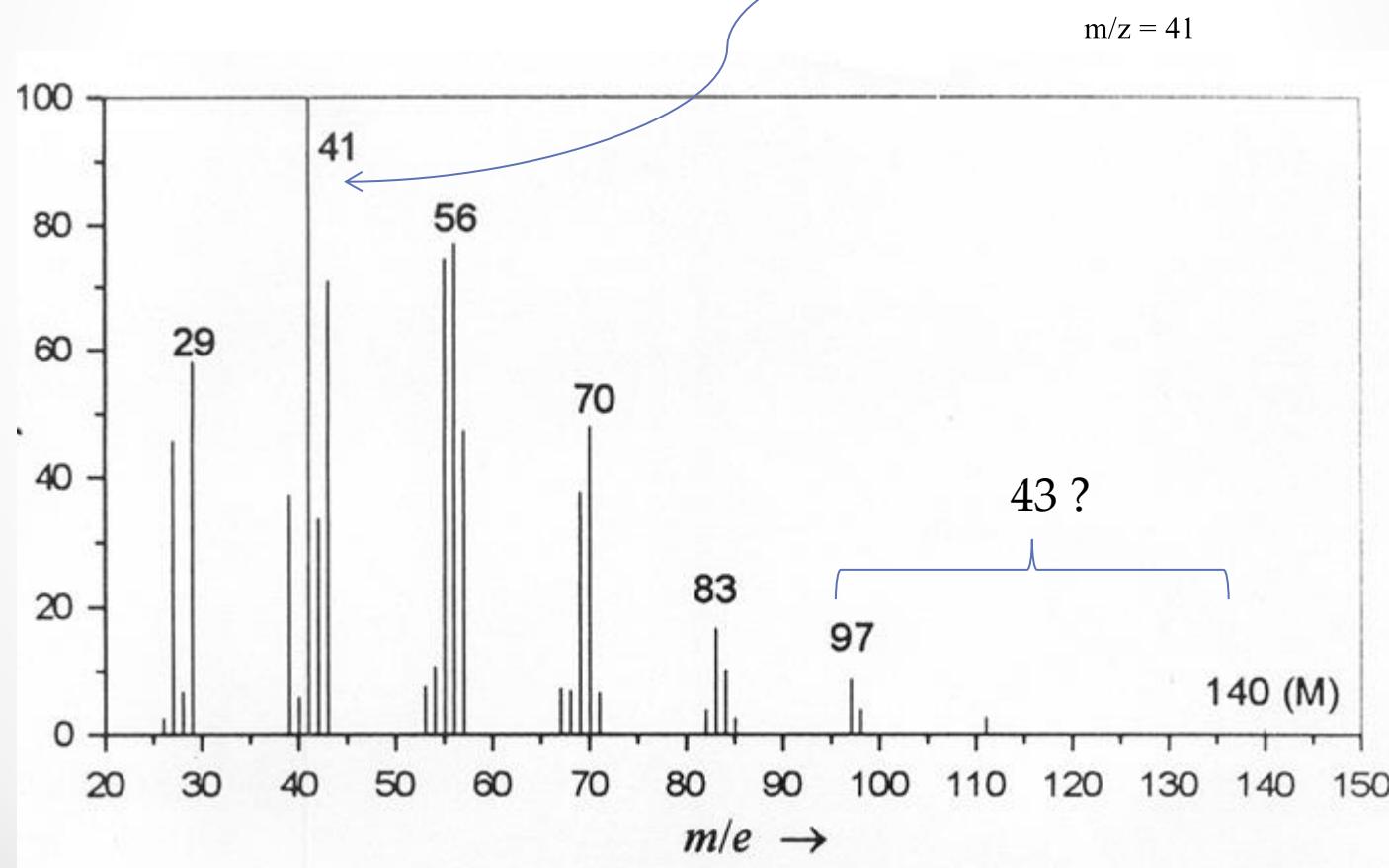
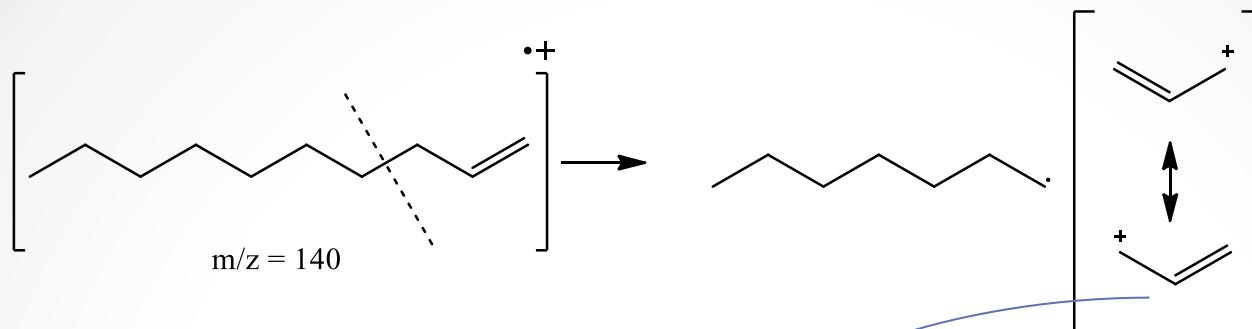
M=57



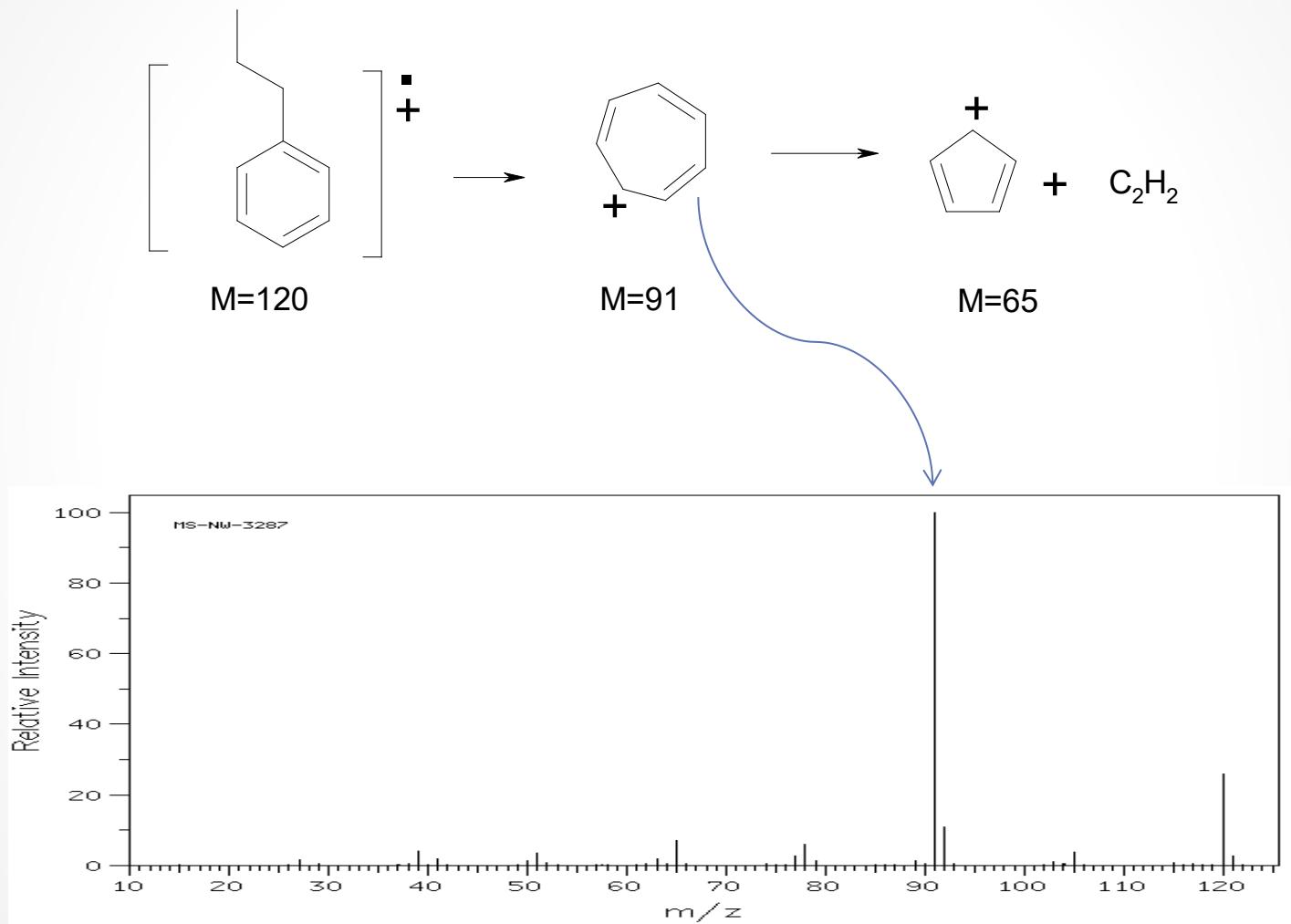
# Alkanes



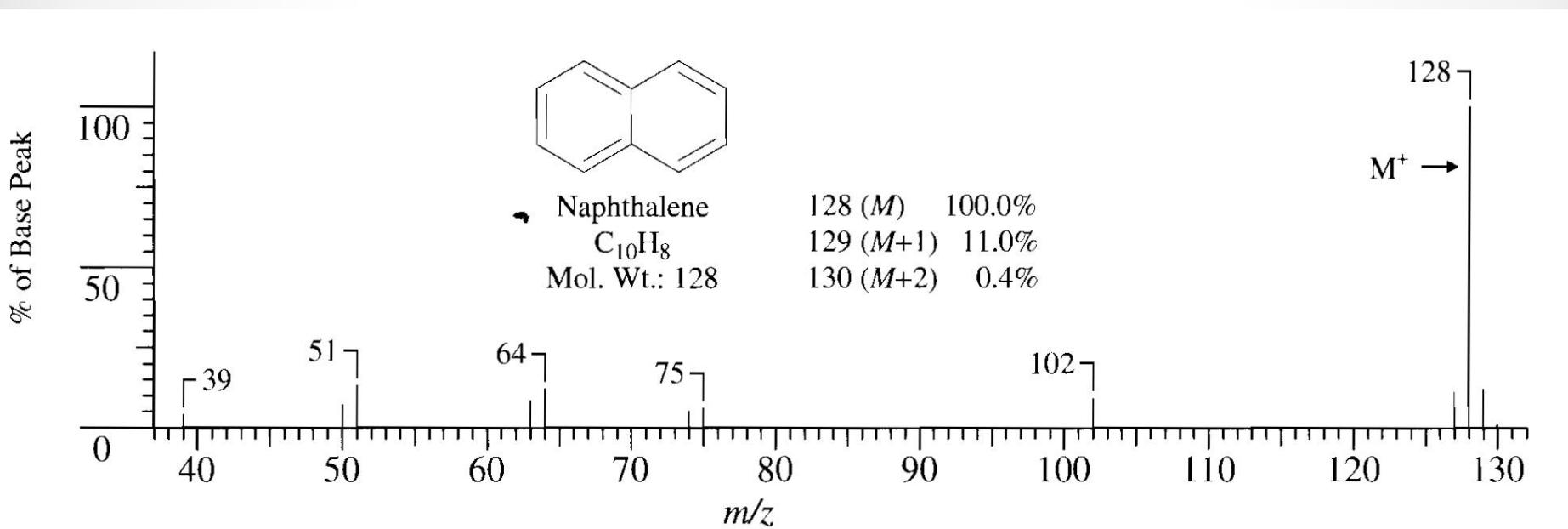
# Alkenes



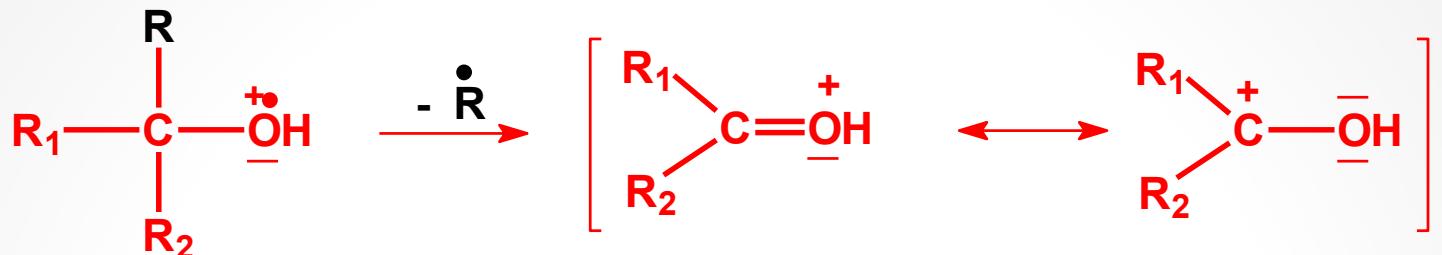
# Aromatic hydrocarbons



# Aromatic hydrocarbons



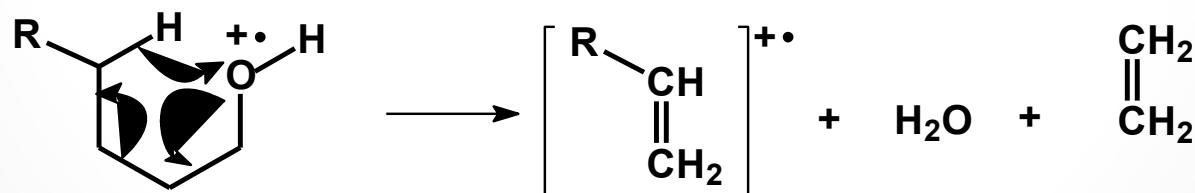
# Alkohols



$\alpha$ -Cleavage

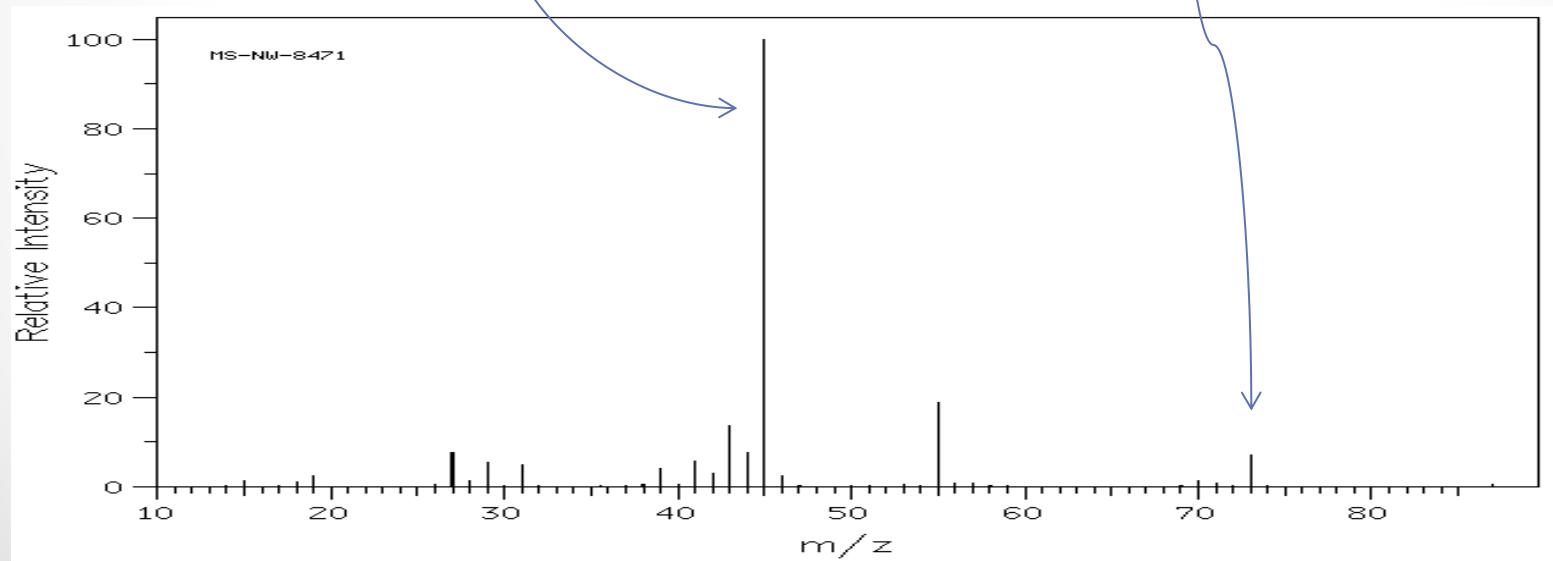
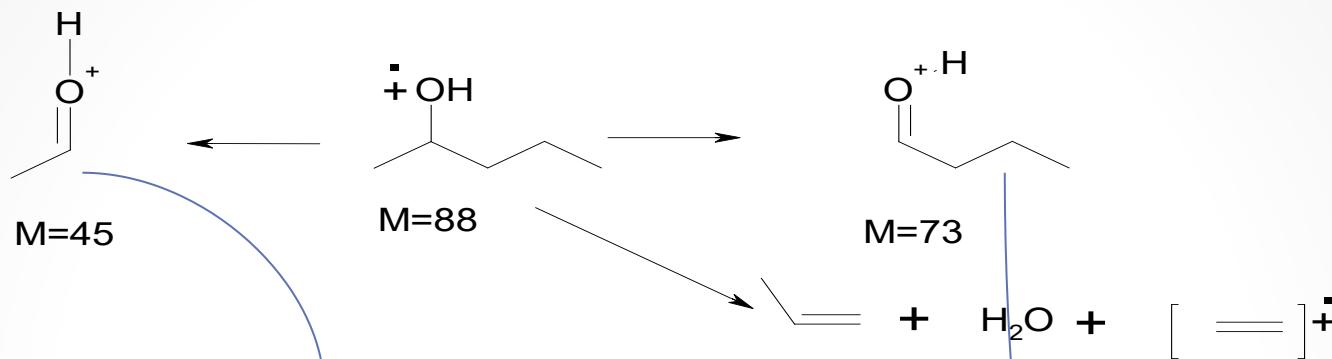
$m/z$ for $R_1 = R_2 = H$	31
$R_1 = CH_3$	45
$R_1 = CH_3CH_2$	59
$R_1 = CH_3CH_2CH_2$	73

Alkohols with chain length at least four carbons .

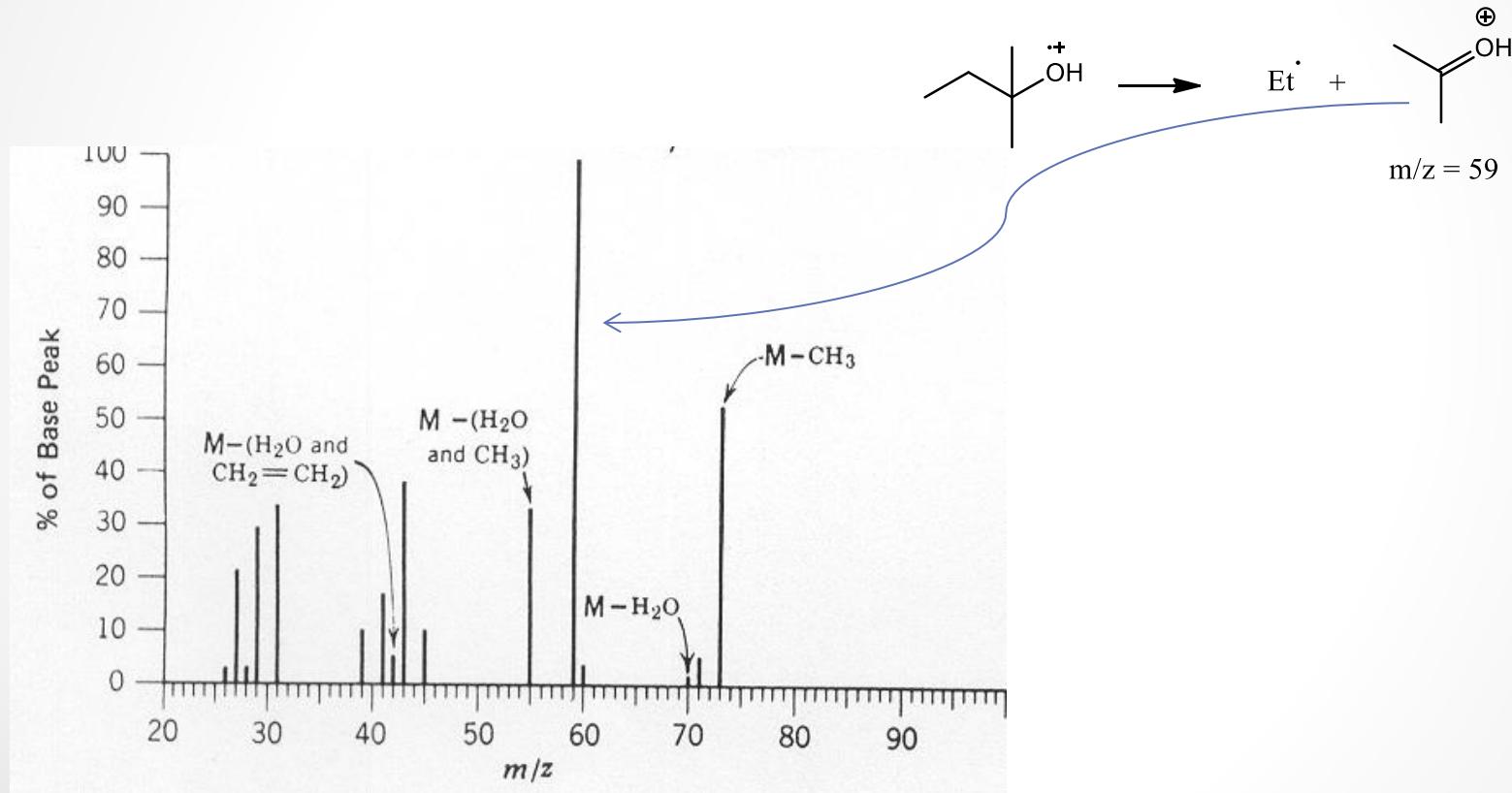


$m/z$  28 for n-butanol  
 $m/z$  42 for n-pentanol  
etc....

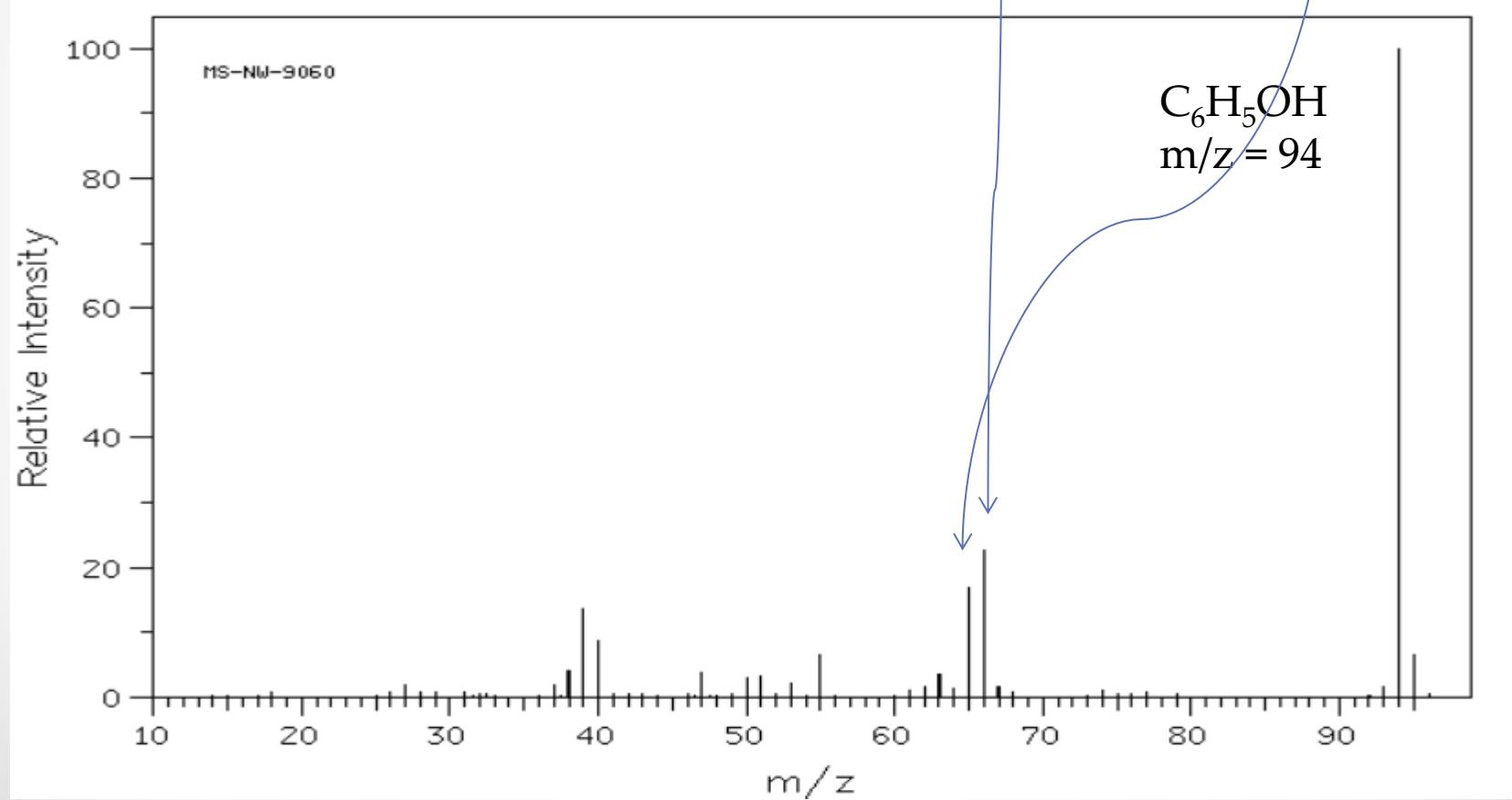
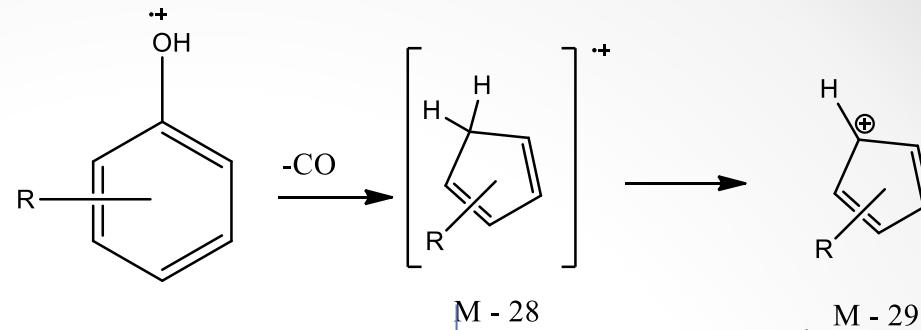
# Alkohols



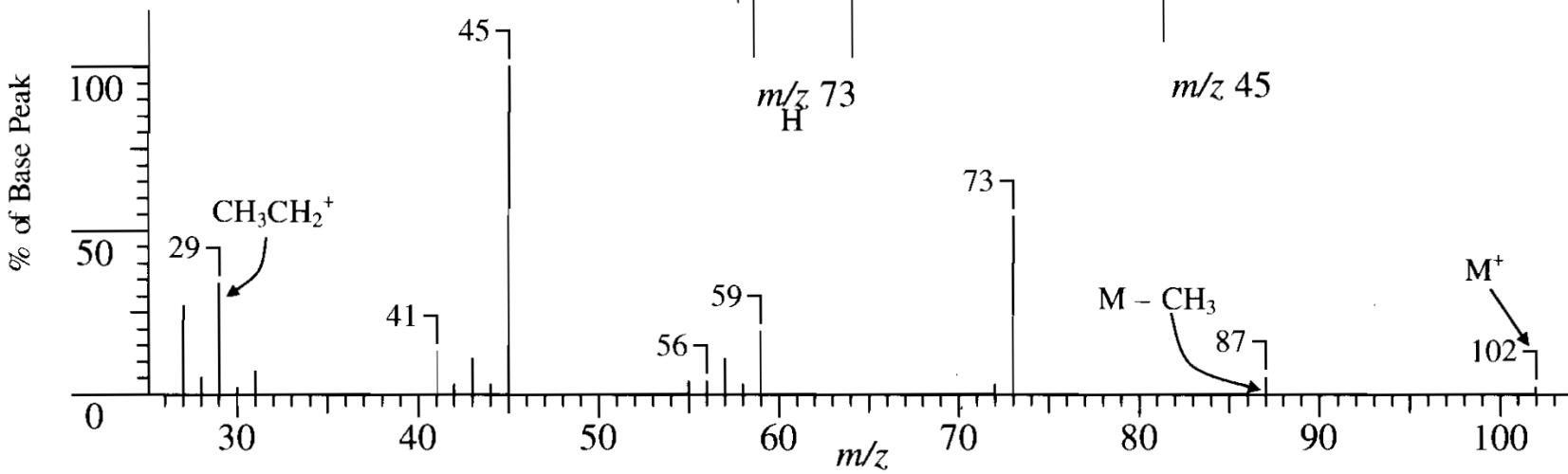
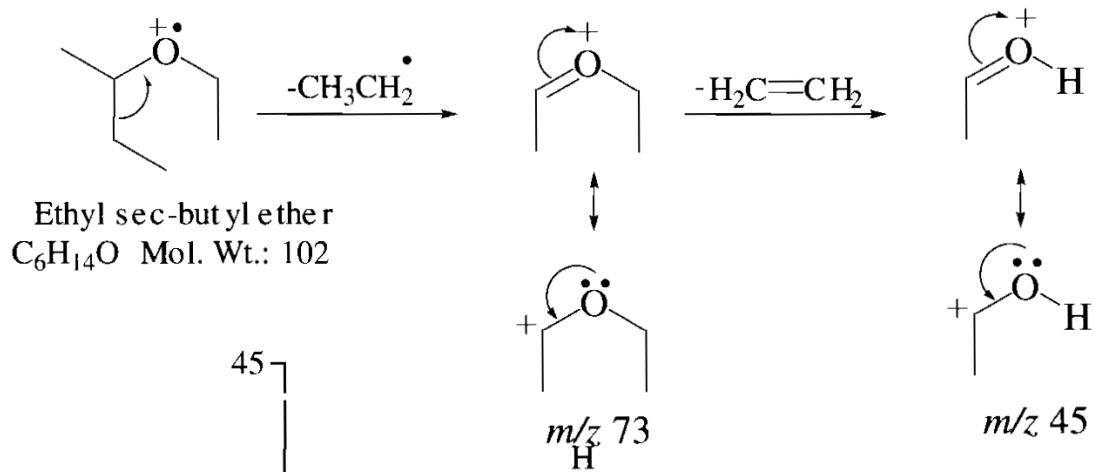
# Alkohols: tert-pentanol



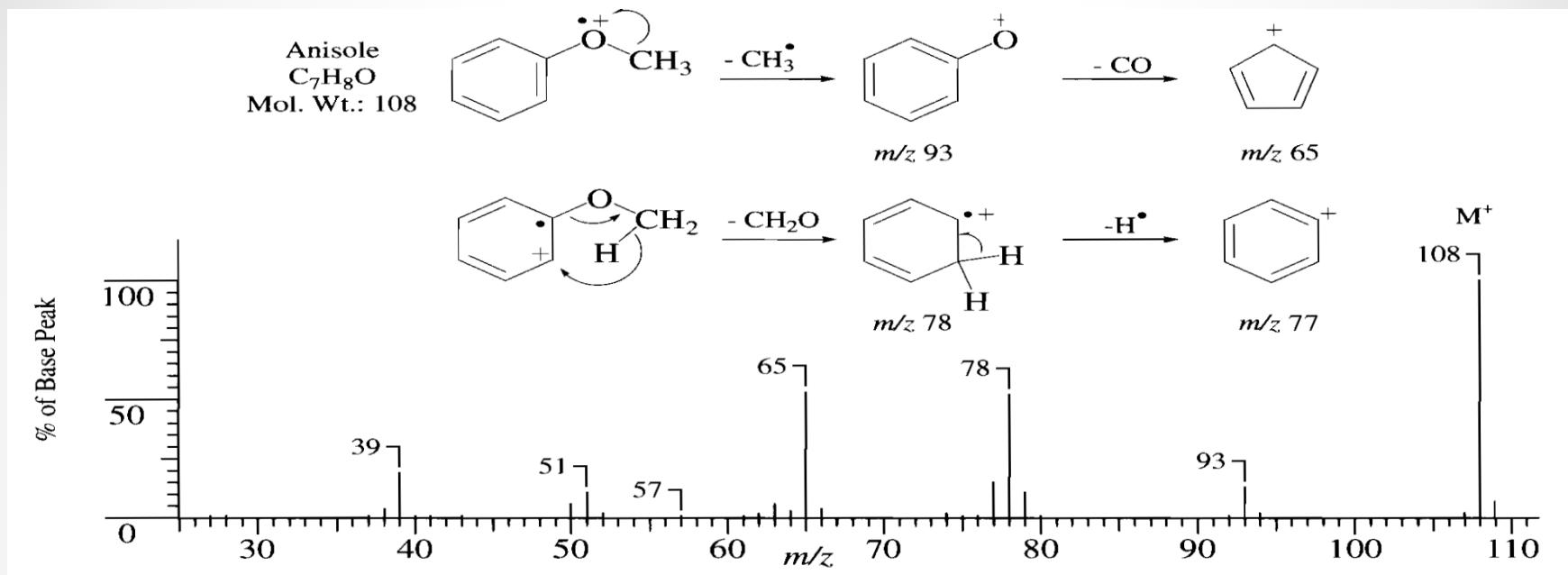
# Phenols



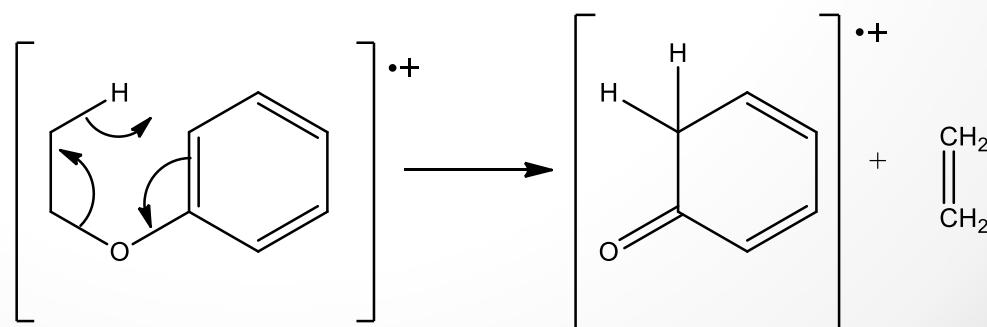
# Ethers $\alpha$ -cleavage



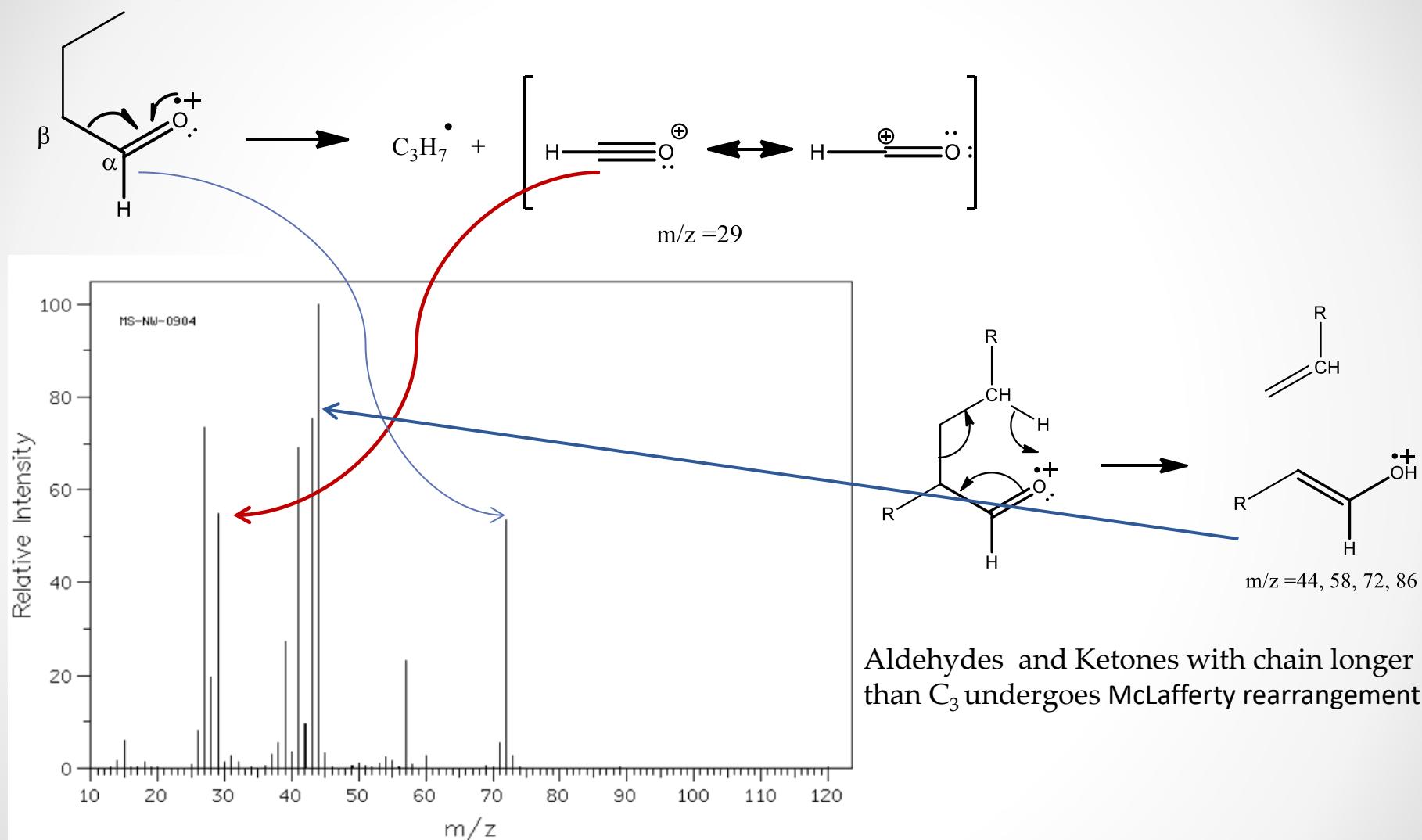
# Alkyl-aryl ethers



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

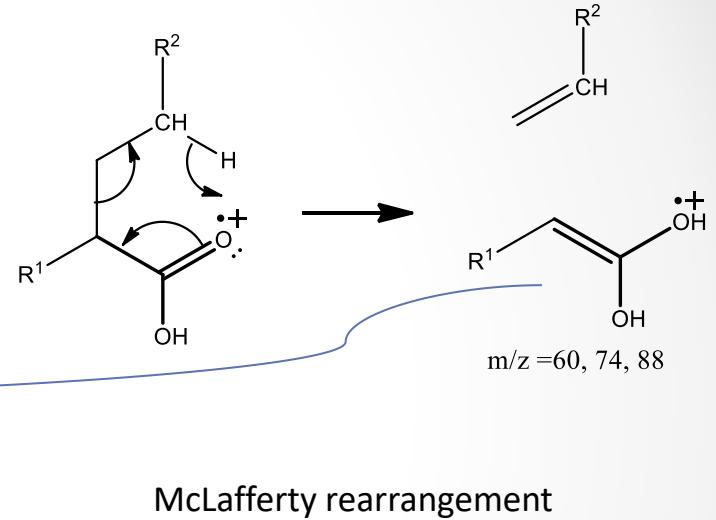
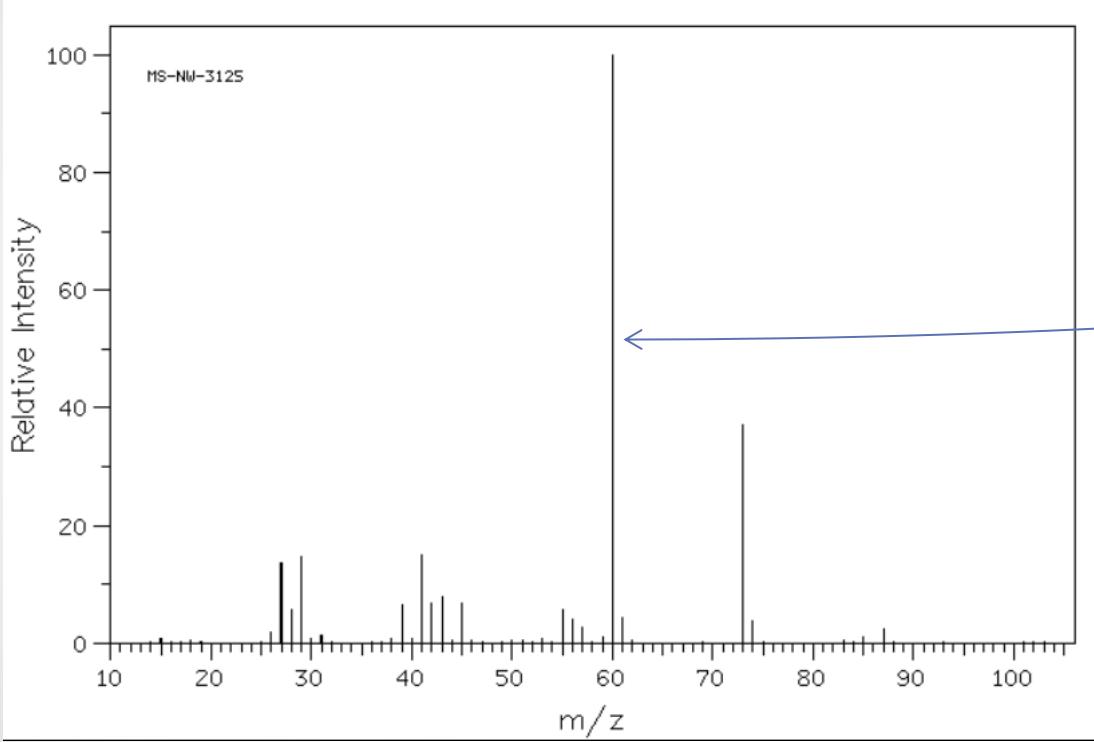


# Aldehydes and ketones



Butyric aldehyde

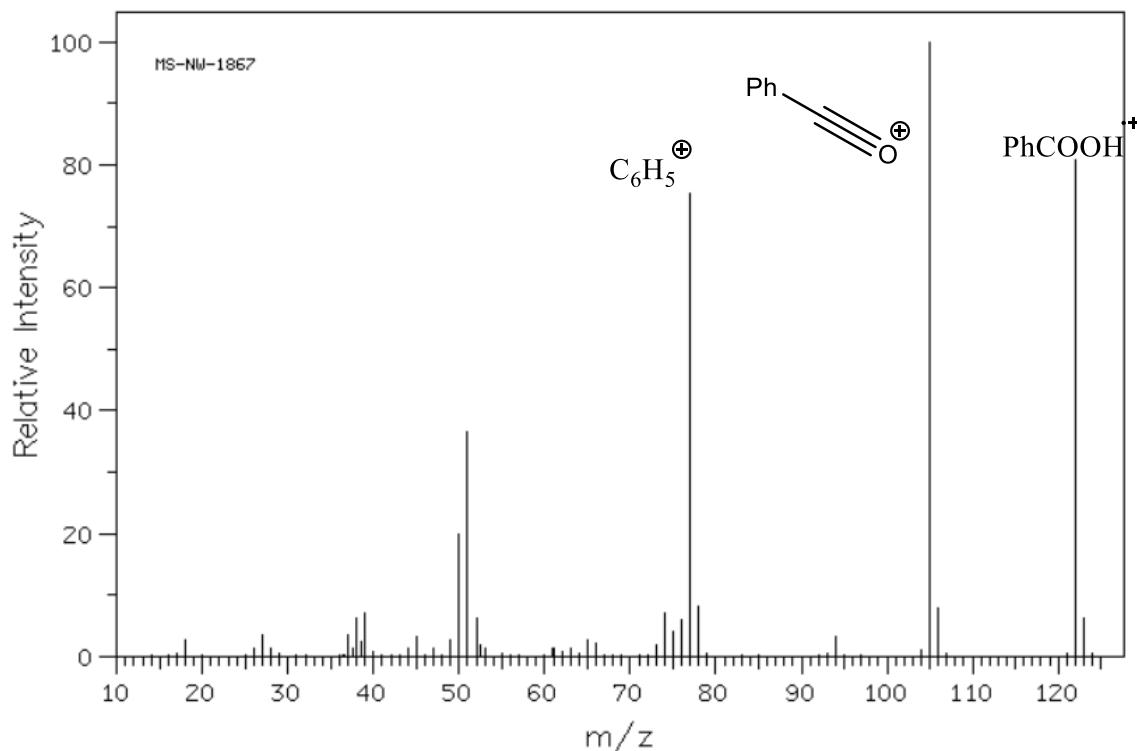
# Carboxylic acids



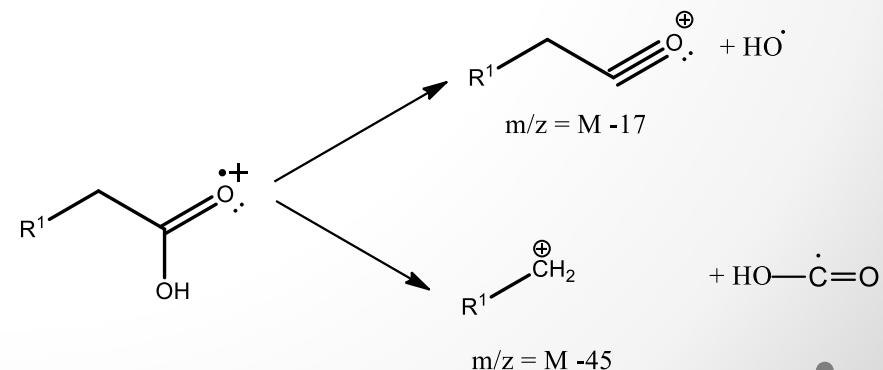
Valeric acid ( pentanoic acid)

# Carboxylic acids - aromatic and aliphatic with short chain

MS-NW-1867                    SDBS NO.        673  
benzoic acid                    (Mass of molecular ion:        122)  
C7H6O2



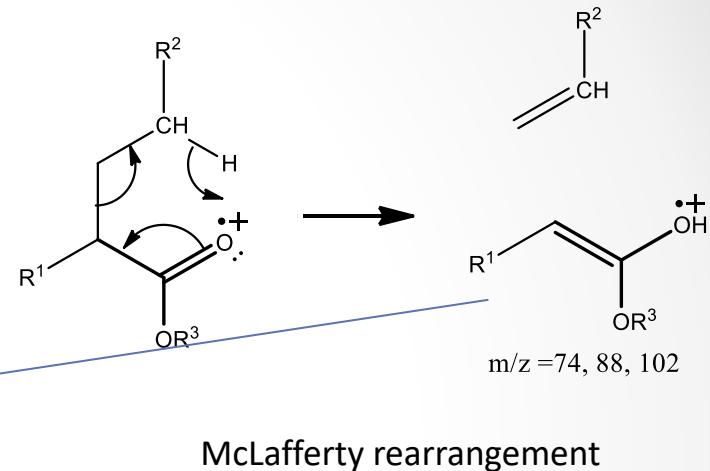
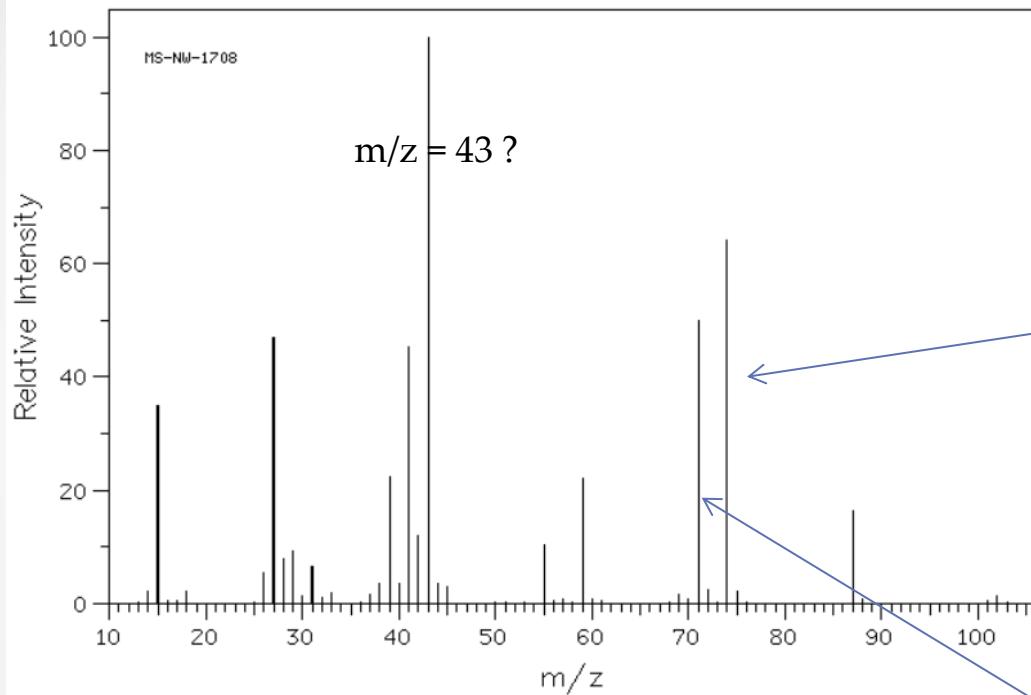
Benzoic acid



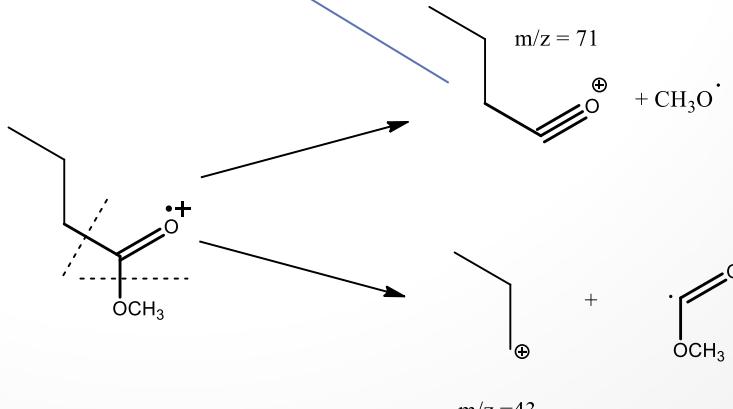
# Carboxylic esters

MS-NW-1708  
methyl butyrate  
C5H10O2

SDBS NO. 2641  
(Mass of molecular ion: 102)



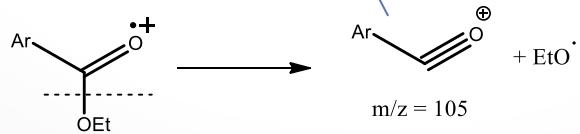
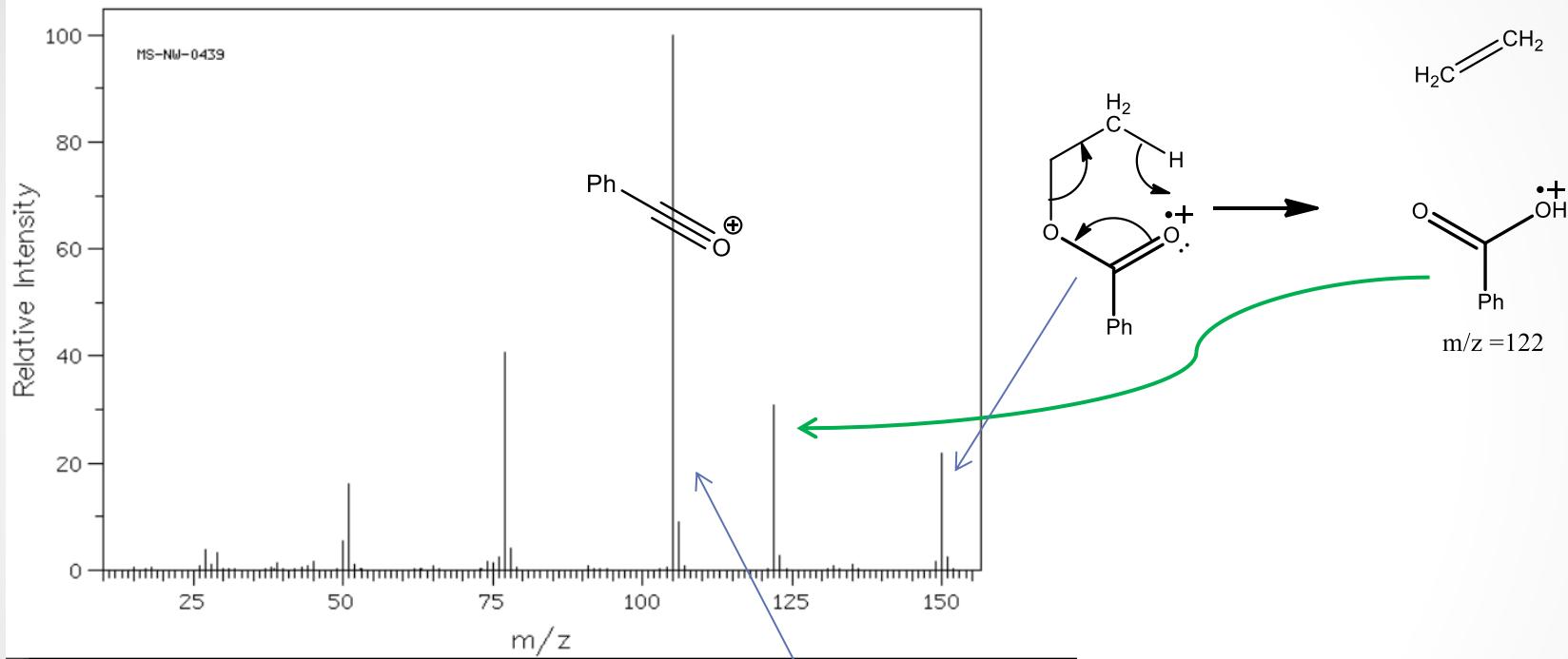
Methyl butyrate



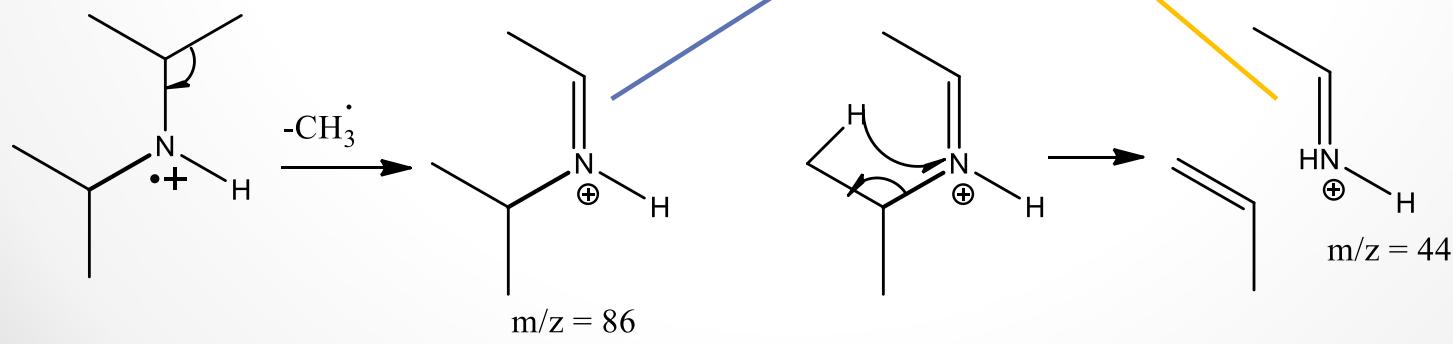
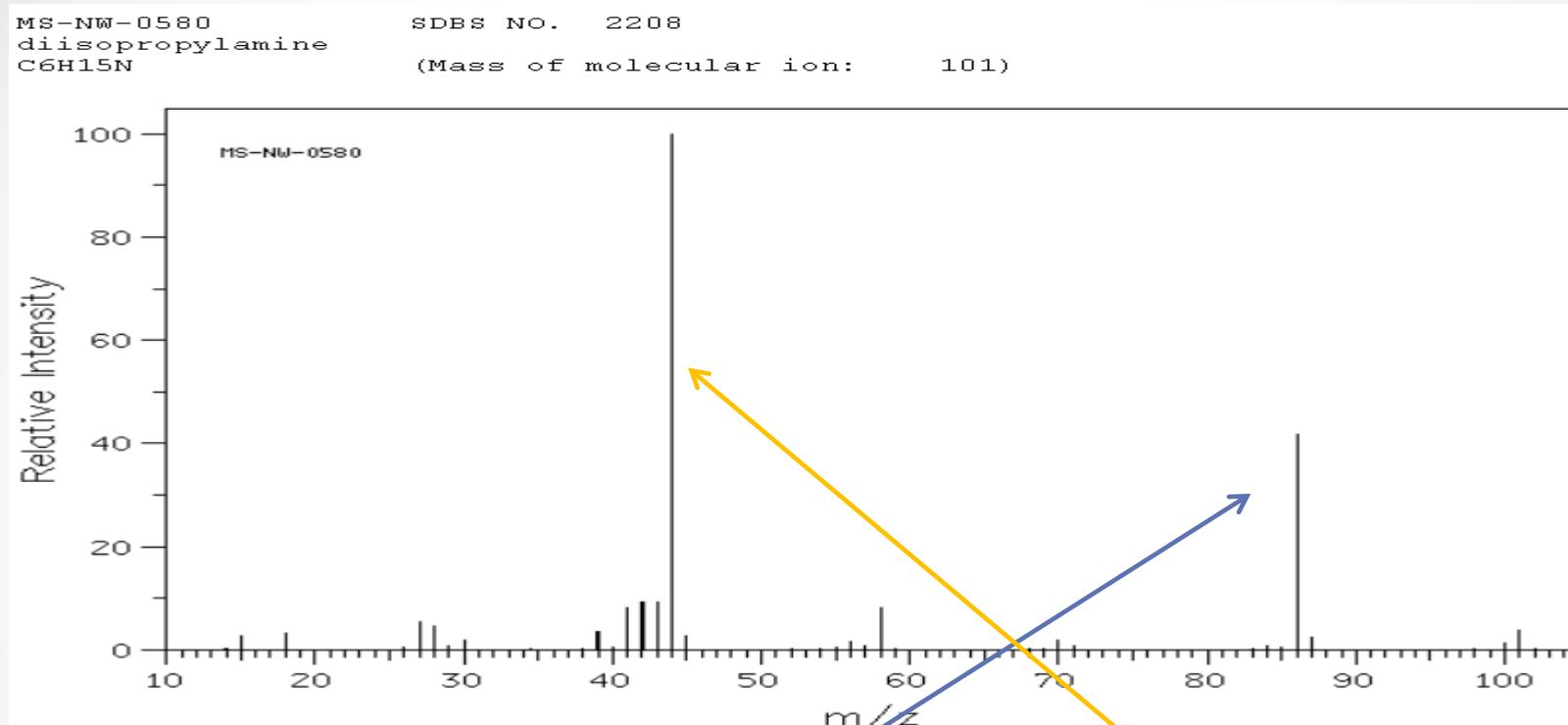
# Carboxylic esters - aromatic and aliphatic with short chain

MS-NW-0439  
ethyl benzoate  
C9H10O2

SDBS NO. 1460  
(Mass of molecular ion: 150)



# Amines

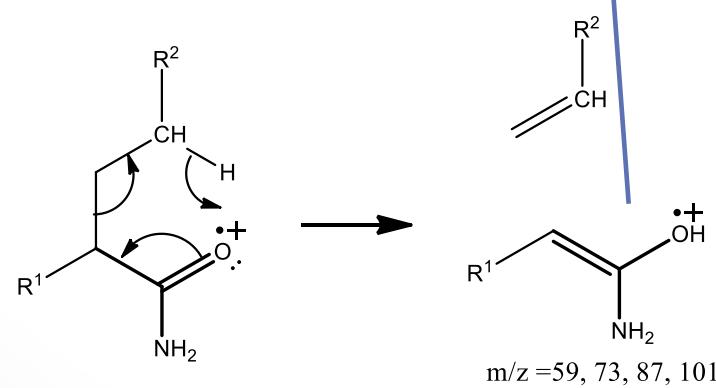
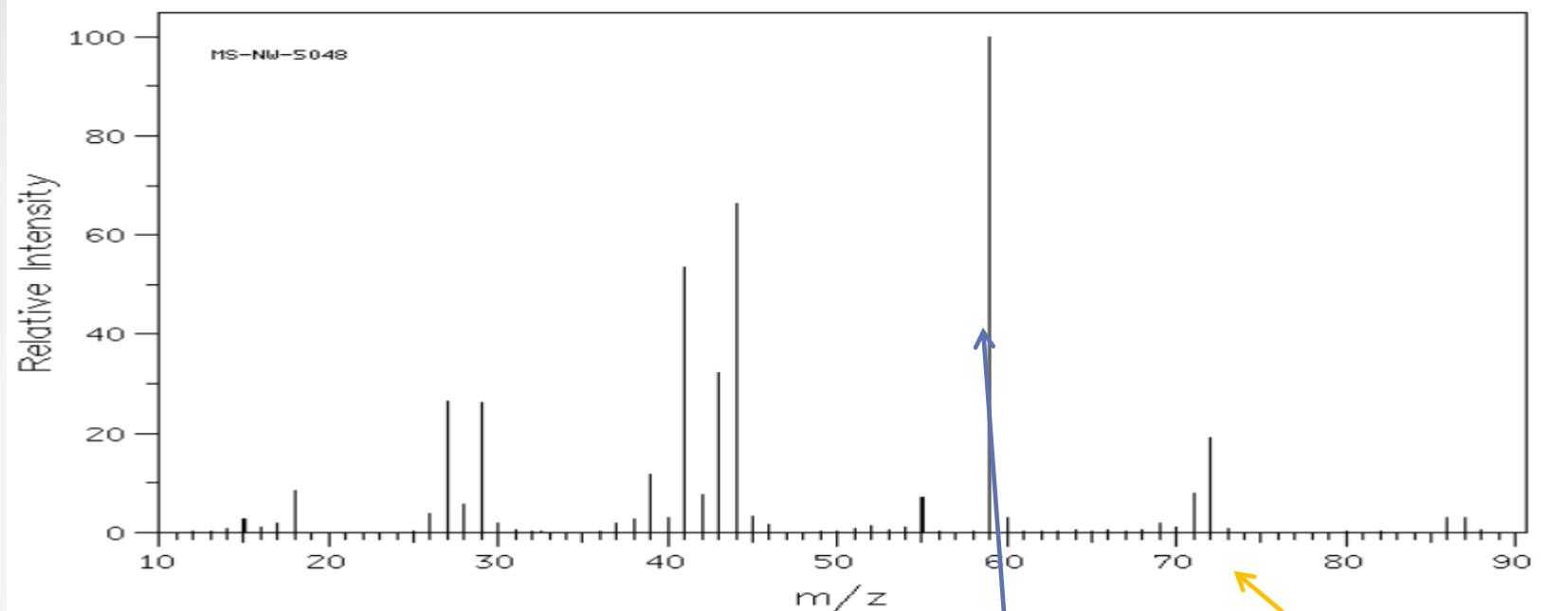


•  $\alpha$  - Cleavage

# Amides

MS-NW-5048  
butyramide  
C<sub>4</sub>H<sub>9</sub>NO

SDBS NO. 3782  
(Mass of molecular ion: 87)

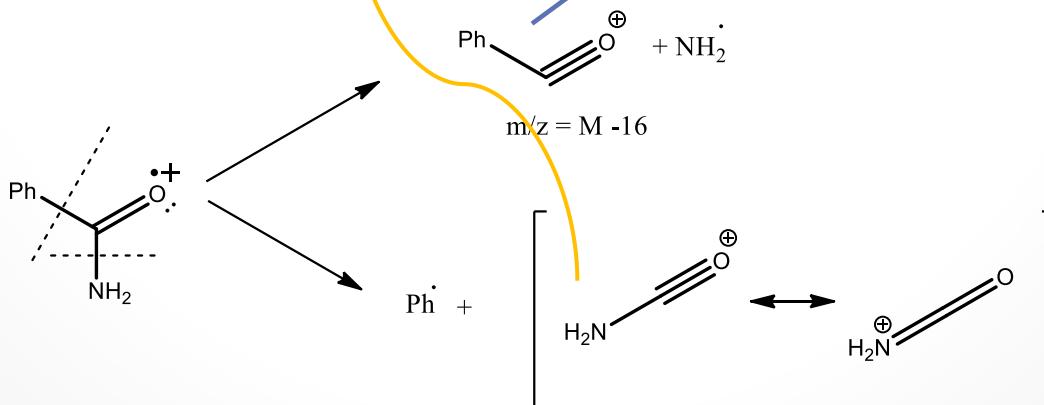
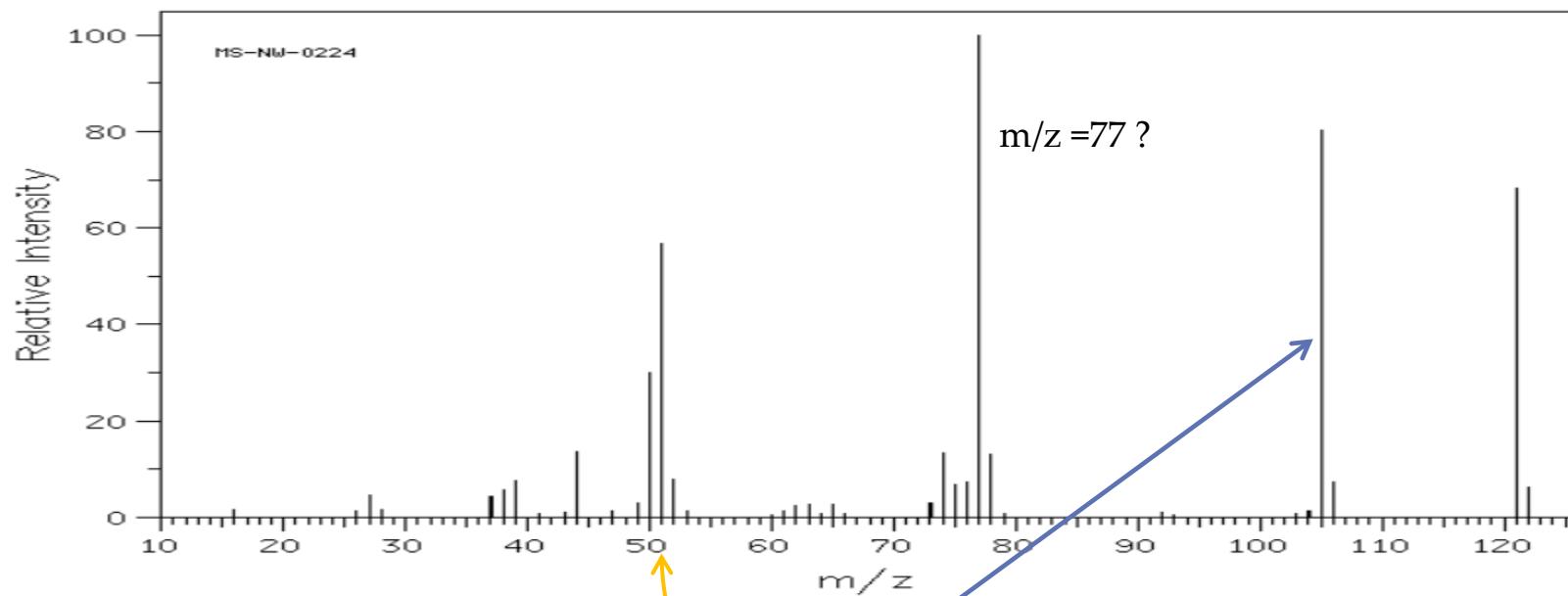


McLafferty rearrangement

# Amides – aromatic and with short chain

MS-NW-0224  
benzamide  
C<sub>7</sub>H<sub>7</sub>NO

SDBS NO. 1689  
(Mass of molecular ion: 121)



$\alpha$  – Cleavage

# Determination of molecular formula

Relative Isotope Abundances of Common Elements.

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



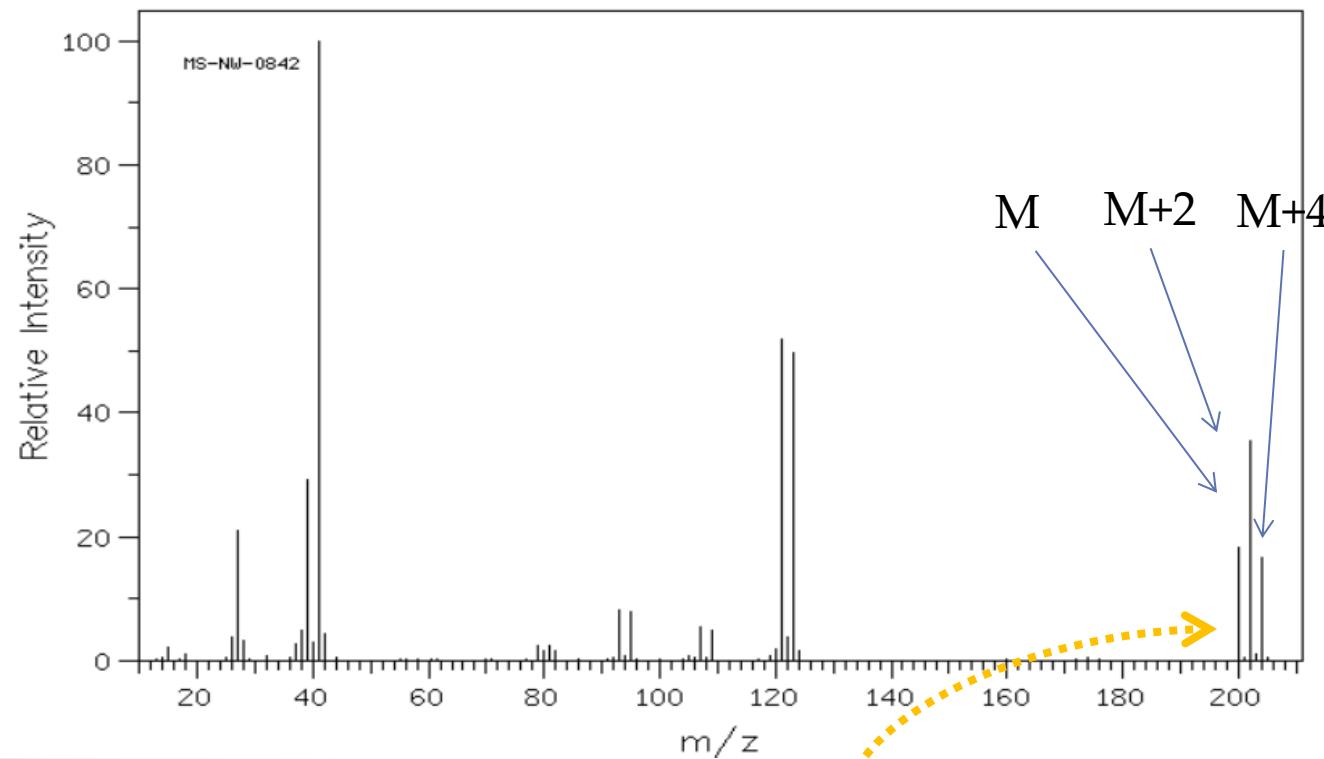
$\text{m/z} = 94$



$\text{m/z} = 96$

Two molecular ions M and M+2

# Determination of molecular formula



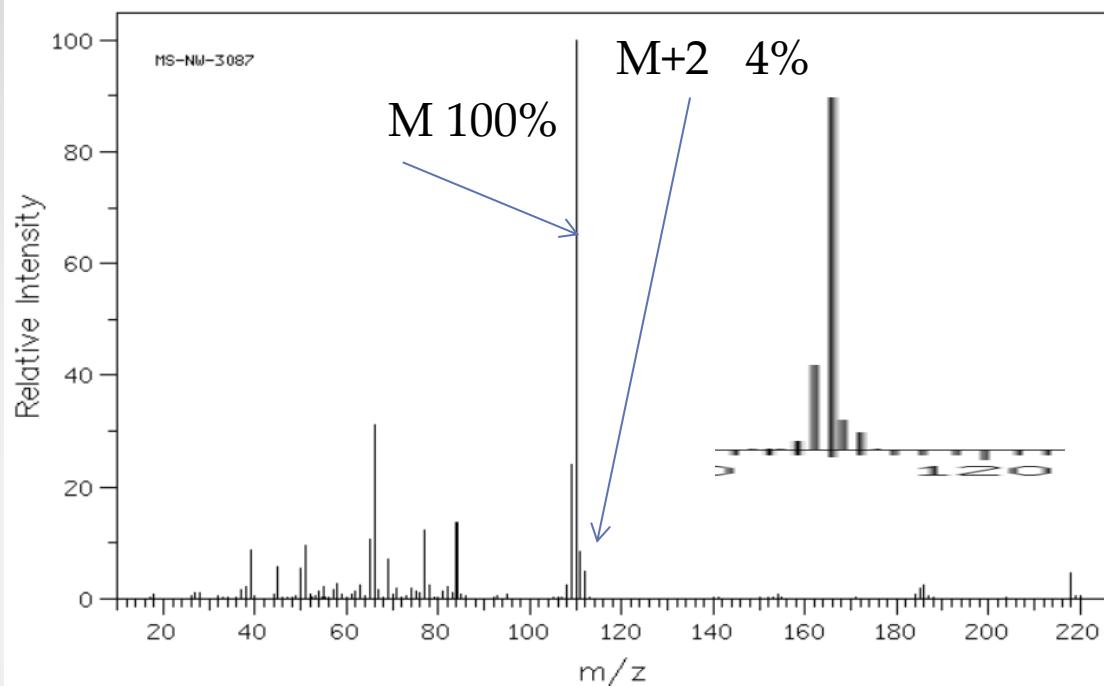
Intensities of Isotope Peaks (Relative to the Molecular Ion) for Combination of Chlorine and Bromine.

Halogen Present	% M+2	% M+4	% M+6	% M+8	% M+10	% M+12
Cl	32.6					
Cl <sub>2</sub>	65.3	10.6				
Cl <sub>3</sub>	97.8	31.9	3.5			
Cl <sub>4</sub>	131.0	63.9	14.0	1.2		
Cl <sub>5</sub>	163.0	106.0	34.7	5.7	0.4	
Cl <sub>6</sub>	196.0	161.0	69.4	17.0	2.2	0.1
Br	97.9					
Br <sub>2</sub>	195.0	95.5				
Br <sub>3</sub>	293.0	286.0	93.4			
BrCl	130.0	31.9				
BrCl <sub>2</sub>	163.0	74.4	10.4			
Br <sub>2</sub> Cl	228.0	159.0	31.2			

If intensity of peaks- M: M+2: M+4 are as  $\approx 1:2:1$   
Molecule contain two bromine atoms

# Determination of molecular formula

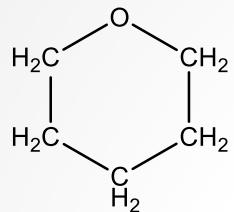
MS-NW-3087      SDBS NO. 558  
 benzenethiol  
 C<sub>6</sub>H<sub>6</sub>S      (Mass of molecular ion: 110)



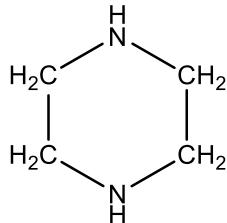
If intensity of peaks, M: M+2: are as  $\approx$  100:4  
 Molecule contain one sulfur atom

Elements	Isotope	Relative Abundance	Isotope	Relative Abundance	Isotope	Relative Abundance
Fluorine	<sup>19</sup> F	100				
Silicon	<sup>28</sup> Si	100	<sup>29</sup> Si	5.1	<sup>30</sup> Si	3.35
Phosphorus	<sup>31</sup> P	100				
Sulfur	<sup>32</sup> S	100	<sup>33</sup> S	0.78	<sup>34</sup> S	4.4

# High resolution Molecular ion



Chemical Formula: C<sub>5</sub>H<sub>10</sub>O  
Exact Mass: 86.07  
Molecular Weight: 86.13



Chemical Formula: C<sub>4</sub>H<sub>10</sub>N<sub>2</sub>  
Exact Mass: 86.08  
Molecular Weight: 86.14

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

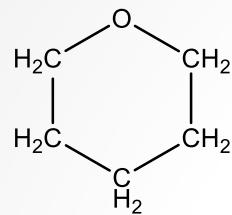
Exact mass: mass of molecule calculated with exact masses of most abundant isotopes.

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

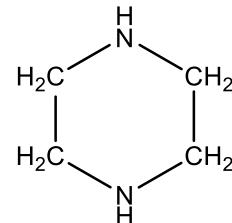


Exact Mass: 157.84  
Molecular Weight:  
159.81

# High resolution Molecular ion



Chemical Formula: C<sub>5</sub>H<sub>10</sub>O  
Exact Mass: 86.07  
Molecular Weight: 86.13



Chemical Formula: C<sub>4</sub>H<sub>10</sub>N<sub>2</sub>  
Exact Mass: 86.08  
Molecular Weight: 86.14

High resolution exact mass:  
for C<sub>5</sub>H<sub>10</sub>O is 86.073165  
for C<sub>4</sub>H<sub>10</sub>N<sub>2</sub> is 86.084398

Exact Masses of Isotopes.

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

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

- This approach is usually used instead of elemental analysis.