Mass Spectrometry - Background

- In mass spectrometry, a substance is bombarded with an electron beam having sufficient energy to fragment the molecule. The positive fragments which are produced (cations and radical cations) are accelerated in a vacuum through a magnetic field and are sorted on the basis of mass-to-charge ratio. Since the bulk of the ions produced in the mass spectrometer carry a unit positive charge, the value m/e is equivalent to the molecular weight of the fragment.

- The analysis of mass spectroscopy information involves the re-assembling of fragments, working backwards to generate the original molecule. A schematic representation of a mass spectrometer is shown below:
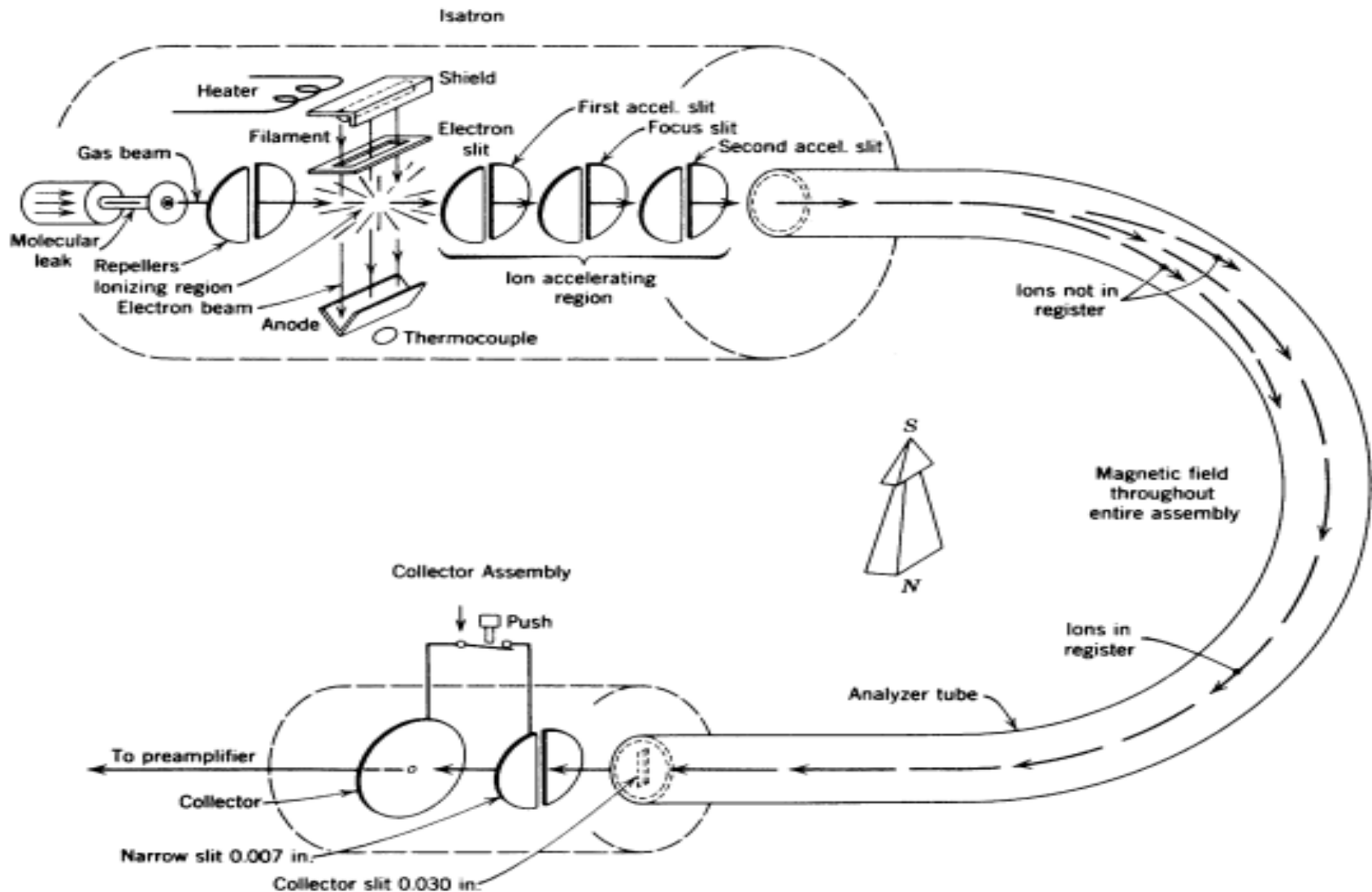
FIGURE 2.2. Schematic diagram of a single-focusing, 180° sector mass analyzer. The magnetic field is perpendicular to the page. The radius of curvature varies from one instrument to another.

• A very low concentration of sample molecules is allowed to leak into the ionization chamber (which is under a very high vacuum) where they are bombarded by a high-energy electron beam.
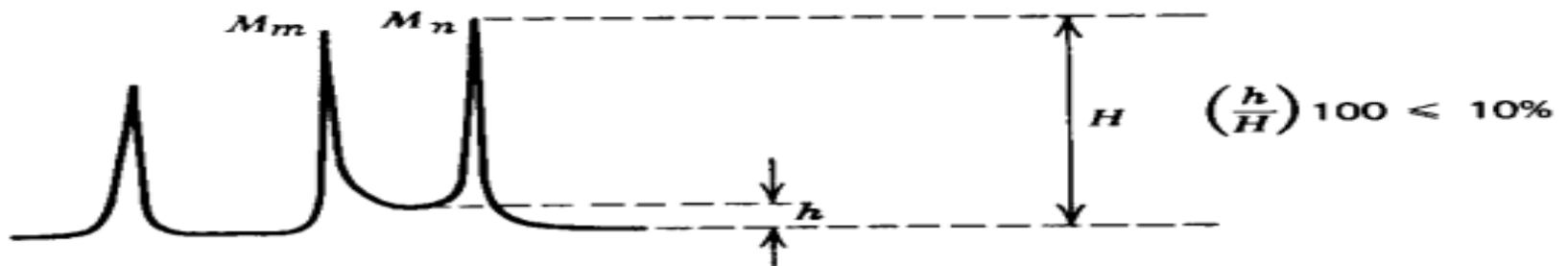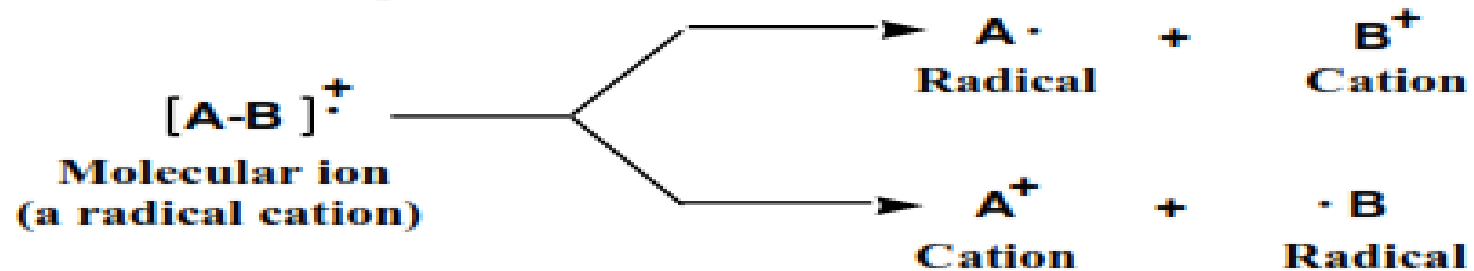
The path of the charged molecules is bent by an applied magnetic field. Ions having low mass (low momentum) will be deflected most by this field and will collide with the walls of the analyzer.

• Likewise, high momentum ions will not be deflected enough and will also collide with the analyzer wall.

Ions having the proper mass-to-charge ratio, however, will follow the path of the analyzer, exit through the slit and collide with the Collector.

This generates an electric current, which is then amplified and detected. By varying the strength of the magnetic field, the mass-to-charge ratio which is analyzed can be continuously varied.

- **Fragmentation of a molecular ion, M, produces a radical and a cation. Only the cation is detected by MS**

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

Molecular ion
(a radical cation)

$$A\cdot \quad + \quad B^+$$
Radical   Cation

$$A^+ \quad + \quad \cdot B$$
Cation   Radical

M_m M_n

$$\left(\frac{h}{H}\right) 100 < 10\%$$

To determine the resolution of an instrument, consider two adjacent peaks of approximately equal intensity. These peaks should be chosen so that the height of the valley between the peaks is less than 10% of the intensity of the peaks. The resolution (R) is

$$R = \frac{M_n}{M_n - M_m}$$

where M_n is the higher mass number of the two adjacent peaks, and M_m is the lower mass number.

There are two important categories of magnetic-deflection mass spectrometers: low (unit) resolution and high resolution. Low-resolution instruments can be defined arbitrarily as the instruments that separate unit masses up to m/z 2000 $[R = 2000/(2000 - 1999) = 2000]$. A high-resolution instrument with $R = 20,000$ can distinguish between $C_{16}H_{26}O_2$ and $C_{15}H_{24}NO_2$:

$$R = \frac{250.1933}{250.1933 - 250.1807} \simeq 20,000$$

This important class of mass spectrometers can measure the mass of an ion with sufficient accuracy to determine its atomic composition.

Mass spectrometers for structure elucidation can be classified according to the method of separating the charged particles:

A. Magnetic Field Deflection
   1. Magnetic Field Only (Unit Resolution)
   2. Double Focusing (Electrostatic Field and Magnetic Field, High Resolution)

B. Quadrupole Mass Spectrometry
   1. Quadrupole Mass Filter
   2. Quadrupole Ion Storage (Ion Trap)

C. Time of Flight

D. FT-ICR (Ion Cyclotron Resonance)

E. MS/MS (Tandem Mass Spectrometry)

The mass spectrum:

- Mass spectra (EI) are routinely obtained at electron beam energy of 70 eV. The simplest event that occurs is the removal of a single electron from the molecule in the gas phase by an electron of the electron beam to form the molecular ion, which is a radicalcation ($M^{·+}$)

The energy of the electrons is ~ 1600 kcal (or 70 eV).

-Since it takes ~100 kcal of energy to cleave a typical s bond, 1600 kcal is an enormous amount of energy to come into contact with a molecule.

- The output of the mass spectrometer shows a plot of relative intensity vs the mass-to-charge ratio (m/e).
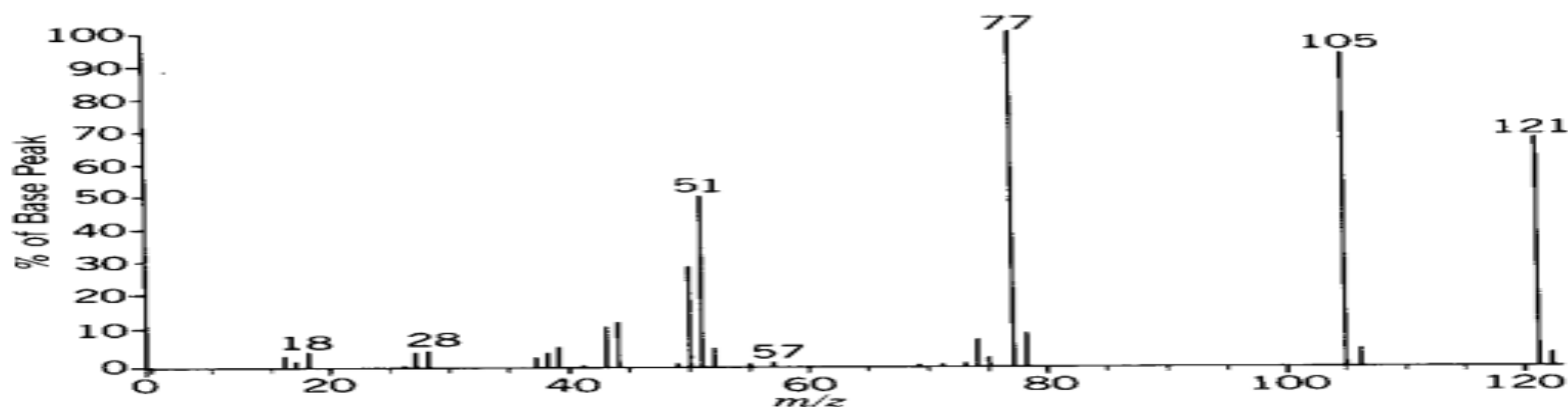

Figure 2.1. Computer-generated, electron-impact (EI) mass spectrum of benzamide $\left(C_6H_5 - \overset{\overset{\displaystyle O}{\|}}{C} - NH_2 \right)$ in bar graph form.

- ➤ The most intense peak in the spectrum is termed the base peak and all others are reported relative to its intensity.
- ➤ Electron is first removed from site with lowest ionization potential
- ➤ non-bonding electrons > pi bond electrons > sigma bond electrons   NB > π > σ
- ➤ Only CHARGED species are detected
- ➤ Determination of molecular formula:

The unit mass of the molecular ion of C_7H_7NO (Fig. 2.1) is m/z 121 _that is, the sum of the unit masses of the most abundant isotopes:

$$7 \times {}^{12}C = 84$$
$$7 \times {}^{1}H = 7$$
$$1 \times {}^{14}N = 14$$
$$1 \times {}^{16}O = 16$$

In addition, molecular species exist that contain the less abundant isotopes, and these give use to the "isotope peaks" at M + 1, M + 2, etc. In Figure 2.1, the M + 1 peak is approximately 8% of the intensity of the molecular ion peak, which for this purpose, is assigned

an intensity of 100%. Contributing to the M + 1 peak are the isotopes, ^{13}C, ^{2}H, ^{15}N, and ^{17}O. Table 2.1 gives the abundances of these isotopes relative to those of the most abundant isotopes. The only contributor to the M + 2 peak of C_7H_7NO is ^{18}O, whose relative abundance is very low; thus the M + 2 peak is undetected.

Table 2.1 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	0.20
Fluorine	^{19}F	100				
Silicon	^{28}Si	100	^{29}Si	5.10	^{30}Si	3.35
Phosphorus	^{31}P	100				
Sulfur	^{32}S	100	^{33}S	0.78	^{34}S	4.40
Chlorine	^{35}Cl	100			^{37}Cl	32.5
Bromine	^{79}Br	100			^{81}Br	98.0
Iodine	^{127}I	100				

High-Resolution Molecular Ion

A unique molecular formula (or fragment formula) can often be derived from a sufficiently accurate mass measurement alone (high-resolution mass spectrometry). This is possible because the nuclide masses are not integers (see Table 2.2). For example, we can distinguish at a unit mass of 28 among CO, N_2, CH_2N, and C_2H_4.

^{12}C 12.0000	$^{14}N_2$ 28.0062	^{12}C 12.0000	$^{12}C_2$ 24.0000
^{16}O 15.9949		$^1H_2$ 2.0156	$^1H_4$ 4.0312
27.9949		^{14}N 14.0031	28.0312
		28.0187	

Thus, the mass observed for the molecular ion of CO, for example, is the sum of the exact formula masses of the most abundant isotope of carbon and of oxygen. This differs from a molecular weight of CO based on atomic weights that are the average of weights of all natural isotopes of an element (e.g., C = 12.01, O = 15.999).

Table 2.2 gives the masses to four or five decimal places for the common nuclides; it also gives the familiar atomic weights (average weights for the elements).

Table 2.2 Exact Masses of Isotopes

Element	Atomic Weight	Nuclide	Mass
Hydrogen	1.00794	^1H	1.00783
		$D(^2H)$	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	32.066	^{32}S	31.9721
		^{33}S	32.9715
		^{34}S	33.9679
Chlorine	35.4527	^{35}Cl	34.9689
		^{37}Cl	36.9659
Bromine	79.9094	^{79}Br	78.9183
		^{81}Br	80.9163
Iodine	126.9045	^{127}I	126.9045

Recognition of the Molecular Ion Peak:

- The process of fragmentation follows simple and predictable chemical pathways and the ions which are formed will reflect the most stable cations and radical cations which that molecule can form.

molecular ion (M+): is the highest molecular weight peak observed in a spectrum will typically represent the parent molecule, minus an electron,

Problems in recognition of the molecular ion peak:

- 1.   Generally, small peaks are also observed above the calculated molecular weight due to the natural isotopic abundance of ^{13}C, ^{2}H, etc.

- 2.   under electron impact (EI) recognition of the molecular ion peak (M) poses a problem:
- ❖ a/ the peak may be very weak or may not appear at all
- ❖ b/ we cannot be sure that is the molecular ion peak and not a fragment peak or an impurity.
- The best solution is to obtain a chemical ionization spectrum. The usual result is an intense peak at M + 1 and little fragmentation.

- 3.   Many molecules with especially labile protons do not display molecular ions; an example of this is alcohols, where the highest molecular weight peak occurs at m/e one less than the molecular ion (m - 1).

Nitrogen rule:

a molecule of even-numbered molecular weight must contain either no nitrogen or an even number of nitrogen atoms; an odd-numbered molecular weight requires an odd number of nitrogen atoms.

The intensity of the molecular ion peak depends on the stability of the molecular ion. The most stable molecular ions are those of purely aromatic systems. If substituents that have favorable modes of cleavage are present, the molecular ion peak will be less intense, and the fragment peaks relatively more intense.

In general:

the following group of compounds will, in order of decreasing ability, give prominent molecular ion peaks:

aromatic compounds > conjugated alkenes > cyclic compounds > organic sulfides > short, normal alkanes

Recognizable molecular ions are usually

produced for these compounds in order of decreasing ability: ketones > amines > esters > ethers > carboxylic acids ~ aldehydes ~ amides ~ halides. The molecular ion is frequently not detectable in aliphatic alcohols, nitrites, nitrates, nitro compounds, nitriles, and in highly branched compounds.

Fragments can be identified by their mass-to-charge ratio, but it is often more informative to identify them by the mass which has been lost. That is:

The presence of an M − 15 peak (loss of CH_3), or an M − 18 peak (loss of H_2O), or an M − 31 peak (loss of OCH_3 from methyl esters), and so on, is taken as confirmation of a molecular ion peak. An M − 1 peak is common, and occasionally an M − 2 peak (loss of H_2 by either fragmentation or thermolysis), or even a rare M − 3 peak (from alcohols) is reasonable. Peaks in the

- Peaks in the range of M_ 3 to M_14, however, indicate that:

- 1. Contaminants may be present.

- 2. The presumed molecular ion peak is actual...

Losses of fragments of masses 19 _ 25 are also unlikely (except for loss of F = 19 or HF = 20 from fluorinated compounds). Loss of 16 (O), 17 (OH), or 18 (H_2O) are likely only if an oxygen atom is in the molecule.

the next step is *chemical ionization* (CI), which usually yields a prominent $[M + H]^+$ peak with little fragmentation.

These CI $[M + H]^+$ ions *(quasimolecular ions)* are often prominent. Chemical ionization spectra sometimes have prominent $[M - H]^+$ ions because of hydride ion abstraction from the $M^{\cdot+}$ ion by CH_5^+. Since the $[M + H]^+$ ions are chemically produced, they do not have the great excess of energy associated with ionization by electron impact, and they undergo less fragmentation. For example, the EI spectrum of 3,4-dimethox-

Index of Hydrogen deficiency

The index of hydrogen deficiency is the number of *pairs* of hydrogen atoms that must be removed from the corresponding "saturated" formula to produce the molecular formula of the compound of interest.

index is thus the sum of the number of rings, the number of double bonds, and twice the number of triple bonds.

For the generalized molecular formula $\alpha_I \beta_{II} \gamma_{III} \delta_{IV}$, the index $= IV - \frac{1}{2}I + \frac{1}{2}III + 1$, where

α is H, D, or halogen (i.e., any monovalent atom)

β is O, S, or any other bivalent atom

γ is N, P, or any other trivalent atom

δ is C, Si, or any other tetravalent atom

The numerals I–IV designate the numbers of the mono-, di-, tri-, and tetravalent atoms, respectively.

the compound C_7H_7NO

The rule of thirteen

The "Rule of Thirteen" can be used to identify possible molecular formulas for an unknown hydrocarbon, C_nH_m.

Step 1 : n = M^+ / 13 [integer only, use remainder (R) in step2]

Step 2 : m = n + remainder from step 1

Example: The formula for a hydrocarbon with M^+ =106 can be found :

   Step 1: n = 106 / 13 = 8 (r = 0.**153** * 13 = 2)

   Step 2: m = 8 + 2 = 10

   Formula: C_8H_{10}

106 / 13 = 8.**153**

- If a heteroatom is present,
   - Subtract the mass of each heteroatom from the MW
   - Calculate the formula for the corresponding hydrocarbon
   - Add the heteroatoms to the formula.

Example: A compound with a molecular ion peak at m/z=102 has a strong peak at 1739 cm^{-1} in its IR spectrum. Determine its molecular formula.

Or It Contain 2 heteroatom of O
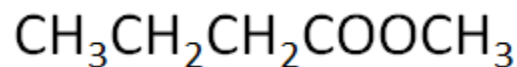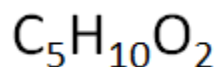
Step 1: Due to the compound has a strong peak at 1739 cm-1 in its IR spectrum therefore it contain ester group (COO-), so it Contain 2 heteroatom O. (2 * 16 = 32)

102-32 =70 MW of corresponding hydrocarbon (C & H).

Step 2: 70/13 = 5.384   n = 5   ➡   (R= 0.384 X 13 = 5)

Step 3: m = n + remainder from step 1 ➡ m = 5+5 =10

Step 4: Chemical formula = CnHm + heteroatom

$$C_5H_{10}O_2 \qquad CH_3CH_2CH_2COOCH_3$$

Isotopes

- Mass spectrometers are capable of separating and detecting individual ions even those that only differ by a single atomic mass unit.

- As a result molecules containing different isotopes can be distinguished.

- 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.

- molecular ion peak is diff. from a M+2 peak by 2   halogen.

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

- "M+1" peaks are seen due the presence of ^{13}C in the sample.
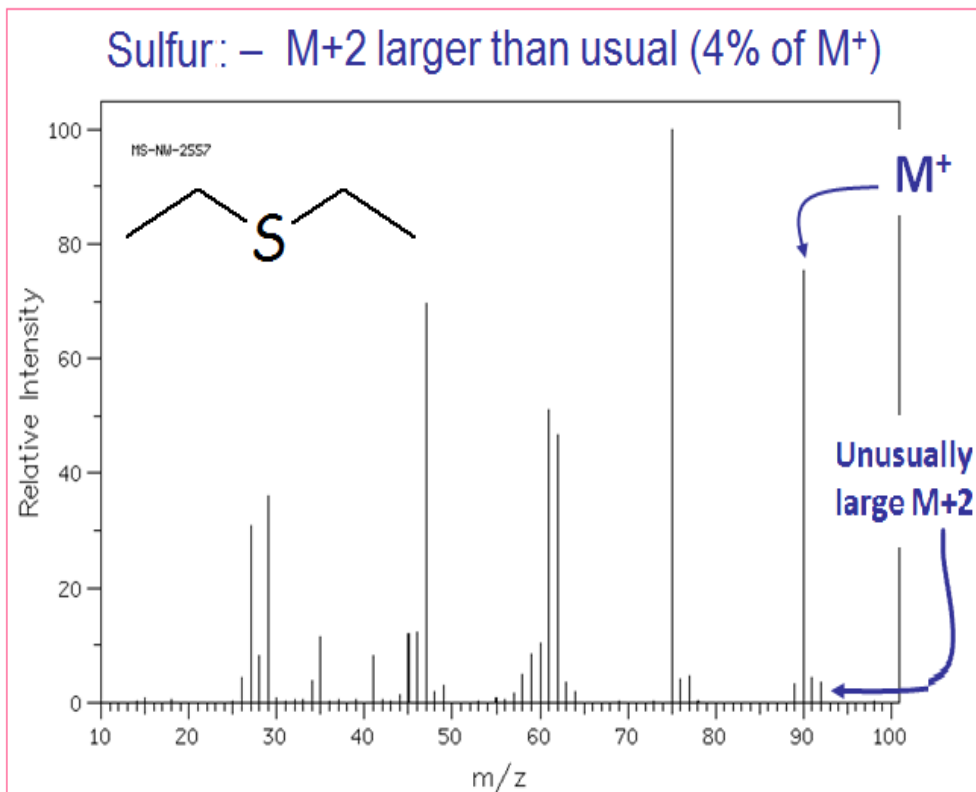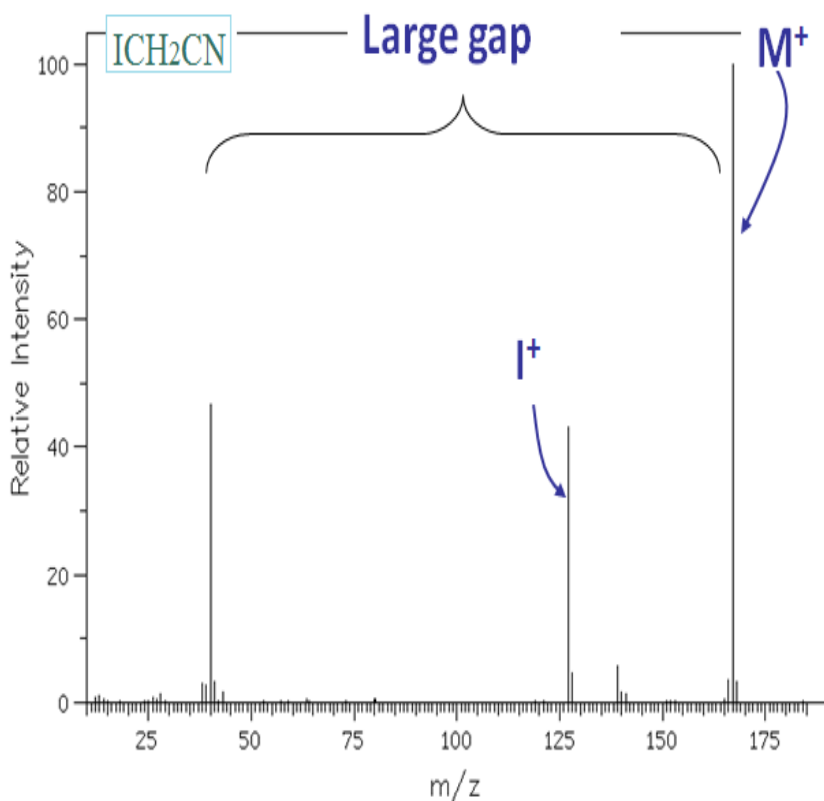
For Easily Recognized Elements in MS

Nitrogen: Nitrogen Rule

Halides

Bromine : $M^+ \sim M+2$ (50.5% ^{79}Br/49.5% ^{81}Br)

Chlorine : - M+2 is $\sim 1/3$ as large as M^+

Iodine : * I^+ at 127   * Large gap

ICH2CN   Large gap M^+

I^+

Sulfur: – M+2 larger than usual (4% of M^+)

S

M^+

Unusually large M+2

MS-NW-2557

Q. / A compound gives a mass spectrum with peaks at $m/z = 77$ (40%), 112 (100%), 114 (33%), and essentially no other peaks. Identify the compound.

First, your molecular ion peak is 112 and you have a M+2 peak at 114. Therefore, you have a halogen.

- Now, your molecular ion peak and M+2 peak are in a 3 to 1 ratio. This means chlorine.

- So, 112 - 35 = 77 (C & H)

- C's   77/12 = 6 carbons

- 6 x 12 = 72

- 77 – 72 = 5

- C_6H_5Cl. DOUS (2(6)+2-5-1)/2 = 4