Applied Spectroscopy

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References

1: Introduction to spectroscopy; 3rd , 4th and 5th edition, Donald L. Pavia
2: Elementary Organic Spectroscopy; Y.R. Sharma
3: Spectroscopic Identification of Organic Compounds
   7th edition, Robert M. Silverstein
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Mass Spectrometry

There are a number of different instrumental techniques (IR, NMR, UV, and Mass) for identifying organic compounds. These techniques can be performed quickly on small amounts of a compound and can provide much more information about the compound's structure than simple chemical tests can provide.

Mass spectrometry is the only one that does not involve electromagnetic radiation. Thus, it is called spectrometry, whereas the others are called spectroscopy.

Mass spectrometry is a powerful technique , that allows us to determine the **molecular mass** and the **molecular formula** of a compound, as well as certain **structural features** of the compound.

The molecular weight of a compound can be determined by its **vapor density** or its **freezing-point** depression, and molecular formulas were determined by **elemental analysis**. These were long and tedious techniques that required relatively large amounts of a very pure sample , while a rapid determination and use of a very small amount of a sample can be performed by mass spectrometry .

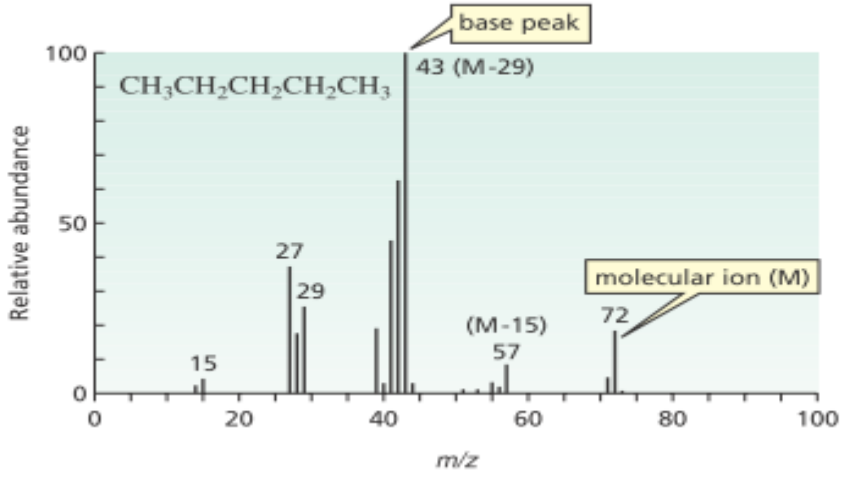
Principle of operation

In mass spectrometry, a small sample of a compound is introduced into an instrument called a mass spectrometer, where it is **vaporized** and then **ionized** by bombarding a molecule with high energy electron beam (70 eV) which knocks out an electron, producing a molecular ion, which is a radical cation $(M^{\bullet}_{+})$.

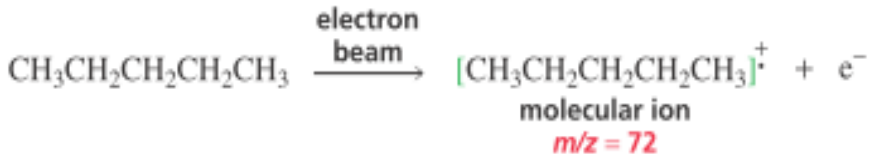
The symbol (+.): indicates that the molecule has lost an electron, it has unpaired electron and is positively charged. The molecular ion in turn produces a series of fragment ions.

Therefore, many of the molecular ions break apart into cations, radicals, neutral molecules, and other radical cations. The bonds most likely to break are the weakest ones and those that result in the formation of the most stable products. All the positively charged fragments of the molecule accelerated toward collector and to the detector .Whereas, neutral fragments are not accelerated, they are eventually pumped out of the spectrometer. The mass spectrometer records a mass spectrum a graph of the relative abundance of each fragment plotted against its (m/z**)** value. Because the charge (z) on essentially all the fragments that reach the collector plate is (+1), (m/z) is the molecular mass (m) of the fragment. Remember that only positively charged species reach the collector.

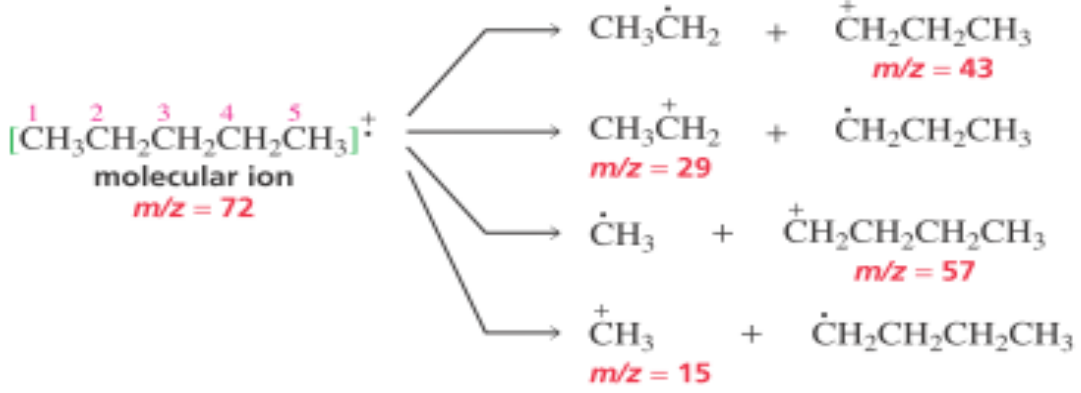
For example: The mass spectrum of pentane, shown as a bar graph and in tabular form. The **base peak** (relative intensity of 100%) represents the fragment that appears in greatest abundance. The (m/z) value of the molecular ion gives the molecular mass of the compound.



m/z	Relative abundance
73	0.52
72	18.56
71	4.32
57	11.20
43	100.00
42	55.27
41	37.93
39	12.44
29	26.65
28	17.75
27	31.22
15	4.22
14	2.56



The way a molecular ion fragments depends on the strength of its bonds and the stability of the fragments.



Instrumentation:

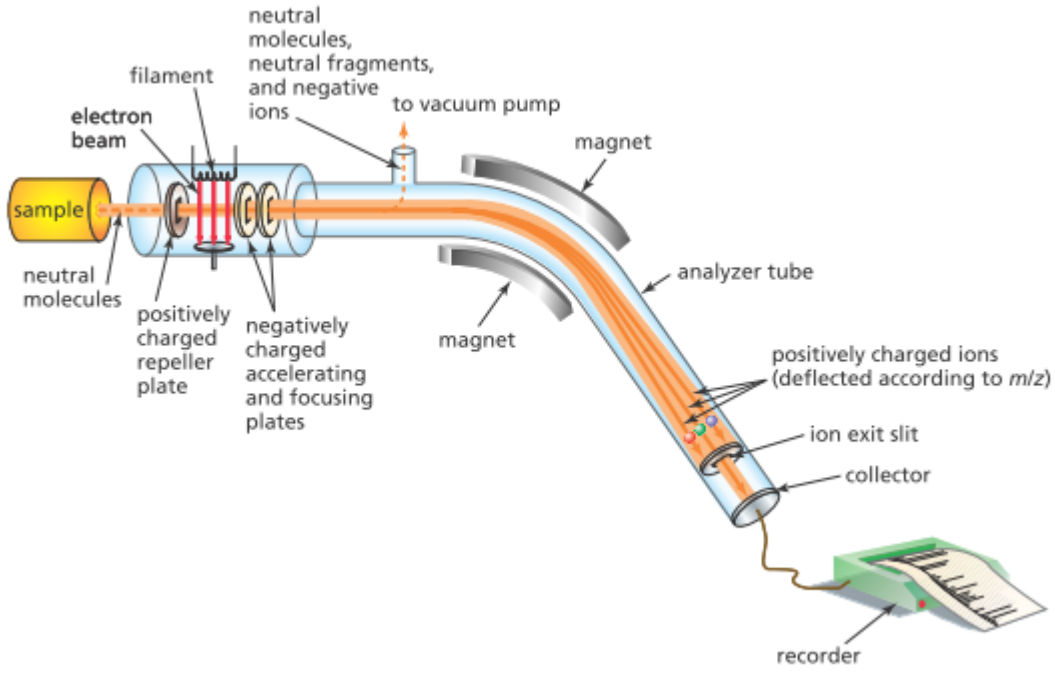
There are different types of mass spectrometer; each has different advantages, drawbacks and applications. All consist of five major sections linked together (figure below):

Inlet – Ionization source – Analyzer – Detector- Computer system.

All sections usually maintained under high vacuum.

The first component of the mass spectrometer is the sample **inlet**, which brings the sample from the laboratory environment (1atm) to the lower pressure (few millimeters) of the mass spectrometer.

The sample inlet leads to the ion source where the sample molecules are transformed into gas phase ions. The ions are then accelerated by an electromagnetic field, toward the mass analyzer, which separate the sample ions depending on their mass-to-charge (m/z) ratio. The ions then are counted by the detector, and the signal is recorded and processed by the data system. The output from the data system is the mass spectrum a graph of the number of ions detected as a function of their (m/z) ratio.



A sample studied by mass spectrometry may be a **gas**, a **liquid**, or a **solid**. Enough of the sample must be converted to the vapor state to obtain the stream of molecules that must flow into the ionization chamber. With gases, of course, the substance is already vaporized, so a simple inlet system can be used.

The same system can be used for volatile liquids or solids. For less-volatile materials, the system can be designed to fit within an oven, which can heat the sample to increase the vapor pressure of the sample. Care must be taken not to heat any sample to a temperature at which it might decompose.

With nonvolatile samples, other sample inlet systems must be used. A common one is the direct probe method. The sample is placed on a thin wire loop or pin on the tip of the probe, which is then inserted through a vacuum lock into the ionization chamber. The sample probe is positioned close to the ion source. The probe can be heated, thus causing vapor from the sample to be evolved in proximity to the ionizing beam of electrons.
The most versatile sample inlet systems are constructed by connecting a chromatograph to the mass spectrometer such as:
Gas chromatography–mass spectrometry (GC-MS) and high-performance liquid chromatography–mass spectrometry (HPLC-MS, or more simply LC-MS).

Isotope ratio data

A method of determining molecular formulas is to examine the relative intensities of the peaks due to the molecular ion and related ions that bear one or more heavy isotopes (the molecular ion cluster), determination of M+1, M+2, and M+4……depending on the relative abundances (table-1). The advantages of this method is that, does not require the much more expensive high-resolution instrument. This method is useless, of course, when the molecular ion peak is very weak or does not appear.
Table-1:

NATURAL ABUNDANCES OF COMMON ELEMENTS AND THEIR ISOTOPES

Element	Relative Abundance					
Hydrogen	^{1}H	100	^{2}H	0.016		
Carbon	^{12}C	100	^{13}C	1.08		
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				

The relative intensities of M + 1 and M + 2 peaks can be estimated quickly using simplified calculations. The formula to calculate the M + 1 peak intensity (relative to $M^+ = 100$) for a given formula is found in Equation (1) . Similarly, the intensity of an M + 2 peak intensity (relative to $M^+ = 100$) may be found by using equation (2):

$$[M+1] = (\text{number of C} \times 1.1) + (\text{number of H} \times 0.015) + (\text{number of N} \times 0.37) + (\text{number of O} \times 0.04) + (\text{number of S} \times 0.8) + (\text{number of Si} \times 5.1) \quad \text{.....1}$$

$$[M+2] = \frac{(\text{number of C} \times 1.1)^2}{200} + (\text{number of O} \times 0.2) + (\text{number of S} \times 4.4) + (\text{number of Si} \times 3.4) \quad \ldots\ldots\ldots 2$$

Q: Calculate the M+1 and M+2 of propene and diazimethane, CO, N_2 and ethene?

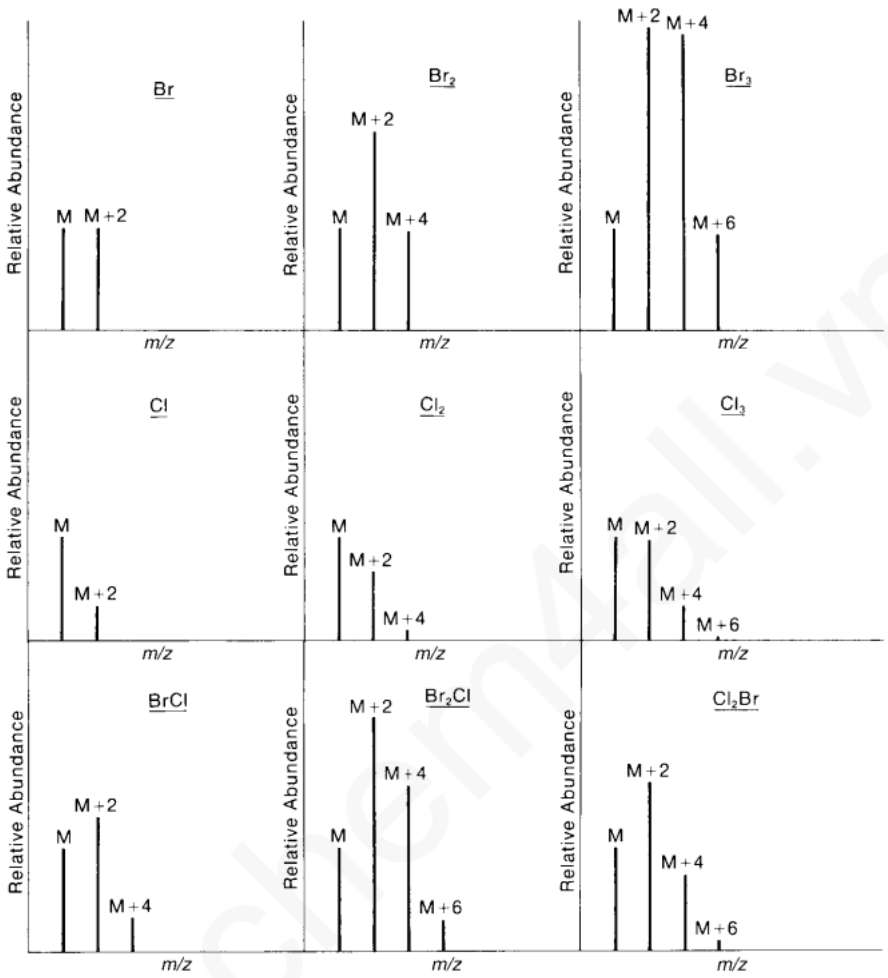
Problem: Calculate the M+1 and M+2 of C_7H_{16} ? Mw= 100.
M+1% = 1.1x 7 + 16x 0.015 = 7.7
M+2%= $(M+1)^2/200 = 7.7^2/200 = 0.3$
Thus: base peak 100 100%
M+1 101 7.7%
M+2 102 0.3%

Q/ Mw=18, M+1=0 and M+2=0.2 deduce the structure?
Q/ Calculate the M+1 and M+2 of C_3H_6O?

When chlorine or bromine is present, the M + 2 peaks become very significant. The heavy isotope of each of these elements is two mass units heavier than the lighter isotope. The natural abundance of ^{37}Cl is 32.5% that of ^{35}Cl, and the natural abundance of ^{81}Br is 98.0% that of ^{79} Br. When either of these elements is present, the M + 2peak become quite intense. If a compound contains two chlorine or bromine atoms a distinct M+2 and M+4 should be observed. Table-2:

	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

Various combinations of bromine and chlorine



Q/ Calculate the M+1 and M+2 of MeBr?

Q/ Deduce the MF of the compound that has: Mw=138 = 24.4%, 139= 1.3%,

140=15.8%, 142=2.6%

Q/ Deduce the MF of the compound that has Mw=158 =100% , 159=8.264%,

160=33% Answer: C_7H_9NOCl.

$(a+b)^2 = a^2 + 2ab + b^2$ $(3+1)^2 = 9 + 6 + 1$

$(a+b)^3 = a^3 + 3a^2b + 3b^2a + b^3$ $(3+1)^3 = 27 + (3 \times 3^2 \times 1) + (3 \times 1^2 \times 3) + 1^3$

$27 + 27 + 9 + 1$

$(1+1)^3 = 1 + 2 + 1.$

Determination of molecular weight and molecular formula

A: Precise Mass Determination

The most important application of high-resolution mass spectrometers is the determination of very precise molecular weights of substances. We are accustomed to thinking of atoms as having integral atomic masses for example, H= 1, C = 12, and O = 16. However, if we determine atomic masses with sufficient precision, we find that this is not true. In l923, Aston discovered that every isotopic mass is characterized by a small "mass defect." The mass of each atom actually differs from a whole mass number by an amount known as the nuclear packing fraction. Table below gives the actual masses of some atoms.

PRECISE MASSES OF SOME COMMON ELEMENTS

Element	Atomic Weight	Nuclide	Mass
Hydrogen	1.00797	^{1}H	1.00783
		^{2}H	2.01410
Carbon	12.01115	^{12}C	12.0000
		^{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	28.086	^{28}Si	27.9769
		^{29}Si	28.9765
		^{30}Si	29.9738
Phosphorus	30.974	^{31}P	30.9738
Sulfur	32.064	^{32}S	31.9721
		^{33}S	32.9715
		^{34}S	33.9679
Chlorine	35.453	^{35}Cl	34.9689
		^{37}Cl	36.9659
Bromine	79.909	^{79}Br	78.9183
		^{81}Br	80.9163
Iodine	126.904	^{127}I	126.9045

Depending on the atoms contained in a molecule, it is possible for particles of the same nominal mass to have slightly different measured masses when precise determinations are

possible. To illustrate, a molecule with a molecular weight of 60.1 g/mole could be C_3H_8O, $C_2H_8N_2$, $C_2H_4O_2$, or CH_4N_2O (Table below). Thus, a low-resolution mass spectrum (LRMS) will not be able to distinguish between these formulas.

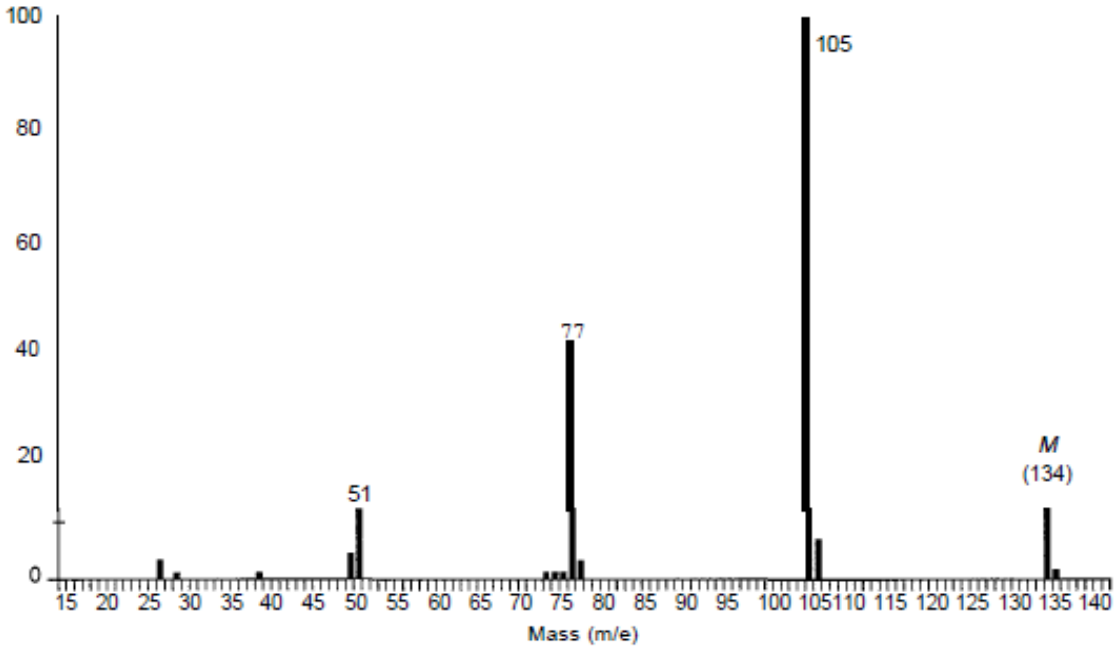
SELECTED COMPARISONS OF MOLECULAR WEIGHTS AND PRECISE MASSES

Molecular Formula (MF)	Molecular Weight (MW) (g/mole)	Precise 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

Q: Depending on table of (precise masses) how could you distinguish between CO, N_2, CH_2N, and C_2H_4 with M.F of 28?

Problem/ An unknown compound has the mass spectrum shown. The infrared spectrum of the unknown shows significant peaks at.There is also a band from aliphatic C-H stretching from 2879 to 2979 cm-1

3102 cm^{-1}	3087	3062	3030	1688
1598	1583	1460	1449	1353
1221	952	746	691	



General principles that govern fragmentation processes

In mass spectrometry, the molecule absorbs a considerable amount of extra energy during bombardment by high-energy electron beams. The excited molecular ion may be unstable, it may lose some of its extra energy by breaking apart into fragments. If the lifetime of the molecular ion is greater than 10^{-5} sec, a peak corresponding to the molecular ion will appear in the mass spectrum. However, molecular ions with lifetimes less than 10^{-5} sec break apart into fragments before they are accelerated within the ionization chamber and enter the mass analyzer. As a result, in a typical mass spectrum one observes peaks corresponding to both the molecular ion and the fragment ions.

Thus, the relative abundances of fragment ions depend on:

Stability of the (M+) carbocation's, electronegativity, polarizability, resonance delocalization, the octet rule, and stability of neutral fragments that does not appear in the mass spectrum, but its existence can be deduced by noting the difference in masses of the fragment ion and the original molecular ion. Again, processes that lead to the formation of a more stable neutral fragment are favored over those that lead to less-stable neutral fragments. Thus, stability of ions and its formation increases in the following order:

$$\underset{\text{DIFFICULT}}{H_3C^+ < RCH_2^+ < R_2CH^+ < R_3^+ < H_2C=CHCH_2^+ \sim HC\equiv CCH_2^+ < C_6H_5CH_2^+}\ \underset{\text{EASY}}{}$$

Important features of the parent molecular ion (M+.):

1: The **(M+.)** peak in aromatic compounds is relatively much intense due to the presence π- electron system.

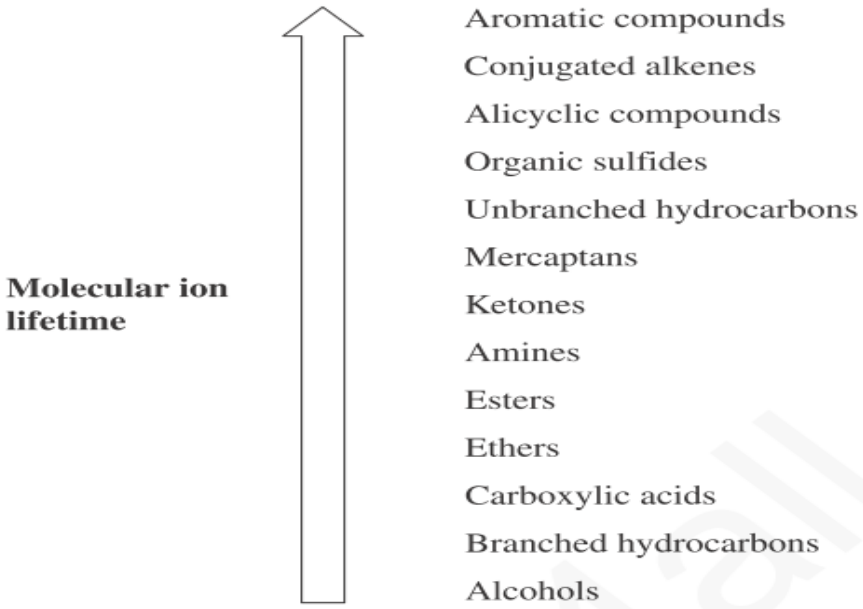
2: Conjugated alkenes show intense **(M+.)** peak than non-conjugated alkenes.

3: Unsaturated compounds give more intense **(M+.)** peak than saturated.

4: The relative abundance of the saturated hydrocarbon is more than the corresponding branched chain.

5: The substituent groups like (OH, NH_2, OR etc.) which lower the ionization potential increase the relative abundance of **(M+.)** in case of aromatic compounds , while the groups like (NO_2 , CN etc.) decrease the relative abundance of the aromatic compounds.

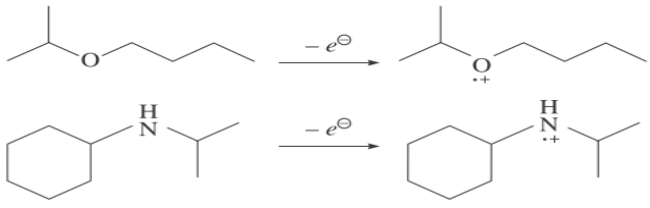
6: Absence of **(M+.)** means that the compound is highly branched or tertiary alcohols.



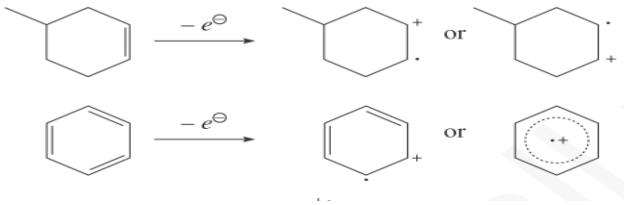
General Fragmentation Modes

There are different **fragmentation modes**, **bond cleavage** and **initial ionization** processes depending on the molecular structure:

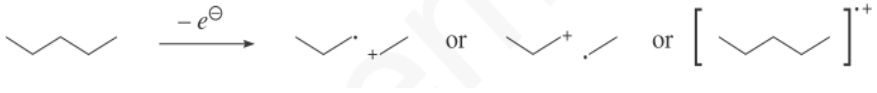
The electrons most likely to be ejected during the ionization event are the ones that are in the highest potential energy molecular orbitals, that is, the electrons held least tightly by the molecule. Thus, it is easier to remove an electron from a nonbonding orbital (n) than it is to strip an electron from a (π orbital). Similarly, it is much easier to eject an electron from a (π orbital) in comparison to σ orbital. Some examples of loss of an electron are shown below:



(n) Remove an electron from a nonbonding



(π) Remove from π electron

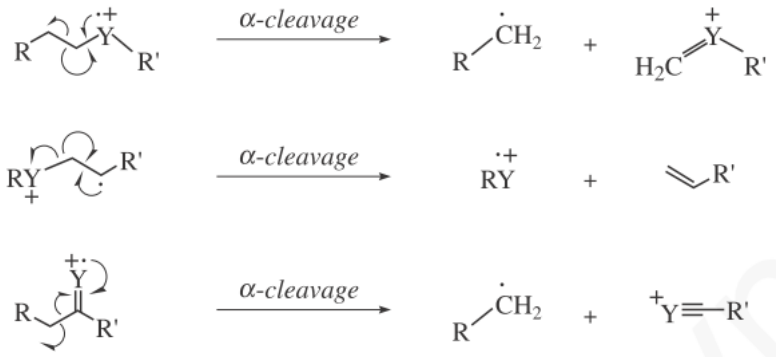


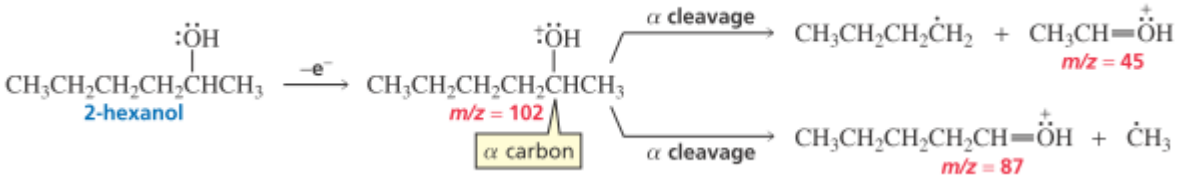
σ Remove from σ electron.

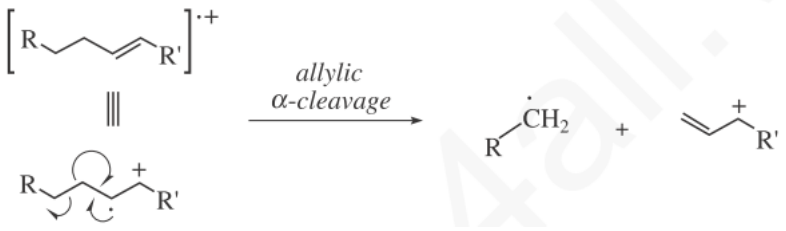
Bond cleavage: homolytic and heterolytic fission

A: Radical-site-initiated cleavage (α–cleavage) (Homolytic): is one of the most common one-bond cleavages and is more commonly called an (α-cleavage). The term α -cleavage is confusing to some because the bond that is broken is not directly attached to the radical site but is rather the bond to the next neighboring atom (the α -position).

α Cleavages may occur at saturated or unsaturated sites that may or may not involve a heteroatom.

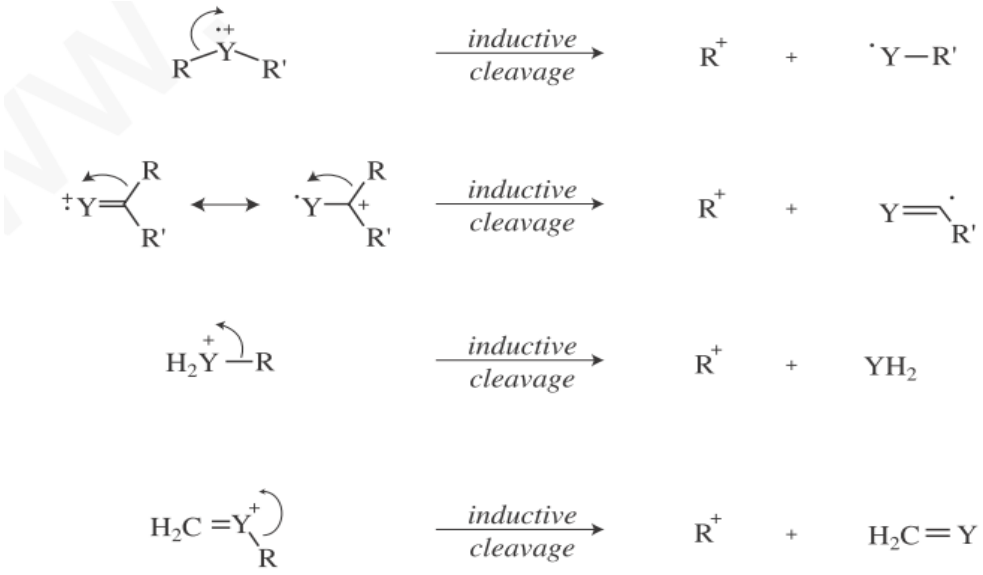






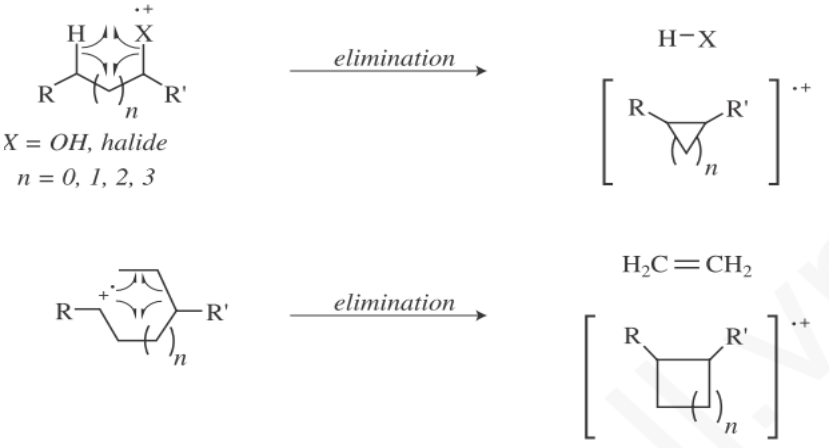
B: Charge-Site-Initiated Cleavage: Inductive Cleavage (heterolytic)

Another common one-bond cleavage is charge-site-initiated or inductive cleavage, often indicated in a fragmentation mechanism by the symbol (i). Inductive cleavage involves the attraction of an electron pair by an electronegative heteroatom that ends up as a radical or as a closed-shell neutral:

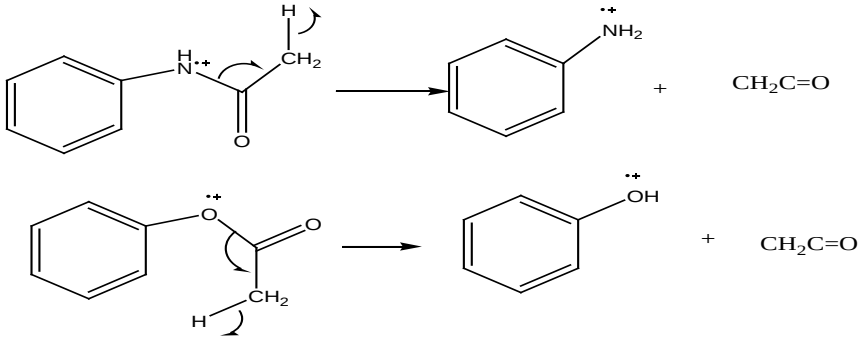


C: Two-Bond Cleavage

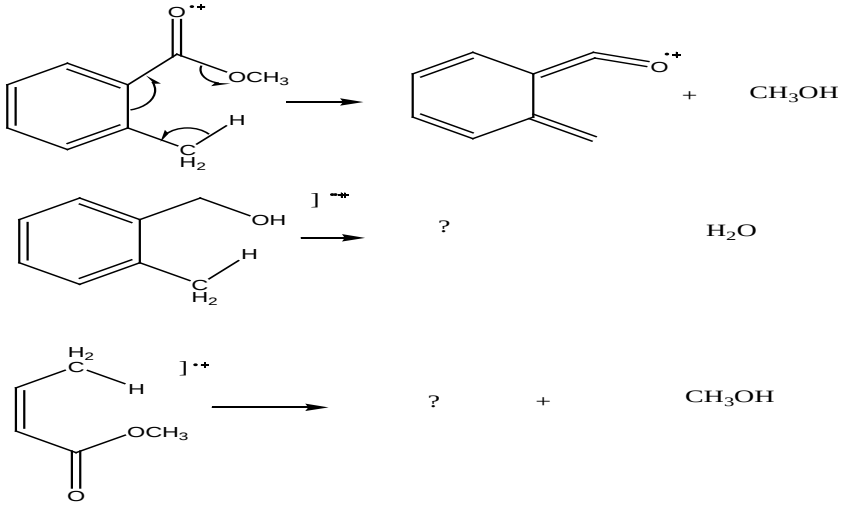
In this process, an elimination occurs, usually a stable small molecule of some type: H_2O, a hydrogen halide, or an alkene. Some examples of two-bond cleavages of this type are shown below:



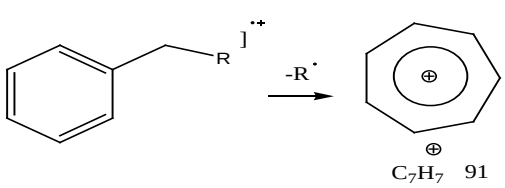
The elimination of ketene is a characteristic fragmentation mode of n-alkyl amide and o-acetates of phenols.

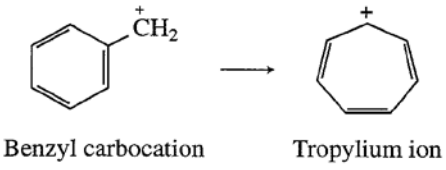


In ortho substituted aromatic compounds or in cis- olefines the substituent and a hydrogen atom can come close proximity so as to eliminate a neutral molecule:



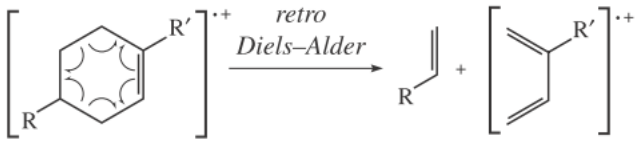
D: Benzylic cleavage: is an energetically preferred fragmentation mode. It involves the cleavage of a C-C bond which is beta to the aromatic ring (β-cleavage), belongs to the stabilization of tropylium ion (m/e=





E: Retro Diels–Alder Cleavage

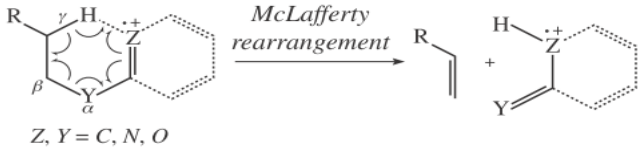
This is characteristic for cyclic alkenes, the ring can undergo a retro Diels–Alder fragmentation, and it involves the cleavage of two bonds of the cyclic system to produce the radical cation of a diene and a neutral alkene.



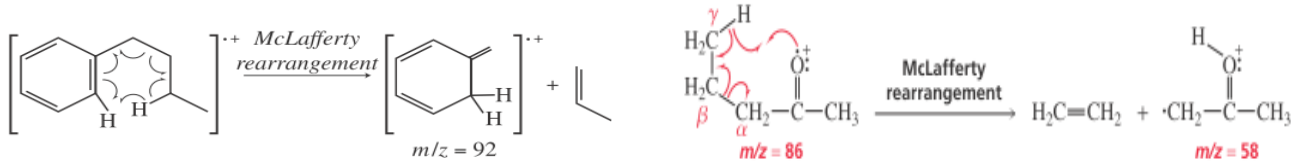
A retro Diels–Alder fragmentation.

F: McLafferty Rearrangement:

In the McLafferty rearrangement, a hydrogen atom on γ- carbon atom away from the radical cation of an alkene, arene, carbonyl, or imine (a so-called γ -hydrogen) is transferred to the charge site via a six-membered transition state, with concurrent cleavage of the sigma bond between the α and β positions. This forms a new radical cation and an alkene with a π- bond between what were the original β and γ carbons.



The McLafferty rearrangement.



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In addition to these processes, fragmentation processes involving rearrangements, migrations of groups, and secondary fragmentations of fragment ions are also possible. These modes of fragmentation occur less often than the important cases that already described.

Metastable ion peak:

Ions with lifetimes on the order of 10^{-6} sec are accelerated in the ionization chamber before they have an opportunity to disintegrate. These ions may disintegrate into fragments while they are passing into the analyzer region of the mass spectrometer. The fragment ions formed at that point have considerably lower energy than normal ions since the uncharged portion of the original ion carries away some of the kinetic energy that the ion received as it was accelerated. As a result, the fragment ion produced in the analyzer follows an abnormal flight path on its way to the detector.

This 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 an ion gives rise to what is termed a metastable ion peak in the mass spectrum. Metastable ion peaks are usually broad peaks, and they frequently appear at non integral values of m/z. The equation that relates the position of the metastable ion peak in the mass spectrum to the mass of the original ion is

$$m_1^+ \longrightarrow m_2^+ \quad , \quad m^* = \frac{(m_2)^2}{m_1}$$

Were m* is the apparent mass of the metastable ion in the mass spectrum, m_1 is the mass of the original ion from which the fragment formed, and m_2 is the mass of the new fragment ion. A metastable ion peak is useful in some applications since its presence definitively links two ions together. Metastable ion peaks can be used to prove a proposed fragmentation pattern or to aid in the solution of structure proof problems.

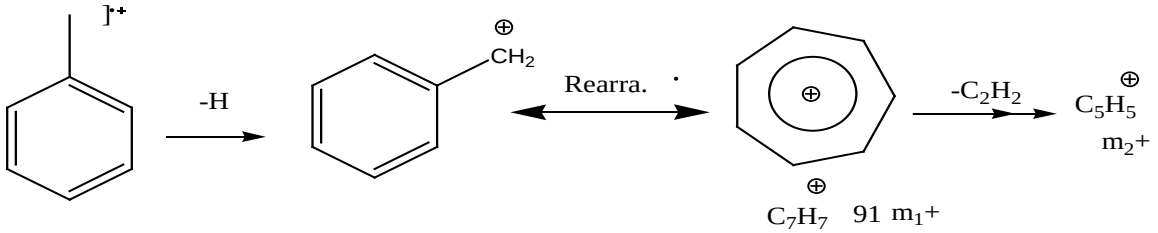
Notes:

It is important to remember that for a reaction $m_1^+ \rightarrow m_2^+$, m* has a distance below m_2 on the mass scale. The distance is approximately similar to the distance that m_2 lies

below m_1. The relative abundance of m* is often on the order of 10^{-2} or less compared the parent ion.

Example:

Consider the mass spectrum of toluene. Two strong peaks at m/e 91 and 65 are formed. The peak at 91 is due the tropylium cation (base peak) which loses a molecule of acetylene (26) to give $C_5H_5^+$ (m/e 65).

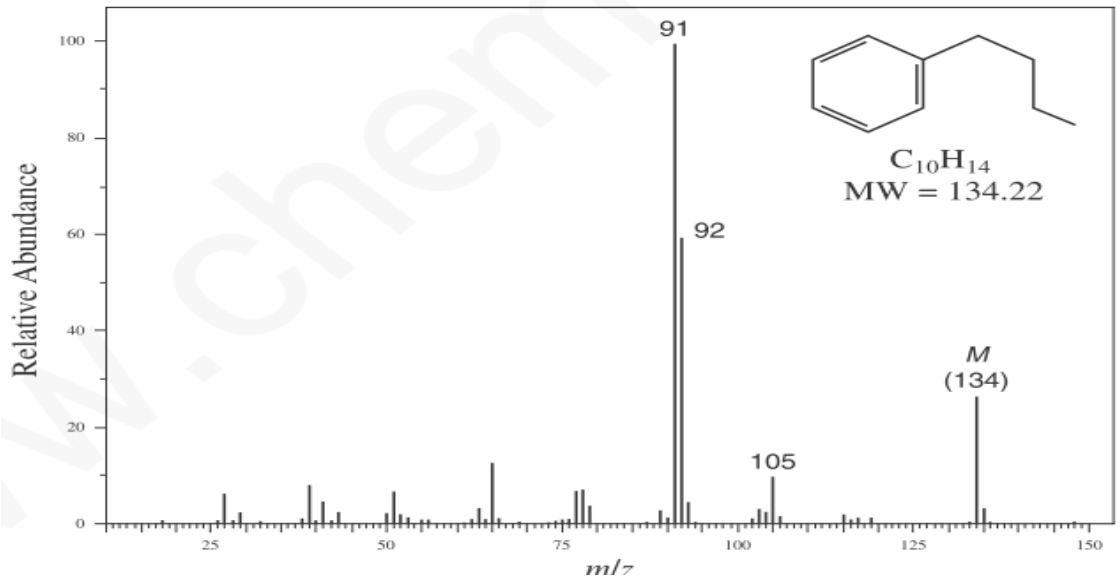


$$m^* = \frac{(m1)^2}{m1} = \frac{65 \times 65}{91} = 46.4$$

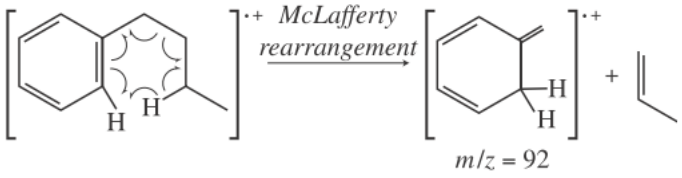
Q:

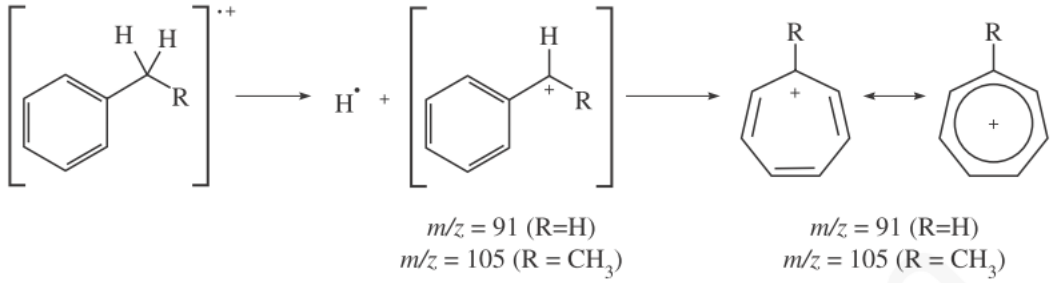
The mass spectra of two very stable cycloalkanes both show a molecular ion peak at $m/z = 98$. One spectrum shows a base peak at $m/z = 69$, the other shows a base peak at $m/z = 83$. Identify the cycloalkanes.

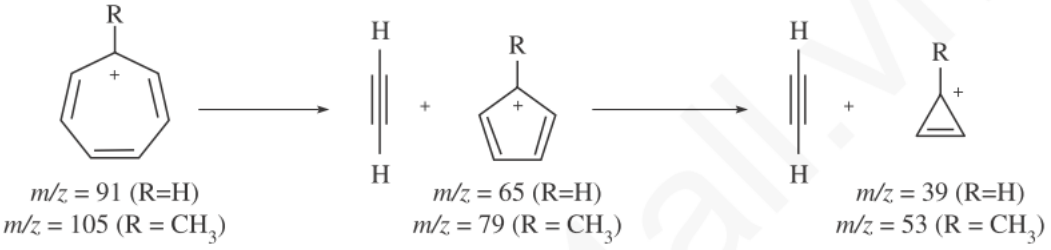
Example: (fragmentation pattern)



peak at $m/z = 92$ in their mass spectra from the McLafferty rearrangement. Using butylbenzene as an example, this rearrangement is depicted below.







IONIZATION METHODS

Regardless of the method of sample introduction, once the stream of sample molecules has entered the mass spectrometer, the sample molecules must be converted to charged particles by the ion source before they can be analyzed and detected.

There are different types of ionization sources depending on the sample properties:

1: Electron Ionization (EI) (the simplest and most common one).

2: Chemical Ionization (CI).

The sample molecules are combined with a stream of ionized reagent gas that is present in great excess relative to the sample.

3: Desorption Ionization Techniques (SIMS, FAB, and MALDI).

Both EI and CI methods require a relatively volatile (low molecular weight) sample. More recently developed ionization techniques allow the analysis of large, nonvolatile molecules by mass spectrometry. Three of these methods, secondary ion mass spectrometry (SIMS), fast atom bombardment (FAB), and matrix-assisted laser desorption ionization (MALDI) are all desorption ionization (DI) techniques.

4: Electrospray Ionization (ESI):

An even more useful technique for studying high molecular weight biomolecules and other labile or nonvolatile compounds is electrospray ionization (ESI) and its cousin thermo spray ionization (TSI). In ESI, a solution containing the sample molecules is sprayed out the end of a fine capillary into a heated chamber that is at nearly atmospheric pressure.

MASS ANALYSIS

Once the sample has been ionized, the beam of ions is accelerated by an electric field and then passes into the mass analyzer, Analyzer is the section of instrument that separates ions of different m/z, Just like there are many different ionization methods for different applications, there are also several types of mass analyzers.

1: The Magnetic Sector Mass Analyzer

2: Double-Focusing Mass Analyzers

3: Quadrupole Mass Analyzer

4: Time-of-Flight Mass Analyzer (TOF)