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Northern Border University
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Department of Chemistry

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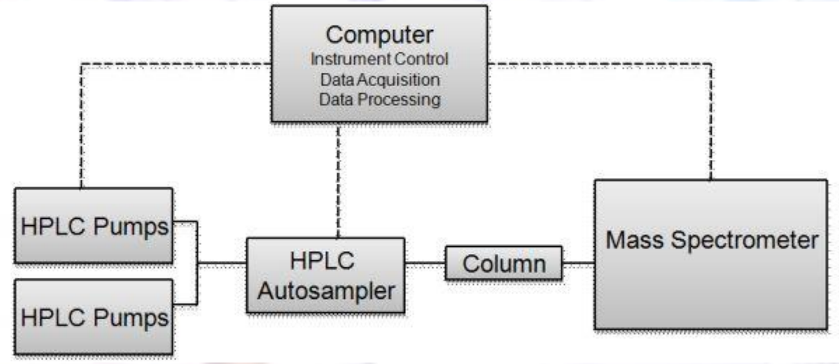
Application of Mass Spectrometry in Analytical Chemistry

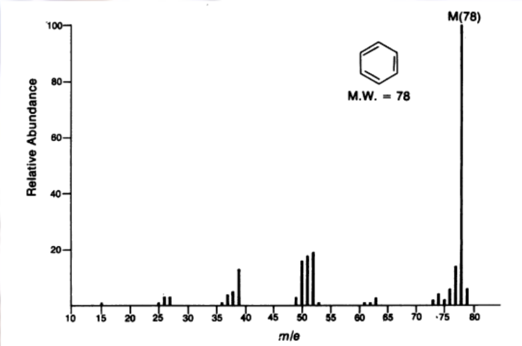
(Theory)

(1102-415)

(تطبيق طيف الكتلة في الكيمياء التحليلية)

(نظري)





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APPLICATIONS OF MASS SPECTROMETRY IN ANALYTICAL CHEMISTERY

1102-415

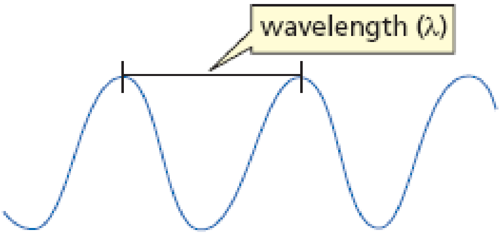
MASS SPECTRSCOPY

Spectroscopy and the Electromagnetic Spectrum

Spectroscopy is the study of the interaction of matter and *electromagnetic radiation*.

Electromagnetic radiation is radiant energy having the properties of both particles and waves. A continuum of different types of electromagnetic radiation—each type associated with a particular energy range—constitutes the electromagnetic spectrum. Visible light is the type of electromagnetic radiation with which we are most familiar, but it represents only a fraction of the range of the entire electromagnetic spectrum. X-rays and radio waves are other types of familiar electromagnetic radiation.

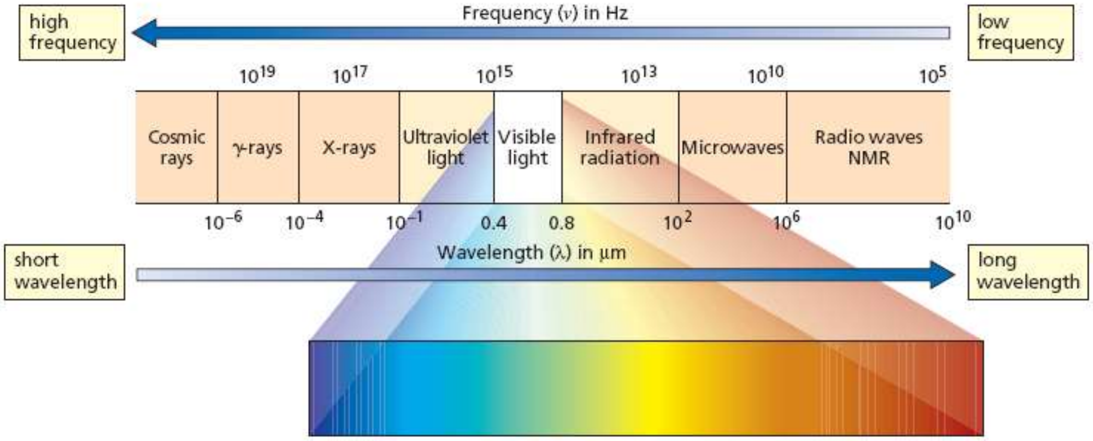
A particle of electromagnetic radiation is called a *photon*. We may think of electromagnetic radiation as photons traveling at the speed of light. Because electromagnetic radiation has both particle-like and wave-like properties, it can be characterized by either its frequency (υ) or its wavelength (λ). **Frequency** is defined as the number of wave crests that pass by a given point in one second. Frequency has units of hertz (Hz). **Wavelength** is the distance from any point on one wave to the corresponding point on the next wave. Wavelength is generally measured in micrometers or nanometers. One micrometer (μm) is 10^{-6} of a meter; one nanometer (nm) is of 10^{-9} a meter.



The frequency of electromagnetic radiation, therefore, is equal to the speed of light *c*) divided by the radiation’s wavelength: hort wavelenths have high frequencies, and long wavelengths have low frequencies.

$$\upsilon = c/\lambda \qquad\qquad c = 3 \text{ x } 10^{10} \text{ cm/s}$$

Short wavelenths have high frequencies, and long wavelengths have low frequencies.



The electromagnetic spectrum

The relationship between the energy (*E*) of a photon and the frequency (or the wavelength) of the electromagnetic radiation is described by the equation

$$E = h\nu = \frac{hc}{\lambda}$$

Mass spectroscopy

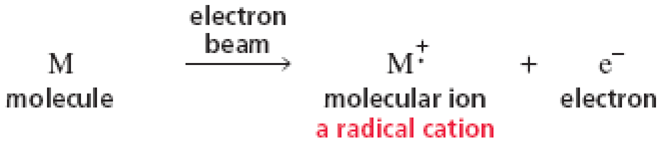
Scientists search the world for new compounds with physiological activity. If a promising compound is found, its structure needs to be determined. Without knowing its structure, chemists cannot design ways to synthesize the compound, nor can they undertake studies to provide insights into its biological behavior. Today, a number of different instrumental techniques are used to identify 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. We will look at two more instrumental techniques: mass spectrometry and infrared (IR) spectroscopy.

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

At one time, the molecular weight of a compound was determined by its vapor density r its freezing-point depression, and molecular formulas were determined by lemental analysis, a technique that determined the relative proportions of the elements present in the compound. These were long and tedious techniques that required relatively large amounts of a very pure sample. Today, molecular weights and molecular formulas can be rapidly determined by mass spectrometry from a very small amount of a sample.

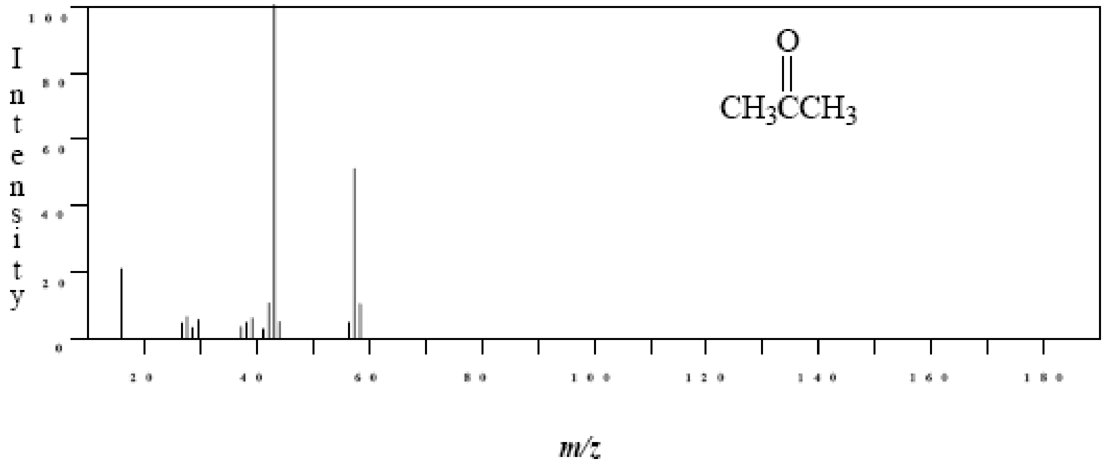
In mass spectrometry, a small sample of a compound is introduced into an instrument called a mass spectrometer, where it is vaporized and then ionized as a result of an electron’s being removed from each molecule. Ionization can be accomplished in several ways. The most common method bombards the vaporized molecules with a beam of high-energy electrons.

The energy of the electron beam can be varied, but a beam of about 70 electron volts (eV) is commonly used. When the electron beam hits a molecule, it knocks out an electron, producing a **molecular ion,** which is a **radical cation**—a species with an unpaired electron and a positive charge.

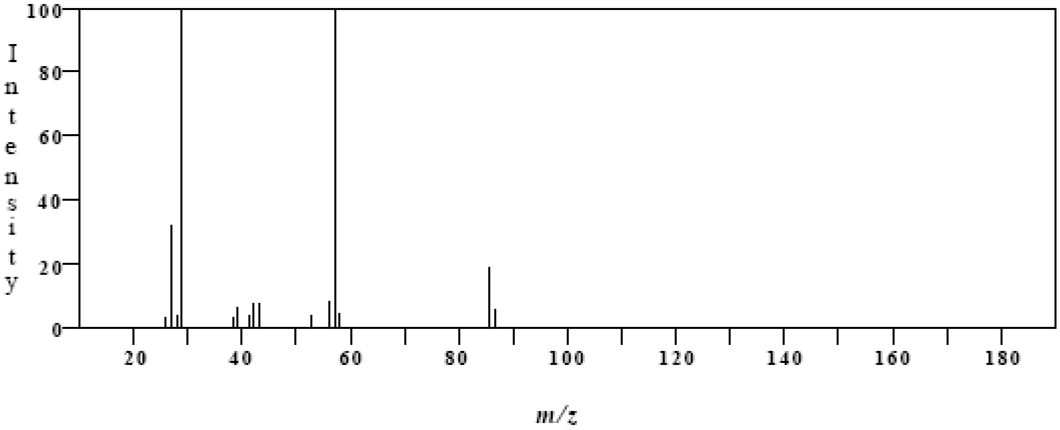


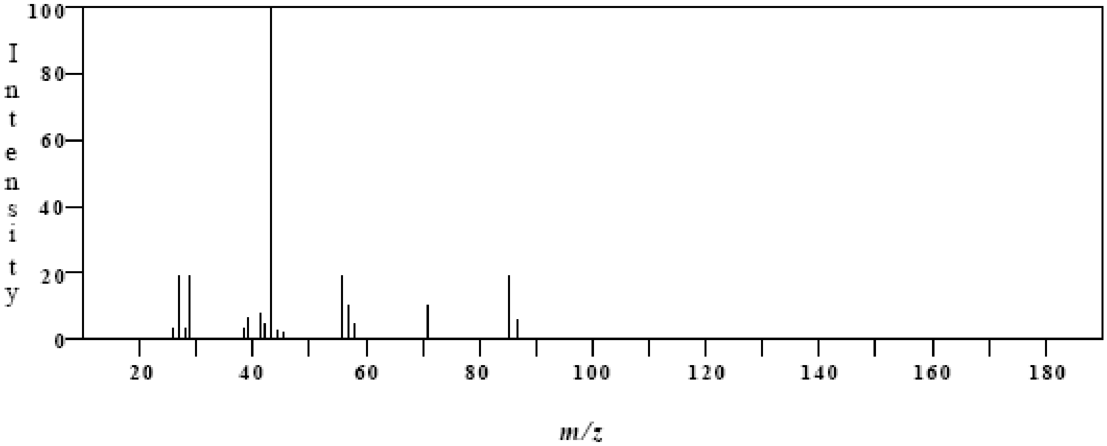
The Molecular Ion:

From an MS, chemists determine the structure of the fragments represented by the peaks, then assemble these fragments into the structure of the molecule. However, this task is complex and beyond the scope of this book. For you, the beginning organic chemist, the most useful information that you can gather from an MS is from the peaks representing the molecular ion and its isotopes. You can learn the molecular weight of the compound, and you can tell if the compound contains certain elements, such as nitrogen or a halogen.



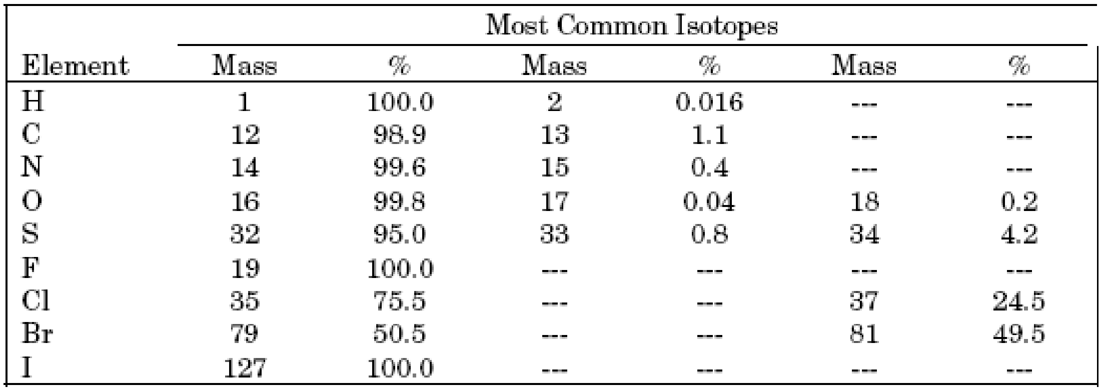
The mass spectrum of acetone.





Notice that the previous mass spectra each have a small peak to the right of the peak representing the molecular ion.

These peaks, which occur at *m/z* 59 and *m/z* 87, respectively, arise from the presence of isotopes of hydrogen, carbon, and oxygen. Most elements have more than one isotope, with the various isotopes present in varying amounts. The heavier isotopes give rise to the small peaks at a higher mass than the molecular ion peak. A peak that is one mass unit heavier than the molecular ion is called the M+1 peak. A peak that is two mass units heavier is called the M+2 peak. The following table contains a list of elements common to organic compounds, their isotopic masses, and the ratio of each isotope in a naturally occurring sample of that element. Thus, you can see how each isotope contributes to the M+1 and M+2 peaks.



	Most Common Isotopes					
Element	Mass	%	Mass	%	Mass	%
H	1	100.0	2	0.016	---	---
C	12	98.9	13	1.1	---	---
N	14	99.6	15	0.4	---	---
O	16	99.8	17	0.04	18	0.2
S	32	95.0	33	0.8	34	4.2
F	19	100.0	---	---	---	---
Cl	35	75.5	---	---	37	24.5
Br	79	50.5	---	---	81	49.5
I	127	100.0	---	---	---	---

Natural isotopic abundance of some common elements in organic molecules.

Loss of an electron from a molecule weakens the molecule’s bonds. Therefore, many of the molecular ions break apart into cations, radicals, neutral molecules, and other radical cations. Not surprisingly, 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 pass between two negatively charged plates, which accelerate the fragments into an analyzer tube (Figure 2).

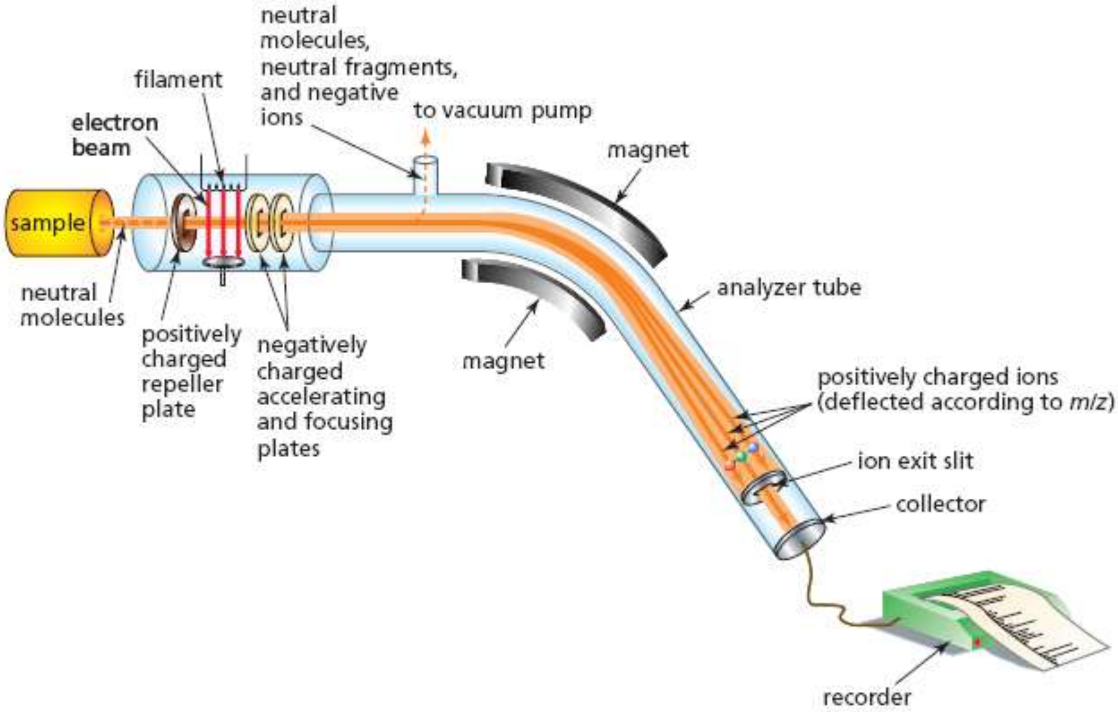


Figure 13.1
Schematic of a mass spectrometer. A beam of high-energy electrons causes molecules to ionize and fragment. Positively charged fragments pass through the analyzer tube. Changing the magnetic field strength allows the separation of fragments of varying mass-to-charge ratio.

Neutral fragments are not attracted to the negatively charged plates and therefore are not accelerated. They are eventually pumped out of the spectrometer.

The analyzer tube is surrounded by a magnet whose magnetic field deflects the positively charged fragments in a curved path. At a given magnetic field strength, the degree to which the path is curved depends on the mass-to-charge ratio (*m z*) of the fragment: The path of a fragment with a smaller *m z*value will bend more than that of a heavier fragment. In this way, the particles with the same *m z*values can be separated from all the others. If a fragment’s path matches the curvature of the analyzer tube, the fragment will pass through the tube and out the ion exit slit. A collector records the relative number of fragments with a particular *m z* passing through the slit. The morestable the fragment, the more likely it will make it to the collector.

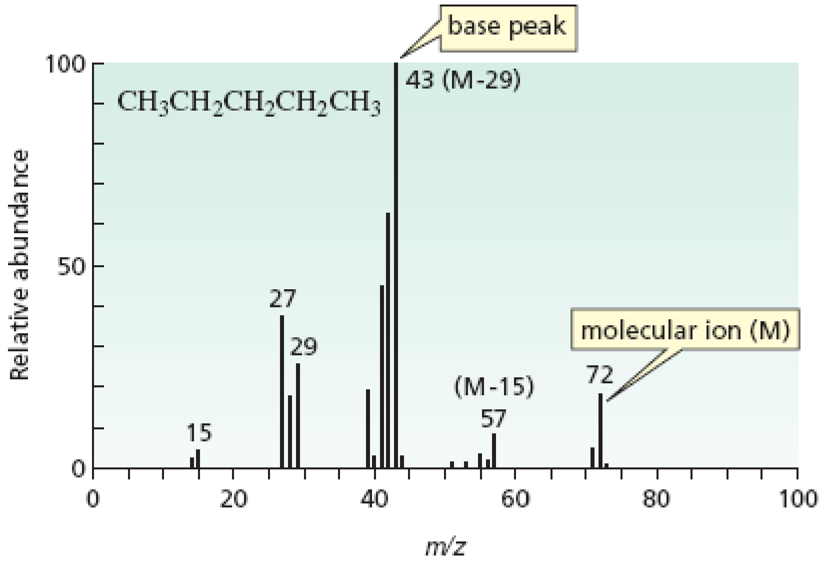
The strength of the magnetic field is gradually increased, so fragments with progressively larger *m z* values are guided through the tube and out the exit slit.

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 *m z*is the molecular mass (*m*) of the fragment. *Remember that only positively charged species reach the collector.*

The Mass Spectrum: Fragmentation

The mass spectrum of pentane is shown in Figure 13.2. Each *m z* value is the **nominal molecular mass** of the fragment—the molecular mass to the nearest whole number is defined to have a mass of 12.000 atomic mass units (amu), and the masses of other atoms are based on this standard. For example, a proton has a mass of 1.007825 amu. Pentane, therefore, has a *molecular mass* of 72.0939 and a *nominal molecular mass* of 72.

The peak with the highest *m z*value in the spectrum—in this case, at — is due to the fragment that results when an electron is knocked out of a molecule of the injected sample—in this case, a pentane molecule. In other words, the peak with the highest *m z* value represents the molecular ion (M) of pentane. (The tiny peak at will be explained later.) Since it is not known what bond loses the electron, the molecular ion is put in brackets and the positive charge and unpaired electron are assigned to the entire structure. *The m z value of the molecular ion gives the molecular mass of the* *compound*. Peaks with smaller *m z* values—called **fragment ion peaks**—represent positively charged fragments of the molecule.



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

Figure 3

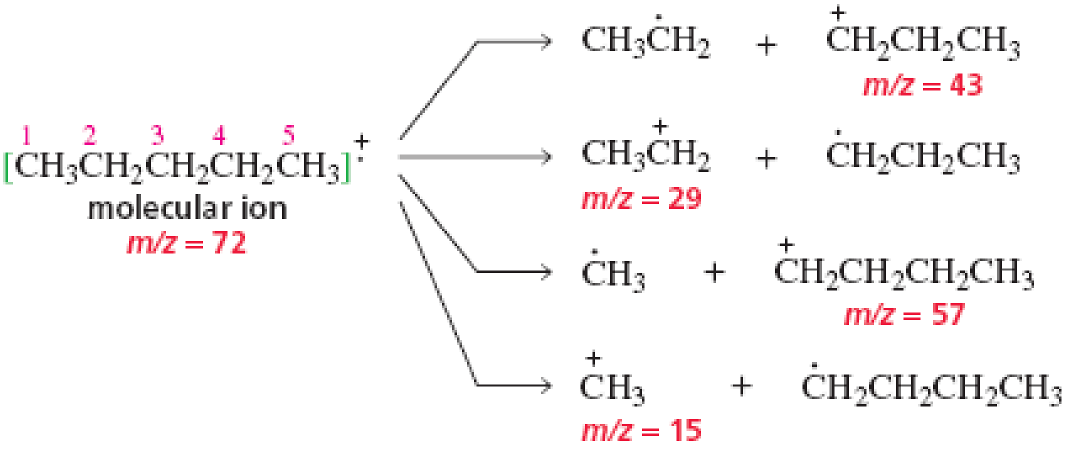
The mass spectrum of pentane, shown as a bar graph and in tabular form. The base peak represents the fragment that appears in greatest abundance. The value of the molecular ion gives the molecular mass of the compound.

$$CH_3CH_2CH_2CH_2CH_3 \xrightarrow{\text{electron beam}} \underset{\substack{\text{molecular ion} \\ m/z = 72}}{[CH_3CH_2CH_2CH_2CH_3]^{+\cdot}} + e^-$$

The **base peak** is the peak with the greatest intensity, due to its having the greatest relative abundance. The base peak is assigned a relative intensity of 100%, and the relative intensity of each of the other peaks is reported as a percentage of the base peak.

Mass spectra can be shown either as bar graphs or in tabular form.

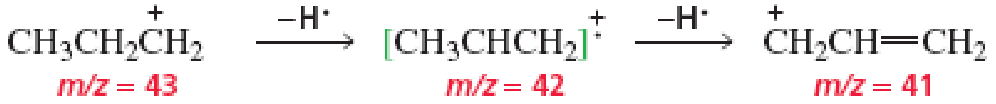
A mass spectrum gives us structural information about the compound because *the* m/z *values and the relative abundances of the fragments depend on the strength of the molecular ion's bonds and the stability of the fragments*. Weak bonds break in preference to strong bonds, and bonds that break to form more stable fragments break in preference to those that form less stable fragments. For example, the C-C bonds in the molecular ion formed from pentane have about the same strength. However, the C-1 – C-2 bond is more likely to break than the C-2 – C-3 bond because fragmentation leads to a *primary* carbocation and a *primary* radical, which together are more stable than the *primary* carbocation and *methyl* radical (or *primary* radical and *methyl* cation) obtained from C-1 – C-2 fragmentation. C-2 – C-3 fragmentation forms ions with or 29, and C-1 – C-2 fragmentation forms ions with or 15. The base peak of 43 in the mass spectrum of pentane indicates the preference for fragmentation C-2 – C-3.



A method commonly used to identify fragment ions is to determine the difference between the *m z* value of a given fragment ion and that of the molecular ion. For example, the ion with m/z = 43 in the mass spectrum of pentane is 29 units smaller than the molecular ion (M - 29 = 43). An ethyl radical (CH_3CH_2) has a molecular mass of 29 (because the mass numbers of C and H are 12 and 1, respectively), so the peak at 43 can be attributed to the molecular ion minus an ethyl radical. Similarly, the peak at m/z = 57 (M – 15) can be attributed to the molecular ion minus a methyl radical.

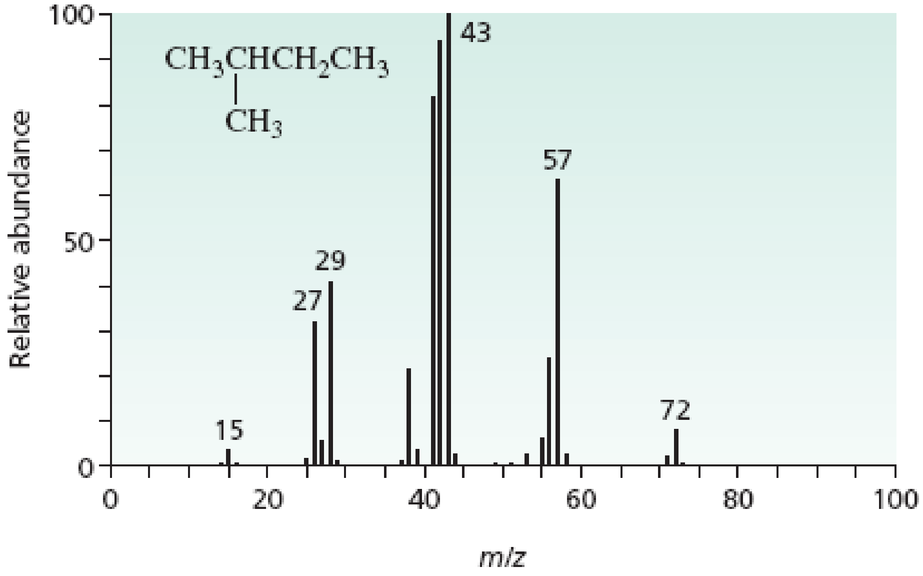
Peaks at m/z = 15 m/z = 29 and are readily recognizable as being due to methyl and ethyl cations, respectively.

Peaks are commonly observed at *m/ z* values one and two units less than the *m /z* values of the carbocations because the carbocations can undergo further fragmentation—losing one or two hydrogen atoms.

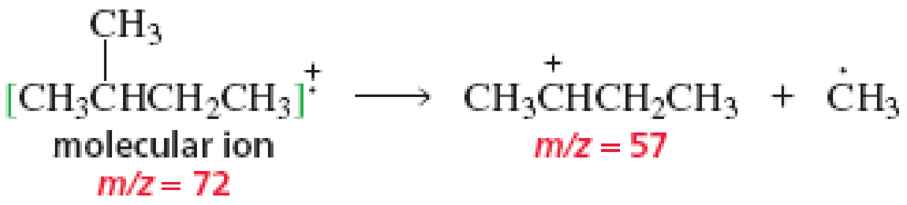


$$\underset{m/z\,=\,43}{CH_3CH_2\overset{+}{C}H_2} \xrightarrow{-H^\bullet} \underset{m/z\,=\,42}{[CH_3CHCH_2]^{+\bullet}} \xrightarrow{-H^\bullet} \underset{m/z\,=\,41}{\overset{+}{C}H_2CH{=}CH_2}$$

2-Methylbutane has the same molecular formula as pentane, so it, too, has a molecular ion with m/z = 72 Its mass spectrum is similar to that of pentane, with one notable exception: The peak at m/z = 57 (M – 15) is much more intense.



2-Methylbutane is more likely than pentane to lose a methyl radical, because, when it does, a *secondary* carbocation is formed. In contrast, when pentane loses a methyl radical, a less stable *primary* carbocation is formed.



$$[\mathrm{CH_3\overset{\overset{CH_3}{|}}{C}HCH_2CH_3}]^{+\cdot} \longrightarrow \mathrm{CH_3\overset{+}{C}HCH_2CH_3} + \dot{\mathrm{C}}\mathrm{H_3}$$

Problem 1: What *m/z* value is most likely for the base peak in the mass spectrum of 3-methylpentane?

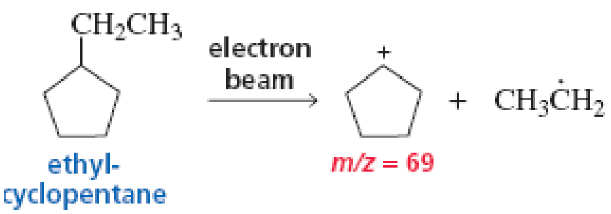
Problem 2:

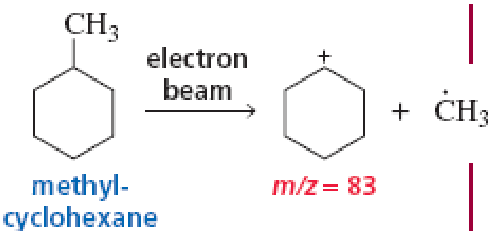
The mass spectra of two very stable cycloalkanes both show a molecular ion peak at m/z = 98. One spectrum shows a base peak a m/z = 69, the other shows a base peak at m/z = 83. Identify the cycloalkanes

***SOLUTION*:**

The molecular formula for a cycloalkane is C_nH_{2n}. Because the molecular mass of both cycloalkanes is 98, their molecular formulas must be C_7H_{14} (7 x 12 = 84 + 14 = 98). A base peak of 69 means the loss of an ethyl substituent (98 - 69 = 29), whereas a base peak of 83 means the loss of a methyl substituent Because (98 - 83 = 15).

Because the cycloalkanes are known to be very stable, we can rule out cycloalkanes with three or four-membered rings. A seven-carbon cycloalkane with a base peak signifying the loss of an ethyl substituent must be ethylcyclopentane. A seven-carbon cycloalkane with a base peak signifying the loss of a methyl substituent must be methylcyclohexane.





Isotopes in Mass Spectrometry

Although the molecular ions of pentane and 2-methylbutane both have *m/z* values of 72, each spectrum shows a very small peak at m>z = 73 This peak is called an M + 1 peak because the ion responsible for it is one unit heavier than the molecular ion. The M + 1 peak owes its presence to the fact that there are two naturally occurring isotopes of carbon: 98.89% of natural carbon is ^{12}C and 1.11% is ^{13}C. So 1.11% of the molecular ions contain a ^{13}C instead of a ^{12}C and therefore appear at M + 1

Peaks that are attributable to isotopes can help identify the compound responsible for a mass spectrum. For example, if a compound contains five carbon atoms, the relative intensity of the M + 1 ion should be 5(1.1%) = 5(0.011) multiplied by the relative intensity of the molecular ion. This means that the number of carbon atoms in a compound can be calculated if the relative intensities of the M and M + 1 peaks are known.

$$\text{number of carbon atoms} = \frac{\text{relative intensity of M + 1 peak}}{.011 \times (\text{relative intensity of M peak})}$$

The isotopic distributions of several elements commonly found in organic compounds are shown in Table 13.2. From the isotopic distributions, we see why the M + 1 peak can be used to determine the number of carbon atoms in a compound: It is because the contributions to the M + 1 peak by isotopes of H, O, and the halogens are very small or nonexistent. This formula does not work as well in predicting the number of carbon atoms in a nitrogen-containing compound because the natural abundance of ^{15}N is relatively high.

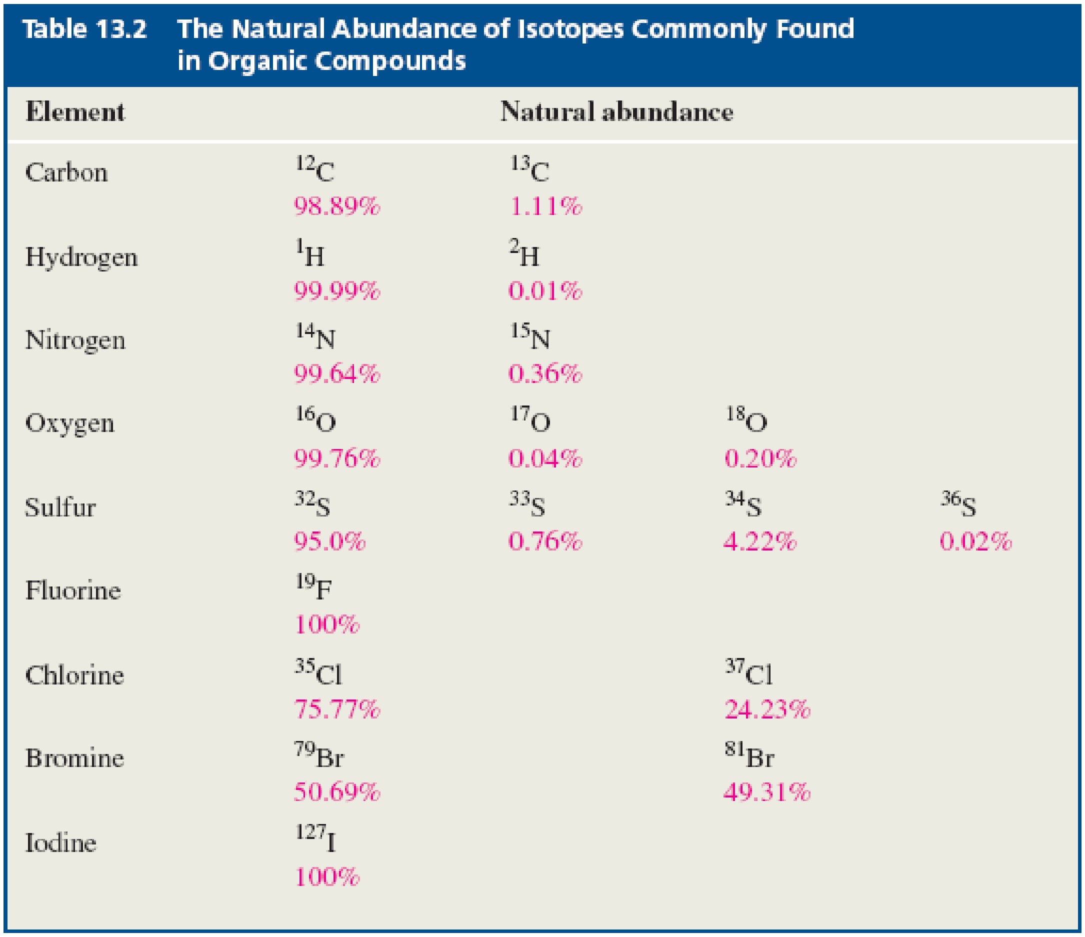


Table 13.2 The Natural Abundance of Isotopes Commonly Found in Organic Compounds

Element	Natural abundance			
Carbon	^{12}C 98.89%	^{13}C 1.11%		
Hydrogen	^{1}H 99.99%	^{2}H 0.01%		
Nitrogen	^{14}N 99.64%	^{15}N 0.36%		
Oxygen	^{16}O 99.76%	^{17}O 0.04%	^{18}O 0.20%	
Sulfur	^{32}S 95.0%	^{33}S 0.76%	^{34}S 4.22%	^{36}S 0.02%
Fluorine	^{19}F 100%			
Chlorine	^{35}Cl 75.77%		^{37}Cl 24.23%	
Bromine	^{79}Br 50.69%		^{81}Br 49.31%	
Iodine	^{127}I 100%			

Mass spectra can show M + 2 peaks as a result of a contribution from ^{18}O or from having two heavy isotopes in the same molecule (say, ^{13}Cand or two ^{13}C). Most of the time, the M + 2 peak is very small. The presence of a large M + 2 peak is evidence of a compound containing either chlorine or bromine, because each of these elements has a high percentage of a naturally occurring isotope that is two units heavier than the most abundant isotope.

From the natural abundance of the isotopes of chlorine and bromine in Table 13.2, one can conclude that if the M + 2 peak is one third the height of the molecular ion peak, then the compound contains one chlorine atom because the natural abundance of ^{37}Cl is one-third that of ^{35}Cl If the M and M + 2 peaks are about the same height, then the compound contains one bromine atom because the natural abundances of ^{79}Br and ^{81}Br are about the same.

In calculating the molecular masses of molecular ions and fragments, the *atomic mass* of a single isotope of the atom must be used (Cl = 35 or 37, etc.); the *atomic weights* in the periodic table (Cl = 35.453) cannot be used because they are the *weighted averages* of all the naturally occurring isotopes for that element, and mass spectrometry measures the *m/z* value of an *individual* fragment.

Determination of Molecular Formulas:

High-Resolution Mass Spectrometry

All the mass spectra shown in this text were determined with a low-resolution mass spectrometer. Such spectrometers give the *nominal molecular mass* of a fragment—the mass to the nearest whole number. High-resolution mass spectrometers can determine the *exact molecular mass* of a fragment to a precision of 0.0001 amu. If we know the exact molecular mass of the molecular ion, we can determine the compound's molecular formula. For example, as the following listing shows, many compounds have a nominal molecular mass of 122 amu, but each of them has a different exact molecular mass.

The exact molecular masses of some common isotopes are listed in Table 13.3. Some computer programs can determine the molecular formula of a compound from the compound's exact molecular mass.

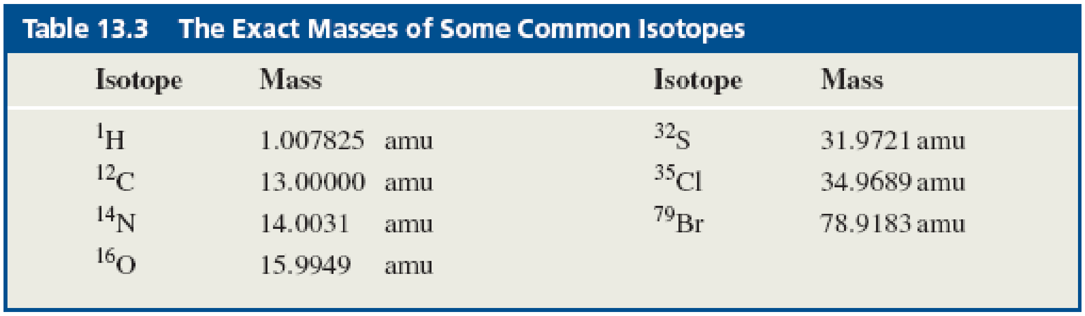


Table 13.3 The Exact Masses of Some Common Isotopes

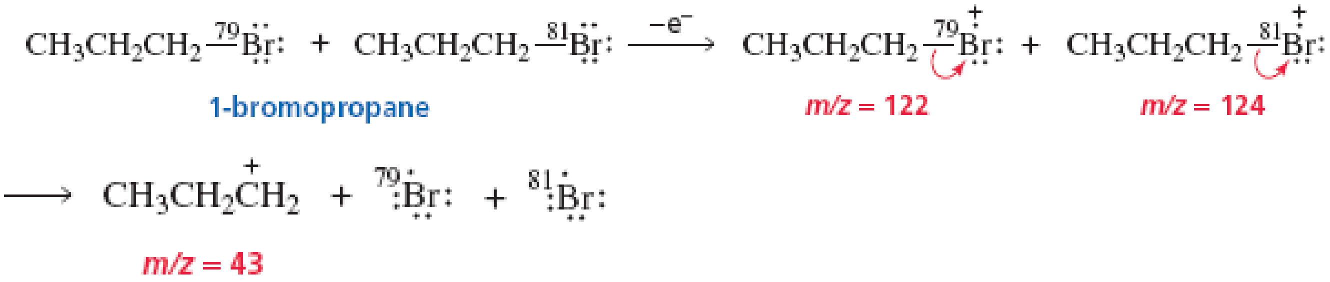
Isotope	Mass	Isotope	Mass
^{1}H	1.007825 amu	^{32}S	31.9721 amu
^{12}C	13.00000 amu	^{35}Cl	34.9689 amu
^{14}N	14.0031 amu	^{79}Br	78.9183 amu
^{16}O	15.9949 amu		

Fragmentation at Functional Groups

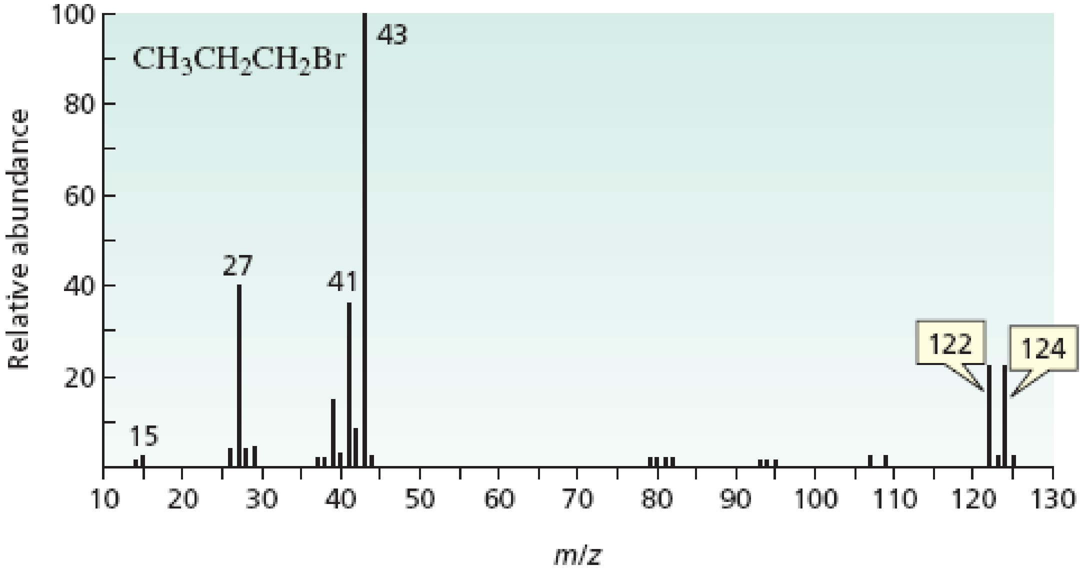
Characteristic fragmentation patterns are associated with specific functional groups; these can help identify a substance based on its mass spectrum. The patterns were recognized after the mass spectra of many compounds containing a particular functional group were studied. We will look at the fragmentation patterns of alkyl halides, ethers, alcohols, and ketones as examples.

Alkyl Halides

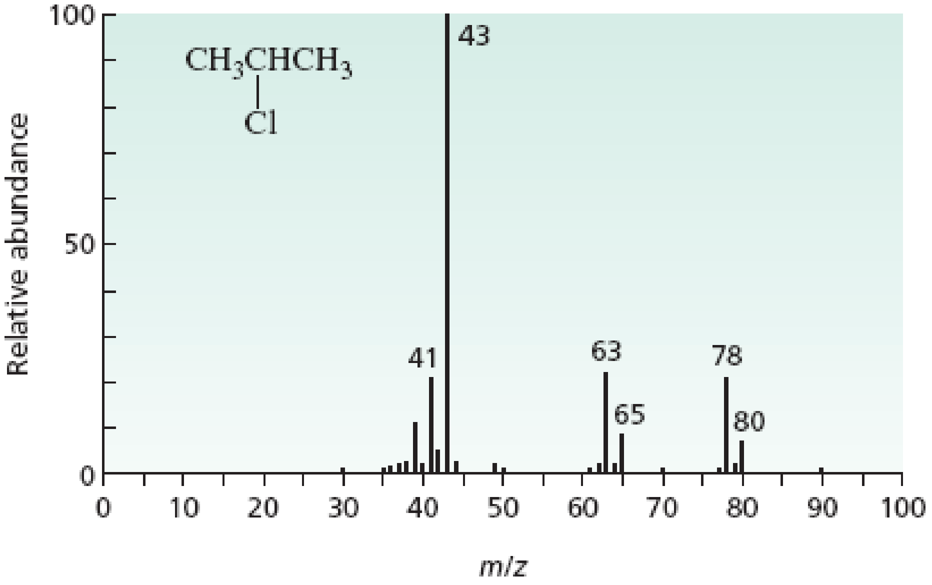
Let’s look first at the mass spectrum of 1-bromopropane. The relative heights of the M and M + 2 peaks are about equal, so we can conclude that the compound contains a bromine atom. Electron bombardment is most likely to dislodge a lone-pair electron if the molecule has any, because a molecule does not hold onto its lone-pair electrons as tightly as it holds onto its bonding electrons. Thus, electron bombardment dislodges one of bromine’s lone-pair electrons.



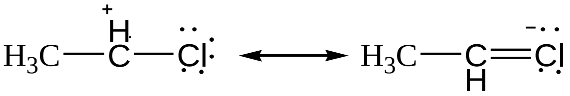
The weakest bond in the resulting molecular ion is C-Br the bond (the C-Br bond dissociation energy is 69 kcal/mol; the C-C bond dissociation energy is 85 kcal/mol; see Table 3.1 on p. 129). The bond breaks heterolytically, with both electrons going to the more electronegative of the atoms that were joined by the bond, forming a propyl cation and a bromine atom. As a result, the base peak in the mass spectrum of 1-bromopropane is at m / z = 43 [M-79, or (M + 2) – 81]. The propyl cation has the same fragmentation pattern it exhibited when it was formed from
the cleavage of pentane.



The mass spectrum of 2-chloropropane is shown in Figure 13.5. We know that the compound contains a chlorine atom, because the M + 2 peak is one-third the height of the molecular ion peak. The base peak at m/z = 43 results from *heterolytic cleavage* of the C-Cl bond. The peaks at m/z = 63 and m/z = 65 have a 3:1 ratio, indicating that these fragments contain a chlorine atom. They result from *hemolytic cleavage* of a C-C bond at the α-carbon (the carbon bonded to the chlorine). This cleavage, known as α **cleavage**, occurs because the C¬Cl (82 kcal/mol) and C¬C

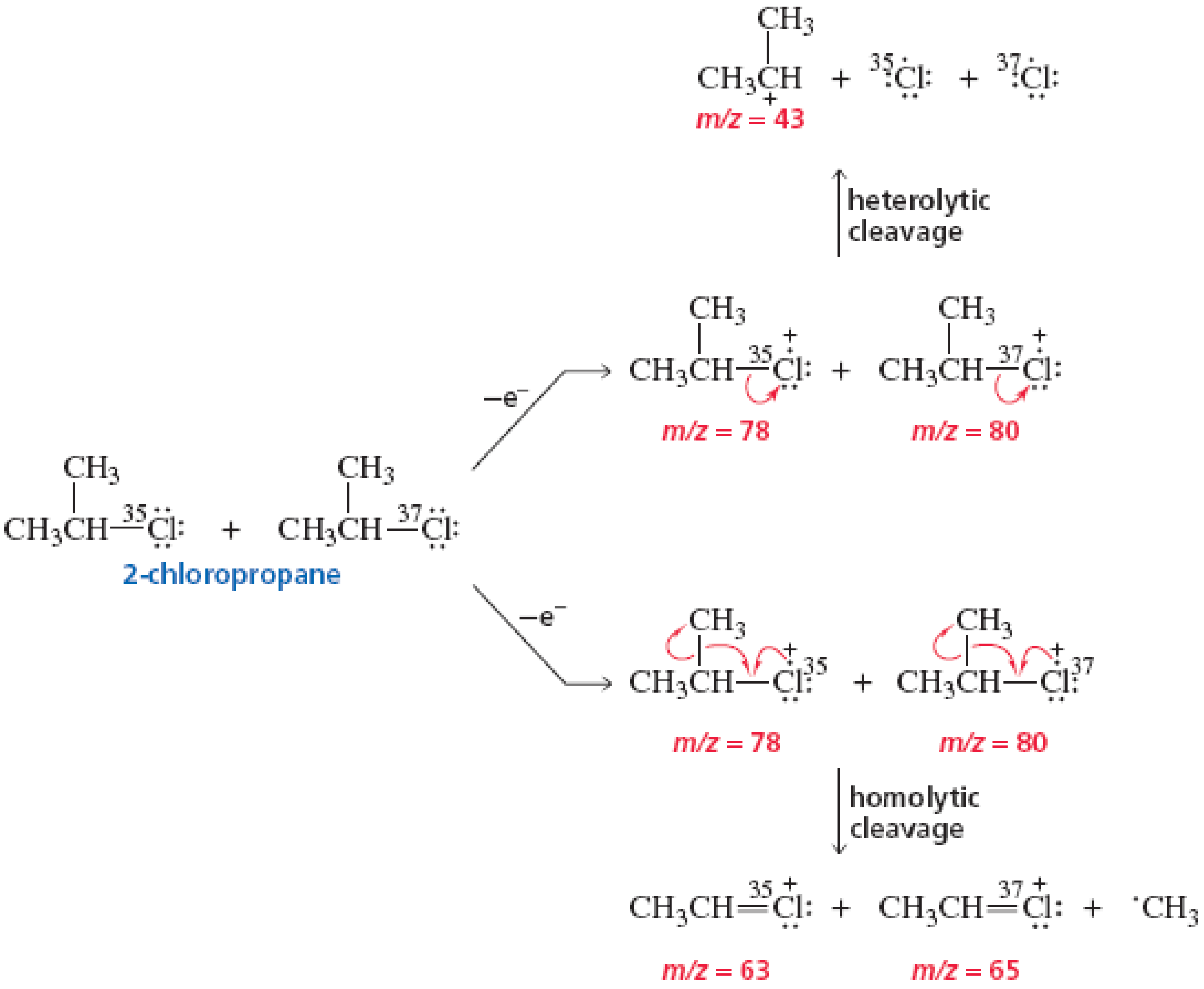


(85 kcal/mol) bonds have similar strengths, and the species that is formed is a relatively stable cation, since its positive charge is shared by two atoms:



$$H_3C-\overset{+}{\overset{H}{C}}-\ddot{Cl}: \longleftrightarrow H_3C-\underset{H}{C}=\overset{-}{\ddot{Cl}}$$

Notice that α cleavage does not occur in alkyl bromides because the C-C bond is much weaker than the C-Br bond



2-chloropropane
−e⁻
m/z = 78
m/z = 80
heterolytic cleavage
m/z = 43
homolytic cleavage
m/z = 63
m/z = 65
·CH3

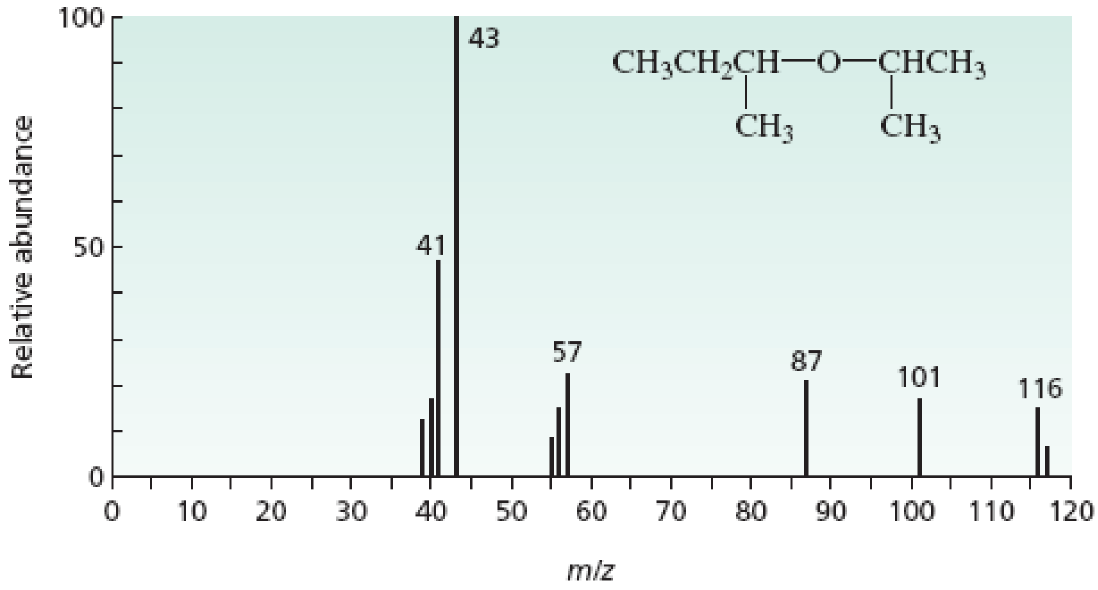
Ethers

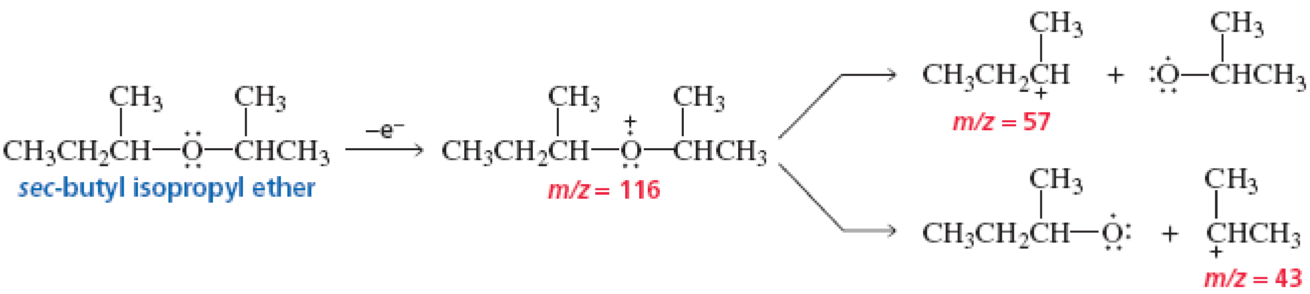
The mass spectrum of *sec*-butyl isopropyl ether is shown in Figure 13.6. The fragmentation pattern of an ether is similar to that of an alkyl halide.

1. Electron bombardment dislodges one of the lone-pair electrons from oxygen.

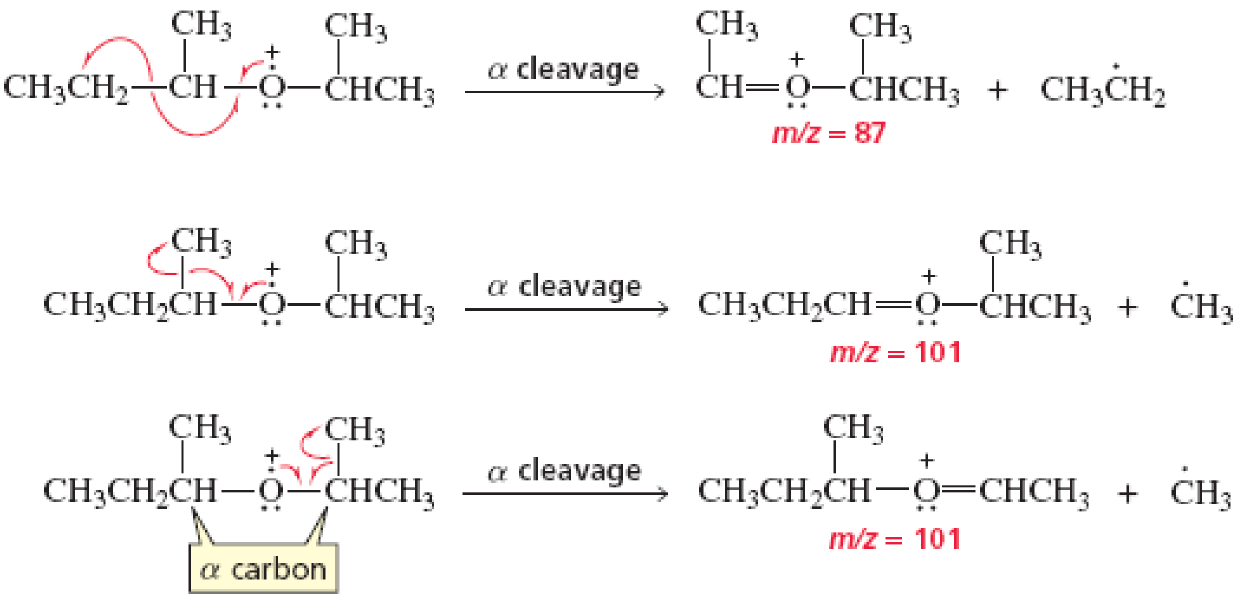
2. Fragmentation of the resulting molecular ion occurs in two principal ways:

a. A C-O bond is cleaved heterolytically, with the electrons going to the more electronegative oxygen atom.





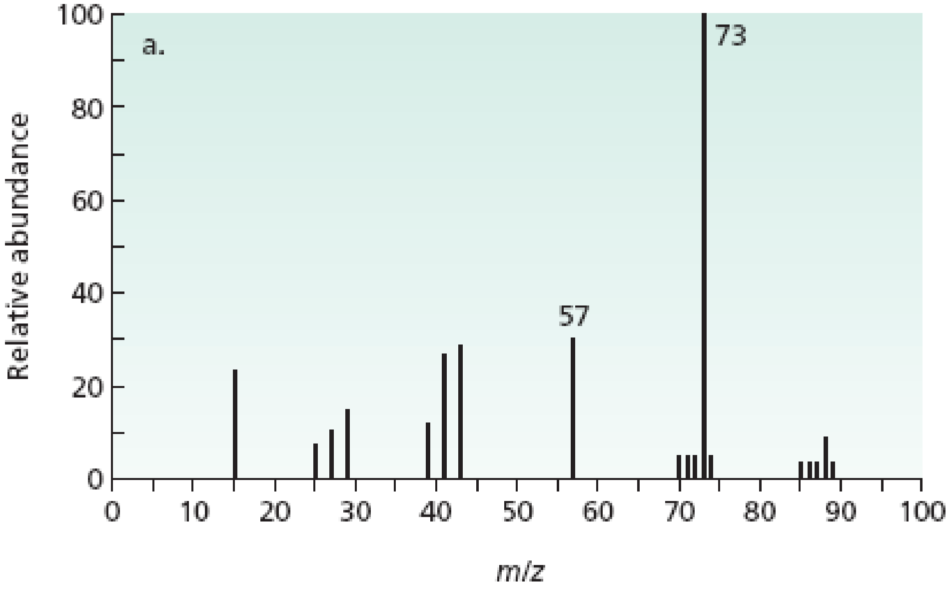
b. A C-C bond is cleaved *homolytically* at the position because it leads to a relatively stable cation in which the positive charge is shared by two atoms (a carbon and an oxygen). The alkyl group leading to the most stable radical is one most easily cleaved. Thus, the peak at m/z = 87 is more abundant than the one at m/z = 101 even though the compound has three methyl groups bonded to α carbons that can be cleaved to produce a peak at because a primary radical is more stable than a methyl radical.

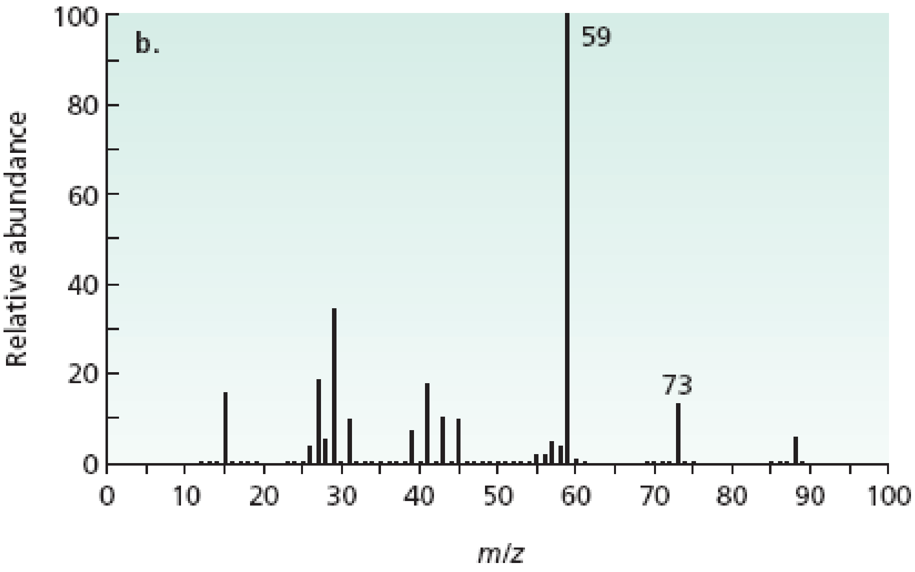


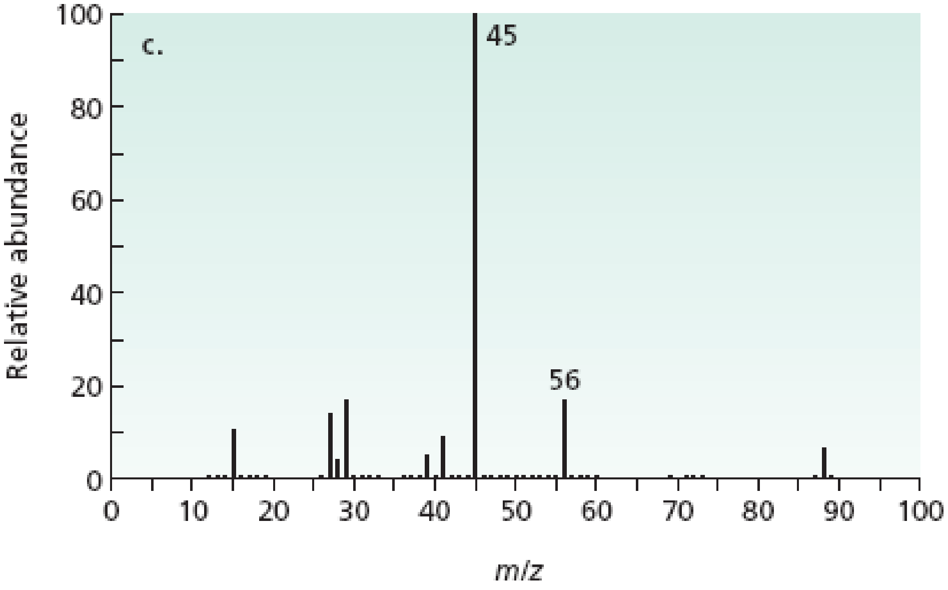
Alcohols

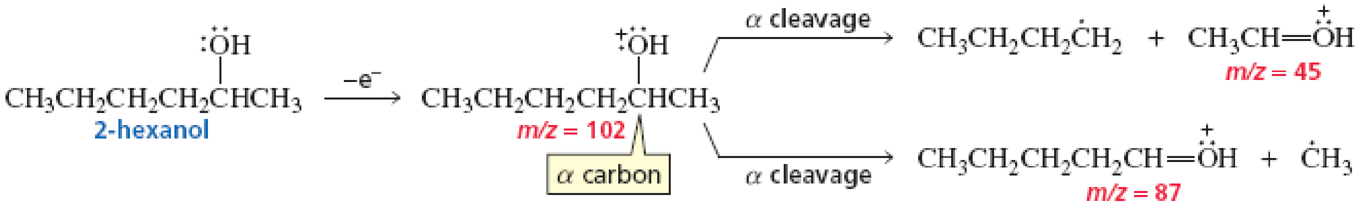
The molecular ions obtained from alcohols fragment so readily that few of them survive to reach the collector. As a result, the mass spectra of alcohols show small molecular ion peaks. Notice the small molecular ion peak at m /z = 102 in the mass spectrum of 2-hexanol.

Like alkyl halides and ethers, alcohols undergo cleavage. Consequently, the mass spectrum of 2-hexanol shows a base peak at m>z = 45 (a cleavage leading to a more stable butyl radical) and a smaller peak at m/z = 87 (αcleavage leading to a less stable methyl radical).



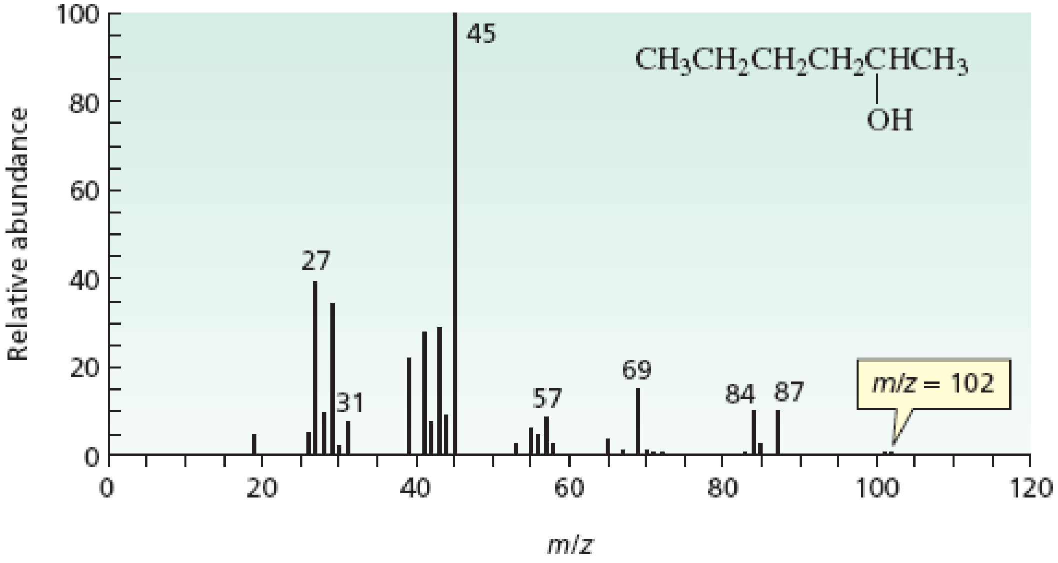




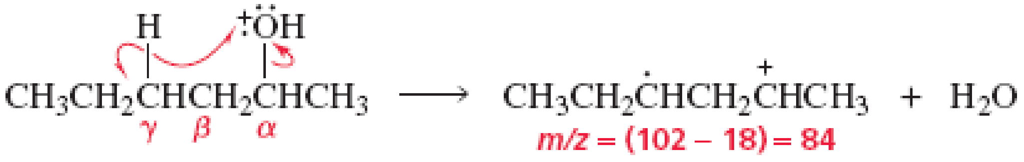


$CH_3CH_2CH_2CH_2CH(\ddot{O}H)CH_3 \xrightarrow{-e^-} CH_3CH_2CH_2CH_2CH(\overset{+}{\ddot{O}}H)CH_3 \rightarrow CH_3CH_2CH_2\dot{C}H_2 + CH_3CH{=}\overset{+}{\ddot{O}}H$

$CH_3CH_2CH_2CH_2CH(\overset{+}{\ddot{O}}H)CH_3 \rightarrow CH_3CH_2CH_2CH_2CH{=}\overset{+}{\ddot{O}}H + \dot{C}H_3$



In all the fragmentations we have seen so far, only one bond is broken. An important fragmentation occurs in alcohols, however, that involves breaking two bonds. Two bonds break because the fragmentation forms a stable water molecule. The water that is eliminated comes from the OH group of the alcohol and a γ hydrogen. Thus, alcohols show a fragmentation peak at m/z = M-18 because of loss of water.



Notice that alkyl halides, ethers, and alcohols have the following fragmentation behavior in common:

1. A bond between carbon and a *more electronegative* atom (a halogen or an oxygen) breaks heterolytically.

2. A bond between carbon and an atom of *similar electronegativity* (a carbon or a hydrogen) breaks homolytically.
3. The bonds most likely to break are the weakest bonds and those that lead to formation of the most stable cation. (Look for fragmentation that results in a cationwith a positive charge shared by two atoms.)

The molecular ions in alcohols and amines are not generally very stable. Frequently, they undergo a reaction and lose a small molecule. In the case of alcohols, the small molecule is water. Thus, the mass spectra for these alcohols and amines may not show any visible molecular ion peak. For example, the mass spectrum of *tert*-butyl alcohol (see Figure 9.26) shows no molecular ion peak at all, and the mass spectrum of 1-butanamine (see Figure 9.27) shows only a very weak molecular ion peak.

$$[(CH_3)_3COH]^{+\bullet} \longrightarrow [(CH_3)_2C{=}CH_2]^{+\bullet} + H_2O$$

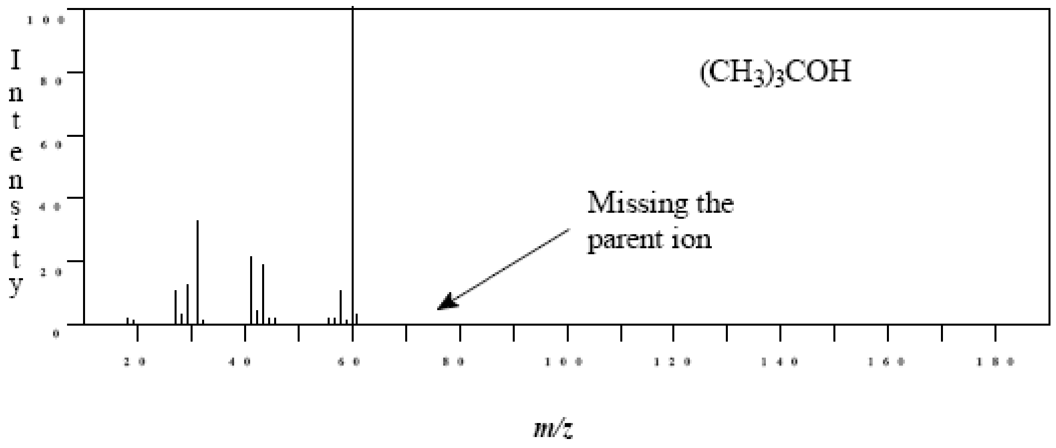
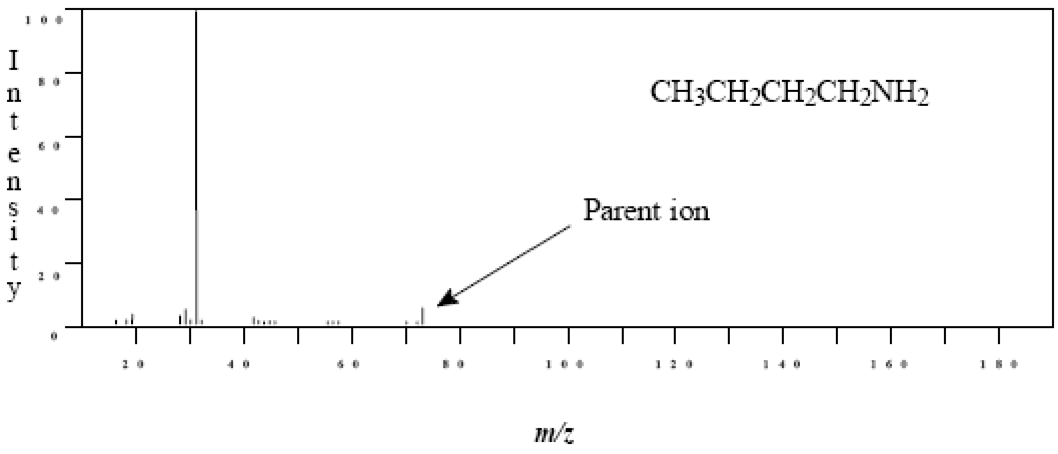
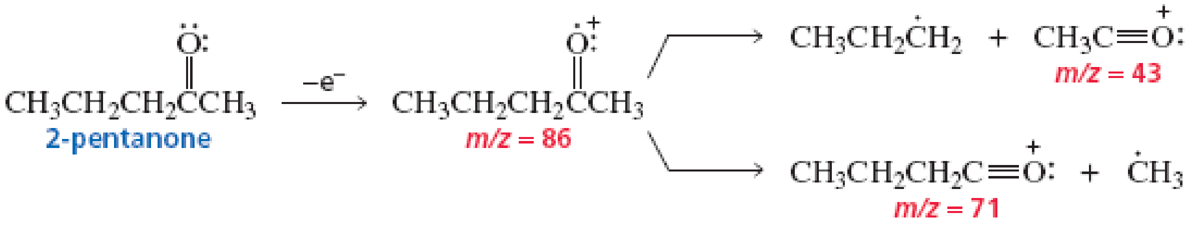


Figure 9.26. The MS of *tert*-butyl alcohol. The molecular ion is expected at m/z 74 but is absent from this spectrum.

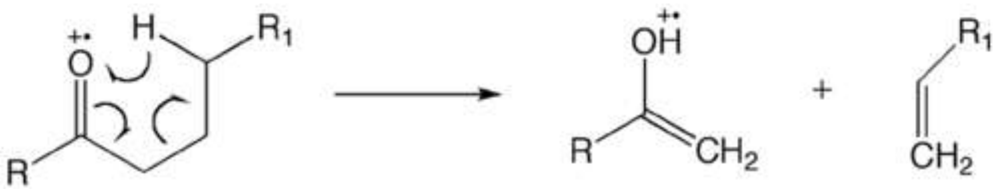


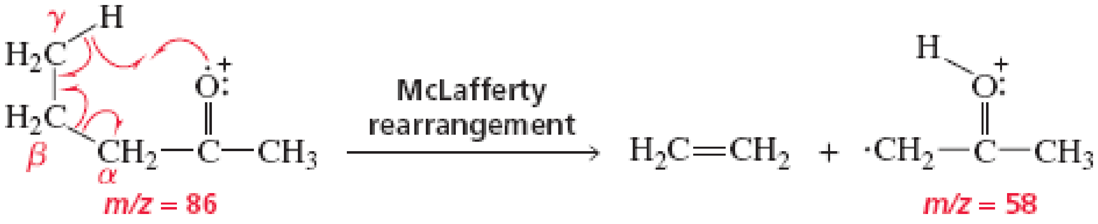
Ketones

The mass spectrum of a ketone generally has an intense molecular ion peak. Ketones fragment homolytically at the C-C bond adjacent to the C=O bond, which results in the formation of a cation with a positive charge shared by two atoms. The alkyl group leading to the more stable radical is the one that is more easily cleaved.



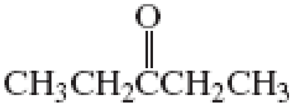
If one of the alkyl groups attached to the carbonyl carbon has a γ hydrogen, a cleavage known as a **McLafferty rearrangement** may occur. The McLafferty rearrangement is a reaction observed in mass spectrometry. It is sometimes found that a molecule containing a keto-group undergoes β-cleavage, with the gain of the γ-hydrogen atom. A description of the reaction was first published by the American chemist Fred McLafferty in 1959. This rearrangement may take place by a radical or ionic mechanism. In this rearrangement, the bond between the α-carbon and the ß-carbon breaks homiletically and a hydrogen atom from the γ-carbon migrates to the oxygen atom. Again, fragmentation has occurred in a way that produces a cation with a positive charge shared by two atoms.

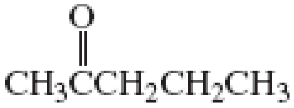


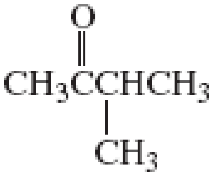


Problem:

How could their mass spectra distinguish the following compounds?

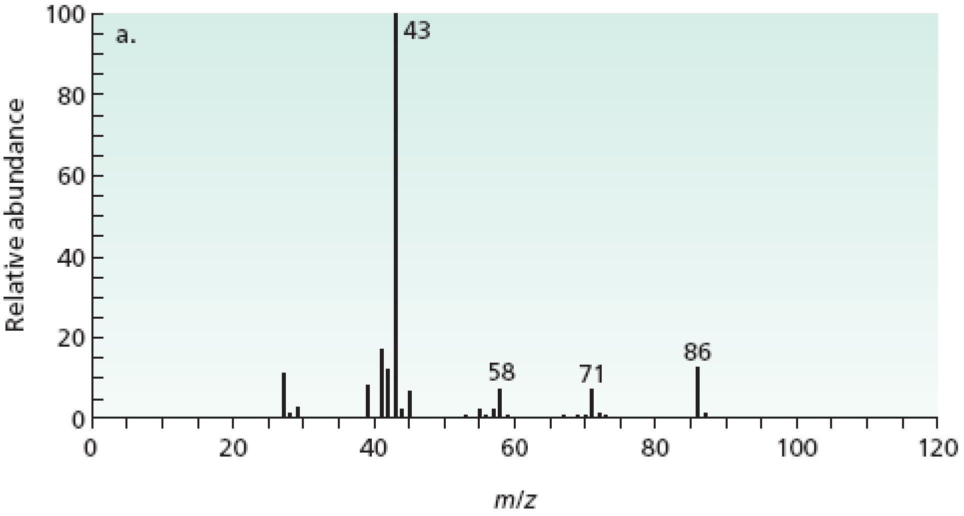

$CH_3CH_2\overset{O}{\overset{\|}{C}}CH_2CH_3$


$CH_3\overset{O}{\overset{\|}{C}}CH_2CH_2CH_3$

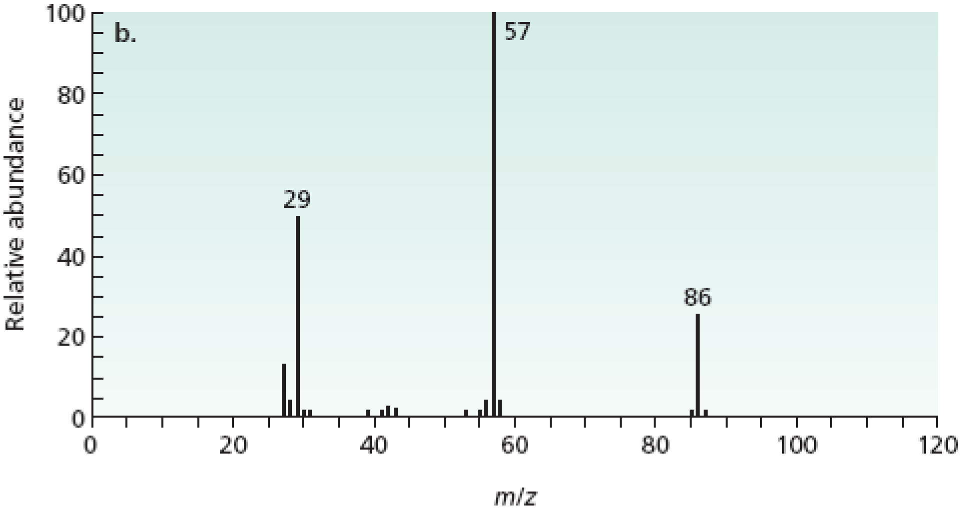

$CH_3\overset{O}{\overset{\|}{C}}\underset{CH_3}{\underset{|}{C}}HCH_3$

Problem:

Identify the ketones that are responsible for the mass spectra shown in the following Figures:


a.
Relative abundance
0
20
40
60
80
100
m/z
0
20
40
60
80
100
120
43
58
71
86

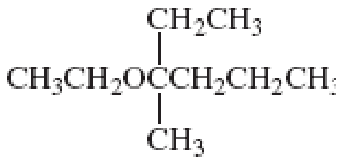
T


b.
Relative abundance
0
20
40
60
80
100
m/z
0
20
40
60
80
100
120
57
29
86

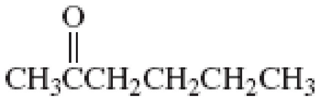
Problem:

Using curved arrows, show the principal fragments that would be observed in the mass spectrum of each of the following compounds:

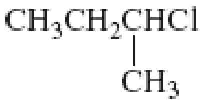
a. $CH_3CH_2CH_2CH_2CH_2OH$

d. 

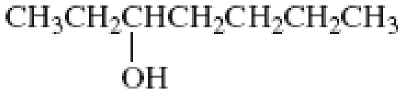
$CH_3CH_2O\overset{CH_2CH_3}{\underset{CH_3}{C}}CH_2CH_2CH$

b. 

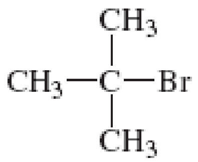
$CH_3\overset{O}{\overset{\|}{C}}CH_2CH_2CH_2CH_3$

e. 

$CH_3CH_2\underset{CH_3}{C}HCl$

c. 

$CH_3CH_2\underset{OH}{C}HCH_2CH_2CH_2CH_3$

f. 

$CH_3-\overset{CH_3}{\underset{CH_3}{C}}-Br$

The molecular ion and the pattern of fragment ion peaks are unique for each compound. A mass spectrum, therefore, is like a fingerprint of the compound. A positive identification of a compound can be made by comparing its mass spectrum with that of a known sample of the compound.

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A mass spectrometer can be considered to comprise four component parts, as follows:

- a method of sample introduction
- a method of ion production
- a method of ion separation
- facilities for ion detection and data manipulation

Before considering these in detail, it is necessary to revisit the inherent incompatibilities between mass spectrometry and liquid chromatography. These are, as discussed previously, that HPLC utilizes a liquid mobile phase, often containing significant amounts of water, flowing typically at 1 ml min^{-1}, while the mass spectrometer must be maintained under conditions of high vacuum, i.e. around 10^{-6} torr ($1.333\,22 \times 10^{-4}$ Pa).

DQ 3.1

Why is the mass spectrometer operated under conditions of high vacuum?

Answer

Ions formed in the source of the mass spectrometer must reach the detector for them to be of any value. The average distance that an ion travels between collisions – the mean free path – at atmospheric pressure is around 10^{-8} m, and it is therefore unlikely that it will reach the detector under these conditions. Since the mean free path is inversely proportional to the pressure, reducing this to 10^{-6} torr will increase the mean free path to around 10 m, and thus allow ions to reach the detector of the mass spectrometer.

The mass spectrometer inlet system for liquid chromatography, often termed the 'interface' between the two component techniques, must therefore remove as much of the unwanted mobile phase as possible while still passing the maximum amount of analyte into the mass spectrometer. This must be done in such a way that the mass spectrometer is still able to generate all of the analytical information of which it is capable.

SAQ 3.1

Calculate the volume of vapour produced when 1 g of (a) methanol, (b) acetonitrile, and (c) water, is vaporized at STP. Calculate the volume if this were to occur at 10^{-6} torr, the operating pressure of the mass spectrometer.

3.2 Ionization Methods

Ionization methods that may be utilized in LC–MS include electron ionization (EI), chemical ionization (CI), fast-atom bombardment (FAB), thermospray (TSP), electrospray (ESI) and atmospheric-pressure chemical ionization (APCI).

EI may be used with the moving-belt and particle-beam interfaces, CI with the moving-belt, particle-beam and direct-liquid-introduction interfaces, and FAB with the continuous-flow FAB interface. A brief description of these ionization methods will be provided here but for further details the book by Ashcroft [8] is recommended.

TSP, ESI and APCI effect ionization from solution and in these cases it is not possible to separate a description of the processes involved in the ionization of an analyte from a description of the interface. These ionization techniques will therefore be described in detail in Chapter 4.

3.2.1 Electron Ionization

In electron ionization (EI), the analyte of interest, in the vapour phase, is bombarded with high-energy electrons (usually 70 eV) (1 ev = $1.602\,177\,33 \times 10^{-19}$ J). Analyte molecules absorb some of this energy (typically around 20 eV) and this causes a number of processes to occur. The simplest of these is where the analyte is ionized by the removal of a single electron. This yields a radical cation, termed the molecular ion ($M^{+\bullet}$), the m/z of which corresponds to the molecular weight of the analyte. This process typically requires some 10 eV of energy and the ion so formed is therefore likely to possess around 10 eV of excess energy which may bring about its fragmentation – bond energies in organic molecules are typically around 4–5 eV. Two types of process may occur, i.e. simple scission of bonds and, when certain spatial arrangements of atoms occur within the molecule, fragmentation after rearrangement of the molecular structure. The latter process produces ions which would not immediately be expected from a simple examination of the structure of the analyte molecule involved. The presence of rearrangement ions within a mass spectrum is usually highly significant in terms of deriving the structure of the analyte concerned. The processes occurring in electron ionization are summarized in Figure 3.1.

Interpretation of an EI spectrum involves a consideration of the chemical significance of the ions observed in the mass spectrum and then using this information to derive an unequivocal structure. For a detailed consideration of the interpretation of EI mass spectra, the text by McLafferty and Turecek [7] is recommended.

3.2.2 Chemical Ionization

One of the major limitations of EI is that the excess energy imparted to the analyte molecule during electron bombardment may bring about such rapid fragmentation that the molecular ion is not observed in the mass spectrum. Under

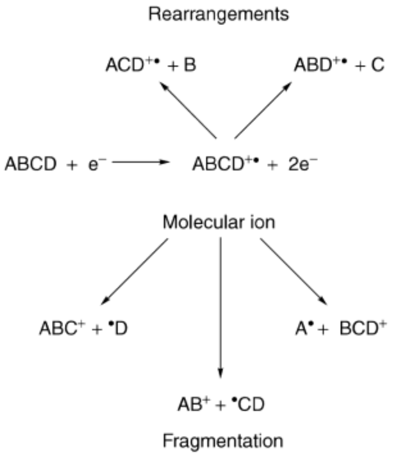

Figure 3.1 Processes occurring in the production of a mass spectrum by electron ionization.

these circumstances, one of the most important pieces of analytical information is lost and the value of mass spectrometry is much reduced.

Chemical ionization (CI) is a technique that has been developed specifically to enhance the production of molecular species, i.e. to reduce the fragmentation associated with ionization. A number of such techniques exist and these are known collectively as 'soft ionization techniques'.

In this approach, analyte molecules, in the vapour phase (as with EI), are introduced into a mass spectrometer source containing a reagent gas. This mixture is then bombarded with electrons, as described above for EI, and ionization occurs. Since the reagent gas is present in vast excess when compared to the analyte molecules (typically $> 1000:1$), it is the reagent gas, almost exclusively, which is ionized. Ion–molecule reactions then take place between the reagent gas ions and the neutral analyte molecules in the high-pressure regime of the mass spectrometer source. The specific reactions which take place depend upon the thermodynamics of the processes that are possible but typically lead to the formation of adducts of reagent ions with analyte molecules in relatively low-energy processes which lead to little fragmentation.

The most commonly used reagent gases are methane, isobutane and ammonia, with the processes involved when methane is used being summarized in Figure 3.2. When interpreting spectra generated in this way it must be remembered that the m/z of the ion observed in the molecular ion region does not give the molecular weight directly as it arises from the combination of the analyte with an adduct. The mass of that adduct, 1 in the case of methane and

$$\begin{array}{lcl}
CH_4 + e^- & \longrightarrow & CH_4^{+\bullet} + 2e^- \\
CH_4^{+\bullet} & \longrightarrow & CH_3^+ + H^\bullet \\
CH_4^{+\bullet} + CH_4 & \longrightarrow & CH_5^+ + CH_3^\bullet \\
CH_3^+ + CH_4 & \longrightarrow & C_2H_5^+ + H_2 \\
CH_5^+ + M & \longrightarrow & MH^+ + CH_4
\end{array}$$

Figure 3.2 Processes occurring in chemical ionization mass spectrometry using methane as the reagent gas.

isobutane, and 18 in the case of ammonia, must be subtracted from the m/z value observed.

SAQ 3.2

The positive-ion CI spectrum, using ammonia as a reagent gas, of an analyte containing carbon, hydrogen, oxygen and two nitrogen atoms, has a molecular species at m/z 222. What is the molecular weight of the compound involved?

CI is not the only ionization technique where this aspect of interpretation must be considered carefully; fast-atom bombardment, thermospray, electrospray and atmospheric-pressure chemical ionization, described below in Sections 3.2.3, 4.6, 4.7 and 4.8, respectively, all produce adducts in the molecular ion region of their spectra.

3.2.3 Fast-Atom Bombardment

Both EI and CI require the analyte of interest to be in the vapour phase before ionization can take place and this precludes the study of a significant number of polar, involatile and thermally labile analytes.

Fast-atom bombardment (FAB) is one of a number of ionization techniques which utilize a matrix material, in which the analyte is dissolved, to transfer sufficient energy to the analyte to facilitate ionization. In FAB, the matrix material is a liquid, such as glycerol, and the energy for ionization is provided by a high-energy atom (usually xenon) or, more recently, an ion (Cs^+) beam. In conventional FAB, the solution of analyte in the matrix material is applied to the end of a probe which is placed in the source of the mass spectrometer where it is bombarded with the atom/ion beam.

When FAB is utilized for LC–MS, often known as continuous-flow FAB, a matrix material is added to the HPLC eluent, either pre- or post-column, and this mixture continuously flows to the tip of a probe inserted into the source of the mass spectrometer where it is bombarded by the atom beam (Figure 3.3).

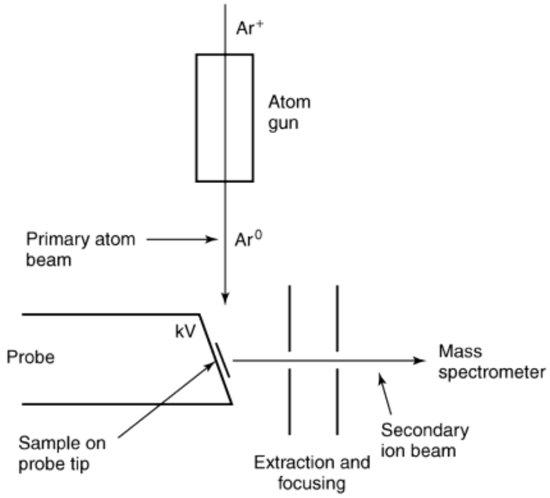

Figure 3.3 Schematic of fast-atom bombardment ionization.

In contrast to conventional FAB where the analyte is dissolved in the matrix material, it has been found that FAB performance can be obtained when the mobile phase contains as little as 5% of the matrix material, thus reducing the chemical background associated with the technique. It should be noted that if the matrix material is added before the column it may have an effect on the separation achieved.

SAQ 3.3

Why might the addition of the FAB matrix to the HPLC mobile phase have an effect on the separation obtained?

3.2.4 Matrix-Assisted Laser Desorption Ionization

Matrix-assisted laser desorption ionization (MALDI) is not yet a technique that has been used extensively for LC–MS applications. It is included here because it often provides analytical information complementary to that obtained from LC–MS with electrospray ionization, as illustrated later in Chapter 5.

MALDI shares many features with FAB in that it employs a matrix which transfers energy to an analyte molecule to facilitate the ionization of polar and thermally labile, high-molecular-weight molecules. In this case, however, the energy is provided by a pulsed laser at a wavelength which may be absorbed by a matrix material such as nicotinic or sinapinic acids. The ability to obtain mass spectra from biomolecules using FAB depends as much on the performance of the mass spectrometer, in particular the range of m/z ratios that it can separate, as it does on the FAB system, the matrix and bombarding species used. The molecular weight limit for FAB is usually taken to be around 10 000 Da. MALDI extends the mass range of compounds that can be successfully ionized and detected to around 500 000 Da.

A major difference between MALDI and FAB is that a solid rather than a liquid matrix is used and a mixture of the analyte and matrix is dried on the laser target. For this reason, the effective combination of HPLC with MALDI is not as readily achieved although, since MALDI is largely free of the suppression effects experienced with FAB, it is able to provide useful analytical data directly from mixtures.

When a pulsed laser is used, ions are only produced for the duration of the pulse, i.e. they are not produced continuously and the mass spectrometer used must be capable of producing a mass spectrum from these 'pulses' of ions. As discussed below in Section 3.3.4, the time-of-flight (ToF) mass analyser is the most appropriate for this purpose and has the added advantage of being able to measure very high m/z ratios. Indeed, the recent dramatic developments in the performance of the ToF mass analyser have largely been occasioned by the requirement to produce useful spectra from MALDI.

3.2.5 Negative Ionization

For many years, electron ionization, then more usually known as electron impact, was the only ionization method used in analytical mass spectrometry and the spectra encountered showed exclusively the positively charged species produced during this process. Electron ionization also produces negatively charged ions although these are not usually of interest as they have almost no structural significance. Other ionization techniques, such as CI, FAB, thermospray, electrospray and APCI, however, can be made to yield negative ions which are of analytical utility.

These arise either by an analogous process to that described above for CI, i.e. the 'adduction' of a negatively charged species such as Cl^-, and the abstraction of a proton to generate an $(M - H)^-$ ion, or by electron attachment to generate an M^- ion. The ions observed in the mass spectrum are dependent on the species generated by the reagent gas and the relative reactivities of these with each other and with the analyte molecule.

Negative-ion CI tends to be a very selective method of ionization which generates reduced levels of background, thus allowing sensitive analyses to be developed for appropriate analytes.

3.3 Ion Separation

A mass spectrum may be considered to be a plot of the number of ions of each m/z ratio produced by an analyte upon ionization. Having produced the ions by

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Examination of the intensity of the $M + 2$ peak is also useful for obtaining information about elements which may be present in the molecular formula. An unusually intense $M + 2$ peak can indicate that sulfur or silicon is present in the unknown substance. The relative abundances of ^{34}S and ^{30}Si are 4.40 and 3.35, respectively. A trained chemist knows that a larger-than-normal $M + 2$ peak can be the first hint that sulfur is present.

8.6 SOME FRAGMENTATION PATTERNS

When a molecule has been bombarded by high-energy electrons in the ionization chamber of a mass spectrometer, besides losing one electron to form an ion, the molecule also absorbs some of the energy transferred in its collision with the incident electrons. This extra energy places the molecular ion in an excited vibrational state. The vibrationally excited molecular ion may be unstable, and 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 appears 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. In such cases, peaks corresponding to the mass-to-charge ratios for these fragments appear in the mass spectrum. For a given compound, not all of the molecular ions formed by ionization have precisely the same lifetime; some may have shorter lifetimes than others. As a result, in a spectrum one usually observes peaks corresponding to both the molecular ion and the fragments.

For most classes of compounds, the mode of fragmentation is somewhat characteristic and hence predictable. This section discusses some of the more important modes of fragmentation.

It is helpful to begin by describing some general principles that govern fragmentation processes. The ionization of the sample molecule forms a molecular ion that not only carries a positive charge but also has an unpaired electron. The molecular ion, then, is actually a radical-cation, and it contains an odd number of electrons.

When fragment ions form in the mass spectrometer, they almost always do so by means of unimolecular processes. The pressure of the sample in the ionization chamber is too low to permit a significant number of bimolecular collisions to occur. The unimolecular processes that are energetically most favorable give rise to the most fragment ions.

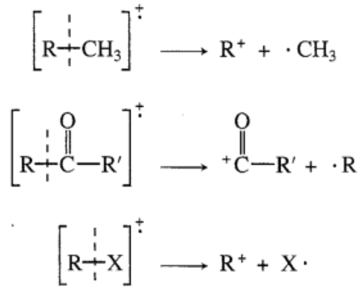
The fragment ions thus formed are cations. A great deal of their chemistry can be explained in terms of what is known about carbocations in solution. For example, alkyl substitution stabilizes fragment ions (and promotes their formation) in much the same way that it stabilizes carbocations. Fragmentation processes that lead to the formation of more stable ions are favored over processes that lead to less stable ions.

Often, fragmentation involves the loss of an electrically neutral fragment. This fragment 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.

The most common mode of fragmentation involves the cleavage of one bond. In this process, the odd-electron molecular ion yields an odd-electron neutral fragment and an even-electron fragment ion. The neutral fragment that is lost is a radical, and the ionic fragment is of the carbocation type. Cleavages which lead to the formation of more stable carbocations are favored. Thus, ease of fragmentation to form ions increases in the order

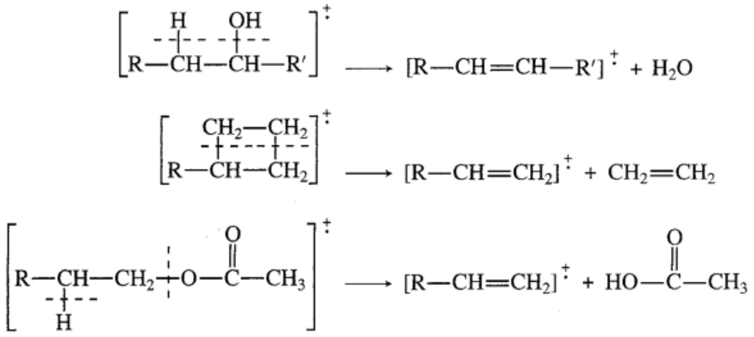
$$\underset{\text{DIFFICULT}}{CH_3^+} < RCH_2^+ < R_2CH^+ < R_3C^+ < CH_2{=}CH{-}CH_2^+ < \underset{\text{EASY}}{C_6H_5{-}CH_2^+}$$

Examples of fragmentation via the cleavage of one bond include the following.



X = halogen, OR, SR, or NR_2
where R = H, alkyl, or aryl

The next most important type of fragmentation involves the cleavage of two bonds. In this process, the odd-electron molecular ion yields an odd-electron fragment ion and an even-electron neutral fragment, usually a small molecule of some type. Examples of this type of cleavage include the following.



In addition to these processes, fragmentation processes involving rearrangements, migrations of groups, and secondary fragmentations of fragment ions are also possible. These latter modes of fragmentation occur less often than the two cases already described, and additional discussion of them will be reserved for the compounds in which they are important.

To assist you in identifying possible fragment ions, Appendix 12 provides a table that lists the molecular formulas for common fragments with masses less than 105. More complete tables may be found in the books by Beynon and by Silverstein and Webster listed in the references at the end of this chapter.

A. Alkanes

For saturated hydrocarbons and organic structures containing large saturated hydrocarbon skeletons, the methods of fragmentation are quite predictable. What is known about the stabilities of carbocations in solution can be used to help us understand the fragmentation patterns of alkanes. The mass spectra of alkanes are characterized by strong molecular ion peaks and a regular series of fragment ion peaks separated by 14 amu.

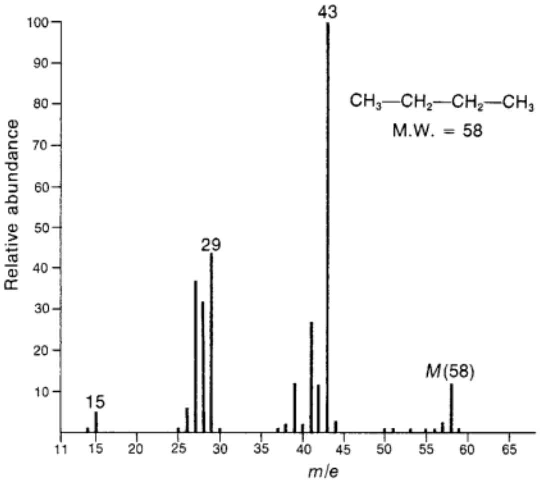


FIGURE 8.5 Mass spectrum of butane.

SPECTRAL ANALYSIS BOX—Alkanes

MOLECULAR ION	FRAGMENT IONS
Strong M^+	Loss of CH_2 units in a series: $M - 14$, $M - 28$, $M - 42$, etc.

For a straight-chain, or "normal," alkane, a peak corresponding to the molecular ion can be observed, as in the mass spectra of butane (Fig. 8.5) and octane (Fig. 8.6). As the carbon skeleton becomes more highly branched, the intensity of the molecular ion peak decreases. You will see this effect easily if you compare the mass spectrum of butane with that of isobutane (Fig. 8.7). The mol-

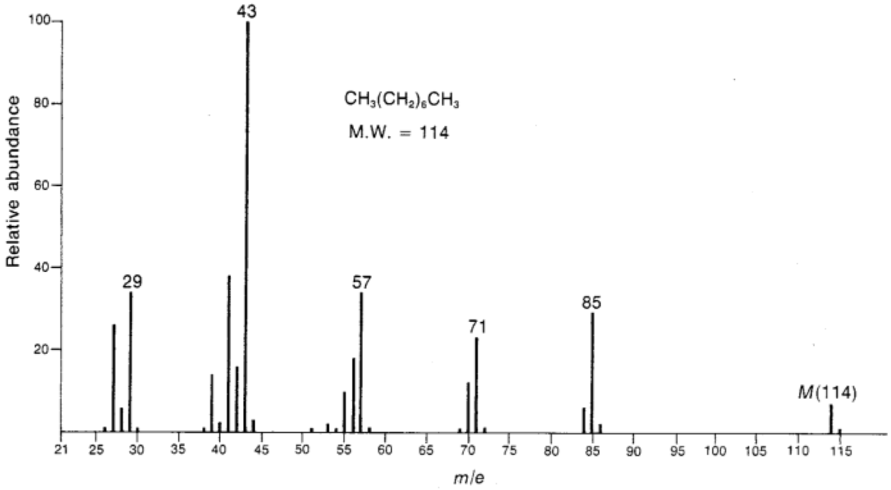


FIGURE 8.6 Mass spectrum of octane.

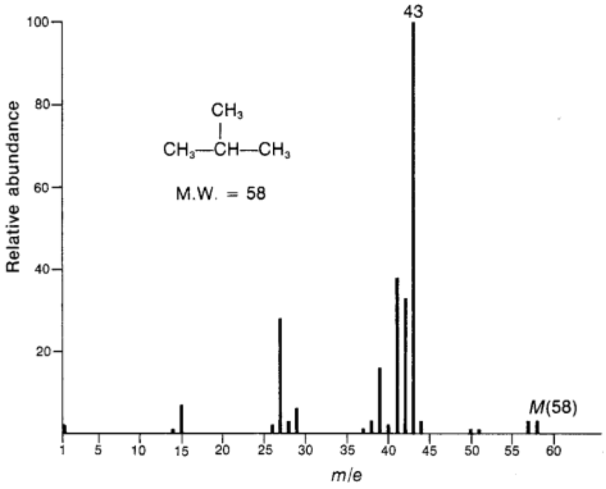


FIGURE 8.7 Mass spectrum of isobutane.

ecular ion peak in isobutane is much less intense than that in butane. Comparison of the mass spectra of octane and 2,2,4-trimethylpentane (Fig. 8.8) provides a more dramatic illustration of the effect of chain branching on the intensity of the molecular ion peak. The molecular ion peak in 2,2,4-trimethylpentane is too weak to be observed, while the molecular ion peak in its straight-chain isomer is quite readily observed.

The effect of chain branching on the intensity of the molecular ion peak can be understood by examining the method by which hydrocarbons undergo fragmentation. Straight-chain hydrocarbons appear to undergo fragmentation by breaking carbon–carbon bonds, resulting in a homologous series of fragmentation products. For example, in the case of butane, cleavage of the C1-to-C2 bond results in the loss of a methyl radical and the formation of the propyl carbocation

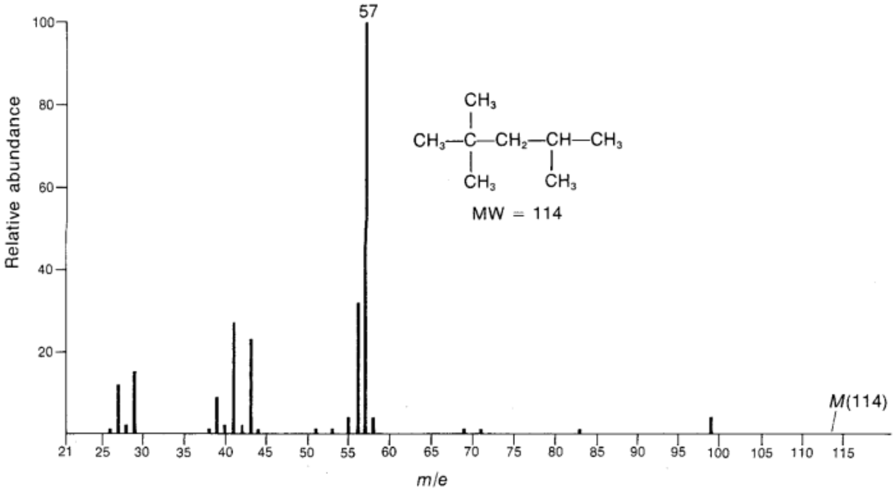
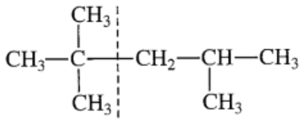


FIGURE 8.8 Mass spectrum of 2,2,4-trimethylpentane ("isooctane").

(*m*/*e* = 43). Cleavage of the C2-to-C3 bond results in the loss of an ethyl radical and the formation of the ethyl carbocation (*m*/*e* = 29). In the case of octane, fragment peaks due to the hexyl ion (*m*/*e* = 85), the pentyl ion (*m*/*e* = 71), the butyl ion (*m*/*e* = 57), the propyl ion (*m*/*e* = 43), and the ethyl ion (*m*/*e* = 29) are observed. Notice that alkanes fragment to form clusters of peaks that are 14 mass units (corresponding to one CH_2 group) apart from each other. Other fragments within each cluster correspond to additional losses of one or two hydrogen atoms. As is evident in the mass spectrum of octane, the three-carbon ions appear to be the most abundant, with the intensities of each cluster uniformly decreasing with increasing fragment weight. Interestingly, for long-chain alkanes, the fragment corresponding to the loss of one carbon atom is generally absent. In the mass spectrum of octane, a seven-carbon fragment should occur at a mass of 99, but it is not observed. Straight-chain alkanes have fragments that are always primary carbocations. Since these ions are rather unstable, fragmentation is not favored. A significant number of the original molecules survive electron bombardment without fragmenting. Consequently, a molecular ion peak of significant intensity is observed.

Cleavage of the carbon–carbon bonds of branched-chain alkanes can lead to secondary or tertiary carbocations. These ions are more stable than primary ions, so fragmentation becomes a more favorable process. A greater proportion of the original molecules undergo fragmentation, so the molecular ion peaks of branched-chain alkanes are considerably weaker or even absent. In isobutane, cleavage of a carbon–carbon bond yields an isopropyl carbocation, which is more stable than a normal propyl ion. Isobutane undergoes fragmentation more easily than butane because of the increased stability of its fragmentation products. With 2,2,4-trimethylpentane, the cleavage shown leads to the formation of a *tert*-butyl carbocation. Since tertiary carbocations are the most stable of the saturated alkyl carbocations, this cleavage is particularly favorable and accounts for the intense fragment peak at *m*/*e* = 57.



$$\mathrm{CH_3-\overset{CH_3}{\underset{CH_3}{C}}-CH_2-\underset{CH_3}{CH}-CH_3}$$

B. Cycloalkanes

Cycloalkanes generally form strong molecular ion peaks. Fragmentation via the loss of a molecule of ethene (*M*–28) is common.

SPECTRAL ANALYSIS BOX — Cycloalkanes

MOLECULAR ION	**FRAGMENT IONS**
Strong M^+	$M - 28$ A series of peaks: $M - 15$, $M - 29$, $M - 43$, $M - 57$, etc.

The typical mass spectrum for a cycloalkane shows a relatively intense molecular ion peak. Fragmentation of ring compounds requires the cleavage of two carbon–carbon bonds, which is a more difficult process than cleavage of one such bond. Therefore, a larger proportion of cycloalkane molecules than of acyclic alkane molecules survive electron bombardment without undergoing fragmentation. In the mass spectra of cyclopentane (Fig. 8.9) and methylcyclopentane (Fig. 8.10), strong molecular ion peaks can be observed.

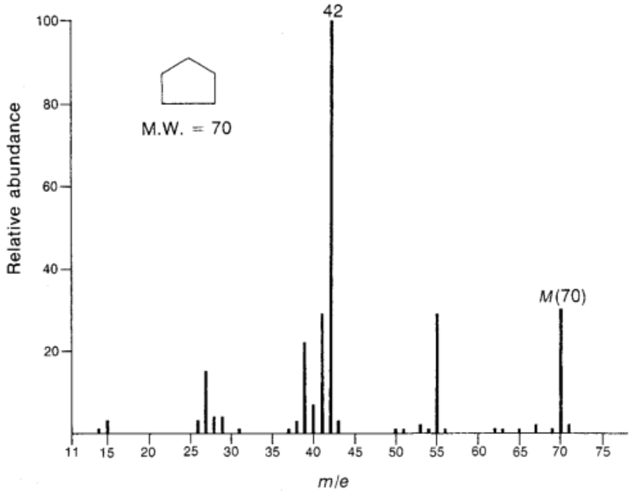


FIGURE 8.9 Mass spectrum of cyclopentane.

The fragmentation patterns of cycloalkanes may show mass clusters arranged in a homologous series, as in the alkanes. However, the most significant mode of cleavage of the cycloalkanes involves the loss of a molecule of ethene, either from the parent molecule or from intermediate radical-ions. The peak at *m/e* = 42 in cyclopentane and the peak at *m/e* = 56 in methylcyclopentane result from the loss of ethene from the parent molecule. Each of these fragment peaks is the most intense in the mass spectrum.

When the cycloalkane bears a side chain, loss of that side chain is a favorable mode of fragmentation. The fragment peak at *m/e* = 69 in the mass spectrum of methylcyclopentane is due to the loss of the CH_3 side chain. A secondary carbocation results from the loss of the methyl group.

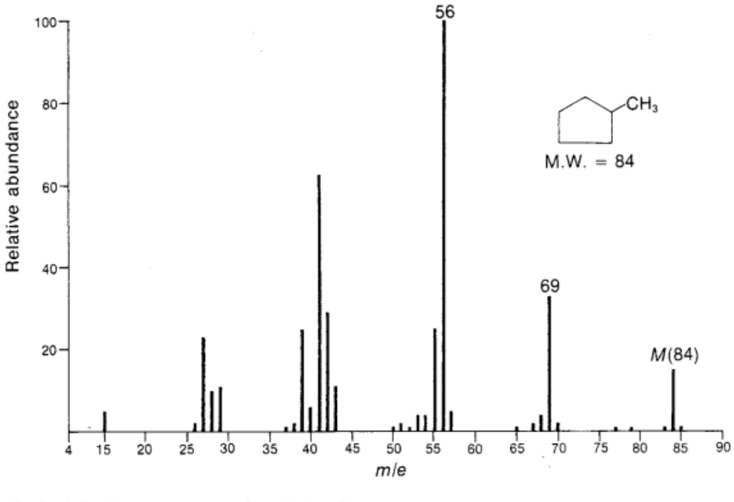


FIGURE 8.10 Mass spectrum of methylcyclopentane.

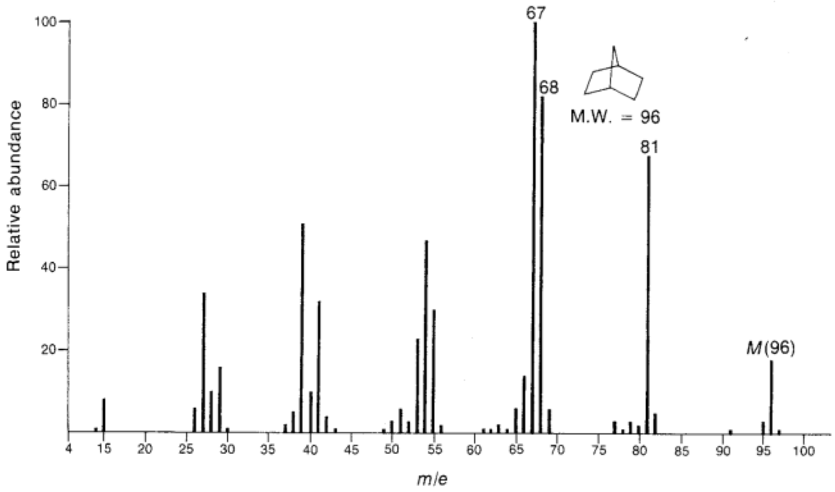


FIGURE 8.11 Mass spectrum of bicyclo[2.2.1]heptane (norbornane).

Applying these pieces of information to the mass spectrum of bicyclo[2.2.1]heptane (Fig. 8.11), we can identify fragment peaks due to the loss of the side chain (the one-carbon bridge, plus an additional hydrogen atom) at $m/e = 81$ and the loss of ethene at $m/e = 68$. The fragment ion peak at $m/e = 67$ is due to the loss of ethene plus an additional hydrogen atom.

C. Alkenes

The mass spectra of most alkenes show distinct molecular ion peaks. Apparently, electron bombardment removes one of the electrons in the π bond, leaving the carbon skeleton relatively undisturbed. Fragmentation to form an allyl cation ($m/e = 41$) is favored.

SPECTRAL ANALYSIS BOX—Alkenes

MOLECULAR ION	**FRAGMENT IONS**
Strong M^+	$m/e = 41$
	A series of peaks: $M - 15$, $M - 29$, $M - 43$, $M - 57$, etc.

The double bond of an alkene is capable of absorbing substantial energy. As a result, the mass spectra of alkenes generally show a strong molecular ion peak. A characteristic of the mass spectra of alkenes is that the mass of the molecular ion should correspond to a molecular formula with an index of hydrogen deficiency equal to at least *one* (see Chapter 1).

When alkenes undergo fragmentation processes, the resulting fragment ions have formulas corresponding to $C_nH_{2n}^+$ and $C_nH_{2n-1}^+$. It is very difficult to locate double bonds in alkenes, since they migrate readily. The similarity of the mass spectra of alkene isomers is readily seen in the mass spectra of two isomers of the formula C_4H_8 (Figs. 8.12 and 8.13). The mass spectra are very nearly identical. Furthermore, *cis*- and *trans*-isomers produce essentially identical mass spectra.

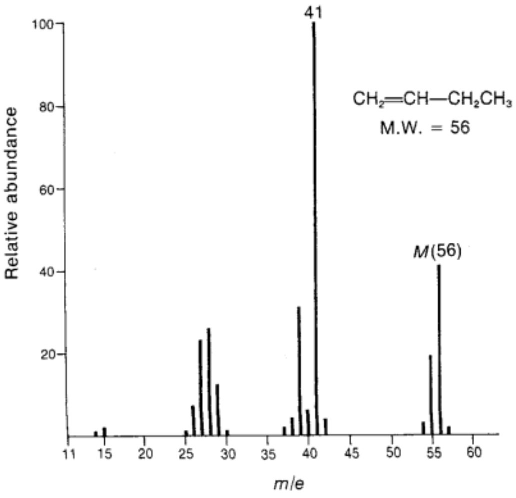
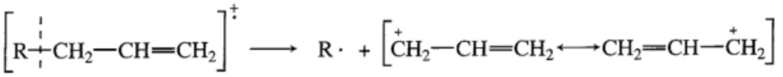


FIGURE 8.12 Mass spectrum of 1-butene.

An important fragment in the mass spectra of terminal alkenes, the allyl carbocation, occurs at an *m/e* value of 41. Its formation is due to cleavage of the type



$$\left[R \vdots CH_2—CH{=}CH_2\right]^{+\cdot} \longrightarrow R\cdot + \left[\overset{+}{C}H_2—CH{=}CH_2 \longleftrightarrow CH_2{=}CH—\overset{+}{C}H_2\right]$$

The mass spectra of cycloalkenes show quite distinct molecular ion peaks. For many cycloalkenes, migration of bonds gives virtually identical mass spectra. Consequently, it may be impossible to locate the position of the double bond in a cycloalkene, particularly a cyclopentene or a cycloheptene.

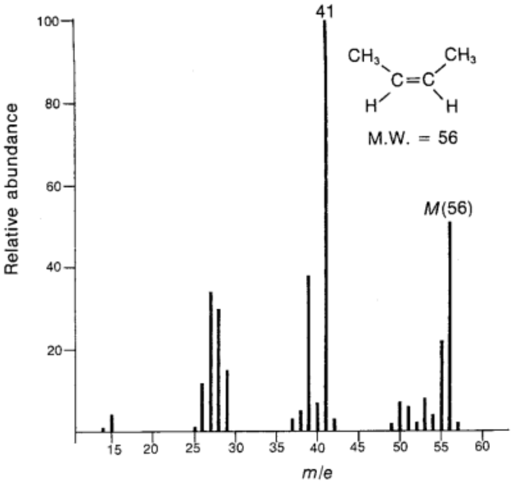


FIGURE 8.13 Mass spectrum of *cis*-2-butene.

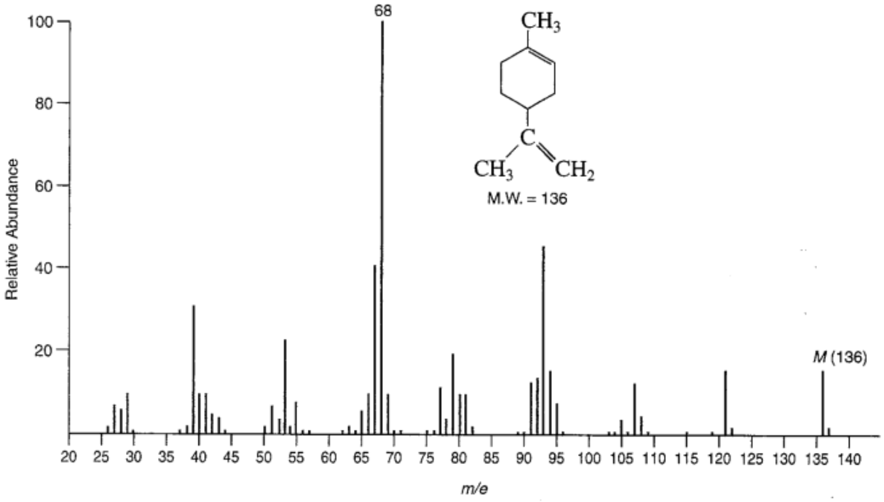
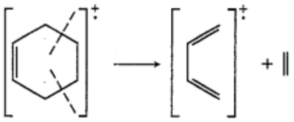
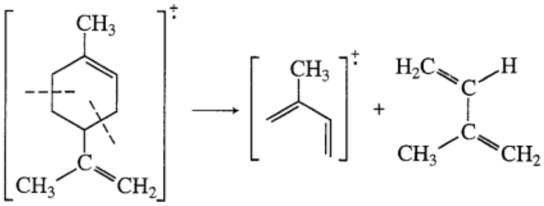


FIGURE 8.14 Mass spectrum of limonene.

Cyclohexenes do have a characteristic fragmentation pattern that corresponds to a reverse Diels–Alder reaction. This cleavage can be illustrated as



In the mass spectrum of limonene (Fig. 8.14), the intense peak at $m/e = 68$ corresponds to the diene fragment arising from the type of cleavage just described.



D. Alkynes

The mass spectra of alkynes are very similar to those of alkenes. The molecular ion peaks tend to be rather intense, and fragmentation patterns parallel those of the alkenes.

SPECTRAL ANALYSIS BOX—Alkynes

MOLECULAR ION	FRAGMENT IONS
Strong M^+	$m/e = 39$ Strong $M - 1$ peak

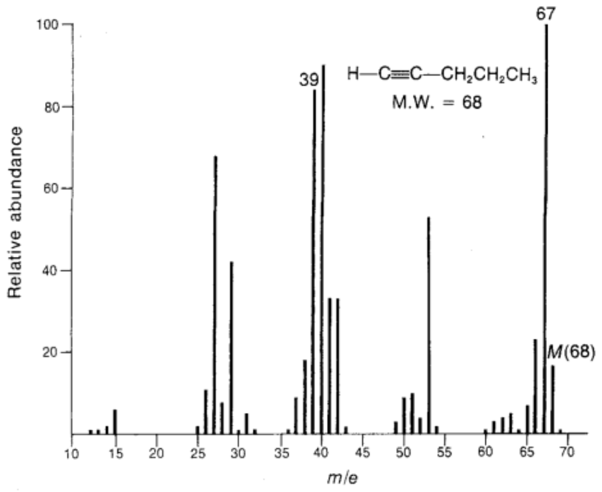
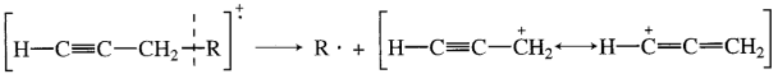


FIGURE 8.15 Mass spectrum of 1-pentyne.

As can be seen from the mass spectrum of 1-pentyne (Fig. 8.15), fragmentation of the type



$$\left[\mathrm{H{-}C{\equiv}C{-}CH_2 \vdots R}\right]^{+\cdot} \longrightarrow \mathrm{R}\cdot + \left[\mathrm{H{-}C{\equiv}C{-}\overset{+}{C}H_2} \longleftrightarrow \mathrm{H{-}\overset{+}{C}{=}C{=}CH_2}\right]$$

to yield the propargyl ion ($m/e = 39$) is important. The formation of the propargyl ion from alkynes is not as important as the formation of the allyl ions from alkenes, since the allyl ion is more stable than the propargyl ion.

Another important mode of fragmentation for terminal alkynes is the loss of the terminal hydrogen, yielding a strong $M - 1$ peak. This peak appears as the base peak ($m/e = 67$) in the spectrum of 1-pentyne.

$$\left[\mathrm{H \vdots C{\equiv}C{-}R}\right]^{+\cdot} \longrightarrow \mathrm{H}\cdot + {}^{+}\mathrm{C{\equiv}C{-}R}$$

E. Aromatic Hydrocarbons

The mass spectra of most aromatic hydrocarbons show very intense molecular ion peaks. As is evident from the mass spectrum of benzene (Fig. 8.16), fragmentation of the benzene ring requires a great deal of energy. Such fragmentation is not observed to any significant extent. When a benzene ring contains side chains, however, a favored mode of fragmentation is cleavage of the side chain to form a **benzyl cation**, which spontaneously rearranges to a **tropylium ion**. When the side chain attached to a benzene ring contains three or more carbons, ions formed by a **McLafferty rearrangement** can be observed.

SPECTRAL ANALYSIS BOX—Aromatic Hydrocarbons

MOLECULAR ION	FRAGMENT IONS
Strong M^+	$m/e = 91$
	$m/e = 92$

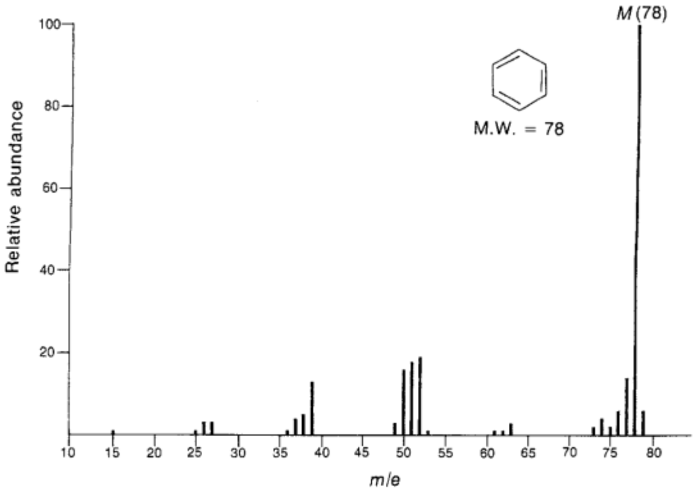


FIGURE 8.16 Mass spectrum of benzene.

When an alkyl group is attached to a benzene ring, preferential fragmentation occurs at a benzylic position to form a fragment ion of the formula $C_7H_7^+$ ($m/e = 91$). In the mass spectrum of toluene (Fig. 8.17), loss of hydrogen from the molecular ion gives a strong peak at $m/e = 91$. Although it might be expected that this fragment ion peak is due to the benzyl carbocation, evidence has accumulated that suggests that the benzyl carbocation actually rearranges to form the **tropylium ion.** Isotope-labeling experiments tend to confirm the formation of the tropylium ion.

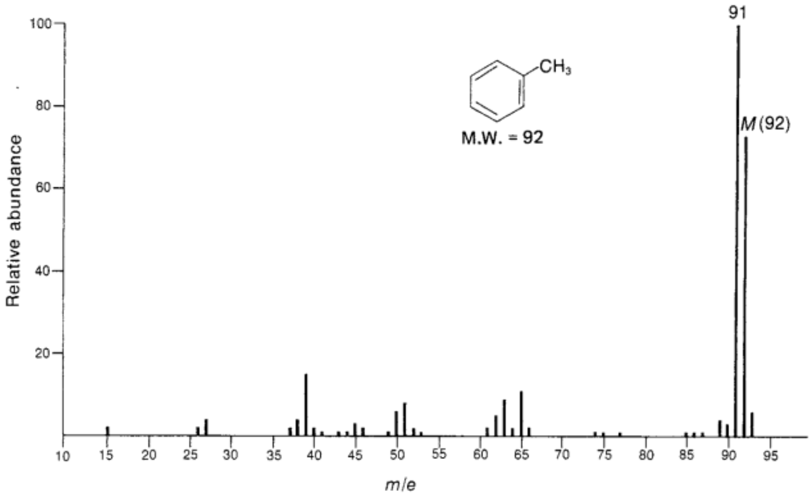


FIGURE 8.17 Mass spectrum of toluene.

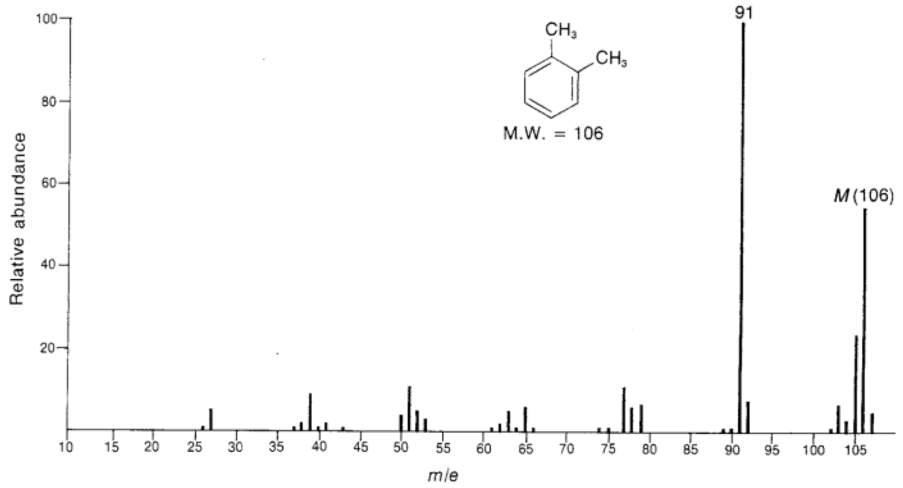
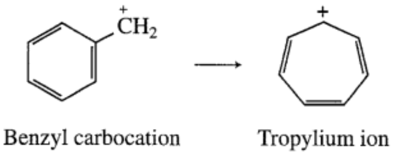
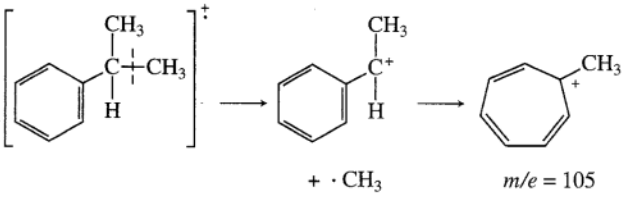


FIGURE 8.18 Mass spectrum of *ortho*-xylene.



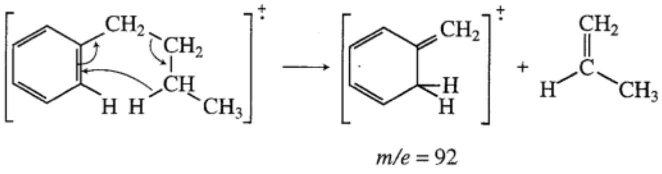
The mass spectra of the xylene isomers (see Fig. 8.18 for an example) show a medium peak at *m/e* = 105, which is due to the methyltropylium ion. More importantly, xylene loses one methyl group to form the unsubstituted $C_7H_7^+$ ion (*m/e* = 91). The mass spectra of *ortho*-, *meta*-, and *para*-disubstituted aromatic rings are essentially identical. As a result, the positions of substitution of polyalkyl-substituted benzenes cannot be determined by mass spectrometry.

The formation of a substituted tropylium ion is typical for alkyl-substituted benzenes. In the mass spectrum of isopropylbenzene (Fig. 8.19), a strong peak appears at *m/e* = 105. This peak corresponds to loss of a methyl group to form a methyl-substituted tropylium ion.



Again in the mass spectrum of propylbenzene (Fig. 8.20), a strong peak due to the tropylium ion appears at *m/e* = 91.

When the alkyl group attached to the benzene ring is a propyl group or larger, an important type of rearrangement, called the **McLafferty rearrangement,** occurs. Using butylbenzene as an example, this arrangement may be depicted as



and leads to a peak at *m/e* = 92. This peak also appears in the mass spectrum of propylbenzene.

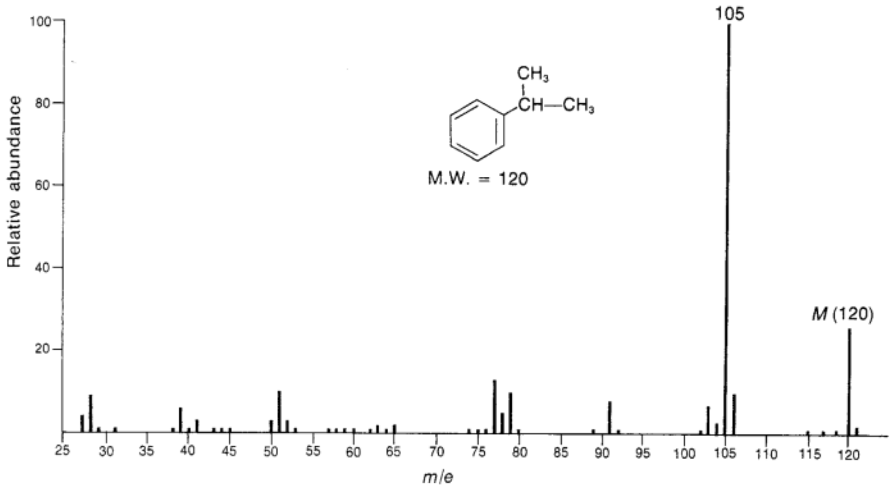


FIGURE 8.19 Mass spectrum of isopropylbenzene (cumene).

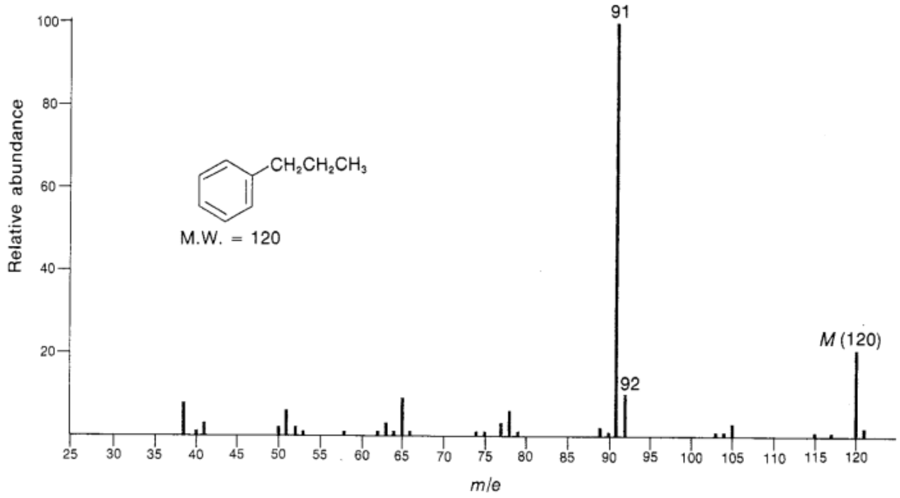


FIGURE 8.20 Mass spectrum of propylbenzene.

F. Alcohols and Phenols

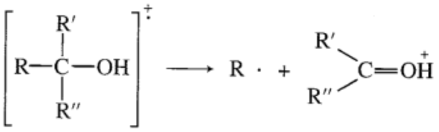
The intensity of the molecular ion peak in the mass spectrum of a primary or secondary alcohol is usually rather low. The molecular ion peak may be entirely absent in the mass spectrum of a tertiary alcohol. Fragmentation involves the loss of an alkyl group or the loss of a molecule of water.

SPECTRAL ANALYSIS BOX—Alcohols

MOLECULAR ION	**FRAGMENT IONS**
M^+ weak or absent	Loss of alkyl group $M - 18$

The mass spectrum of 1-butanol (Fig. 8.21) shows a very weak molecular ion peak at *m/e* = 74, while the mass spectrum of 2-butanol (Fig. 8.22) has a molecular ion peak (*m/e* = 74) that is too weak to be detected. The molecular ion peak for tertiary alcohol, 2-methyl-2-propanol (Fig. 8.23), is entirely absent.

The most important fragmentation reaction for alcohols is the loss of an alkyl group:



$$\left[R-\overset{R'}{\underset{R''}{C}}-OH \right]^{+\cdot} \longrightarrow R\cdot + \begin{matrix} R' \\ R'' \end{matrix}\!\!>C=\overset{+}{O}H$$

The largest alkyl group is most readily lost. In the spectrum of 1-butanol (Fig. 8.21), the intense peak at *m/e* = 31 is due to the loss of a propyl group to form an $H_2C{=}OH^+$ ion. 2-Butanol (Fig. 8.22) loses an ethyl group to form the $CH_3CH{=}OH^+$ fragment at *m/e* = 45. 2-Methyl-2-propanol (Fig. 8.23) loses a methyl group to form the $(CH_3)_2C{=}OH^+$ fragment at *m/e* = 59.

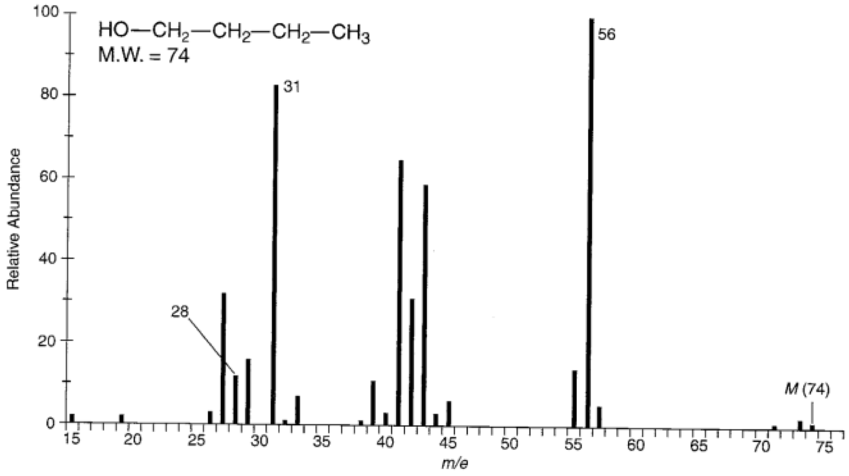


FIGURE 8.21 Mass spectrum of 1-butanol.

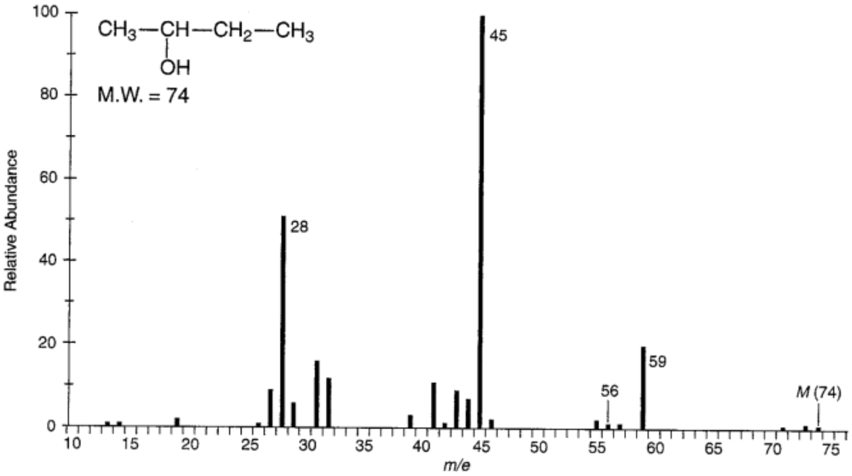


FIGURE 8.22 Mass spectrum of 2-butanol.

A second common mode of fragmentation involves dehydration. The importance of dehydration increases as the chain length of the alcohol increases. While the fragment ion peak resulting from dehydration (*m/e* = 56) is very intense in 1-butanol, it is very weak in the other butanol isomers. However, in the mass spectra of the five-carbon alcohols, this peak due to dehydration of the molecular ion is quite important.

Dehydration may occur by either of two mechanisms. The hot surfaces of the inlet system may stimulate dehydration of the alcohol molecule before the molecule comes in contact with the electrons. In this case, the dehydration is a **1,2-elimination** of water. However, the molecular ion, once it is formed, may also lose water. In that case the dehydration is a **1,4-elimination** of water via a cyclic mechanism:

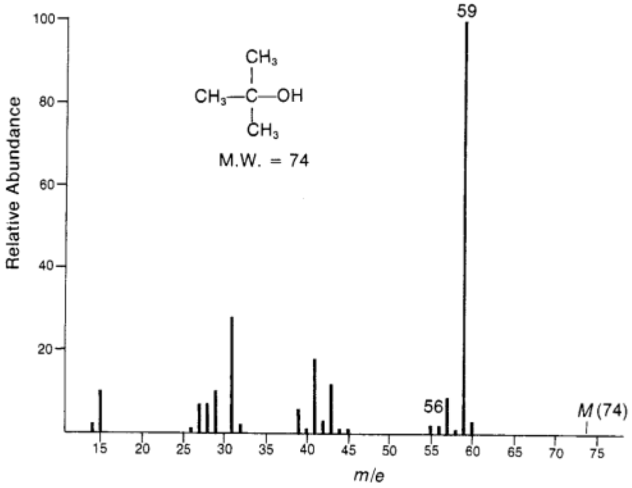
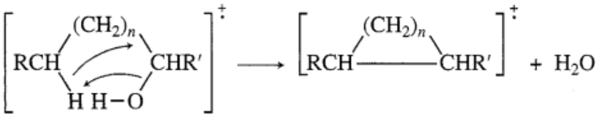
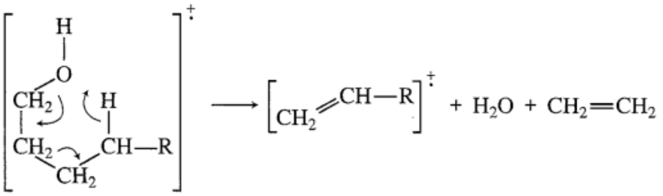


FIGURE 8.23 Mass spectrum of 2-methyl-2-propanol.



n = 1 or 2

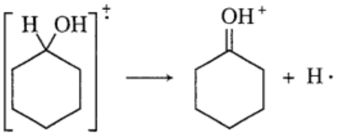
Alcohols containing four or more carbons may undergo the simultaneous loss of both water and ethylene:



In the case of 1-butanol, this fragment ion is responsible for a rather weak peak at $m/e = 28$. However, with 1-pentanol, the corresponding peak is the most intense in the spectrum.

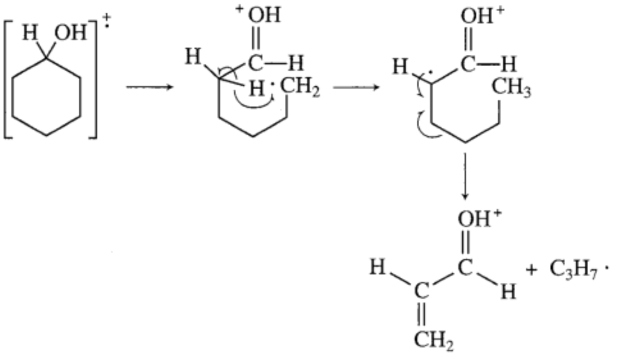
Cyclic alcohols may undergo fragmentation by at least three different pathways:

(1)



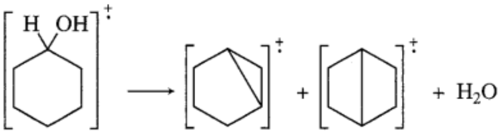
$m/e = 99$

(2)



$m/e = 57$

(3)



$m/e = 82$

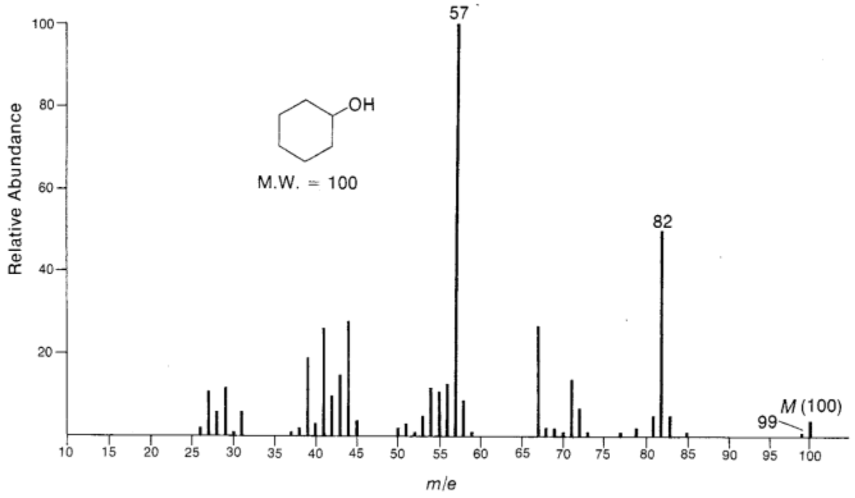
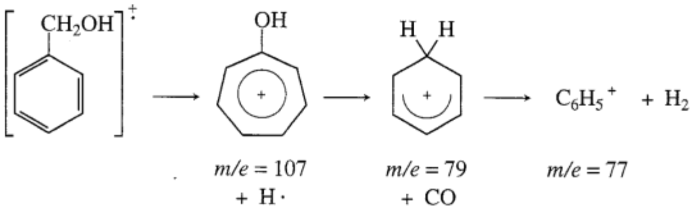


FIGURE 8.24 Mass spectrum of cyclohexanol.

A peak corresponding to each of these fragment ions can be observed in the mass spectrum of cyclohexanol (Fig. 8.24).

Benzylic alcohols exhibit intense molecular ion peaks. The following sequence of reactions illustrates their principal modes of fragmentation.



Peaks arising from these fragment ions can be observed in the mass spectrum of benzyl alcohol (Fig. 8.25).

The mass spectra of phenols show strong molecular ion peaks. Favored modes of fragmentation involve loss of a hydrogen atom, loss of a molecular of carbon monoxide, and loss of a formyl radical.

SPECTRAL ANALYSIS BOX—Phenols

MOLECULAR ION	FRAGMENT IONS
M^+ strong	$M - 1$
	$M - 28$
	$M - 29$

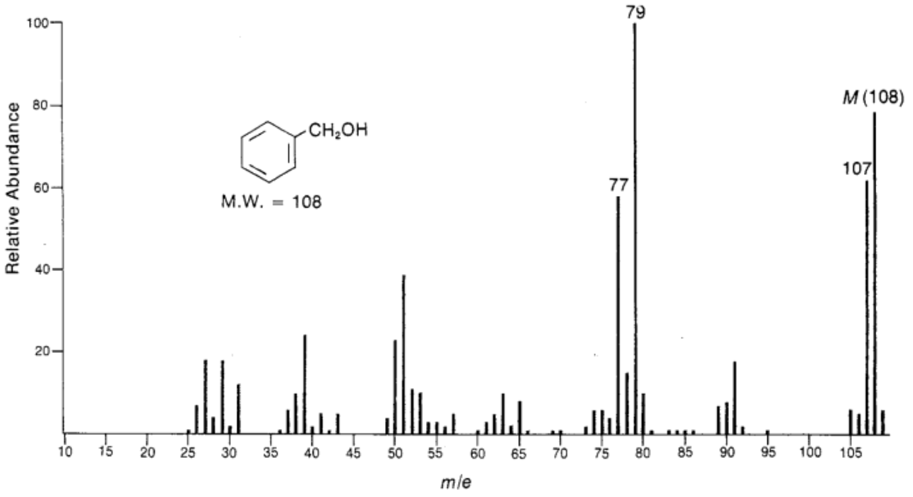


FIGURE 8.25 Mass spectrum of benzyl alcohol.

Phenols typically lose the elements of carbon monoxide to give strong peaks at *m*/*e* values that are 28 mass units below the value for the molecular ion. Such a peak is designated an $M - 28$ peak due to the relationship between its *m*/*e* value and the *m*/*e* value of the molecular ion. Phenols also lose the elements of the formyl radical (HCO·) to give strong $M - 29$ peaks (the fragment ion appears 29 mass units below the molecular ion). An exception appears to be the cresols, for which these fragment peaks are rather weak. Nevertheless, the $M - 28$ peak (*m*/*e* = 80) and the $M - 29$ peak (*m*/*e* = 79) appear in the mass spectrum of 2-methylphenol (Fig. 8.26).

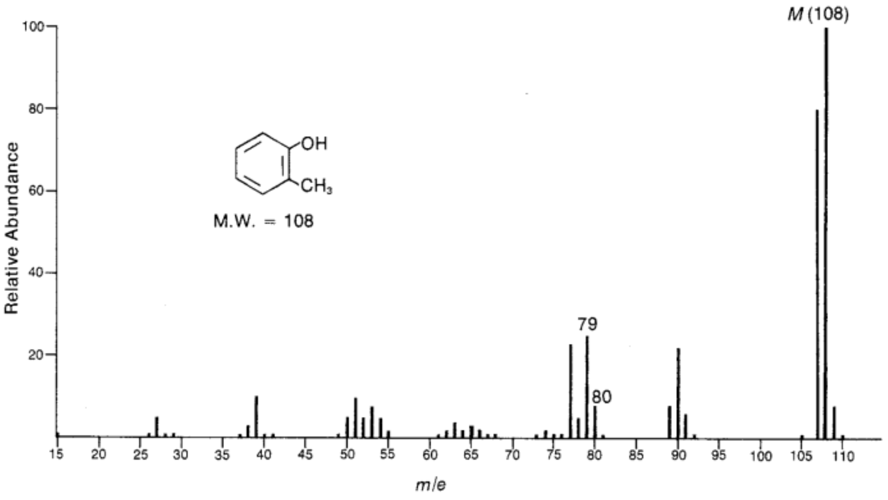


FIGURE 8.26 Mass spectrum of 2-methylphenol (*ortho*-cresol).

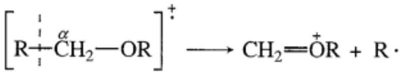
G. Ethers

Aliphatic ethers tend to exhibit molecular ion peaks that are stronger than those of alcohols with the same molecular weights. Nevertheless, the molecular ion peaks of ethers are rather weak. Principal modes of fragmentation include α-cleavage, formation of carbocation fragments, and loss of an alkoxy group.

SPECTRAL ANALYSIS BOX — Ethers

MOLECULAR ION	FRAGMENT IONS
M^+ weak, but observable	α-Cleavage
	$m/e = 43, 59, 73$, etc.
	$M - 31, M - 45, M - 59$, etc.

The fragmentation of the ethers is somewhat similar to that of the alcohols. The carbon–carbon bond to the α carbon may be broken to yield a fragment ion that bears a positive charge on the oxygen.



$$\left[R \,\vdots\, \overset{\alpha}{C}H_2-OR\right]^{+\cdot} \longrightarrow CH_2=\overset{+}{O}R + R\cdot$$

In the mass spectrum of diisopropyl ether (Fig. 8.27), this fragmentation gives rise to a peak at $m/e = 87$, due to the loss of a methyl group.

A second mode of fragmentation involves cleavage of the carbon–oxygen bond of an ether to yield a carbocation. Cleavage of this type in diisopropyl ether is responsible for the $C_3H_7^+$ fragment at $m/e = 43$.

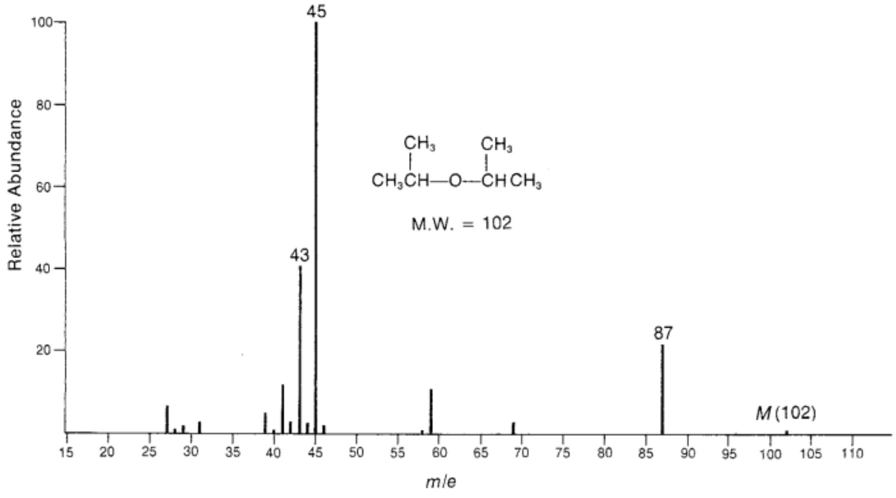
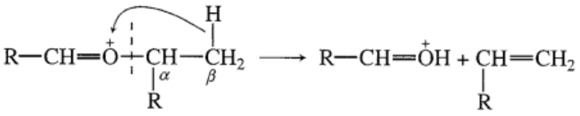


FIGURE 8.27 Mass spectrum of diisopropyl ether.

A third type of fragmentation occurs as a rearrangement reaction taking place on one of the fragment ions, rather than on the molecular ion itself. The rearrangement may be illustrated as follows.



This type of rearrangement is particularly favored when the α carbon of the ether is branched. In the case of diisopropyl ether, this rearrangement gives rise to a $C_2H_5O^+$ fragment ($m/e = 45$).

As a result of these fragmentation processes, the fragment ion peaks of ethers often fall into the series 31, 45, 59, 73, and so on. These fragments are of either the RO^+ or the $ROCH_2{}^+$ type.

Acetals and ketals behave very similarly to ethers. However, fragmentation is even more favorable in acetals and ketals than in ethers, so the molecular ion peak of an acetal or ketal may be either extremely weak or totally absent.

Aromatic ethers may undergo cleavage reactions that involve loss of the alkyl group to form $C_6H_5O^+$ ions. These fragment ions then lose the elements of carbon monoxide to form $C_5H_5{}^+$ ions. In addition, an aromatic ether may lose the entire alkoxy group to yield ions of the types $C_6H_6{}^+$ and $C_6H_5{}^+$.

H. Aldehydes

The molecular ion peak of an aliphatic aldehyde is usually observable, although at times it may be fairly weak. Principal modes of fragmentation include α-cleavage and β-cleavage. If the carbon chain attached to the carbonyl group contains at least three carbons, McLafferty rearrangement is also observed.

SPECTRAL ANALYSIS BOX—Aldehydes

MOLECULAR ION	**FRAGMENT IONS**
M^+ weak, but observable (aliphatic)	Aliphatic:
M^+ strong (aromatic)	$m/e = 29$
	$M - 29$
	$M - 43$
	$m/e = 44$
	Aromatic:
	$M - 1$
	$M - 29$

Cleavage of one of the two bonds to the carbonyl group, sometimes called **α-cleavage,** occurs very commonly. It may be outlined as follows.

$$[R{-}CHO]^{+\cdot} \longrightarrow R{-}C{\equiv}O^+ + H\cdot$$

$$[R{-}CHO]^{+\cdot} \longrightarrow H{-}C{\equiv}O^+ + R\cdot$$

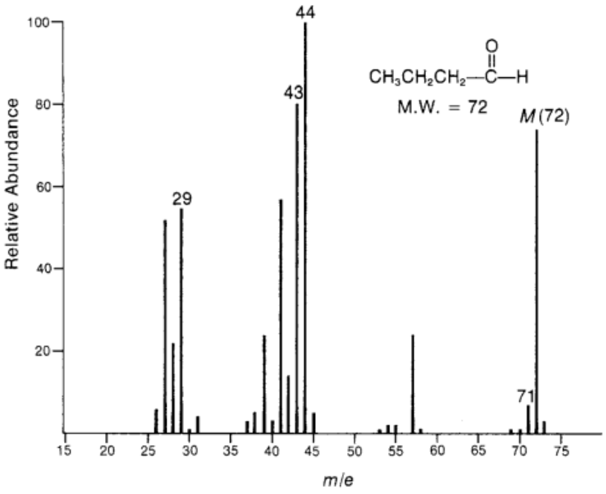


FIGURE 8.28 Mass spectrum of butyraldehyde.

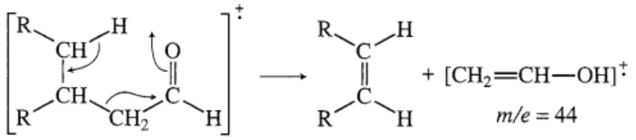
The peak due to the loss of one hydrogen atom is very characteristic of aldehydes. This peak is observed at *m/e* = 71 in the mass spectrum of butyraldehyde (Fig. 8.28). The peak due to the formation of HCO^+ can be observed at *m/e* = 29; this is also a very characteristic peak in the mass spectra of aldehydes.

The second important mode of fragmentation for aldehydes is known as **β-cleavage.**

$$\left[R \vdots CH_2-CHO\right]^{+\cdot} \longrightarrow R^+ + CH_2=CH-O\cdot$$

Coincidentally, the R^+ fragment ion also occurs at *m/e* = 29 in the mass spectrum of butyraldehyde. However, in higher aldehydes it occurs at higher *m/e* values. At any rate, the mass of this peak is 43 mass units less than that of the molecular ion.

The third major fragmentation pathway for aldehydes is the McLafferty rearrangement (see Section 8.6E).



The fragment ion formed in this rearrangement has *m/e* = 44. A peak at *m/e* = 44 is considered to be quite characteristic of the mass spectra of aldehydes. It is the most intense peak in the mass spectrum of butyraldehyde. Note that this rearrangement occurs only if the chain attached to the carbonyl group has three or more carbons.

Aromatic aldehydes exhibit intense molecular ion peaks. The loss of one hydrogen atom via *α*-cleavage is a very favorable process. The resulting $M-1$ peak may be more intense than the molecular ion peak. In the mass spectrum of benzaldehyde (Fig. 8.29), the $M-1$ peak appears at *m/e* = 105. You may also notice a peak at *m/e* = 77, which is due to the loss of the −CHO group to give $C_6H_5^+$.

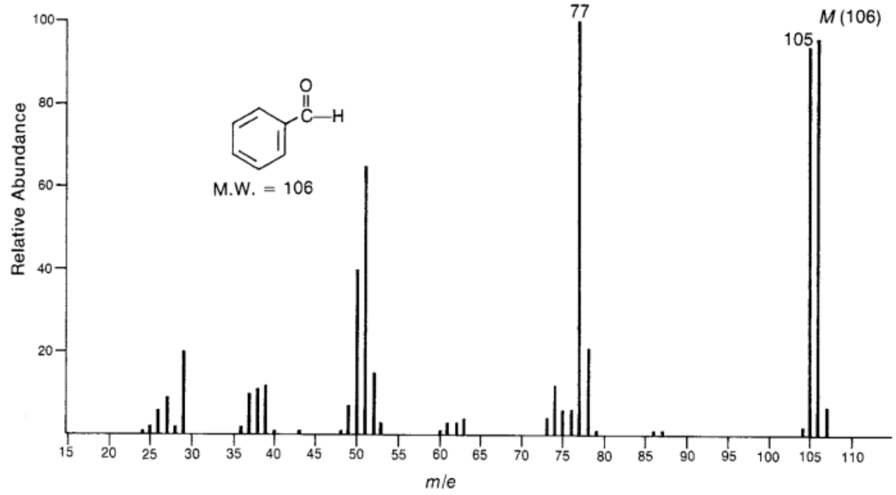


FIGURE 8.29 Mass spectrum of benzaldehyde.

I. Ketones

The mass spectra of ketones show an intense molecular ion peak. Loss of the alkyl groups attached to the carbonyl group is one of the most important fragmentation processes. The pattern of fragmentation is similar to that of aldehydes.

SPECTRAL ANALYSIS BOX—Ketones

MOLECULAR ION	**FRAGMENT IONS**
M^+ strong	Aliphatic: $M - 15$, $M - 29$, $M - 43$, etc. $m/e = 43$ $m/e = 58, 72, 86$, etc. $m/e = 42, 83$ Aromatic: $m/e = 105, 120$

Loss of alkyl groups by means of α-cleavage is an important mode of fragmentation. The larger of the two alkyl groups attached to the carbonyl group appears more likely to be lost. In the mass spectrum of 2-butanone (Fig. 8.30), the peak at $m/e = 43$, due to the loss of the ethyl group, is more intense than the peak at $m/e = 57$, which is due to the loss of the methyl group. Similarly, in the mass spectrum of 2-octanone (Fig. 8.31), loss of the hexyl group, giving a peak at $m/e = 43$, is more important than loss of the methyl group, which gives the weak peak at $m/e = 113$.

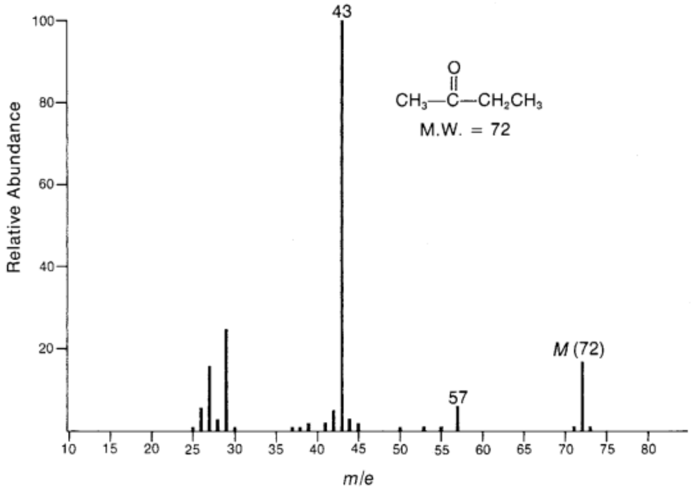


FIGURE 8.30 Mass spectrum of 2-butanone.

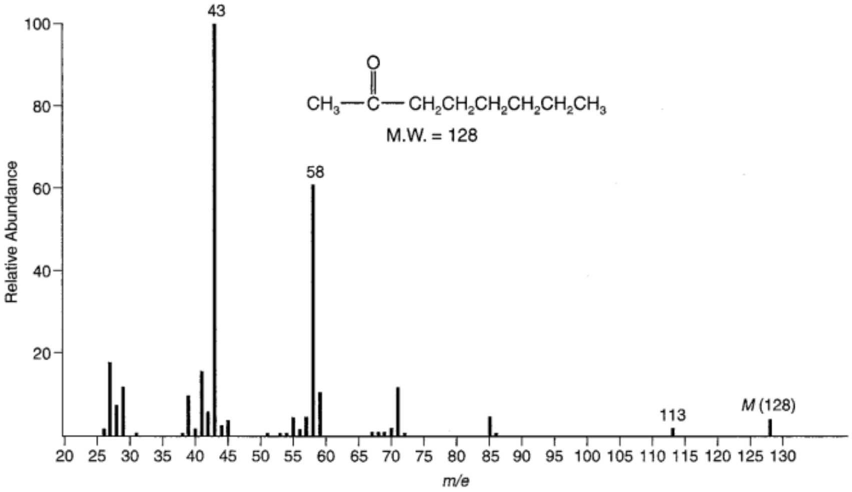
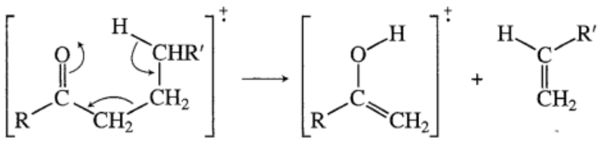


FIGURE 8.31 Mass spectrum of 2-octanone.

When the carbonyl group of a ketone has attached to it at least one alkyl group that is three or more carbon atoms in length, a McLafferty rearrangement is possible. It may be described as follows.



The peak at *m*/*e* = 58 in the mass spectrum of 2-octanone is due to the fragment ion that results from this rearrangement.

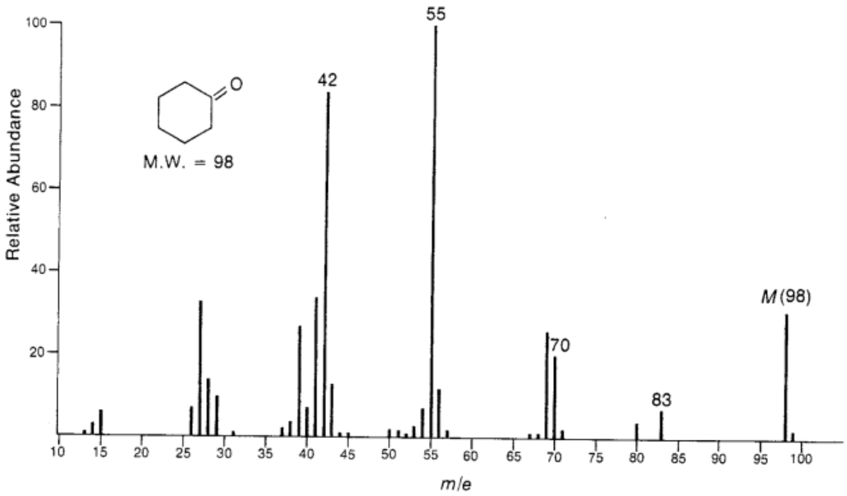
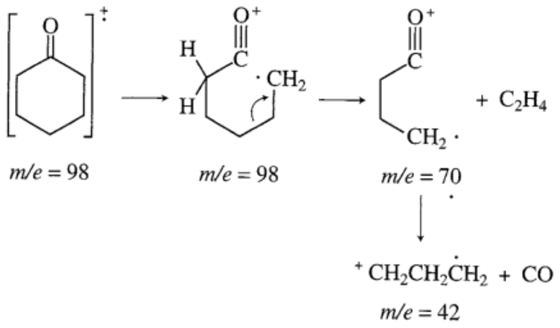
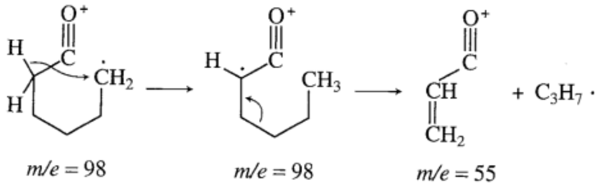
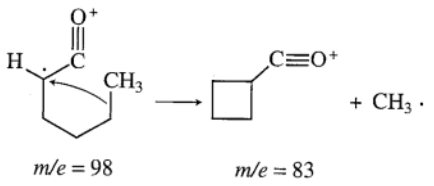


FIGURE 8.32 Mass spectrum of cyclohexanone.

Cyclic ketones may undergo a variety of fragmentation and rearrangement processes. Outlines of these processes for the case of cyclohexanone follow. A fragment ion peak corresponding to each process appears in the mass spectrum of cyclohexanone (Fig. 8.32).







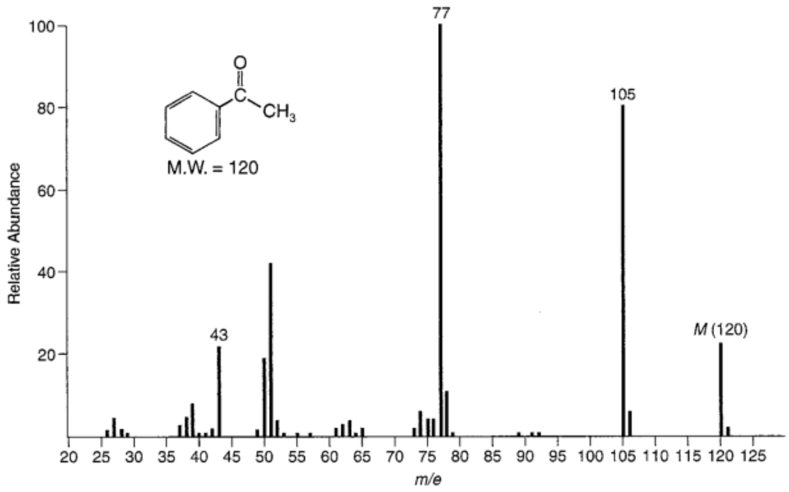
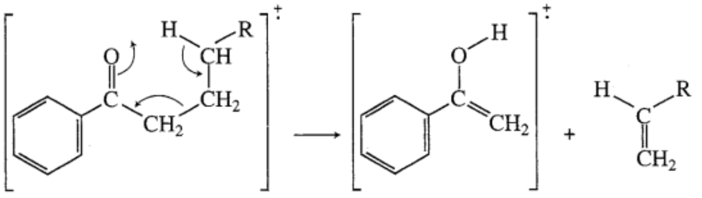


FIGURE 8.33 Mass spectrum of acetophenone.

Aromatic ketones undergo α-cleavage to lose the alkyl group and form the $C_6H_5CO^+$ ion (*m*/*e* = 105). This ion loses carbon monoxide to form the $C_6H_5^+$ ion (*m*/*e* = 77). These peaks appear prominently in the mass spectrum of acetophenone (Fig. 8.33). With larger alkyl groups attached to the carbonyl group of an aromatic ketone, a rearrangement of the McLafferty type is possible, as follows.



m/*e* = 120

The *m*/*e* = 120 fragment ion may undergo additional α-cleavage to yield the $C_6H_5CO^+$ ion at *m*/*e* = 105.

J. Esters

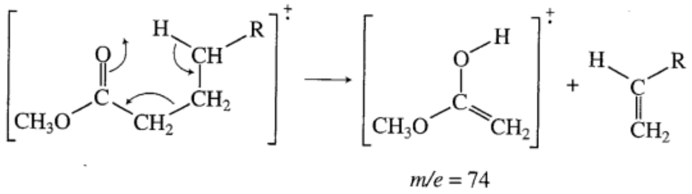
Even though fragmentation readily occurs, it is usually possible to observe weak molecular ion peaks in the mass spectra of methyl esters. The esters of alcohols higher than methanol form much weaker molecular ion peaks. Esters of alcohols larger than four carbons may form molecular ion peaks that are too weak to be observed.

The most important of the α-cleavage reactions involves the loss of the alkoxy group from an ester to form the corresponding acylium ion, RCO^+. The acylium ion peak appears at *m*/*e* = 71 in the mass spectrum of methyl butyrate (Fig. 8.34). The acylium ion peak is a useful diagnostic peak in the mass spectra of esters. A second useful peak results from the loss of the alkyl group from the acyl portion of the ester molecule, leaving a fragment $CH_3{-}O{-}C{=}O^+$ that appears at *m*/*e* = 59. This peak can also be observed in the mass spectrum of methyl butyrate. Again, this *m*/*e* = 59 peak, though less intense than the acylium ion peak, is a useful diagnostic peak for methyl esters. Other fragment ion peaks include the OCH_3^+ fragment and the R^+ fragment from the acyl portion of the ester molecule. These latter ions are much less important than the former two.

SPECTRAL ANALYSIS BOX—Esters

MOLECULAR ION	**FRAGMENT IONS**
M^+ weak, but generally observable	Methyl esters: $M - 31$ $m/e = 59, 74$ Higher esters: $M - 45, M - 59, M - 73$ $m/e = 73, 87, 101$ $m/e = 88, 102, 116$ $m/e = 61, 75, 89$ $m/e = 77, 105, 108$ $M - 32, M - 46, M - 60$

The most important β-cleavage reaction of methyl esters is the McLafferty rearrangement:



This fragment ion peak is very important in the mass spectra of methyl esters, as is evident from its prominence in the mass spectrum of methyl butyrate.

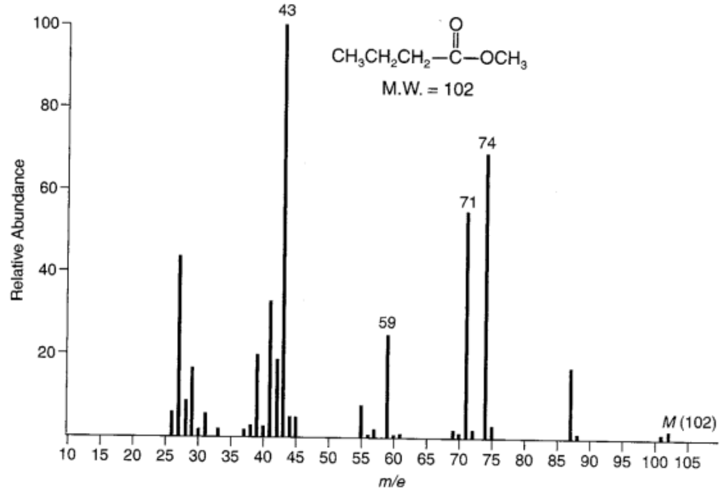
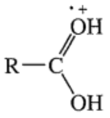


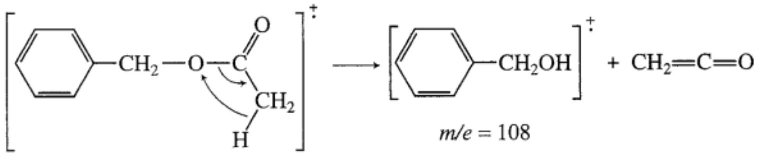
FIGURE 8.34 Mass spectrum of methyl butyrate.

Ethyl, propyl, butyl, and higher alkyl esters also undergo the α-cleavage and McLafferty rearrangements typical of the methyl esters. In addition, however, these esters may undergo a rearrangement of the alkyl portion of the molecule, in which a hydrogen atom from the alkyl portion is transferred to the carbonyl oxygen of the acyl portion of the ester. This rearrangement results in fragments of the type



which appear in the series $m/e = 61, 75, 89$, and so on. These ions may also appear without the extra hydrogen as $RCOOH^+$ fragment ions.

Benzyl esters undergo rearrangement to eliminate the neutral ketene molecule.



The resulting ion is often the most intense peak in the mass spectrum of such a compound.

Alkyl benzoate esters prefer to lose the alkoxy group to form the $C_6H_5CO^+$ ion ($m/e = 105$). This ion may lose carbon monoxide to form the $C_6H_5^+$ ion at $m/e = 77$. Each of these peaks appears in the mass spectrum of methyl benzoate (Fig. 8.35).

Alkyl substitution on benzoate esters appears to have little effect on the mass spectral results unless the alkyl group is in the *ortho* position with respect to the ester functional group. In this case, the alkyl group can interact with the ester function, with the elimination of a molecule of alcohol.

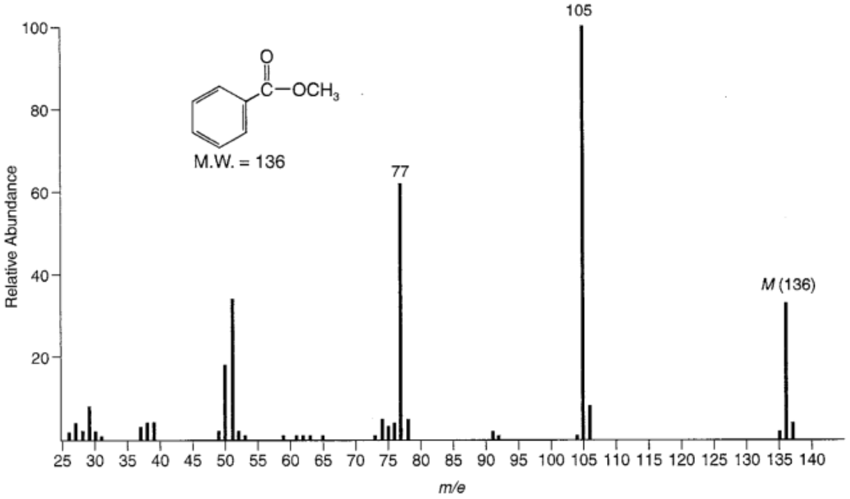
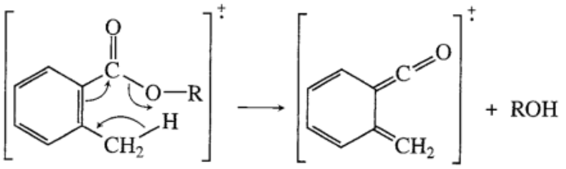


FIGURE 8.35 Mass spectrum of methyl benzoate.



K. Carboxylic Acids

Aliphatic carboxylic acids generally show weak, but observable, molecular ion peaks. Aromatic carboxylic acids, on the other hand, show strong molecular ion peaks. The principal modes of fragmentation resemble those of the methyl esters.

SPECTRAL ANALYSIS BOX — Carboxylic Acids

MOLECULAR ION	**FRAGMENT IONS**
Aliphatic carboxylic acids:	Aliphatic carboxylic acids:
M^+ weak, but observable	$M - 17$, $M - 45$
	$m/e = 45, 60$
Aromatic carboxylic acids:	Aromatic carboxylic acids:
M^+ strong	$M - 17$, $M - 45$
	$M - 18$

With short-chain acids, the loss of OH and COOH through α-cleavage on either side of the C=O group may be observed. In the mass spectrum of propionic acid (Fig. 8.36), loss of OH

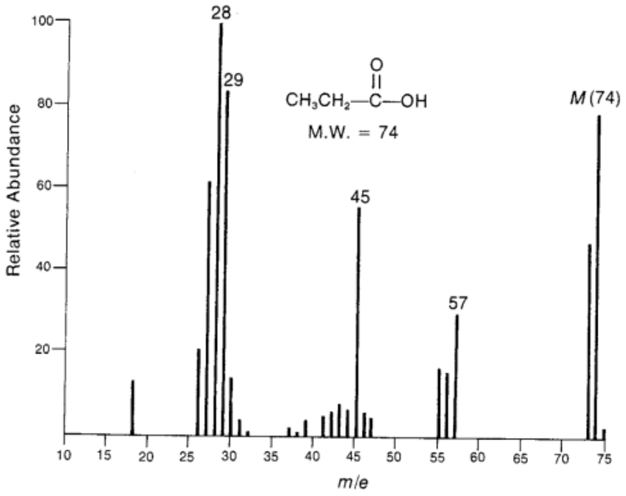


FIGURE 8.36 Mass spectrum of propionic acid.

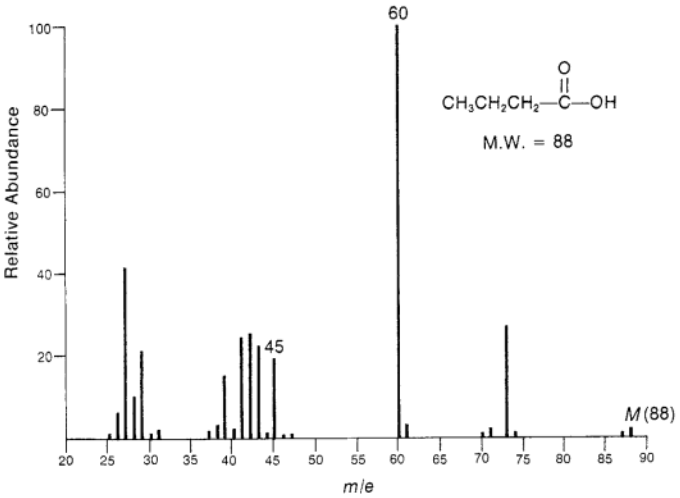
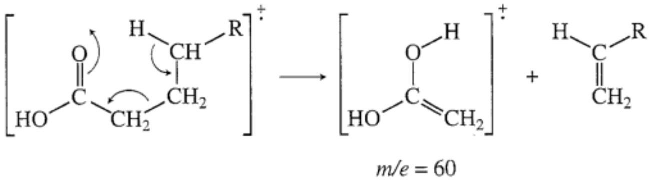


FIGURE 8.37 Mass spectrum of butyric acid.

gives rise to a peak at $m/e = 57$. Loss of COOH gives rise to a peak at $m/e = 29$. The intense peak at $m/e = 28$ is due to further fragmentation of the alkyl portion of the acid molecule. Loss of the alkyl group as a free radical, leaving the $COOH^+$ ion ($m/e = 45$), also occurs. This fragment ion peak appears in the mass spectrum, as well, and is characteristic of the mass spectra of carboxylic acids.

With acids containing γ hydrogens, the principal pathway for fragmentation is the McLafferty rearrangement. In the case of carboxylic acids, this rearrangement produces a prominent peak at $m/e = 60$.



The $m/e = 60$ peak appears in the mass spectrum of butyric acid (Fig. 8.37). You may also notice a peak at $m/e = 45$, corresponding to the $COOH^+$ ion.

Aromatic carboxylic acids produce intense molecular ion peaks. The most important fragmentation pathway involves loss of OH to form the $C_6H_5CO^+$ ion ($m/e = 105$), followed by loss of CO to form the $C_6H_5^+$ ion. In the mass spectrum of *para*-anisic acid (Fig. 8.38), loss of OH gives rise to a peak at $m/e = 135$. Further loss of CO from this ion gives rise to a peak at $m/e = 107$.

Benzoic acids bearing *ortho* alkyl, hydroxy, or amino substituents undergo loss of water through a rearrangement reaction similar to that observed for *ortho*-substituted benzoate esters, as illustrated at the end of Section 8.6J.

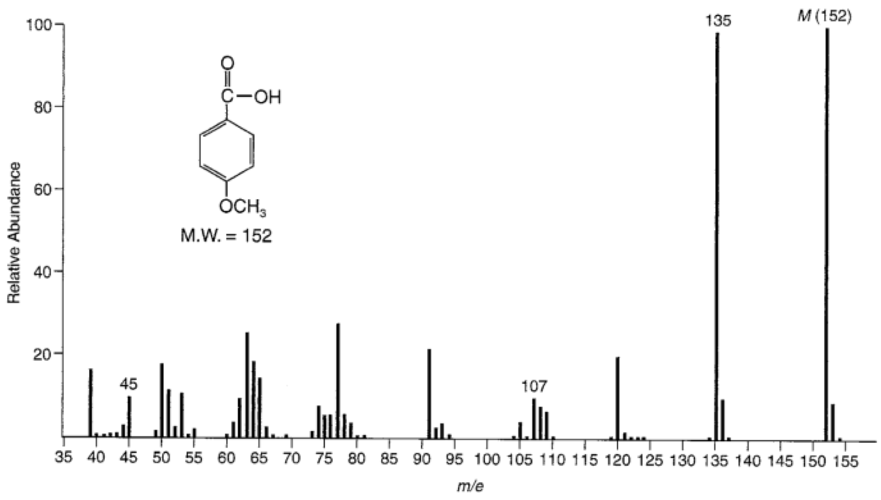


FIGURE 8.38 Mass spectrum of *para*-anisic acid.

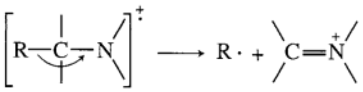
L. Amines

The value of the mass of the molecular ion can be of great help in identifying a substance as an amine. As stated in Section 8.4, a compound with an odd number of nitrogen atoms must have an odd-numbered molecular weight. On this basis, it is possible to quickly determine whether a substance could be an amine. Unfortunately, in the case of aliphatic amines, the molecular ion peak may be very weak or even absent.

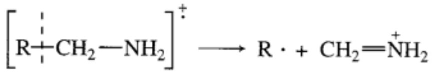
SPECTRAL ANALYSIS BOX—Amines

MOLECULAR ION	**FRAGMENT IONS**
M^+ weak or absent	α-Cleavage
Nitrogen Rule obeyed	$m/e = 30$

The most intense peak in the mass spectrum of an aliphatic amine arises from α-cleavage:



When there is a choice of R groups to be lost through this process, the largest R group is lost preferentially. For primary amines that are not branched at the carbon next to the nitrogen, the most intense peak in the spectrum occurs at $m/e = 30$. It arises from α-cleavage:



$$\left[R-CH_2-NH_2\right]^{+\cdot} \longrightarrow R\cdot + CH_2{=}\overset{+}{N}H_2$$

$m/e = 30$

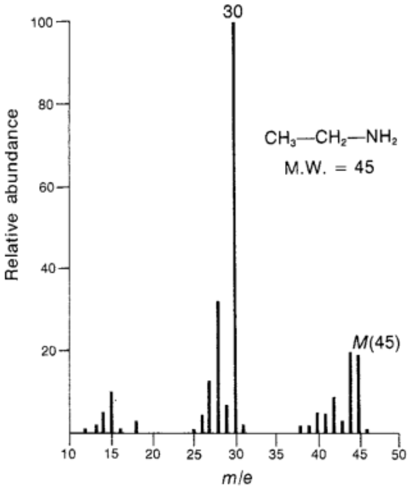


FIGURE 8.39 Mass spectrum of ethylamine.

The presence of this peak is good, although not conclusive, evidence that the test substance is a primary amine. The peak may arise from secondary fragmentation of ions formed from the fragmentation of secondary or tertiary amines, as well. In the mass spectrum of ethylamine (Fig. 8.39), the *m/e* = 30 peak can be seen clearly.

The same β-cleavage peak can also occur for long-chain primary amines. Further fragmentation of the R group of the amine leads to clusters of fragments 14 mass units apart, due to sequential loss of CH_2 units from the R group. Long-chain primary amines can also undergo fragmentation, via the process

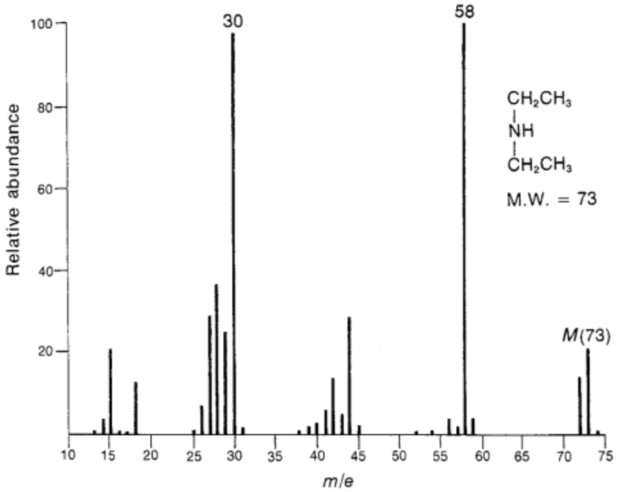
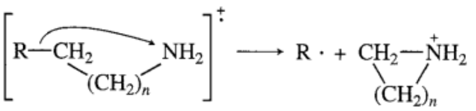


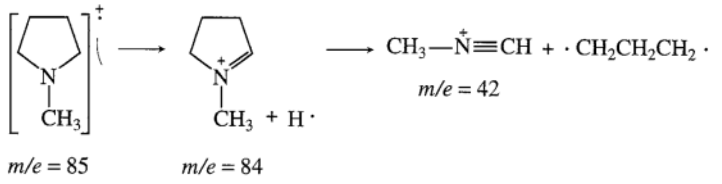
FIGURE 8.40 Mass spectrum of diethylamine.

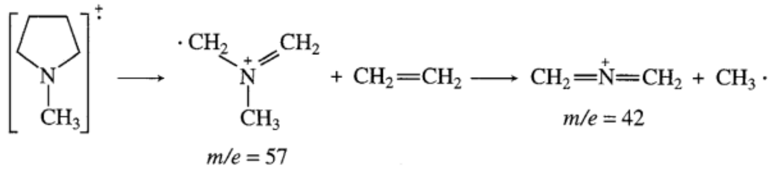


This is particularly favorable when $n = 4$, since a stable six-membered ring results. In this case, the fragment ion appears at $m/e = 86$.

Secondary and tertiary amines also undergo fragmentation processes as described earlier. The most important fragmentation is β-cleavage. In the mass spectrum of diethylamine (Fig. 8.40), the intense peak at $m/e = 58$ is due to loss of a methyl group. Again in the mass spectrum of triethylamine (Fig. 8.41), loss of methyl produces the most intense peak in the spectrum, at $m/e = 86$. In each case, further fragmentation of this initially formed fragment ion produces a peak at $m/e = 30$.

Cyclic aliphatic amines usually produce intense molecular ion peaks. Their principal modes of fragmentation are as follows.





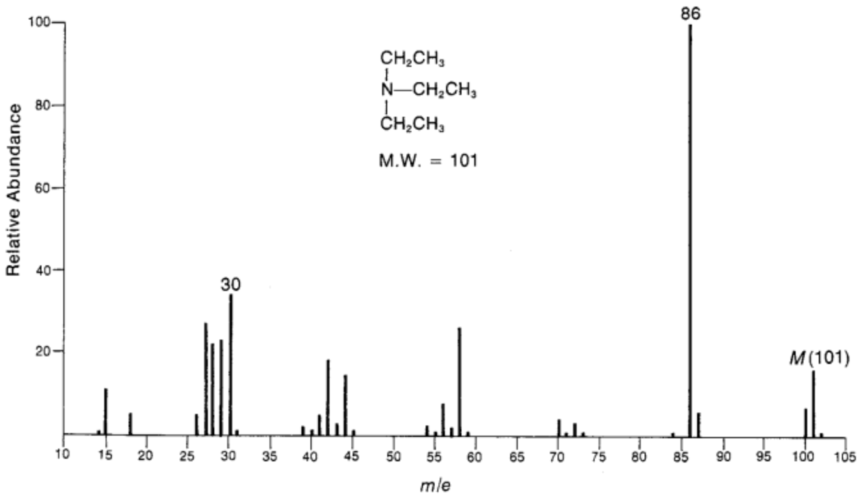
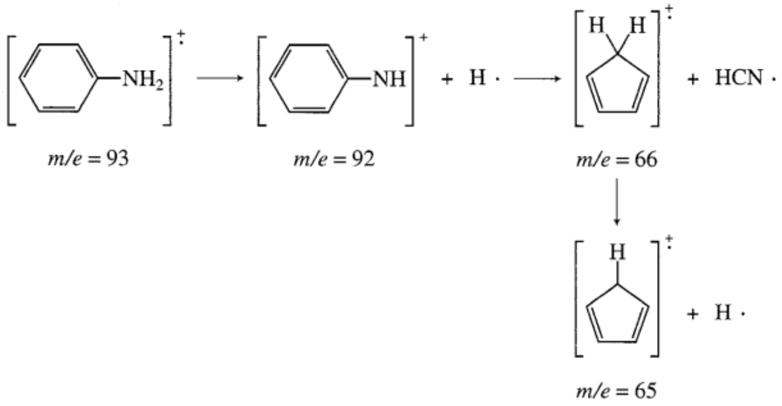


FIGURE 8.41 Mass spectrum of triethylamine.

Aromatic amines show intense molecular ion peaks. A moderately intense peak may appear at an *m/e* value one mass unit less than that of the molecular ion, due to loss of a hydrogen atom. The fragmentation of aromatic amines can be illustrated for the case of aniline:



Very intense molecular ion peaks characterize substituted pyridines. Frequently, loss of a hydrogen atom to produce a peak at an *m/e* value one mass unit less than the molecular ion is also observed.

The most important fragmentation process for the pyridine ring is loss of the elements of hydrogen cyanide. This produces a fragment ion which is 27 mass units lighter than the molecular ion. In the mass spectrum of 3-methylpyridine (Fig. 8.42), you can see the peak due to loss of hydrogen (*m/e* = 92) and the peak due to loss of hydrogen cyanide (*m/e* = 66).

When the alkyl side chain attached to a pyridine ring contains three or more carbons arranged linearly, fragmentation via the McLafferty rearrangement can also occur.

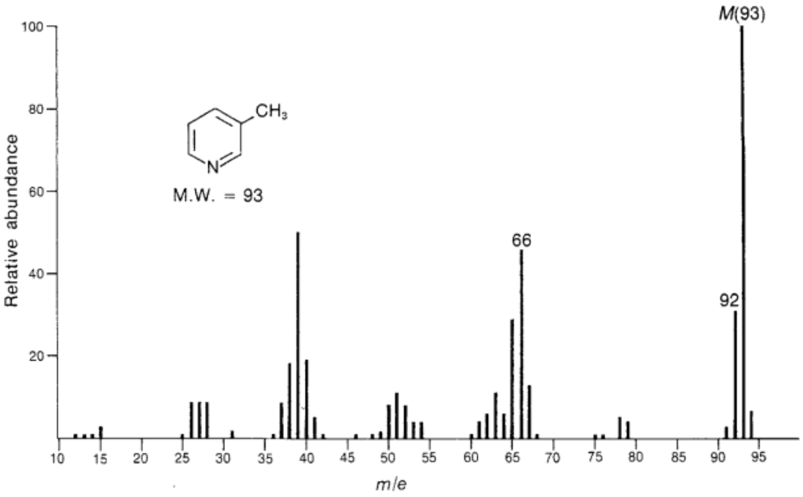
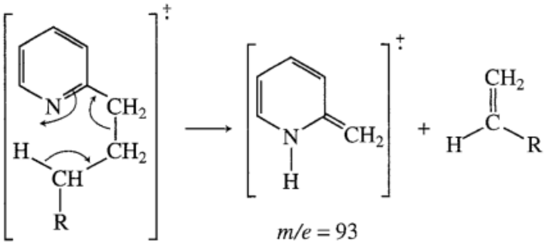


FIGURE 8.42 Mass spectrum of 3-methylpyridine.



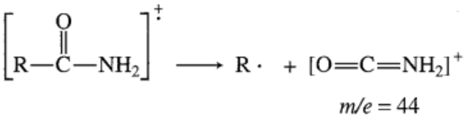
This mode of cleavage is most important for substituents attached to the number 2 position of the ring.

M. Selected Nitrogen and Sulfur Compounds

As is true of amines, nitrogen-bearing compounds such as amides, nitriles, and nitro compounds must follow the Nitrogen Rule (explained more completely in Section 8.4): If they contain an odd number of nitrogen atoms, they must have an odd-numbered molecular weight.

Amides

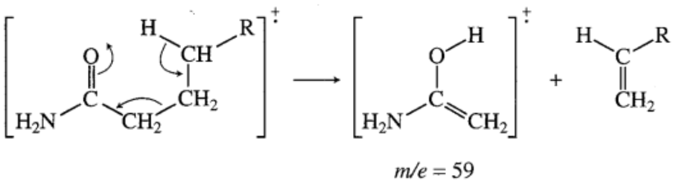
The mass spectra of amides usually show observable molecular ion peaks. The fragmentation patterns of amides are quite similar to those of the corresponding esters and acids. The presence of a strong fragment ion peak at *m/e* = 44 is usually indicative of a primary amide. This peak arises from α-cleavage of the following sort.



$$\left[R-\overset{\displaystyle O}{\overset{\|}{C}}-NH_2\right]^{+\cdot} \longrightarrow R\cdot \; + \; [O{=}C{=}NH_2]^+$$

$m/e = 44$

Once the carbon chain in the acyl moiety of an amide becomes long enough to permit the transfer of a hydrogen attached to the γ position, McLafferty rearrangements become possible. For primary amides, the McLafferty rearrangement gives rise to a fragment ion peak at *m/e* = 59. For *N*-alkylamides, analogous peaks at *m/e* values of 73, 87, 101, and so on often appear.



Nitriles

Aliphatic nitriles usually undergo fragmentation so readily that the molecular ion peak is too weak to be observed. However, most nitriles form a peak due to the loss of one hydrogen atom, producing an ion of the type $R{-}CH{=}C{=}N^+$. Although this peak may be weak, it is a useful diagnostic peak in characterizing nitriles. In the mass spectrum of hexanenitrile (Fig. 8.43), this peak appears at *m/e* = 96.

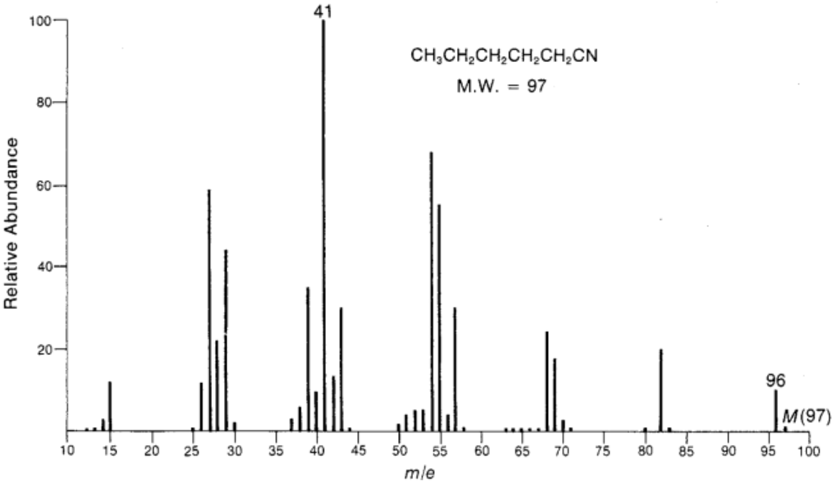
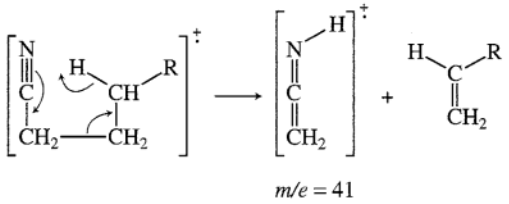


FIGURE 8.43 Mass spectrum of hexanenitrile.

When the alkyl group attached to the nitrile functional group is a propyl group or some longer hydrocarbon group, the most intense peak in the mass spectrum results from a McLafferty rearrangement:



This peak, which appears in the mass spectrum of hexanenitrile, can be quite useful in characterizing an aliphatic nitrile. Unfortunately, as the alkyl group of a nitrile becomes longer, the probability of formation of the $C_3H_5^+$ ion, which also appears at *m/e* = 41, increases. With high-molecular-weight nitriles, most of the fragment ions of mass 41 are $C_3H_5^+$ ions, rather than ions formed as a result of a McLafferty rearrangement.

The strongest peak in the mass spectrum of an aromatic nitrile is the molecular ion peak. Loss of cyanide occurs, giving, in the case of benzonitrile (Fig. 8.44), the $C_6H_5^+$ ion at *m/e* = 77. More important fragmentation involves loss of the elements of hydrogen cyanide. In benzonitrile, this gives rise to a peak at *m/e* = 76.

Nitro Compounds

The molecular ion peak for an aliphatic nitro compound is seldom observed. The mass spectrum is the result of fragmentation of the hydrocarbon part of the molecule. However, the mass spectra of nitro compounds may show a moderate peak at *m/e* = 30, corresponding to the NO^+ ion, and a weaker peak at *m/e* = 46, corresponding to the NO_2^+ ion. These peaks appear in the mass spectrum of 1-nitropropane (Fig. 8.45). The intense peak at *m/e* = 43 is due to the $C_3H_7^+$ ion.

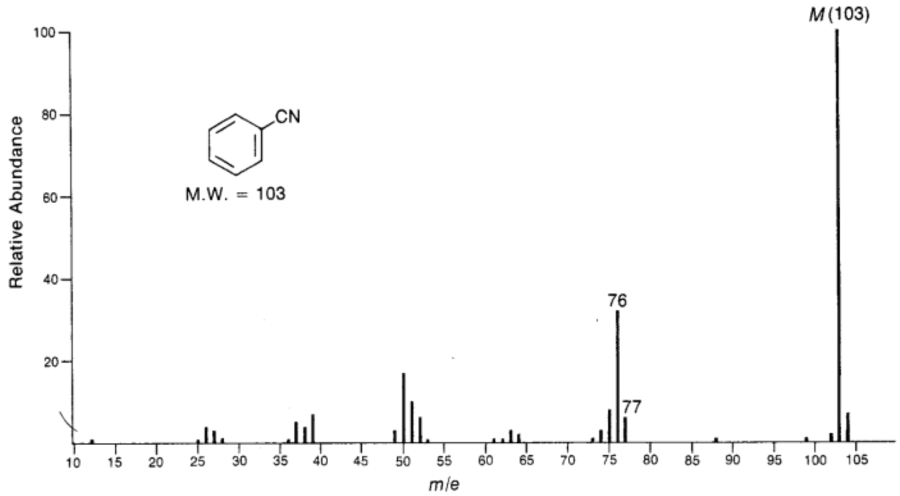


FIGURE 8.44 Mass spectrum of benzonitrile.

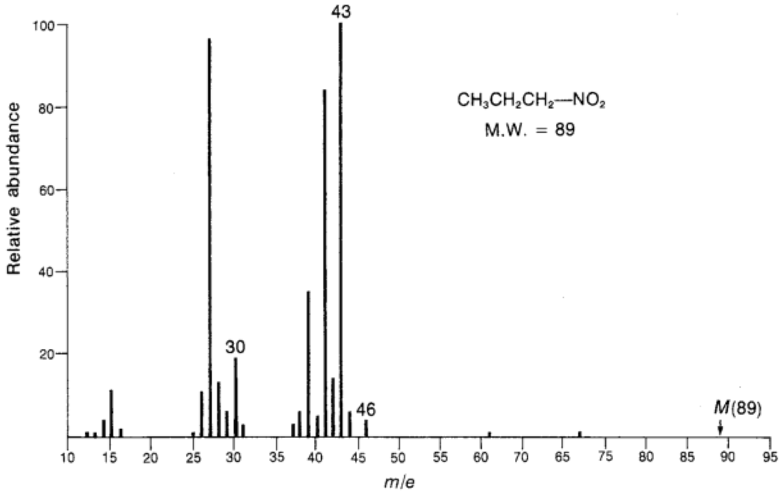


FIGURE 8.45 Mass spectrum of 1-nitropropane.

Aromatic nitro compounds show intense molecular ion peaks. The characteristic NO^+ ($m/e = 30$) and NO_2^+ ($m/e = 46$) peaks appear in the mass spectrum. The principal fragmentation pattern, however, involves loss of all or part of the nitro group. Using nitrobenzene (Fig. 8.46) as an example, this fragmentation pattern may be described as follows.

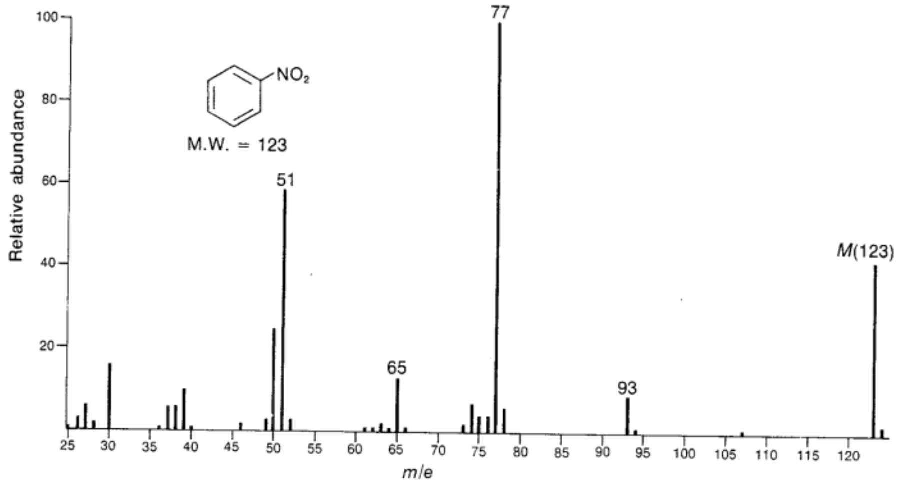
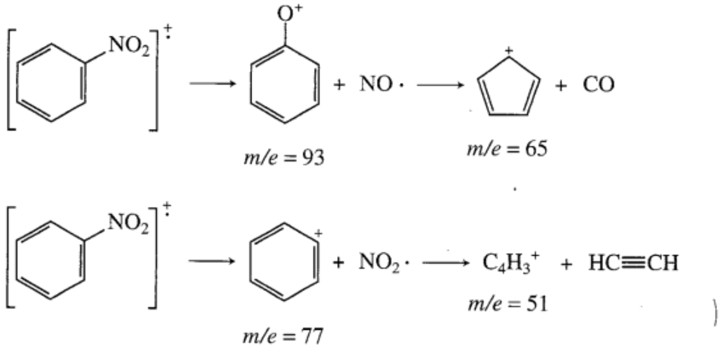


FIGURE 8.46 Mass spectrum of nitrobenzene.



Thiols and Thioethers

Thiols show molecular ion peaks which are more intense than those of the corresponding alcohols. A characteristic feature of the mass spectra of sulfur compounds is the presence of a significant **$M + 2$** peak. This peak arises from the presence of the heavy isotope, ^{34}S, which has a natural abundance of 4.4%.

The fragmentation patterns of the thiols are very similar to those of the alcohols. As alcohols tend to undergo dehydration under some conditions, thiols tend to lose the elements of hydrogen sulfide, giving rise to an **$M - 34$** peak.

Thioethers show mass spectral patterns which are very similar to those of the ethers. As in the case of the thiols, thioethers show molecular ion peaks that tend to be more intense than those of the corresponding ethers.

N. Alkyl Chlorides and Alkyl Bromides

The most dramatic feature of the mass spectra of alkyl chlorides and alkyl bromides is the presence of an important $\mathbf{M + 2}$ peak. This peak arises because both chlorine and bromide are present in nature in two isotopic forms, each with a significant natural abundance.

For aliphatic halogen compounds, the molecular ion peak is strongest with alkyl iodides, less strong for alkyl bromides, weaker for alkyl chlorides, and weakest for alkyl fluorides. Furthermore, as the alkyl group increases in size or as the amount of branching at the [α] position increases, the intensity of the molecular ion peak decreases.

SPECTRAL ANALYSIS BOX—Alkyl Halides

MOLECULAR ION	**FRAGMENT IONS**
Strong $\mathbf{M + 2}$ peak	Loss of Cl or Br
(for Cl, $M/M + 2 = 3:1$;	Loss of HCl
for Br, $M/M + 2 = 1:1$)	α-Cleavage

There are several important fragmentation mechanisms for the alkyl halides. Perhaps the most important is the simple loss of the halogen atom, leaving a carbocation. This fragmentation is most important where the halogen is a good leaving group. Therefore, this type of fragmentation is most prominent in the mass spectra of the alkyl iodides and the alkyl bromides. In the mass spectrum of 1-bromohexane (Fig. 8.47), the peak at $m/e = 85$ is due to the formation of the hexyl ion. This ion undergoes further fragmentation to form a $C_3H_7^+$ ion at $m/e = 43$. The corresponding heptyl ion peak at $m/e = 99$ in the mass spectrum of 2-chloroheptane (Fig. 8.48) is quite weak.

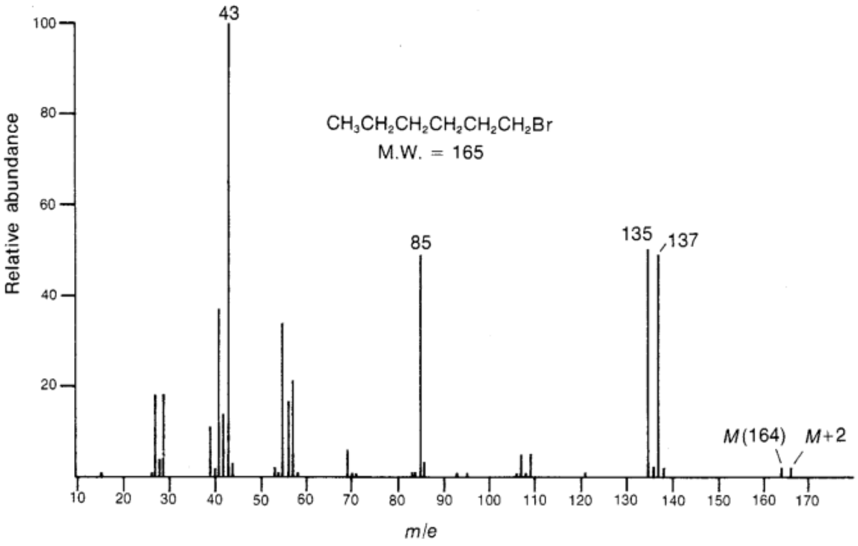


FIGURE 8.47 Mass spectrum of 1-bromohexane.

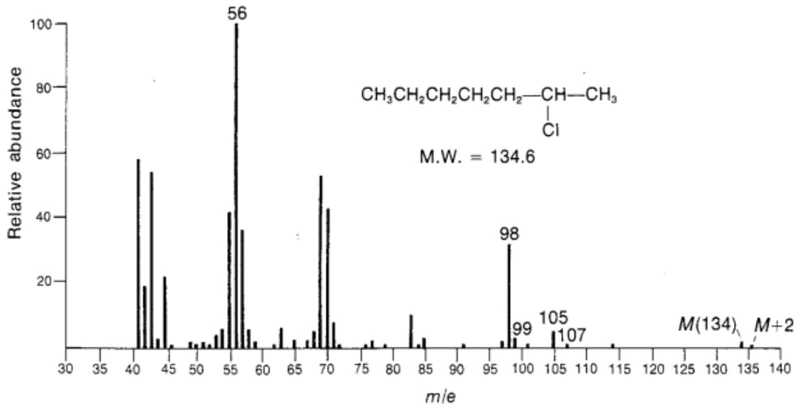


FIGURE 8.48 Mass spectrum of 2-chloroheptane.

Alkyl halides may also lose a molecule of hydrogen halide according to the process

$$[R{-}CH_2{-}CH_2{-}X]^{+\cdot} \longrightarrow [R{-}CH{=}CH_2]^{+\cdot} + HX$$

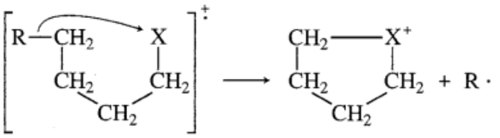
This mode of fragmentation is most important for alkyl fluorides and chlorides and is less important for alkyl bromides and iodides. In the mass spectrum of 1-bromohexane, the peak corresponding to the loss of hydrogen bromide at *m/e* = 84 is very weak. However, for 2-chloroheptane, the peak corresponding to the loss of hydrogen chloride at *m/e* = 98 is quite intense.

A less important mode of fragmentation is α-cleavage, for which a fragmentation mechanism might be

$$\left[R \,\vdots\, CH_2{-}X\right]^{+\cdot} \longrightarrow R\cdot + CH_2{=}X^+$$

In cases where the α position is branched, the heaviest alkyl group attached to the α carbon is lost with greatest facility. The peaks arising from α-cleavage are usually rather weak.

A fourth fragmentation mechanism involves rearrangement and loss of an alkyl radical:



The corresponding cyclic ion can be observed at *m/e* = 135 and 137 in the mass spectrum of 1-bromohexane and at *m/e* = 105 and 107 in the mass spectrum of 2-chloroheptane. Such fragmentation is important only in the mass spectra of long-chain alkyl chlorides and bromides.

The molecular ion peaks in the mass spectra of benzyl halides are usually of sufficient intensity to be observed. The most important fragmentation involves loss of halogen to form the $C_7H_7^+$ ion. When the aromatic ring of a benzyl halide carries substituents, a substituted phenyl cation may also appear.

The molecular ion peak of an aromatic halide is usually quite intense. The most important mode of fragmentation involves loss of halogen to form the $C_6H_5^+$ ion.

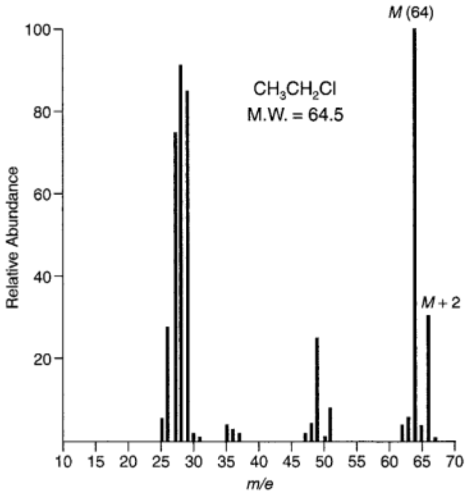


FIGURE 8.49 Mass spectrum of ethyl chloride.

Although the fragmentation patterns we have described are well characterized, the most interesting feature of the mass spectra of chlorine- and bromine-containing compounds is the presence of two molecular ion peaks. As Section 8.4 indicated, chlorine occurs naturally in two isotopic forms. The natural abundance of chlorine of mass 37 is 32.5% that of chlorine of mass 35. The natural abundance of bromine of mass 81 is 98.0% that of ^{79}Br. Therefore, the intensity of the $M + 2$ peak in a chlorine-containing compound should be 32.5% of the intensity of the molecular ion peak, and the intensity of the $M + 2$ peak in a bromine-containing compound should be almost equal to the intensity of the molecular ion peak. These pairs of molecular ion peaks (sometimes called doublets) appear in the mass spectra of ethyl chloride (Fig. 8.49) and ethyl bromide (Fig. 8.50).

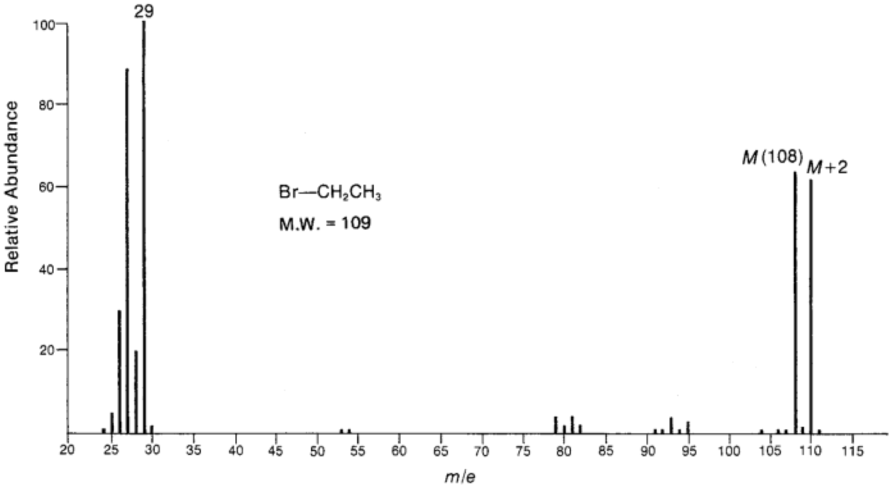


FIGURE 8.50 Mass spectrum of ethyl bromide.

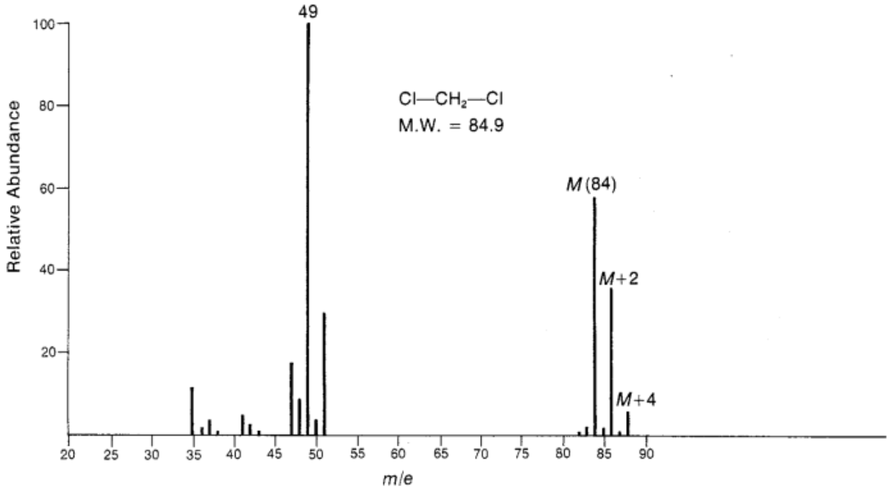


FIGURE 8.51 Mass spectrum of dichloromethane.

Table 8.6 in Section 8.5 can be used to determine what the ratio of the intensities of the molecular ion and isotopic peaks should be when more than one chlorine or bromine is present in the same molecule. The mass spectra of dichloromethane (Fig. 8.51), dibromomethane (Fig. 8.52), and 1-bromo-2-chloroethane (Fig. 8.53) are included here to illustrate some of the combinations of halogens listed in Table 8.6.

Unfortunately, it is not always possible to take advantage of these characteristic patterns to identify halogen compounds. Frequently the molecular ion peaks are too weak to permit accurate measurement of the ratio of the intensities of the molecular ion and isotopic peaks. However, it is often possible to make such a comparison on certain fragment ion peaks in the mass spectrum of a halogen compound. The mass spectrum of 1-bromohexane (Fig. 8.47) may be used to illustrate this method. The presence of bromine can be determined using the fragment ion peaks at *m/e* values of 135 and 137.

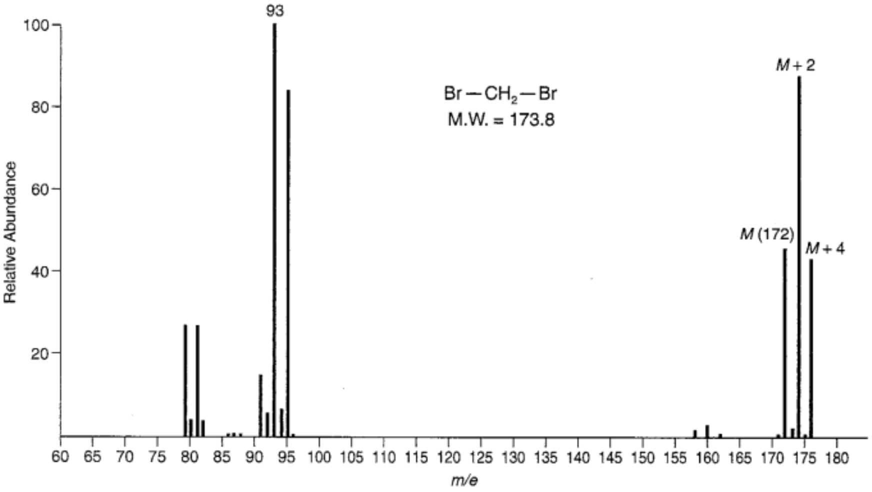


FIGURE 8.52 Mass spectrum of dibromomethane.

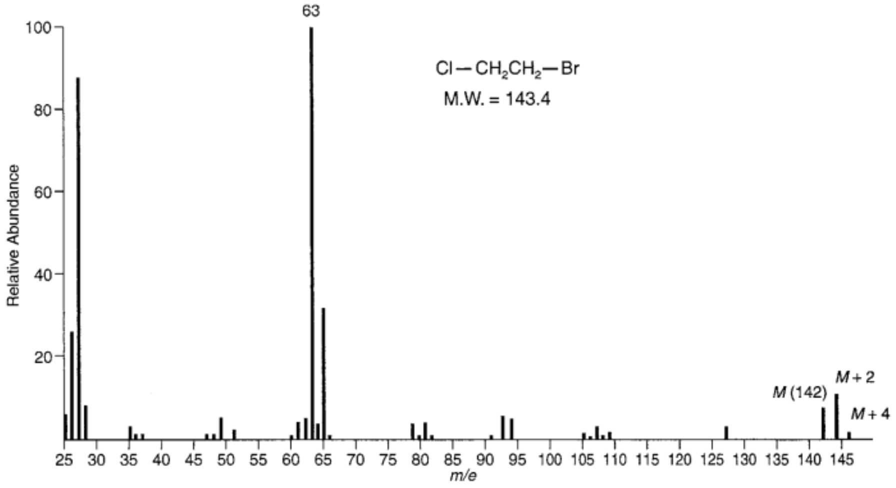


FIGURE 8.53 Mass spectrum of 1-bromo-2-chloroethane.

Since iodine and fluorine exist in nature in the form of only one isotope each, their mass spectra do not show isotopic peaks. The presence of halogen must be deduced either by noting the unusually weak $M + 1$ peak or by observing the mass difference between the fragment ions and the molecular ion.

8.7 ADDITIONAL TOPICS

As you may have observed from the discussions in Section 8.6, the molecular ions of many classes of compounds are so unstable that they decompose before they can reach the detector of the mass spectrometer. As a result, the molecular ion peaks for these classes of compounds are either very weak or totally absent. In such cases, two specialized techniques are useful for studying the molecular ion: field ionization and chemical ionization.

Field ionization involves passing the sample molecules very close to a thin wire that carries a high electrical potential. The strong electric field in the vicinity of this wire ionizes the molecules of sample. Molecular ions formed in this manner do not possess the high degree of vibrational energy found in molecular ions formed by electron impact. Consequently, the ions formed in field ionization are considerably more stable. The molecular ions formed in this manner are much more abundant than those formed by other means of ionization, and the molecular ion peak is usually fairly intense. In some cases, a molecular ion peak may be observed easily through the use of field ionization, whereas no peak would be observed after electron bombardment.

In **chemical ionization,** the sample is introduced into the ionization chamber along with 1 or 2 mm Hg of some reagent gas, usually methane. Essentially all of the electrons ionize methane molecules rather than sample molecules. Once the methane molecules are ionized, a series of ion–molecule collisions yields, among other species, the ions CH_5^+, $C_2H_5^+$, and $C_3H_5^+$. These ions act as strong Lewis acids and can react with sample molecules to produce their corresponding conjugate acids. A sample chemical equation illustrates this behavior:

$$CH_5^+ + R{-}H \longrightarrow RH_2^+ + CH_4$$

These protonated molecules, being cations, are accelerated in the usual way, giving rise to peaks with masses one unit higher than those of the expected molecular ions. Chemical ionization mass spectra show

Liquid Chromatography (LC), LCMS, GCMS

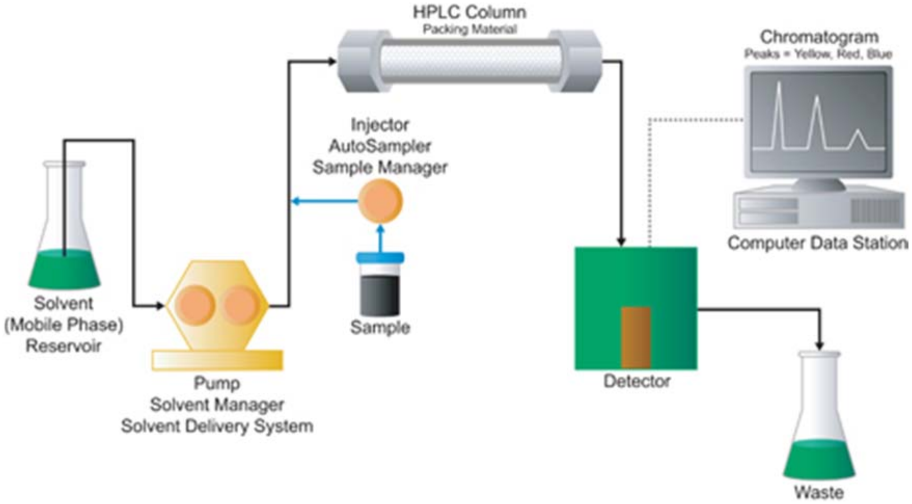


Figure E: High-Performance Liquid Chromatography [HPLC] System

Figure F: A Typical HPLC [Waters Alliance] System

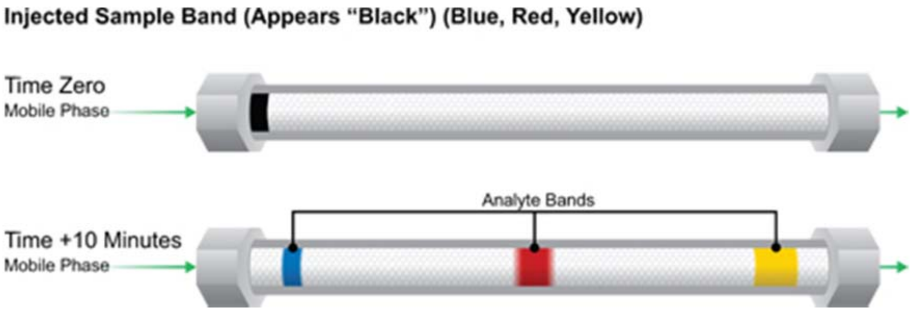


Figure G: Understanding How a Chromatographic Column Works – Bands

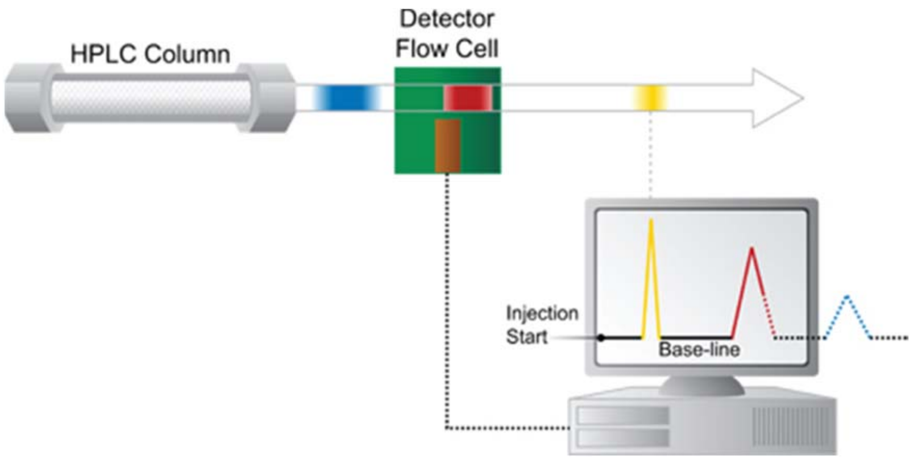
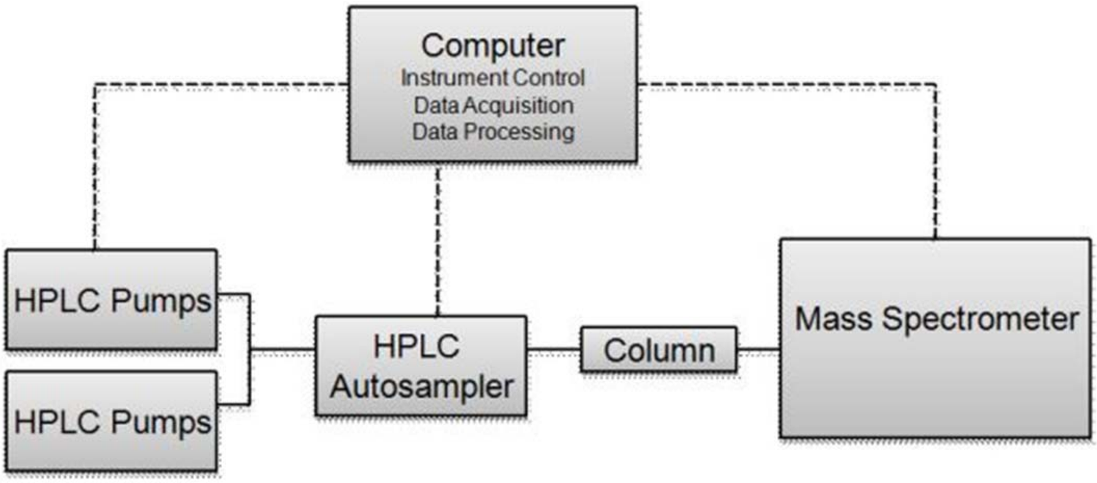


Figure H: How Peaks Are Created

Liquid Chromatography coupled to Mass Spectrometry (LCMS)



Gas Chromatography coupled to Mass Spectrometry (GCMS)

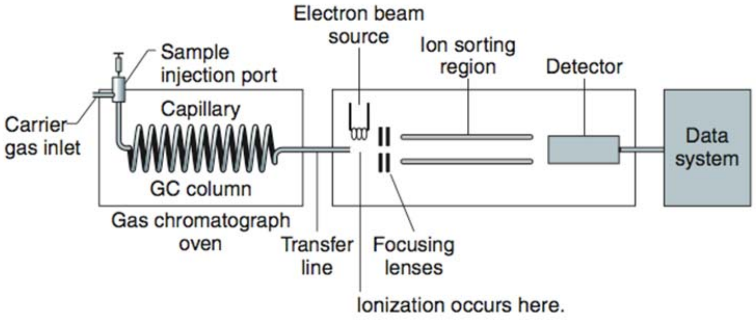


Figure 9.45 Schematic of a typical capillary gas chromatograph/mass spectrometer (GC/MS).

What is the working principle of LCMS and GCMS, how they differ and what types of analysis can be performed with these two?

1. The working principle of both is more or less the same, the affinity for the stationary phase of a given molecule will determine its retention time (and as such the separation).
A very crude definition could be:
LCMS (Liquid Chromatography coupled to Mass Spectrometry) is applied mainly for the analysis of thermally unstable molecules in complex samples. (Example, analysis biological fluids)
GCMS (Gas Chromatography coupled to Mass Spectrometry) is applied mainly for the analysis of volatile compounds in complex samples. (example: analysis of gasoline and petroleum products)

When you couple a separation technique to mass spectrometry you can do quantification of a given compound (or compounds) and identification of unknown species. Of course you can do a lot more with these techniques, basically it depends on what do you want to do.

2. In LCMS,the analyte must be soluble in the mobile phase that is used as mobile phase or carrier solvent, while in GCMS , gas phase is used to elute the analyse. This means that the analyte used for LCMS must be soluble in the mobile phase, while analyte for GCMS must be volatile and does not decompose upon heating. The detection principles are almost similar, using the mass fragments that resulted from ionisation of the molecules. Basically, for volatile compounds, GCMS is very useful, while for polar compounds, LCMS is more useful.

3. LC-MS -MS works on soft ionization technique where as GC-MS works on hard ionization technique. These is a basic differences between two. LC-MS-MS is useful for non volatile compounds , vitamins, amino acids, protein and pep tides having molecular weight in Kilo Dalton. It is very much useful for studies purity and impurity profiles in drugs. So these tool has wide application in R & D in pharmaceutical industries.

   GC-MS is useful for identification volatile compounds having molecular weight less than 1200 amu. It useful in petro chemical , pesticides industries and in field of perfumery .