UNIT - MASS SPECTROMETRY

THE MASS SPECTROMETER: OVERVIEW

In its simplest form, the mass spectrometer has five components (Fig. 3.1), and each will be discussed separately in this chapter. The first component of the mass spectrometer is the **sample inlet** (Section 3.2), which brings the sample from the laboratory environment (1 atm) to the lower pressure of the mass spectrometer. Pressures inside the mass spectrometer range from a few millimeters of mercury in a chemical ionization source to a few micrometers of mercury in the mass analyzer and detector regions of the instrument. The sample inlet leads to the ion source (Section 3.3), where the sample molecules are transformed into gas phase ions. Some instruments have been developed recently that combine the sample inlet and ion source under ambient conditions, thereby greatly simplifying sample preparation. The ions are then accelerated by an electromagnetic field. Next, the **mass analyzer** (Section 3.4) separates the sample ions based on their **mass-to-charge (m/z) ratio**. The ions then are counted by the **detector** (Section 3.5), and the signal is recorded and processed by the **data system**, typically a personal computer (PC). The output from the data system is the **mass spectrum**—a graph of the number of ions detected as a function of their *m/z* ratio.

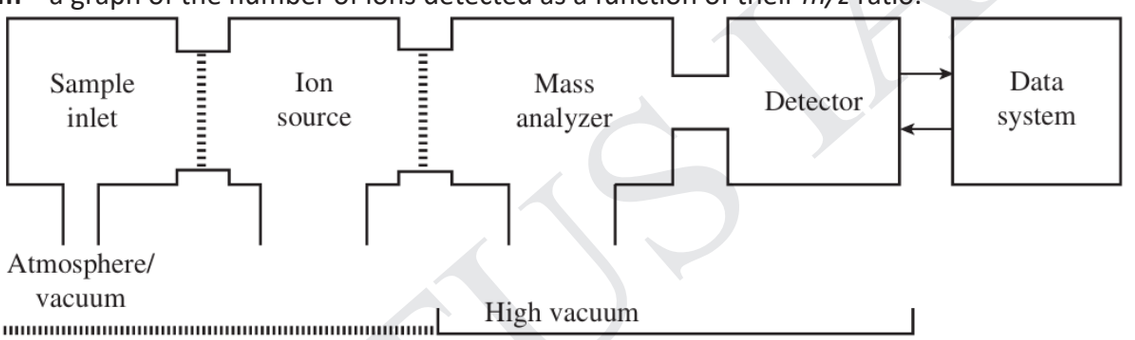

Fig. 3.1: The components of a mass spectrometer. (Adapted from Gross, J. H., *Mass Spectrometry: A Textbook*, Springer, Berlin, 2004. Reprinted by permission.)

DETECTION AND QUANTITATION: THE MASS SPECTRUM

The **detector** of a typical mass spectrometer consists of a counter that produces a current that is proportional to the number of ions that strike it. This sounds quite reasonable until one pauses to think about exactly how many ions will strike the detector in a typical experiment. Consider a typical application—analysis of a small organic molecule (MW = 250) by EI GC-MS. A 1.0-µL injection of a 1.0 mg/mL sample contains 3.6×10^{15} molecules. If the GC is running in split mode with a 1:100 ratio, only 3.6×10^{13} molecules enter the chromatographic column. A mass spectrum acquired at the top of the GC peak may only account for 10% of the material that elutes, and if only 1 in 1000 molecules is converted to an ion, just 3.6 billion ions are available. This still sounds like a lot of charged particles, but wait! In a scanning spectrometer, most of these ions never reach the detector; as the mass analyzer sweeps through the range of 35 to 300 m/z, most of the ions discharge on the quadrupole rods, for example. In a case like this, an ion of any given m/z value makes it through the analyzer only 1 time out of 300. Clearly, each peak in the mass spectrum represents a very small electrical signal, and the detector must be able to amplify this tiny current.

Through the use of **electron multiplier** circuits, this current can be measured so accurately that the current caused by just one ion striking the detector can be measured. These detectors are based on the simple concept of the Faraday cup, a metal cup that is in the path of ions emanating from the mass analyzer. When an ion strikes the surface of the electron multiplier two electrons are ejected. The approximately 2-kV potential difference between the opening and end of the detector draws the electrons further into the electron multiplier, where each electron strikes the surface again, each causing the ejection of two more electrons. This process continues until the end of the electron multiplier is reached, and the electrical current is analyzed and recorded by the data system. The signal amplification just described will be 2^n, where n is the number of collisions with the electron multiplier surface. Typical electron multipliers provide a signal increase of 10^5–10^6. Two configurations of electron multipliers are shown in Figure 3.17. A curved electron multiplier shortens the ion path and results in a signal with less noise. Photomultiplier detectors operate on a similar principle as the electron multiplier, except ion collisions with the fluorescent screen in the photomultiplier result in photon emission

proportional to the number of ion collisions. The intensity of the light (rather than electrical current) is then analyzed and recorded by the data system.

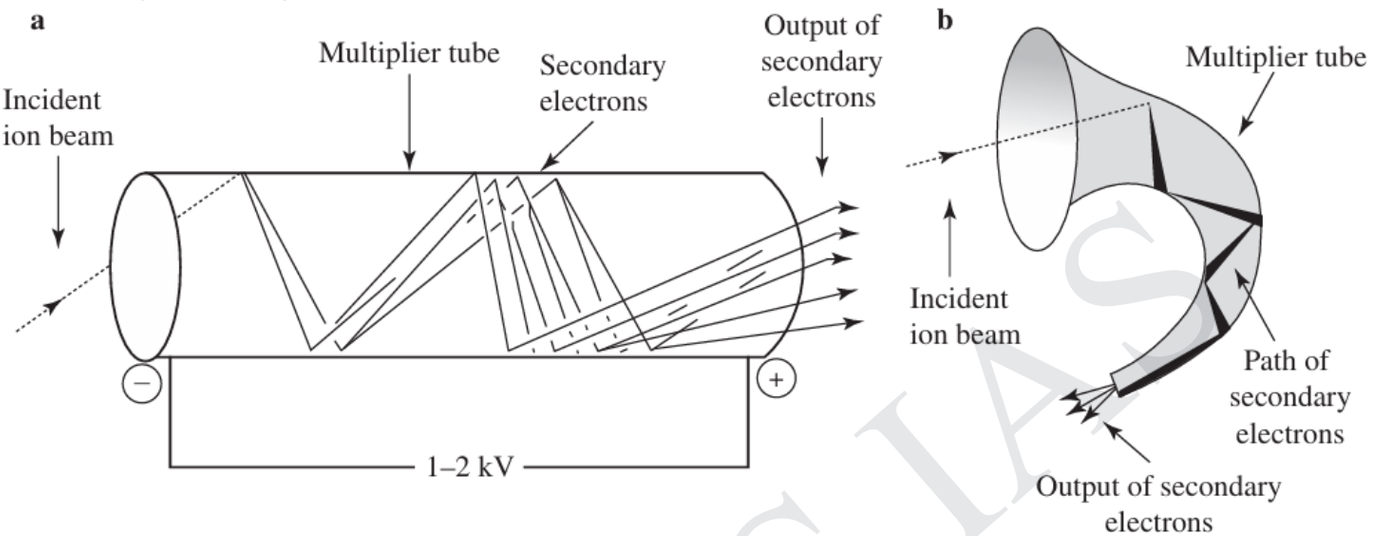

Fig. 3.17: Schematic representation of a linear channel electron multiplier (a) and a curved channel electron multiplier (b). (Based on, J. H., *Mass Spectrometry: A Textbook*, Spinger, Berlin, 2004.)

The signal from the detector is fed to a **recorder**, which produces the mass spectrum. In modern instruments, the output of the detector is fed through an interface to a computer. The computer can store the data, provide the output in both tabular and graphic forms, and compare the data to standard spectra, which are contained in spectra libraries that are also stored in the computer.

Figure 3.18 is a portion of a typical mass spectrum—that of dopamine, a substance that acts as a neurotransmitter in the central nervous system. The *x*-axis of the mass spectrum is the m/z ratio, and the *y*-axis is ion abundance. Mass spectral results may also be presented in tabular form, as in Table 3.2. The most abundant ion formed in the ionization chamber gives rise to the tallest peak in the mass spectrum, called the **base peak**. In the mass spectrum of dopamine, the base peak is indicated at an m/z value of 124. The spectral intensities are normalized by setting the base peak to relative abundance 100, and the rest of the ions are reported as percentages of the base peak intensity. The low end of the m/z range is typically 35 or 40 to eliminate the very large peaks from low-mass fragments from background ions arising from gases and small alkyl fragments. When acquiring data under CI conditions, the low end of the m/z range is set higher to eliminate the large peaks from the reagent gas ions.

As discussed earlier, in EI-MS, the beam of electrons in the ionization chamber converts some of the sample molecules to positive ions. The simple removal of an electron from a molecule yields an ion with weight that is the actual molecular weight of the original molecule. This is the **molecular ion,** which is usually represented by M^+ or $M^{\bullet+}$. Strictly speaking, the molecular ion is a **radical cation** since it contains an unpaired electron as well as a positive charge. The value of m/z at which the molecular ion appears on the mass spectrum, assuming that the ion has only one electron missing, gives the molecular weight of the original molecule. If you can identify the molecular ion peak in the mass spectrum, you will be able to use the spectrum to determine the molecular weight of an unknown substance. Ignoring heavy isotopes for the moment, the molecular ion peak is the peak in the mass spectrum with the largest m/z value; it is indicated in the graphic presentation in Figure 3.18 (m/z = 153).

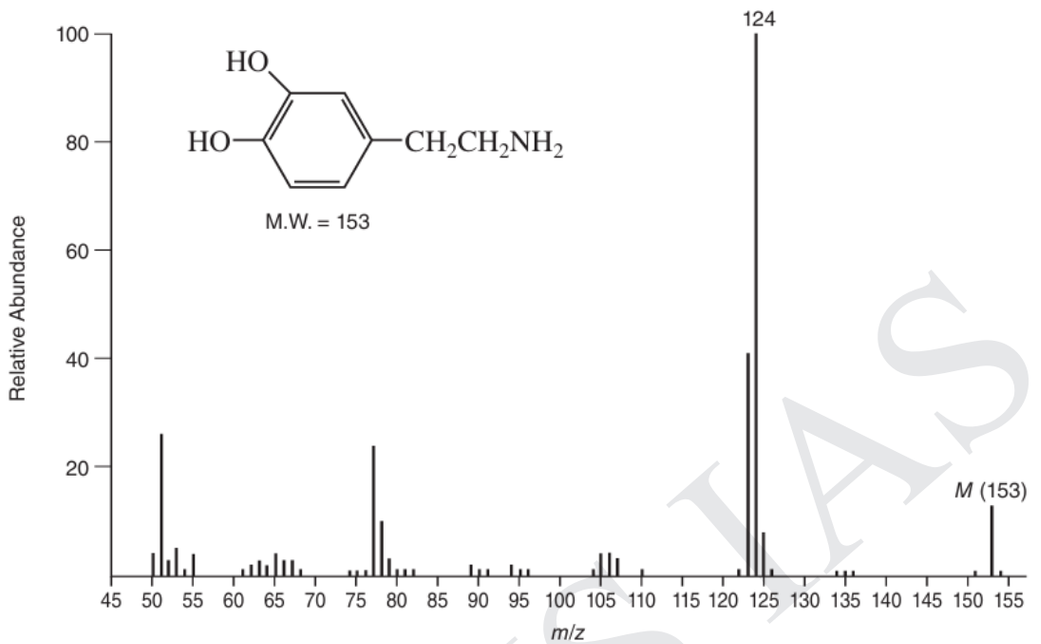

Fig. 3.18: Partial EI-MS of dopamine.

Molecules in nature do not occur as isotopically pure species. Virtually all atoms have heavier isotopes that occur in characteristic natural abundances. Hydrogen occurs largely as ^{1}H, but about 0.02% of hydrogen atoms are the isotope ^{2}H. Carbon normally occurs as ^{12}C, but about 1.1% of carbon atoms are the heavier isotope ^{13}C. With the possible exception of fluorine and a few other elements, most elements have a certain percentage of naturally occurring heavier isotopes. Peaks caused by ions bearing those heavier isotopes also appear in mass spectra. The relative abundances of such isotopic peaks are proportional to the abundances of the isotopes in nature. Most often, the isotopes occur one or two mass units above the mass of the "normal" atom. Therefore, besides looking for the molecular ion (M^+) peak, one would also attempt to locate $M + 1$ and $M + 2$ peaks. As Section 3.6 will demonstrate, the relative abundances of the $M + 1$ and $M + 2$ peaks can be used to determine the molecular formula of the substance being studied. In Figure 3.18, the isotopic peaks are the low-intensity peaks at m/z values (154 and 155) higher than that of the molecular ion peak (see also Table 3.2).

We have seen that the beam of electrons in the ionization chamber can produce the molecular ion. This beam is also sufficiently powerful to break some of the bonds in the molecule, producing a series of molecular fragments. The positively charged fragments are also accelerated in the ionization chamber, sent through the analyzer, detected, and recorded on the mass spectrum. These **fragment ions** appear at m/z values corresponding to their individual masses. Very often, a fragment ion, rather than the parent ion, is the most abundant ion produced in the mass spectrum. A second means of producing fragment ions exists if the molecular ion, once it is formed, is so unstable that it disintegrates before it can pass into the accelerating region of the ionization chamber. Lifetimes less than 10^{-6} sec are typical in this type of fragmentation. The fragments that are charged then appear as fragment ions in the mass spectrum. A great deal of structural information about a substance can be determined from an examination of the fragmentation pattern in the mass spectrum. Chapter 4 will examine some fragmentation patterns for common classes of compounds.

Table 3.2: EI-MS of Dopamine. Tabular Representation of the Data in Figure 3.18

m/z	Relative Abundance	m/z	Relative Abundance	m/z	Relative Abundance
50	4.00	76	1.48	114	0.05
50.5	0.05	77	24.29	115	0.19
51	25.71	78	10.48	116	0.24

51.5	0.19	79	2.71	117	0.24
52	3.00	80	0.81	118	0.14
52.5	0.62	81	1.05	119	0.19
53	5.43	82	0.67	120	0.14
53.5	0.19	83	0.14	121	0.24
54	1.00	84	0.10	122	0.71
55	4.00	85	0.10	123	41.43
56	0.43	86	0.14	124	100.00 (base peak)
56.5	0.05 (metastable peak)	87	0.14	125	7.62
57	0.33	88	0.19	126	0.71
58	0.10	89	1.57	127	0.10
58.5	0.05	89.7	0.10 (metastable peak)	128	0.10
59	0.05	90	0.57	129	0.10
59.5	0.05	90.7	0.10 (metastable peak)	131	0.05
60	0.10	91	0.76	132	0.19
60.5	0.05	92	0.43	133	0.14
61	0.52	93	0.43	134	0.52
61.5	0.10	94	1.76	135	0.52
62	1.57	95	1.43	136	1.48
63	3.29	96	0.52	137	0.33
64	1.57	97	0.14	138	0.10
65	3.57	98	0.05	139	0.10
65.5	0.05	99	0.05	141	0.19
66	3.14	100.6	0.19 (metastable peak)	142	0.05
66.5	0.14	101	0.10	143	0.05
67	2.86	102	0.14	144	0.05
67.5	0.10	103	0.24	145	0.05
68	0.67	104	0.76	146	0.05
69	0.43	105	4.29	147	0.05
70	0.24	106	4.29	148	0.10
71	0.19	107	3.29	149	0.24
72	0.05	108	0.43	150	0.33
73	0.14	109	0.48	151	1.00
74	0.67	110	0.86	152	0.38
74.5	0.05	111	0.10	153	13.33 (molecular ion)
75	1.00	112	0.05	154	1.48
75.5	0.14	113	0.05	155	0.19

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

As a result, the fragment ion produced in the analyzer follows an abnormal flight path on its way to the detector. This ion appears at an m/z ratio that depends on its own mass, as well as the mass of the original ion from which it formed. Such an ion gives rise to what is termed a **metastable ion peak** in the mass spectrum. Metastable ion peaks are usually broad peaks, and they frequently appear at non-integral values of m/z. The equation that relates the position of the metastable ion peak in the mass spectrum to the mass of the original ion is

$$m_1^+ \longrightarrow m_2^+$$ **Equation 3.25**

and

$$m^* = \frac{(m_2)^2}{m_1}$$ **Equation 3.26**

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

Isotope Ratio Data

The preceding section described a method of determining molecular formulas using data from high-resolution mass spectrometers. Another method of determining molecular formulas is to examine the relative intensities of the peaks due to the molecular ion and related ions that bear one or more heavy isotopes (the molecular ion cluster). This method would not be commonly used by researchers who have a high-resolution mass spectrometer at their disposal or are able to submit their samples to a service laboratory for exact mass analysis. Use of the molecular ion cluster can be useful, though, for a relatively quick determination of the molecular formula that does not require the much more expensive high-resolution instrument. This method is useless, of course, when the molecular ion peak is very weak or does not appear. Sometimes the isotopic peaks surrounding the molecular ion are difficult to locate in the mass spectrum, and the results obtained by this method may at times be rendered ambiguous.

The example of ethane (C_2H_6) can illustrate the determination of a molecular formula from a comparison of the intensities of mass spectral peaks of the molecular ion and the ions bearing heavier isotopes. Ethane has a molecular weight of 30 when it contains the most common isotopes of carbon and hydrogen. Its molecular ion peak should appear at a position in the spectrum corresponding to m/z = 30. Occasionally, however, a sample of ethane yields a molecule in which one of the carbon atoms is a heavy isotope of carbon, ^{13}C. This molecule would appear in the mass spectrum at m/z = 31. The relative abundance of ^{13}C in nature is 1.08% of the ^{12}C atoms. In the tremendous number of molecules in a sample of ethane gas, one of the carbon atoms of ethane will turn out to be a ^{13}C atom 1.08% of the time. Since there are two carbon atoms in the molecule, an ethane with mass 31 will turn up (2 × 1.08) or 2.16% of the time. Thus, we would expect to observe a peak at m/z = 31 with an intensity of 2.16% of the molecular ion peak intensity at m/z = 30. This mass 31 peak is called the $M + 1$ peak since its mass is one unit higher than that of the molecular ion. You may notice that a particle of mass 31 could form in another manner. If a deuterium atom, ^2H, replaced one of the hydrogen atoms of ethane, the molecule would also have a mass of 31. The natural abundance of deuterium is only 0.016% of the abundance of ^1H atoms. The intensity of the $M + 1$ peak would be (6 × 0.016) or 0.096% of the intensity of the molecular ion peak if we consider only contributions due to deuterium. When we add these contributions to those of ^{13}C, we obtain the observed intensity of the $M + 1$ peak, which is 2.26% of the intensity of the molecular ion peak. An ion with m/z = 32 can form if both of the carbon atoms in an ethane molecule are ^{13}C. The probability that a molecule of formula $^{13}C_2H_6$ will appear in a natural sample of ethane is (1.08 × 1.08)/100, or 0.01%.

A peak that appears two mass units higher than the mass of the molecular ion peak is called the $M + 2$ peak. The intensity of the $M + 2$ peak of ethane is only 0.01% of the intensity of the molecular ion peak. The contribution due to two deuterium atoms replacing hydrogen atoms would be (0.016 × 0.016)/100 = 0.00000256%, a negligible amount. To assist in the determination of the ratios of molecular ion, $M + 1$, and $M + 2$ peaks, Table 3.5 lists the natural abundances of some common elements and their isotopes. In this table, the relative abundances of the isotopes of each element are calculated by setting the abundances of the most common isotopes equal to 100.

Table 3.4: Precise Masses of Some Common Elements

Element	Atomic Weight	Nuclide	Mass
Hydrogen	1.00797	^{1}H ^{2}H	1.00783 2.01410
Carbon	12.01115	^{12}C ^{13}C	12.0000 13.00336
Nitrogen	14.0067	^{14}N ^{15}N	14.0031 15.0001
Oxygen	15.9994	^{16}O ^{17}O ^{18}O	15.9949 16.9991 17.9992
Fluorine	18.9984	^{19}F	18.9984
Silicon	28.086	^{28}Si ^{29}Si ^{30}Si	27.9769 28.9765 29.9738
Phosphorus	30.974	^{31}P	30.9738
Sulfur	32.064	^{32}S ^{33}S ^{34}S	31.9721 32.9715 33.9679
Chlorine	35.453	^{35}Cl ^{37}Cl	34.9689 36.9659
Bromine	79.909	^{79}Br ^{81}Br	78.9183 80.9163
Iodine	126.904	^{127}I	126.9045

Table 3.5: Natural Abundances of Common Elements and their Isotopes

Element			Relative Abundance			
Hydrogen	^{1}H	100	^{2}H	0.016		
Carbon	^{12}C	100	^{13}C	1.08		
Nitrogen	^{14}N	100	^{15}N	0.38		
Oxygen	^{16}O	100	^{17}O	0.04	^{18}O	0.20
Fluorine	^{19}F	100				
Silicon	^{28}Si	100	^{29}Si	5.10	^{30}Si	3.35
Phosphorus	^{31}P	100				
Sulfur	^{32}S	100	^{33}S	0.78	^{34}S	4.40
Chlorine	^{35}Cl	100			^{37}Cl	32.5
Bromine	^{79}Br	100			^{81}Br	98.0
Iodine	^{127}I	100				

To demonstrate how the intensities of the $M + 1$ and $M + 2$ peaks provide a unique value for a given molecular formula, consider two molecules of mass 42, propene (C_3H_6) and diazomethane (CH_2N_2). For propene, the intensity of the $M + 1$ peak should be (3 × 1.08) + (6 × 0.016) = 3.34%, and the intensity of the $M + 2$ peak should be 0.05%. The natural abundance of ^{15}N isotopes of nitrogen is 0.38% of the abundance of ^{14}N atoms. In diazomethane, we expect the relative intensity of the $M + 1$ peak to be 1.08 + (2 × 0.016) + (2 × 0.38) = 1.87% of the intensity of the molecular ion peak, and

the intensity of the *M* + 2 peak would be 0.01% of the intensity of the molecular ion peak. Table 3.6 summarizes these intensity ratios. It shows that the two molecules have nearly the same molecular weight, but the relative intensities of the *M* + 1 and *M* + 2 peaks that they yield are quite different.

As an additional illustration, Table 3.7 compares the ratios of the molecular ion, *M* + 1, and *M* + 2 peaks for three substances of mass 28: carbon monoxide, nitrogen, and ethene. Again, notice that the relative intensities of the *M* + 1 and *M* + 2 peaks provide a means of distinguishing among these molecules.

As molecules become larger and more complex, the number of possible combinations that yield *M* + 1 and *M* + 2 peaks grows. For a particular combination of atoms, the intensities of these peaks relative to the intensity of the molecular ion peak are unique. Thus, the isotope ratio method can be used to establish the molecular formula of a compound. Examination of the intensity of the *M* + 2 peak is also useful for obtaining information about elements that 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 ^{33}S and ^{34}S are 0.78 and 4.40, respectively, and the relative abundance of ^{30}Si is 3.35. A trained chemist knows that a larger-than-normal *M* + 2 peak can be the first hint that sulfur or silicon is present. Chlorine and bromine also have important *M* + 2 isotopes, and they are discussed separately below.

Table 3.6: Isotope Ratios for Propene and Diazomethane

		Relative Intensities		
Compound	**Molecular Mass**	***M***	***M* + 1**	***M* + 2**
C_3H_6	42	100	3.34	0.05
CH_2N_2	42	100	1.87	0.01

Table 3.7: Isotope Ratios For CO, N_2, and C_2H_4

		Relative Intensities		
Compound	**Molecular Mass**	***M***	***M* + 1**	***M* + 2**
CO	28	100	1.12	0.2
N_2	28	100	0.76	
C_2H_4	28	100	2.23	0.01

Tables of possible combinations of carbon, hydrogen, oxygen, and nitrogen and intensity ratios for the *M* + 1 and *M* + 2 peaks for each combination have been developed. More extensive tables of intensity ratios for the *M* + 1 and *M* + 2 peaks may be found in specialized books on interpreting mass spectra. Accurate calculation of the relative intensities of isotope peaks in a molecular ion cluster for compounds containing several elements with isotopes is time consuming to do by hand as it requires polynomial expansions. Fortunately, many websites dealing with mass spectrometry have isotope calculators that make this a trivial task. Some of these sites may be found in the references at the end of this chapter.

For compounds containing only C, H, N, O, F, Si, P, and S, the relative intensities of *M* + 1 and *M*+ 2 peaks can be estimated quickly using simplified calculations. The formula to calculate the *M* + 1 peak intensity (relative to M^+ = 100) for a given formula is found in Equation 3.27. Similarly, the intensity of an *M* + 2 peak intensity (relative to M^+ = 100) may be found by using Equation 3.28.

$$[M+1] = (\text{number of C} \times 1.1) + (\text{number of H} \times 0.015) + (\text{number of N} \times 0.37) + (\text{number of O} \times 0.04) + (\text{number of S} \times 0.8) + (\text{number of Si} \times 5.1) \quad \textbf{Equation 3.27}$$

$$[M+2] = \frac{(\text{number of C} \times 1.1)^2}{200} + (\text{number of O} \times 0.2) + (\text{number of S} \times 4.4) + (\text{number of Si} \times 3.4) \quad \textbf{Equation 3.28}$$

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

a case, it is important to exercise caution in identifying the molecular ion peak in the mass spectrum. Section 4.9 will discuss the mass spectral properties of the organic halogen compounds in greater detail. Table 3.8 gives the relative intensities of isotope peaks for various combinations of bromine and chlorine atoms, and Figure 3.19 illustrates them.

Table 3.8: Relative Intensities of Isotope Peaks for Various Combinations of Bromine and Chlorine

	Relative Intensities			
Halogen	***M***	***M* + 2**	***M* + 4**	***M* + 6**
Br	100	97.7		
Br_2	100	195.0	95.4	
Br_3	100	293.0	286.0	93.4
Cl	100	32.6		
Cl_2	100	65.3	10.6	
Cl_3	100	97.8	31.9	3.47
BrCl	100	130.0	31.9	
Br_2Cl	100	228.0	159.0	31.2
Cl_2Br	100	163.0	74.4	10.4

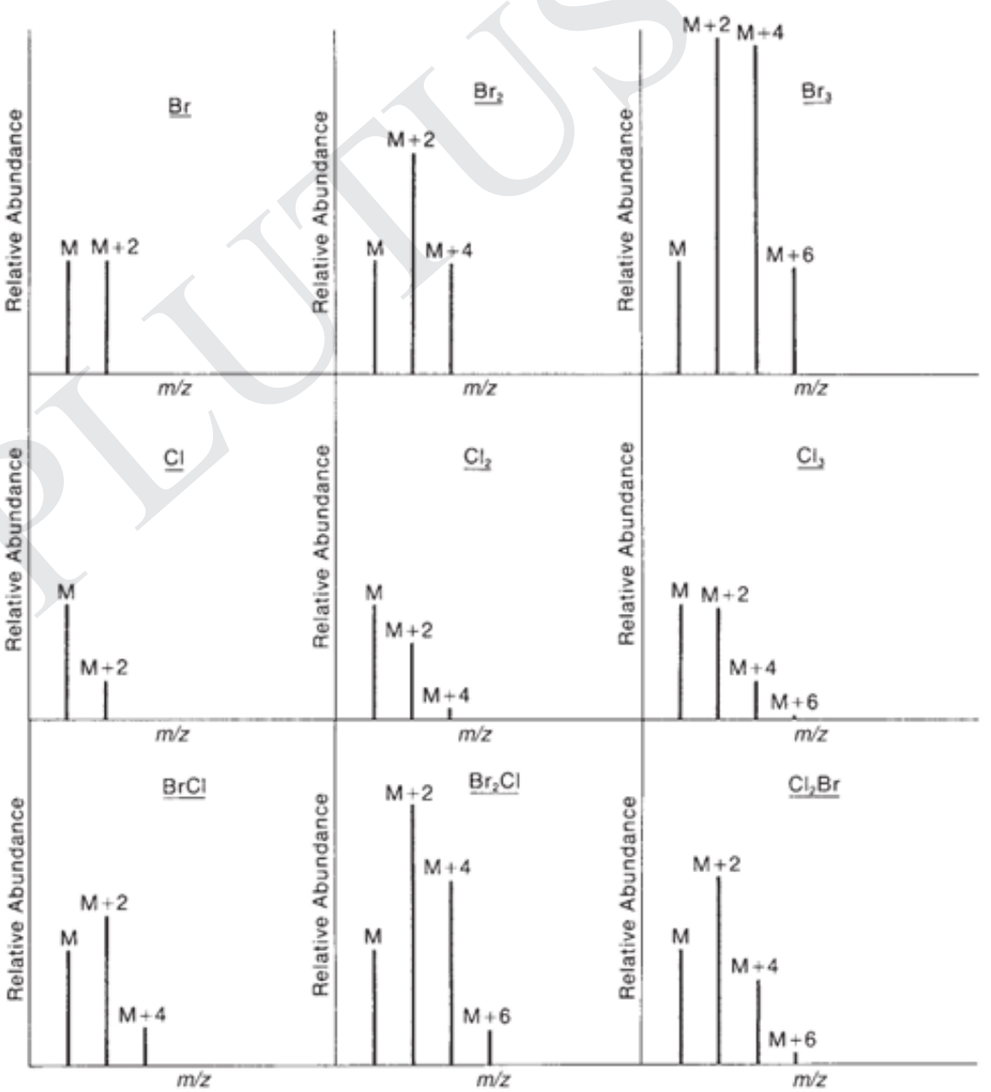

Figure 3.19: Mass spectra expected for various combinations of bromine and chlorine.

McLafferty Rearrangements

Another very common fragmentation that can occur with many substrates is the **McLafferty rearrangement** (Fig. 4.5). This fragmentation was first described by Fred McLafferty in 1956 and is one of the most predictable fragmentations,next to the simple α-cleavage. In the McLafferty rearrangement,a hydrogen atom on a carbon 3 atoms away from the radical cation of an alkene, arene, carbonyl,or imine (a so-called ϒ-hydrogen) is transferred to the charge site via a six-membered transition state,with concurrent cleavage of the sigma bond between the α and β positions of the tether.

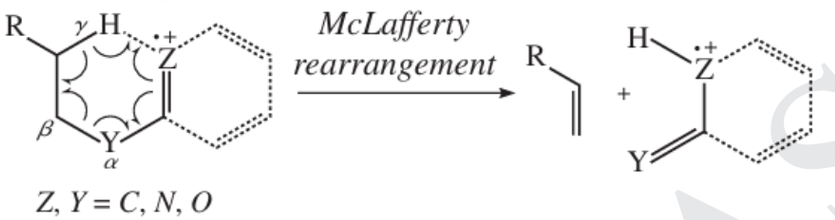

Figure 4.5: The McLafferty rearrangement.

This forms a new radical cation and an alkene with a π bond between what were the original β and ϒ carbons. For simplicity, the mechanism of the McLafferty rearrangement is usually drawn as a concerted process, as in Figure 4.5. There is experimental evidence, however, that the fragmentation is in fact stepwise, and as a general rule fragmentations that involve breaking more than one bond are probably stepwise. The McLafferty rearrangement is readily observed in the mass spectra of many organic functional groups, and several examples will be shown in the remaining sections of this chapter.