Mass Spectrometry II
## Spectral Interpretation and Applications

J.J. Thompson in 1913 first used mass spectrometry (MS) to demonstrate that neon gas consisted of a mixture of nonradioactive isotopes, ^{20}Ne and ^{22}Ne. The atomic weight of neon listed in a modern periodic table is 20.18. Thompson obtained two peaks in the mass spectrum of neon, at masses of 20 and 22 with a roughly 10:1 intensity ratio, but no peak at mass 20.18. This work was revolutionary because it demonstrated that elements existed as isotopes with different atomic weights and simultaneously explained why the apparent atomic weight of an element based on chemical reactions was not a whole number. Neon in fact has three natural isotopes, but ^{21}Ne is present in much smaller amounts than the other two isotopes. In 1923, Francis W. Aston used a higher-resolution instrument he designed to determine the atomic weights of the elements and the isotope ratios of each particular element. This was extremely useful to inorganic chemists and helped solve many of the problems concerning the position of elements in the periodic table at that time. During World War II, Nier at the University of Minnesota developed the high-resolution double-focusing instrument that permitted the analysis and separation of ^{235}U from ^{238}U, aiding in the development of the atomic bomb.

In the 1940s, the first commercial mass spectrometers were developed for petroleum analysis. Subsequent instrument developments, many of them only in the past decade, have led to the widespread use of MS in many branches of science. It has been estimated (Busch) that a billion mass spectra are recorded daily.

MS is a powerful analytical tool with vast applications in organic, inorganic, environmental, polymer, and physical chemistry, physics, geology, climatology, paleontology, archaeology, materials science, biology, and medicine. Advances in MS instrumentation have made possible major advances in our understanding of the human genome, protein structure, and drug metabolism. For example, intact viruses of millions of daltons have been analyzed by MS using electrospray ionization (ESI) with retention of viral activity and structure (Fuerstenau et al.). Commercial hyphenated gas chromatography (GC)-MS^n and liquid chromatography (LC)-MS^n systems permit rapid, sensitive biomonitoring of humans for exposure to chemicals, including chemicals used by terrorists. It is impossible to cover all applications of MS in one chapter, but examples of important uses of both molecular and atomic MS will be presented. In addition, this chapter introduces the interpretation of simple mass spectra for the identification of molecules.

10.1 INTERPRETATION OF MASS SPECTRA: STRUCTURAL DETERMINATION OF SIMPLE MOLECULES

The major reasons for learning to interpret mass spectra of molecules are so that the structure of an unknown compound can be deduced and an unknown molecule can be completely and unambiguously identified. For even fairly small organic molecules, a thorough knowledge of structural organic
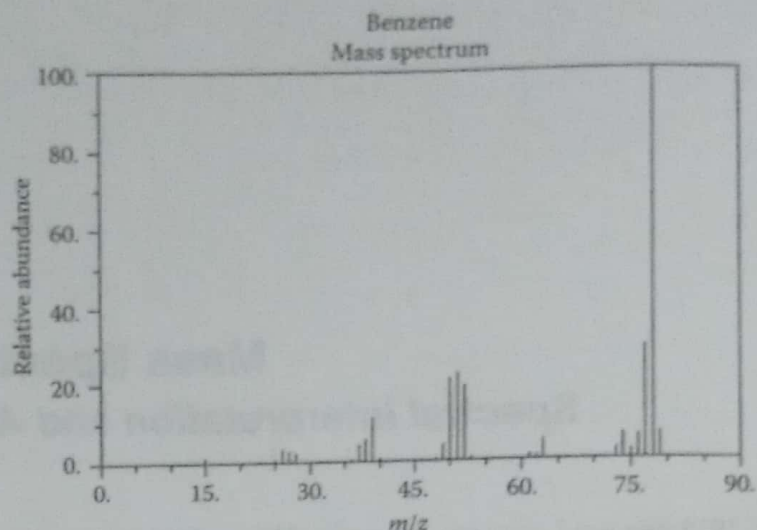
Figure 10.1 A mass spectrum of benzene, C_6H_6. (From the NIST Mass Spectrometry Data Center, accessed via http://webbook.nist.gov. © 2011 U.S. Secretary of Commerce for the United States of America. All rights reserved. Used with permission.)

chemistry and lots of practice are required to do this. Fortunately, simple mass spectra often can be interpreted using basic arithmetic and some topics you learned in general chemistry: chemical bonding, valence, isotopes, atomic weights, and molecular weight (MW) calculations. For completely unknown molecules, more than one mass spectral technique may need to be combined with other analytical techniques to positively identify a molecule. As will be seen, there are some types of molecules that cannot be identified using MS alone.

A mass spectrum is a plot or table of the mass-to-charge ratio, m/z, of detected ions versus their relative abundance (relative concentration). A typical mass spectral plot for a small organic molecule, benzene, is presented in Figure 10.1. The m/z values are plotted on the x-axis; relative abundance is plotted on the y-axis. The *most abundant peak* in the spectrum is called the **base peak**. The base peak is assigned an abundance of 100% and the other peak heights are plotted as percentages of that base peak. A tabular form of benzene mass spectral data is given in Table 10.1. The tabular data have the advantage that very low abundance ions can be listed, such as the ions at $m/z = 64$ and 80, which are too small to be seen on the normalized plot.

Table 10.1 Mass Spectral Data for Benzene

m/z	Relative Abundance	m/z	Relative Abundance
37	4.0	53	0.80
37.5	1.2	63	2.9
38	5.4	64	0.17
38.5	0.35	73	1.5
39	13	74	4.3
39.5	0.19	75	1.7
40	0.37	76	6.0
48	0.29	77	14
49	2.7	78	100
50	16	79	6.4
51	18	80	0.18
52	19		

The mass spectra we will study have been obtained by electron ionization (EI) since other techniques enhance the molecular ion but greatly reduce fragmentation, making structural analysis difficult. Consequently, the following discussion is concerned mostly with EI spectra of pure compounds. The ions that appear in these spectra are all positively charged ions. These are also low-resolution mass spectra; the m/z values are measured only to the units place. (We will explain the half-integral values in Table 10.1 later in this chapter.) Exact MWs, which are not integer values, are obtained by high-resolution MS, also discussed later in this chapter.

There are two ways to interpret such spectra. The first is to compare the spectrum you have with those in a searchable computerized mass spectral database. The second is to evaluate the spectrum using the interpretation procedure described subsequently. In either case, once an unknown compound has been "identified" from its mass spectrum, the pure compound should be obtained and analyzed under the same conditions as the sample for confirmation. Over 10 million chemical compounds have been identified. No mass spectral database contains spectra for every possible compound, although mass spectral databases of over 719,000 compounds are available, such as the Wiley Registry of Mass Spectral Data, 10th edition (www.wiley.com/go/databases), ~$8,000 on DVD-ROM. The mass spectral database from the US National Institute for Standards and Technology (NIST) (www.nist.gov/srd/nista.cfm) contains EI spectra for 212,961 compounds in the 2011 release and may be purchased from a number of licensed vendors. Limited mass spectra from NIST are available online in the NIST Chemistry WebBook (http://webbook.nist.gov). Commercial vendors and publishers offer specialized mass spectral libraries of compounds, such as environmental compounds, pharmaceuticals, natural products, and oil industry compounds. As discussed in the following, many compounds of the same empirical formula may exist as multiple structural isomers, with substituents such as halogen or alkyl groups attached at different locations. For example, there are 46 possible different pentachlorobiphenyls. Such isomers may have essentially indistinguishable EI mass spectra. As we will see in Chapter 12, these may be separable by GC coupled to an MS instrument and the isomers distinguished by their GC retention index (RI) (cf. Section 12.9) values. Many MS spectral databases also provide some GC-RI values for this purpose.

In practice, the analyte spectrum is entered into the computer, which compares it to the spectra in the stored database using a search algorithm. There are a number of algorithms currently available, including probability-based matching, designed by Professor F. W. McLafferty and coworkers at Cornell University; the INCOS dot-product algorithm; and the NIST library search algorithm. These algorithms use pattern matching, peak intensities, weighting factors, and other information from the spectrum to compare the candidate spectrum to spectra in the library database. The search will result in a list of possible candidate compounds with a probability attached to the "match." The analyst should visually compare the candidate spectra to that of the analyte. Using knowledge, judgment, and experience, the analyst then chooses which of the candidate compounds matches the unknown compound. This spectral matching method in theory requires little training on the part of the analyst to identify the compound but requires pure compounds, a good mass spectrum of the sample, and a comprehensive mass spectral database. The technique of identifying a pure compound by comparing its spectrum with known spectra works well if we already know something about the compound (e.g., odor, color, melting point, functional groups present from an infrared (IR) spectrum, and elements present from combustion analysis) and if the compound's mass spectrum is in our database. This enables us to make an informed deduction as to the identity of the compound by direct comparison with the library spectrum. Unfortunately, this is not always the case; more frequently, the nature of the sample is not known. Furthermore, the sample may not be pure, and therefore, direct comparison of spectra will not constitute a valid confirmation of the compound's identity. It is possible that spectra in a database are not identified correctly. It is not prudent to rely completely on a library match, especially for a complete unknown, since there are many cases of multiple compounds with very similar mass spectra; for example, polychlorinated biphenyls (PCBs), dioxins, and polychlorinated diphenyl ethers (PCDEs) with the same number of Cl substituents each

have many structural isomers, and these often have nearly indistinguishable mass spectra. Refer also to Figure 10.30 at the end of Section 10.2, which displays an unusual example of two radically different molecular formulas and structures yielding essentially identical mass spectra. In these circumstances, it is necessary to take the spectrum and deduce the information from it. Therefore, an analyst should understand how to identify the compound from the information in the mass spectrum; this ability will also enable the analyst to evaluate the validity of the spectral database search results. The procedure for interpreting a mass spectrum consists of the following steps:

1. Identify the molecular ion if present.
2. Apply the "nitrogen rule."
3. Evaluate for "A + 2" elements.
4. Calculate "A + 1" and "A" elements.
5. Look for reasonable loss peaks from the molecular ion.
6. Look for characteristic low-mass fragments.
7. Postulate a possible formula.
8. Calculate "rings + double bonds."
9. Postulate a reasonable structure.

We will define the terms in quotation marks as we go through the process. Interpretation of mass spectra is much like solving a jigsaw puzzle: all or most of the pieces are there, but putting them together involves a lot of educated guesswork followed by confirmation.

10.1.1 Molecular Ion and Fragmentation Patterns

When a molecule is ionized by EI, a **molecular ion** forms by loss of an electron from the molecule. This radical cation, symbolized as $M^{+\cdot}$, has the same mass as the neutral molecule, because the loss in mass of one electron is too small to measure. The molecular ion generally has absorbed excess energy in the ionization process. The excess energy causes the molecular ion to break apart. The fragments formed may be ions, neutral molecules, radicals, and the like. Fragments may undergo more fragmentation into even smaller pieces. The ions that appear in the mass spectrum are called fragment ions. If the ionization conditions are kept constant, a given molecule will always produce the same fragments in the same proportions. The mass and abundance of these fragments are called the molecule's **fragmentation pattern**. Each molecule has its own characteristic fragmentation pattern under particular ionization conditions.

Using low-resolution MS, the molecular ion, if present, will provide the MW of the compound to unit mass accuracy, a very valuable piece of information. High-resolution spectra with high mass accuracy can reduce the number of possible elemental formulas for the molecular ion. The identification of the molecular ion is important because from the MW, we can immediately conclude a great deal about the compound. For example, for an organic compound with an MW = 54, we would be able to conclude that the compound can only have a limited number of empirical formulas, such as $C_2H_2N_2$, $C_2H_2N_2$, C_3H_2O, C_3H_4N, or C_4H_6. Some of these possibilities can be eliminated by inspection, because it is not possible to have a neutral molecule with certain empirical formulas. For example, C_2NO cannot exist as a neutral molecule. Thus, if we know the MW, we have considerable information about the possible empirical formula of the molecule. MS is one of the most rapid and reliable methods of determining the MW of compounds. Unfortunately, in EI spectra, the molecular ion is often of low abundance and is not always observed, or it cannot be distinguished from ions originating from low-level, unrelated background contaminants. In short, the m/z value of the molecular ion indicates the MW of the molecule; the fragmentation pattern is used to deduce the structure of the molecule. The molecular ion is not necessarily the base peak in the spectrum; particularly in EI spectra, the base peak is usually not the molecular ion.
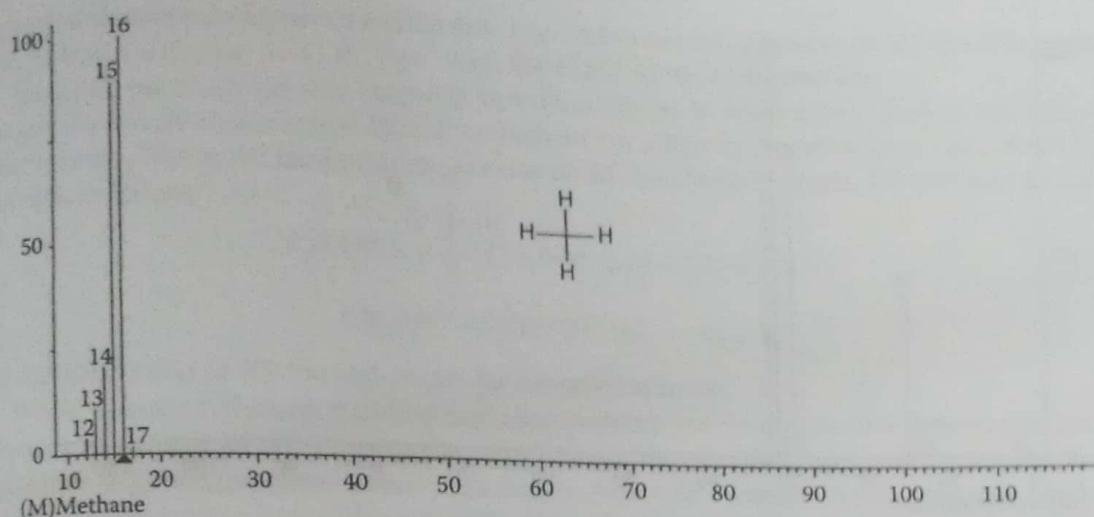
Figure 10.2 A mass spectrum of methane, CH_4.

Table 10.2 Mass Spectral Data for Methane

m/z	Relative Abundance
1	3.1
2	0.17
12	1.0
13	3.9
14	9.2
15	85
16	100
17	1.11

In this chapter, we shall emphasize compounds with molecular ions that can be identified or deduced with reasonable certainty. If the molecular ion is present, it must have the highest m/z in the spectrum, excluding the effects of isotopes. Examples are shown for methane (Figure 10.2, Table 10.2), methanol (Figure 10.3, Table 10.3), and benzene (Figure 10.4, Table 10.1). In each case, the molecular ion was very abundant and not difficult to identify. This is not always the case, as will be seen in later examples. The student should note that in most of the mass spectra used as examples, the molecular ion m/z value is marked by a black triangle on the x-axis. The x-axis is in units of m/z, while the y-axis is relative abundance, even though these units are not marked on the spectra. The most intense peak is set to 100%, and the rest of the peaks are normalized to that peak. The structure of the compound is also shown on the spectrum, using a shorthand method that does not show the hydrogen atoms or the carbon atoms. The methanol spectrum (Figure 10.3) demonstrates clearly that the molecular ion is not the base peak in the spectrum; the fragment ion at m/z = 31 is the most abundant ion.
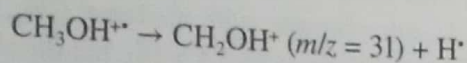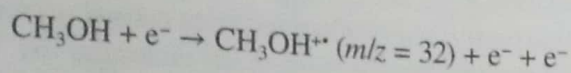
When a molecule is ionized by electron impact, it undergoes the reaction

$$M + e^- \rightarrow M^{+\bullet} + e^- + e^-$$
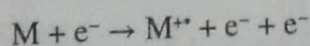
Thus, a molecular ion is always a radical cation, usually with a single positive charge. It is evident that if an organic molecule loses an electron, it must be left with an unpaired electron (i.e., it is a radical ion), as shown by the dot representing the unpaired electron. This radical ion with an unpaired

electron is termed an *odd electron* (OE) *ion. The molecular ion is always an OE ion.* For example, in the reaction $C_2H_6 + e^- \rightarrow C_2H_6^{+\cdot} + e^- + e^-$, the $C_2H_6^{+\cdot}$ is the molecular ion.

However, the molecule may fragment in such a way as to leave a pair of electrons behind on one ion. This is an *even electron* (EE) *ion.* Such an ion arises by *fragmentation* and *cannot* be the molecular ion. Hence, the molecular ion is *never* an EE ion. As an example, CH_3OH may ionize and fragment as follows:

$$CH_3OH + e^- \rightarrow CH_3OH^{+\cdot} \ (m/z = 32) + e^- + e^-$$

$$CH_3OH^{+\cdot} \rightarrow CH_2OH^+ \ (m/z = 31) + H^\cdot$$

The CH_2OH^+ ion is an EE ion and cannot be the molecular ion.

While a detailed discussion of fragmentation patterns and how to identify a molecular ion peak is beyond the scope of this chapter, there are certain classes of organic compounds that tend to give stable molecular ions and classes that do not. Aromatic compounds and conjugated hydrocarbons give more intense molecular ion peaks than alkanes; aliphatic alcohols, nitrates, and highly branched compounds tend not to give peaks for the molecular ion. Texts listed in the bibliography such as those by Silverstein and Webster, McLafferty and Tureček, Pavia et al., or Lambert et al. should be consulted for more details.

10.1.2 Nitrogen Rule

The "nitrogen rule" can be used to help decide if a peak is the molecular ion peak. Because of the atomic weights and valences of elements commonly present in organic molecules, it transpires that the m/z value of the molecular ion *is always an even number if the molecular ion contains either no nitrogen atoms or an even number of nitrogen atoms.* If the molecular ion contains an odd number of nitrogen atoms, the m/z value (and the MW) must be an odd number. This is a very important rule to remember, and it is valid for organic compounds containing C, H, N, O, S, the halogens, P, Si, and many other elements. If the highest m/z value in a mass spectrum is an odd number, the ion must contain an odd number of nitrogen atoms if it is the molecular ion. To use this rule, MWs are calculated using the most abundant isotope and unit atomic masses. This calculation is discussed in the next section. Some examples of the rule follow:

Compound	Number of Nitrogen Atoms	MW	
CH_4	0	16	Even
HCN	1	27	Odd
H_2NNH_2	2	32	Even
C_2H_5OH	0	46	Even
C_6H_6	0	78	Even
$C_2H_5NH_2$	1	45	Odd
$C_6H_5NH_2$	1	93	Odd
$C_6H_4(NH_2)_2$	2	108	Even
C_9H_7N	1	129	Odd

10.1.3 Molecular Formulas and Isotopic Abundances

If you look at the mass spectrum for methane (Figure 10.2) and the corresponding Table 10.2, you note from the marked peak on the spectrum that the molecular ion is at $m/z = 16$. The MW to the units place for methane is, of course, equal to the atomic weight of carbon plus four times the atomic weight of hydrogen, or $(1)(12) + (4)(1) = 16$. The table shows the presence of an additional small peak

at $m/z = 17$. The peak at 17 exists because the element carbon has more than one isotope. The two stable isotopes of carbon are ^{12}C and ^{13}C. ^{12}C constitutes 98.90% of all naturally occurring carbon atoms; ^{13}C is 1.10% of all naturally occurring carbon. (A third carbon isotope, ^{14}C, is radioactive and does not need to be accounted for in organic structure determination but is very important for other applications, such as radiocarbon dating.) Therefore, in a compound containing one carbon atom, 98.90% of the molecules will have a ^{12}C atom, while 1.10% will have a ^{13}C atom.

In every organic compound (natural or synthesized from natural sources), the ^{13}C will result in a peak that is one mass number greater than the mass of the molecular ion. This ^{13}C-containing peak is generally designated as (M + 1). For example, with methane, CH_4, the MW is 16, assuming that carbon has an atomic weight of 12 (i.e., $^{12}C^1H_4 = 12 + 4 = 16$). Let the abundance of this peak be 100. But the abundance of ^{13}C is 1.1% of ^{12}C; therefore, $^{13}C^1H_4 = 13 + 4 = 17$, and its abundance is 1.1% of $^{12}C^1H_4$, as shown in the following:

Peak Designation	Isotopic Formula	MW	Rel. Abundance
M	$^{12}C^1H_4$	16	100
M + 1	$^{13}C^1H_4$	17	1.1

Similarly, for ethane, C_2H_6, which has two carbon atoms, we can designate the MS pattern as follows:

Peak Designation	Isotopic Formula	MW	Rel. Abundance
M	$^{12}CH_3\ ^{12}CH_3$	30	100
M + 1	$^{12}CH_3\ ^{13}CH_3$	31	1.1
M + 1	$^{13}CH_3\ ^{12}CH_3$	31	1.1
Total M + 1		31	2.2
M + 2	$^{13}CH_3\ ^{13}CH_3$	32	0.012

With C_2H_6, we have $^{12}C_A\ ^{12}C_B\ ^1H_6$ for an MW of 30 and a corresponding $m/z = 30$ for the molecular ion. But we also will have ions of $^{13}C_A\ ^{12}C_B\ ^1H_6$ ($m/z = 31$) and $^{12}C_A\ ^{13}C_B\ ^1H_6$ ($m/z = 31$). Both combinations are equally possible. If the abundance at $m/z = 30$ is 100, then $^{13}C\ ^{12}C\ ^1H_6$ is 1.1% of that and $^{12}C\ ^{13}C\ ^1H_6$ is also 1.1% of that, so the total abundance at $m/z = 31$ is 2.2% of $m/z = 30$. The small peak at two mass units above the molecular ion peak occurs when both carbon atoms are ^{13}C atoms.

If our molecule contains only carbon and hydrogen, since there is 1.1% relative natural abundance of ^{13}C compared to 100% ^{12}C, and our mass spectrum shows a 1.1% abundance of (M + 1) to M, only one carbon atom can be present in the molecule. That is, if we are dealing with a hydrocarbon and only one carbon atom is present, then the ratio (M + 1)/M = 1.1%. If two carbons are present in the molecule, as there are in ethane, the probability of ^{13}C being present is twice as great and (M + 1)/M = 2.2%.

10.1.3.1 Counting Carbon Atoms

There is a definite relationship between the peaks at (M + 1) and M that is directly related to the number of carbons present in hydrocarbon molecules:

$$\frac{M+1}{M} = 1.1\% \times \text{Number of C atoms in the molecule} \qquad (10.1)$$

This relationship is very valuable in characterizing an unknown compound. The calculation just presented is for hydrocarbons (compounds containing only carbon and hydrogen) and ignores the contribution from the ^2H isotope of hydrogen, called deuterium, due to its low relative abundance of 0.016%. In rigorous calculations, the contribution from deuterium would be taken into account.

Table 10.4 Relative Natural Isotope Abundances of Common Elements in Organic Compounds

Isotope	Mass	Relative Abundance	Mass	Relative Abundance	Mass	Relative Abundance
H	1	100	2	0.016		
C	12	100	13	1.11		
N	14	100	15	0.37		
O	16	100	17	0.04	18	0.20
F	19	100				
Si	28	100	29	5.07	30	3.36
P	31	100				
S	32	100	33	0.78	34	4.39
Cl	35	100			37	32.7
Br	79	100			81	97.5
I	127	100				

For benzene, C_6H_6 (Table 10.1), the molecular ion $m/z = 78$, and the (M + 1) $m/z = 79$. The relative abundance of the (M + 1) peak is 6.6%; dividing 6.6% by 1.1% confirms that there are six carbons in the molecule.

Using a similar process for C_3H_8, MW = 44, the abundance of an (M + 1) peak at $m/z = 45$ will be predicted to be $3 \times 1.1\%$ of the abundance at $m/z = 44$. The same calculation can be made for any hydrocarbon.

A list of common isotopes found in organic compounds and their relative abundance in nature is given in Table 10.4. A complete table of the relative abundance of natural isotopes for all elements is located in Appendix 10.A. As can be seen in Table 10.4, the natural abundance of deuterium is only 0.016% of the ^1H abundance, so it can usually be ignored in calculations.

However, there are a number of common isotopes that we cannot ignore for many organic molecules, such as those of oxygen, nitrogen, sulfur, and the halogens. This gives rise to the question of what atomic weights to use when calculating MWs for MS so that we have the correct mass for the molecular ion.

There are three expressions for mass that are important to know. The **integer mass** of the most abundant naturally occurring stable isotope is the **nominal mass** of the element. The **exact mass** of an isotope is determined by high-resolution MS; the exact mass of ^{35}Cl is 34.9689 Da and that for ^{37}Cl is 36.9659 Da. The **atomic weight** is the **average mass** of the element. For example, if we have a compound containing chlorine, most periodic tables list the atomic weight of chlorine as 35.45 or 35.5. The MW of CH_2Cl_2 is therefore equal to $12 + 2(1) + 2(35.5) = 85$. But a mass spectrum of dichloromethane does not have an intense peak at $m/z = 85$. The "atomic weight" of chlorine in the periodic table is a weighted average of the exact masses of ^{35}Cl and ^{37}Cl, and ^{35}Cl is about three times as abundant as ^{37}Cl. If chlorine were analyzed by MS, there would be a peak at $m/z = 35$ and a peak about one-third as intense at $m/z = 37$, but no peak at $m/z = 35.5$. For MS, MWs are calculated using the most abundant isotope of the element with its mass rounded to the nearest integer value; that is, the nominal mass is used. For most organic compounds, the most abundant isotope is the lowest mass isotope. Therefore, the MW of dichloromethane would be calculated as $12 + 2(1) + 2(35) = 84$. There is a significant peak at $m/z = 84$ in the mass spectrum of dichloromethane. At what higher values of m/z would you expect to see peaks in this spectrum based on the isotopes present?

10.1.3.2 Counting Carbon, Nitrogen, and Sulfur Atoms

From Table 10.4, it is clear that not only carbon but also other elements contribute to the (M + 1) peak intensity, notably nitrogen and sulfur when they are present. Silicon obviously will impact the

(M + 1) peak in organosilicon compounds such as silicone polymers. A more general equation for the ratio of the (M + 1) to M peak is

$$\frac{M+1}{M} = 1.1(\#\text{C atoms}) + 0.016(\#\text{H atoms}) + 0.37(\#\text{N atoms}) + 0.78(\#\text{S atoms}) + \cdots \quad (10.2)$$

where #C atoms is the number of carbon atoms in the molecule and so on. The general expression for compounds containing only C, H, N, and O would use only the terms for carbon and nitrogen. This equation would also apply to CHNO compounds containing fluorine, phosphorus, and/or iodine, since these have only one isotope. It can be seen that the formula is of most use in confirming molecular formulas, since we need to know what elements are present to use the equation effectively. We may also have previous information about what elements are present from combustion analysis; that type of analysis would indicate oxygen, nitrogen, or sulfur in the molecule, for example.

10.1.3.3 Counting Oxygen Atoms

Similarly, the (M + 2) peak contains intensity contributions from oxygen, Cl, Br, and multiple heavy isotopes of carbon, hydrogen, and so on. A general formula for compounds that contain only C, H, N, O, F, P, and I can be used to ascertain the number of oxygen atoms, in theory. Examination of Table 10.4 shows that oxygen has two important isotopes, ^{16}O and ^{18}O, separated by 2 Da, with a relative abundance $^{18}O/^{16}O$ of 0.2%. The number of oxygen atoms in a molecular ion can be calculated from

$$\frac{M+2}{M} = 0.20(\#\text{O atoms}) + \frac{(1.1(\#\text{C atoms}))^2}{200} \quad (10.3)$$

This equation again ignores the contribution from hydrogen. Compounds containing the halogens Cl and Br will be discussed later in this chapter. Iodine does not affect the equation, since iodine is monoisotopic.

For example, the mass spectrum of methanol, CH_3OH, is shown in Figure 10.3, and the mass spectral data for methanol are shown in Table 10.3. Figure 10.3 notes with the triangle that the molecular ion is at $m/z = 32$. (This can be deduced with practice; aliphatic alcohols often fragment by loss of a proton or water. Is there an M − 18 peak in the methanol spectrum corresponding to loss of water?) The ratio of (M + 1)/M, the $m/z = 33$ peak to that at $m/z = 32$, is 1.0/66 or 1.5%, indicating not more than one carbon atom. Also, the (M + 2)/M ratio, m/z 34/32, is 0.21%; inserting one carbon atom and 0.21 for the ratio into Equation 10.3 shows us that only one oxygen atom is present in the molecular ion.

There are some other interesting points in the methanol spectrum. For instance, the most abundant peak (the base peak) is at $m/z = 31$, indicating that methanol very easily loses one hydrogen atom and forms CH_3O. Another abundant peak is at $m/z = 29$, indicating that methanol loses three hydrogen atoms to form COH. The next most abundant peak is at 15, which is equivalent to a CH_3 ion, indicating breaking of the OH bond to the methyl group. The fragment that would correspond to the hydroxyl ion would be at $m/z = 17$; it is not abundant, indicating that if OH ions are formed, they do not reach the detector. Since we are detecting positively charged ions in EI MS, is this surprising?

The mass spectrum of water is shown in Figure 10.5 and Table 10.5. To calculate the number of carbon and oxygen atoms in a molecule, we assume that only the elements C, H, N, O, F, P, and I are present and that there are no interferences from other ions. The molecular ion $^1H_2{}^{16}O$ is at $m/z = 18$,
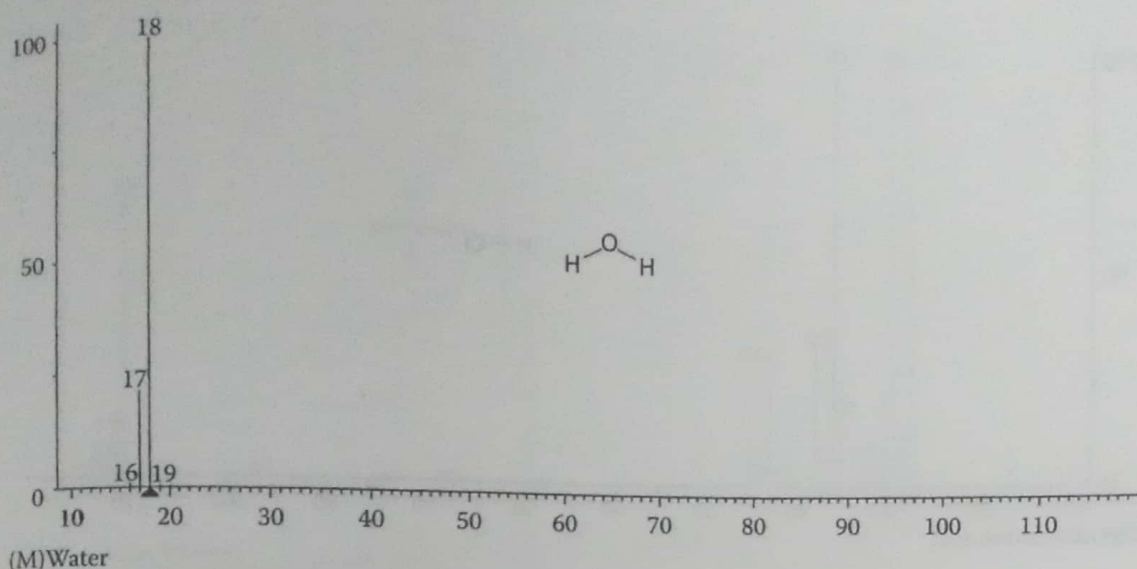
(M)Water

Figure 10.5 A mass spectrum of water, H_2O.

Table 10.5 Mass Spectral Data for H_2O

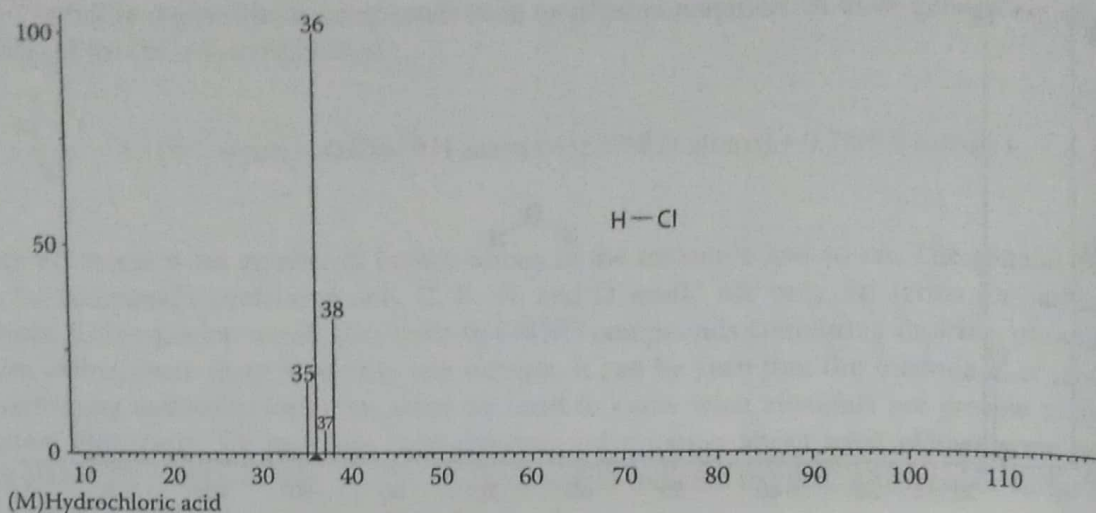| m/z	Relative Abundance
1	<0.1
16	1.0
17	21
18	100
19	0.08
20	0.22

but there is also a peak listed in the table at $m/z = 20$, the (M + 2) peak caused by $^1H_2$ ^{18}O. Its abundance is approximately 0.2% of the abundance of 18 ($^1H_2$$^{16}O$), confirming that only one oxygen atom is present in the molecular ion. The (M + 1) peak is only 0.08% of the M peak, which does not allow for a carbon atom in the molecular ion.

While Equation 10.3 can be used, in theory, to calculate the number of oxygen atoms in a compound, in reality, the ^{18}O isotope is of low abundance, and oxygen-containing compounds often fragment so that the molecular ion is of low intensity or not detected. In practice, the observation of isotope information for oxygen is often difficult.

10.1.4 Compounds with Heteroatoms

The elements shown in Table 10.4 can be classified into three categories, following the recommended nomenclature of McLafferty. "A" elements are those that are monoisotopic, such as F, P, and I. Hydrogen is also classified as an A element, because the natural abundance of deuterium is so low. "A + 1" elements are those with two isotopes, one heavier than the most abundant isotope by 1 Da. Carbon and nitrogen are A + 1 elements. "A + 2" elements have an isotope that is 2 Da heavier than the most abundant isotope. Cl, Br, and O are the three most common of these; S and Si are also A + 2 elements that must be considered if their presence is detected by elemental combustion analysis or other analytical methods.

The A + 2 elements are the easiest to recognize, so they should be looked for first when presented with an unknown mass spectrum. Oxygen has already been discussed, so look at the isotope ratios of Cl and Br from Table 10.4. Cl has two isotopes, ^{35}Cl and ^{37}Cl, in a ratio of 100:33. A compound

(M)Hydrochloric acid

Figure 10.6 A mass spectrum of hydrogen chloride, HCl, showing the characteristic 3:1 ratio of the M/(M + 2) peaks for a compound containing a single Cl atom.

Table 10.6   Mass Spectral Data for HCl

m/z	Relative Abundance
35	12
36	100
37	4.1
38	33
39	0.01

that contains one Cl atom would have an (M + 2) peak approximately one-third as intense as the M peak. Br has two isotopes, ^{79}Br and ^{81}Br, in a ratio of ~1:1. A compound with one Br atom would have approximately equal M and (M + 2) peaks. These are very distinctive patterns.

Figure 10.6 and Table 10.6 show two strong peaks at $m/z = 38$ (M + 2) and 36 (M) in an abundance ratio of ~1/3. (We know that the peak at $m/z = 36$ is the M peak because it is marked by the triangle.) This is typical of the pattern seen when one Cl atom is present. If a chlorine atom is present, then $m/z = 36$ arises from ^{35}Cl plus one ^1H atom (only ^1H could increase the mass from 35 to 36). Then $m/z = 38$ would be due to $^{37}Cl^1H$. This is the mass spectrum of hydrochloric acid, HCl. The peaks at $m/z = 35$ and 37 are of course due to the ionized chorine isotopes that have lost the hydrogen through fragmentation. The ratio of the m/z 37/35 peaks is also 1/3, supporting the identification of a single Cl atom.

For organochlorine compounds with one Cl atom, such as CH_3Cl, we will expect to see a peak at (M + 2) because there will be ^{37}Cl present. The ratio (M + 2)/M will be the same as the natural ratio of ^{37}Cl to ^{35}Cl, that is, 32.7%, resulting in the same one-third pattern seen in HCl.

Looking at Figure 10.7 and Table 10.7, the almost equal intensity of the two peaks at $m/z = 94$ and 96 is characteristic of the presence of one bromine atom. The presence of one Br atom is confirmed by the peaks at $m/z = 79$ and 81 in approximately equal abundance. These two peaks can reasonably be attributed to ^{79}Br and ^{81}Br ions. If the MW is 94 as indicated by the identification of $m/z = 94$ as the molecular ion (note the triangle), a mass of ^{79}Br subtracted from 94 shows that atoms equivalent to a group of mass 15 must be present. This is most likely a methyl group, CH_3-, confirmed by an abundant peak at $m/z = 15$. Based on the m/z 16/15 ratio, which is 0.62/59 = 1.05%, the ion of $m/z = 15$ contains 1 C and is probably CH_3. Consecutive peaks at masses of 14, 13, and 12 confirm 3 hydrogen atoms,

(M)Methane, bromo
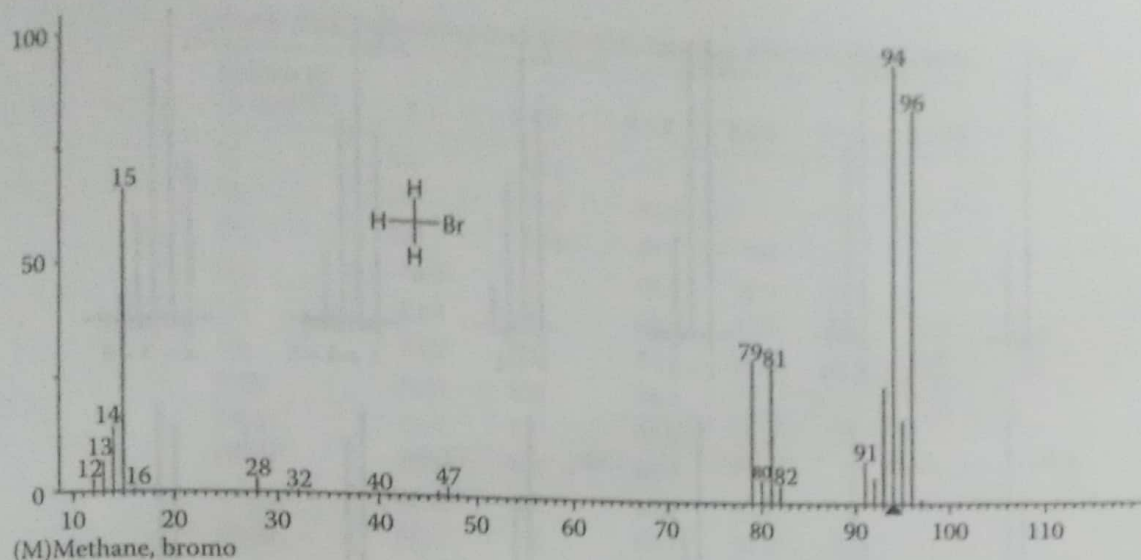
Figure 10.7 A mass spectrum of bromomethane (or methyl bromide), CH_3Br, showing the characteristic 1:1 ratio of the M/(M + 2) peaks for a compound containing a single Br atom.

Table 10.7 Mass Spectral Data for CH_3Br

m/z	Relative Abundance	m/z	Relative Abundance
12	1.2	48	0.95
13	1.4	79	10
14	3.8	81	10
15	59	91	4.2
16	0.62	92	2.4
39.5	0.19	93	6.8
40.5	0.20	94	100
46	1.3	95	0.9
46.5	0.30	96	96
47	2.3	97	1.1
47.5	0.28		

ending in a C ion with no H attached. Hence, the molecule is most likely composed of a methyl group and one Br atom: $Br + CH_3$ or CH_3Br. This is bromomethane (also called methyl bromide).

10.1.5 Halogen Isotopic Clusters

If more than one chlorine atom or bromine atom is present in a molecule, distinctive "isotope cluster patterns" are seen in the mass spectrum. Figure 10.8 gives a graphical representation of the isotope peak intensity patterns for Cl and Br. The numerical values for the peak ratios are given in Table 10.8. The patterns arise as follows:

If we have two Cl atoms present in an ion, the distribution of masses in each ion may be $^{35}Cl^{35}Cl$, $^{37}Cl^{35}Cl$, $^{35}Cl^{37}Cl$, or $^{37}Cl^{37}Cl$. The $^{35}Cl:^{37}Cl$ abundance is 100:33, so the probability of these isotope distributions occurring is approximately 100:66:11.

Consequently, any molecule RCl_2 containing two Cl atoms will exhibit masses of R + 70, R + 72, and R + 74 with a relative abundance due to Cl isotopes of 100:66:11, due to the presence of $R^{35}Cl^{35}Cl$, $R^{37}Cl^{35}Cl$, $R^{35}Cl^{37}Cl$, and $R^{37}Cl^{37}Cl$. This pattern in the mass spectrum is a Cl_2 isotope cluster. The same pattern will be seen not only for the molecular ion but also for any fragment ion that contains both Cl atoms. Similarly, if three chlorine atoms are present (RCl_3), a cluster will occur with masses R + 105, R + 107, R + 109, and R + 111 with approximate abundances of 100:98:32:3.

10.1.6 Rings Plus Double Bonds

The formula for *n*-hexane is C_6H_{14}. The formula for cyclohexane is C_6H_{12}, the same as the formula for hexene. The presence of a ring or double bond results in a change in the ratio of carbon to hydrogen. This change is a general property, and based on it, the following formula is derived.

The number of rings + double bonds in a molecule of formula $C_xH_yN_zO_m$ is

$$x - \frac{1}{2}y + \frac{1}{2}z + 1 \tag{10.4}$$

For example, for *n*-hexane, $x = 6$, $y = 14$, so the number of rings + double bonds is $6 - (1/2 \times 14) + 0 + 1 = 0$. For cyclohexane, $x = 6$, $y = 12$; therefore, rings + double bonds $= 6 - (1/2 \times 12) + 0 + 1 = 1$. For benzene, C_6H_6, rings + double bonds $= 6 - (1/2 \times 6) + 0 + 1 = 4$, that is, three double bonds plus one ring. Acetylene, C_2H_2, contains a triple bond between the two carbon atoms. Equation 10.4 applied to acetylene yields $2 - (1/2 \times 2) + 0 + 1 = 2$. A triple bond is equivalent to two double bonds. The equation does not tell us whether we are dealing with a ring or a double bond or two double bonds versus a triple bond, or, as is the case with benzene, a delocalized molecular orbital that can be represented for bonding purposes as three double bonds. We need to use the equation in conjunction with the molecular formula (and any other information we have from IR, nuclear magnetic resonance (NMR), etc.) to postulate a reasonable structure.

When atoms other than CHNO are present, the heteroatoms are matched according to their lowest absolute valence. Si takes four bonds, like C; P is equivalent to N, while the halides, with a lowest absolute valence of 1, act like H. Hence, the general equation is

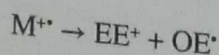$$\text{Rings and double bonds} = x - \frac{1}{2}y + \frac{1}{2}z + 1 \tag{10.5}$$

where
$x = $ C, Si
$y = $ H, F, Cl, Br, I
$z = $ N, P

10.1.7 Common Mass Losses on Fragmentation

When a molecule ionizes, it follows the reaction $M + e^- \rightarrow M^{+\cdot} + e^- + e^-$, as described earlier. When an OE molecular ion fragments, it can do so in a variety of ways, and resulting OE fragment ions can also fragment in similar ways. Simple cleavage results in the expulsion of a neutral fragment that is an OE radical (OE$^\cdot$) and formation of an EE ion (EE$^+$) as shown in the following:

$$M^{+\cdot} \rightarrow EE^+ + OE^\cdot$$

Multicentered fragmentation results in the expulsion of an EE neutral species and formation of an OE ion (OE$^{+\cdot}$):

$$M^{+\cdot} \rightarrow EE + OE^{+\cdot}$$
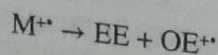
In the first case, we would detect the EE$^+$ ion and, in the second case, the OE$^{+\cdot}$ ion. The neutral species lost on fragmentation can be identified by the difference between the mass of M$^{+\cdot}$ and the fragment ion. Common losses from the molecular ion are given in Table 10.9. Tables 10.10 and 10.11 list more common neutral fragments expelled by simple cleavage and by multicentered fragmentations.