Lecture # 6

C. Desorption Ionization Techniques (SIMS, FAB, and MALDI)

Both EI and CI methods require a relatively volatile (low molecular weight) sample. More recently developed ionization techniques allow the analysis of large, nonvolatile molecules by mass spectrometry. Three of these methods, **secondary ion mass spectrometry** (**SIMS**), **fast atom bombardment** (**FAB**), and **matrix-assisted laser desorption ionization** (**MALDI**) are all **desorption ionization** (**DI**) techniques. In desorption ionization, the sample to be analyzed is dissolved or dispersed in a matrix and placed in the path of a high-energy (1- to 10-keV) beam of ions (SIMS), neutral atoms (FAB), or high-intensity photons (MALDI). Beams of Ar^+ or Cs^+ are often used in SIMS, and beams of neutral Ar or Xe atoms are common in FAB. Most MALDI spectrometers use a nitrogen laser that emits at 337 nm, but some applications use an infrared (IR) laser for direct analysis of samples contained in gels or thin-layer chromatography (TLC) plates. The collision of these ions/atoms/photons

The collision of these ions/atoms/photons

with the sample ionizes some of the sample molecules and ejects them from the surface (Fig. 3.6).

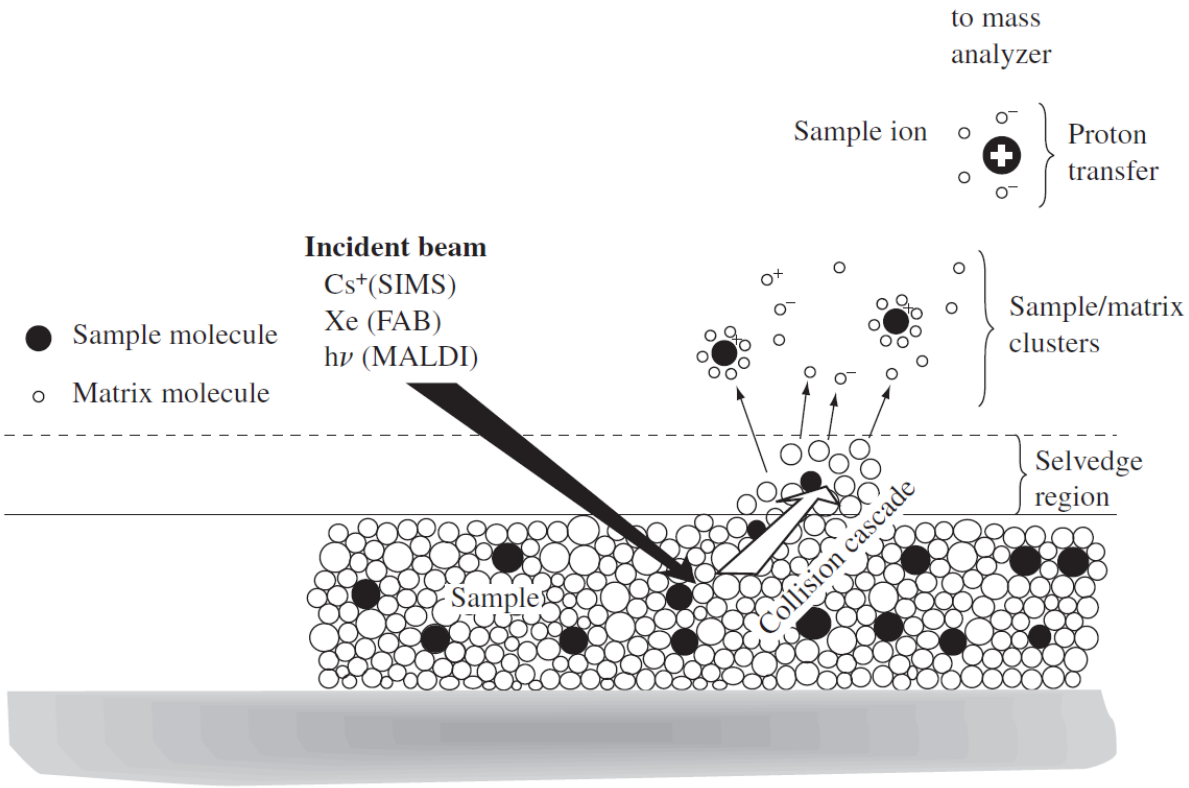


FIGURE 3.6 Schematic representations of desorption ionization techniques.

ejected ions are then accelerated toward the mass analyzer as with other ionization methods. Since FAB uses neutral atoms to ionize the sample, both positive-ion and negative-ion detection are possible. Molecular ions in SIMS and FAB are typically $(M + H)^+$ or $(M - H)^-$, but adventitious alkali metals can create $(M + Na)^+$ and $(M + K)^+$ ions also. SIMS and FAB ionization methods may be used on sample compounds with molecular weights up to about 20,000, such as polypeptides and oligonucleotides.

The matrix should be nonvolatile, relatively inert, and a reasonable electrolyte to allow ion formation. If the matrix compound is more acidic than the analyte, then predominantly $(M + H)^+$ ions will be formed, while mostly $(M - H)^-$ ions will result when the matrix is less acidic than the analyte. The matrix absorbs much of the excess energy imparted by the beam of ions/atoms and produces ions that contribute a large amount of background ions to the mass spectrum. In fact, chemical reactions within the matrix during ionization can contribute background ions in most mass

regions below about 600 *m/z*. Common matrix compounds for SIMS and FAB include glycerol, thioglycerol, 3-nitrobenzyl alcohol, di- and triethanolamine, and mixtures of dithiothreitol (DTT) and dithioerythritol (Fig. 3.7)

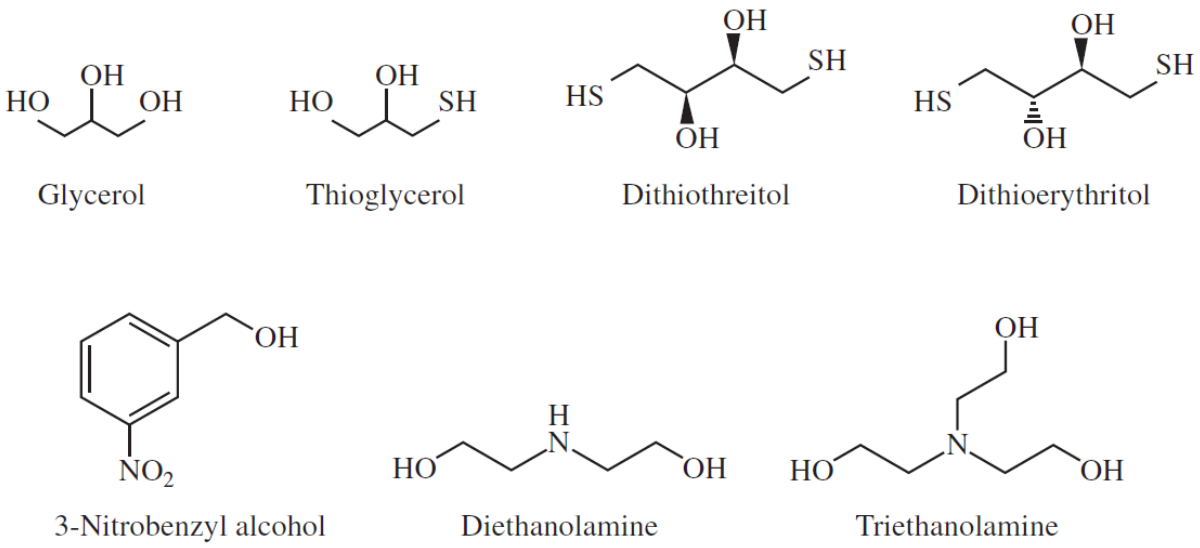


FIGURE 3.7 Common matrices for SIMS and FAB mass spectrometry.

The matrix compounds used in MALDI are chosen for their ability to absorb the ultraviolet (UV) light from a laser pulse (337 nm for N_2 laser). Substituted nicotinic, picolinic, and cinnamic acid derivatives are often used in MALDI techniques (Fig. 3.8). The matrix absorbs most of the energy from the laser pulse, thus allowing for the creation of intact sample ions that are ejected from the matrix. MALDI mass spectrometry is useful for analytes spanning a wide range of molecular weights, from small polymers with average molecular weights of a few thousand atomic mass units (amu) to oligosaccharides, oligonucleotides and polypeptides, antibodies, and small proteins with molecular weights approaching 300,000 amu. Furthermore, MALDI requires only a few femtomoles (1×10^{-15} mole) of sample!

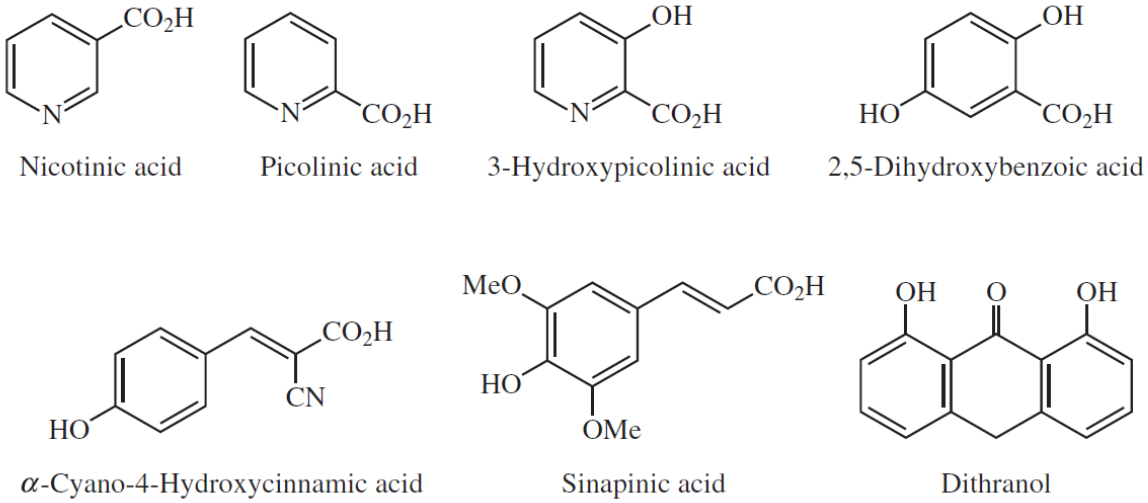


FIGURE 3.8 Common matrices for MALDI applications.

D. Electrospray Ionization (ESI)

An even more useful technique for studying high molecular weight biomolecules and other labile or nonvolatile compounds is **electrospray ionization** (**ESI**) and its cousin **thermospray ionization** (**TSI**). In ESI, a solution containing the sample molecules is sprayed out the end of a fine capillary into a heated chamber that is at nearly atmospheric pressure. The capillary through which the sample solution passes has a high voltage potential across its surface, and small, charged droplets are expelled into the ionization chamber. The charged droplets are subjected to a counterflow of a drying gas (usually nitrogen) that evaporates solvent molecules from the droplets. Thus, the charge density of each droplet increases until the electrostatic repulsive forces exceed the surface tension of the droplet (the Rayleigh limit), at which point the droplets break apart into smaller droplets.

This process continues until solvent-free sample ions are left in the gas phase (Fig. 3.9). TSI occurs by a similar mechanism but relies on a heated capillary rather than one with an electrostatic potential to initially form the charged droplets. Negative ions may also be formed in ESI by loss of protons from the sample to basic species in solution. ESI has become much more common than TSI over the last decade or two, and because it relies on a sample in solution, ESI is the most logical method to be employed in LC-MS systems.

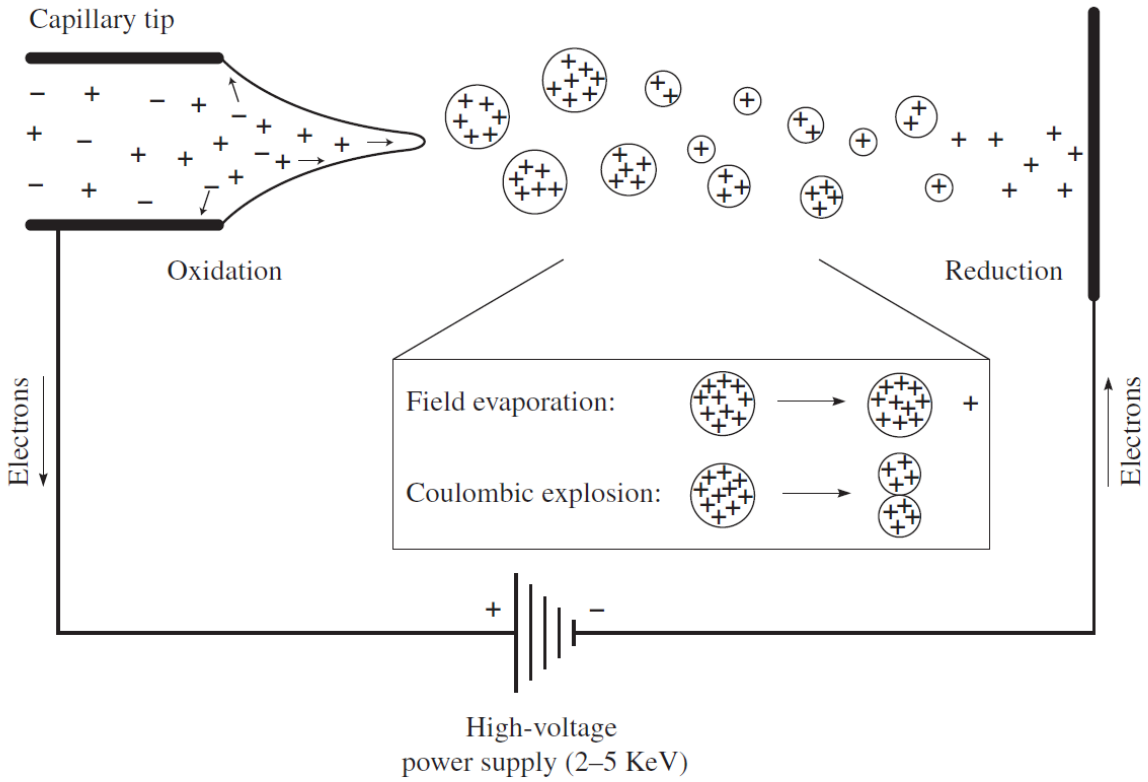


FIGURE 3.9 Schematic representation of electrospray ionization (ESI) showing both field evaporation and coulombic explosion. (From Gross, J. H., *Mass Spectrometry: A Textbook*, Springer, Berlin, 2004. Reprinted by permission.)

The charges of the ions generated using ESI do not necessarily reflect the charge state of the sample in solution. The charge transferred to the sample molecules (usually in the form of protons) arises from a combination of charge concentration in the droplets during evaporation of the aerosol and electrochemical processes stemming from the electrostatic potential of the capillary.

The sample ions may bear a single charge or multiple charges. Figure 3.10 shows the ESI-MS of lysozyme from chicken egg white in the absence and presence of dithiothreitol. In the first spectrum, ions are observed representing protein molecules bearing 10^+, 11^+, 12^+, and 13^+ charges. The latter spectrum shows even more highly charged ions—including a peak from protein bearing a 20^+ charge. The formation of multiply charged ions is particularly useful in the MS analysis of proteins. Typical proteins can carry many protons due to the presence of basic amino acid side chains, resulting in peaks at m/z = 600–2000 for proteins with a molecular weight that approaches 200,000 amu.

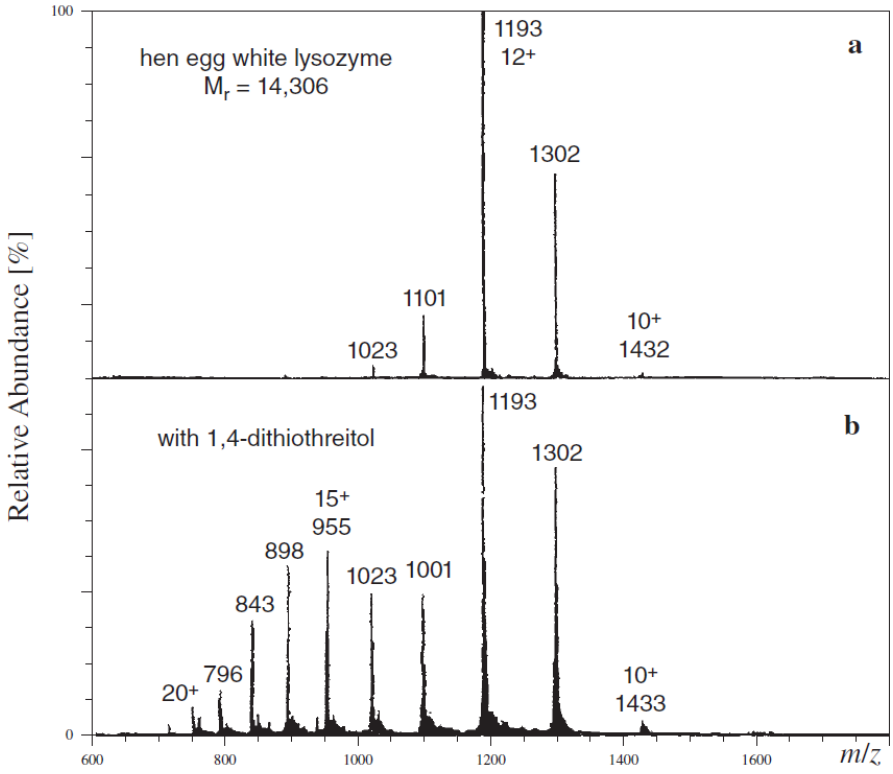


FIGURE 3.10 ESI-MS of proteins. Chicken egg white lysozyme in the absence (top) and presence (middle) of dithiothreitol. (From Gross, J. H., *Mass Spectrometry*: *A Textbook*, Spinger, Berlin, 2004. Reprinted with permission.)

The data shown in Figure 3.10 can be used to calculate the molecular mass for lysozyme. The mass is calculated by multiplying the charge on the lysozyme by the *m/z* value shown on the chromatogram. For example:

$$(10)(1432) = 14{,}320 \text{ AMU}$$
$$(12)(1193) = 14{,}316$$
$$(15)(955) = 14{,}325$$

Thus, the molecular mass of lysozyme is about 14,320 AMU.

ESI-MS is not limited to the study of large biomolecules, however. Many small molecules with molecular weight in the 100–1500 range can be studied by ESI-MS. Compounds that are too nonvolatile to be introduced by direct probe methods or are too polar or thermally labile to be introduced by GC-MS methods are ideal for study by LC-MS using ESI techniques.

Desorption electrospray ionization (DESI) combines the soft ionization of the electrospray technique with desorption of the sample ions from a surface. Unlike MALDI, however, no matrix is needed. The DESI technique uses electrosprayed aqueous aerosols to ionize and desorb analyte ions.

Developed around the same time as the DART technique (Section 3.3B), DESI interfaces with a mass analyzer using a heated ion transfer tube that is in some cases flexible and can be held in the researcher's hand directly above the sample surface.

Soft ionization techniques like DESI and DART coupled with the appropriate mass analyzer can produce accurate mass spectra to determine exact elemental composition (Section 3.7). The open ion source configuration allows the sampling from a number of matrices and surfaces as widely varied as intact plant material, cloth, concrete, and even human skin. Moving the sample in the ion beam provides spatial resolution, allowing one to observe differing compositions in different areas of the same sample. These techniques are useful for forensic and public safety applications with the ability to detect pictograms of material including biological molecules and explosive residues.

3.4 MASS ANALYSIS

Once the sample has been ionized, the beam of ions is accelerated by an electric field and then passes into the **mass analyzer,** the region of the mass spectrometer where the ions are separated according to their mass-to-charge (m/z) ratios. Just like there are many different ionization methods for different applications, there are also several types of mass analyzers. While some mass analyzers are more versatile than others, none of the options are one-size-fits-all.

A. The Magnetic Sector Mass Analyzer

The kinetic energy of an accelerated ion is equal to

$$\frac{1}{2}mv^2 = zV \qquad \textbf{Equation 3.16}$$

where m is the mass of the ion, v is the velocity of the ion, z is the charge on the ion, and V is the potential difference of the ion-accelerating plates. In the **magnetic sector** mass analyzer (Fig. 3.11), the ions are passed between the poles of a magnet. In the presence of a magnetic field, a charged particle describes a curved flight path. The equation that yields the radius of curvature of this path is

$$r = \frac{mv}{zB} \qquad \textbf{Equation 3.17}$$

where r is the radius of curvature of the path, and B is the strength of the magnetic field. If these two equations are combined to eliminate the velocity term, the result is

$$\frac{m}{z} = \frac{B^2r^2}{2V} \qquad \textbf{Equation 3.18}$$

As can be seen from Equation 3.18, the greater the value of m/z, the larger the radius of the curved path. The analyzer tube of the instrument is constructed to have a fixed radius of curvature. A particle with the correct m/z ratio can negotiate the curved analyzer tube and reach the detector. Particles with m/z ratios that are either too large or too small strike the sides of the analyzer tube and do not reach the detector. The method would not be very interesting if ions of only one mass could be detected. Therefore, the magnetic field strength is continuously varied (called a *magnetic field scan*) so that all of the ions produced in the ionization chamber can be detected. The record produced from the detector system is in the form of a plot of the numbers of ions versus their m/z values.

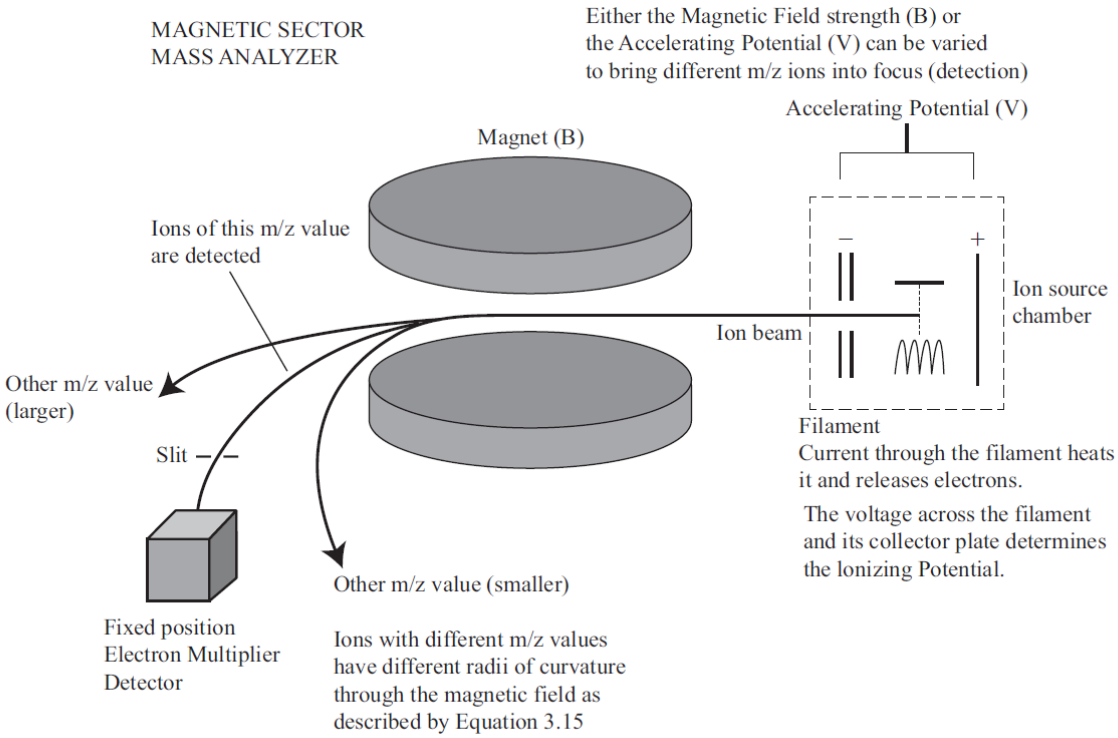


FIGURE 3.11 Schematic of a magnetic sector mass analyzer.

An important consideration in mass spectrometry is **resolution,** defined according to the relationship

$$R = \frac{M}{\Delta M} \qquad \textbf{Equation 3.19}$$

where R is the resolution, M is the mass of the particle, and ΔM is the difference in mass between a particle of mass M and the particle of next higher mass that can be resolved by the instrument. A magnetic sector analyzer can have R values approaching 10,000, depending on the radius of curvature and slit widths.

B. Double-Focusing Mass Analyzers

For many applications, much higher resolution is needed and can be achieved through modifications of this basic magnetic sector design. In fact, magnetic sector analyzers are used today only in **double-focusing mass spectrometers.** The particles leaving the ionization chamber do not all have precisely the same velocity, so the beam of ions passes through an electric field region before or after the magnetic sector (Fig. 3.12). In the presence of an electric field, the particles all travel at the same velocity. The particles describe a curved path in each of these regions, and the resolution of the mass analyzer improves—by a factor of 10 or more over the magnetic sector alone.

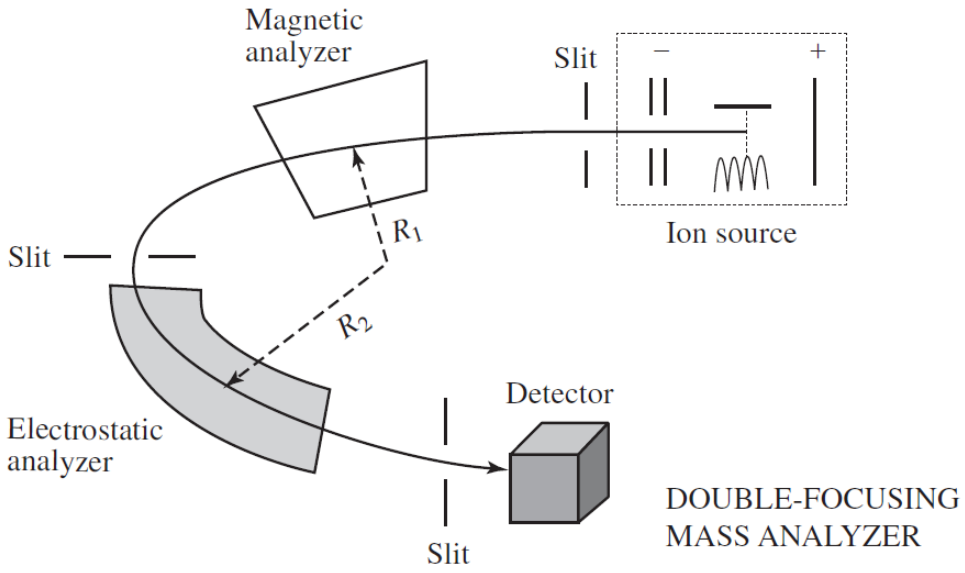


FIGURE 3.12 Schematic of a double-focusing mass analyzer.

C. Quadrupole Mass Analyzers

In a **quadrupole mass analyzer** (Fig. 3.13), a set of four solid rods is arranged parallel to the direction of the ion beam. The rods should be hyperbolic in cross section, although cylindrical rods may be used. A direct-current (DC) voltage and a radiofrequency (RF) is applied to the rods, generating an oscillating electrostatic field in the region between the rods. Depending on the ratio of the RF amplitude to the DC voltage, ions acquire an oscillation in this electrostatic field. Ions of an incorrect *m/z* ratio (too small or too large) undergo an unstable oscillation. The amplitude of the oscillation continues to increase until the particle strikes one of the rods. Ions of the correct mass-to-charge ratio undergo a stable oscillation of constant amplitude and travel down the quadrupole axis with a "corkscrew"-type trajectory. These ions do not strike the quadrupole rods but pass through the analyzer to reach the detector. Like the magnetic sector an-

Like the magnetic sector an-

alyzer, the quadrupole can be scanned from high to low values of *m/z*. A quadrupole mass analyzer is found in most "benchtop" GC-MS systems and typically has a *m/z* range from 0 to 1000, although quadrupole analyzers are available on some LC-MS systems with *m/z* ranges that approach 2000. Quadrupole mass spectrometers are low-resolution instruments (R~3000) incapable of providing exact elemental composition of the sample but their relatively low cost makes them popular for many applications. An additional drawback is their relatively slow acquisition rate due to their scanning nature.

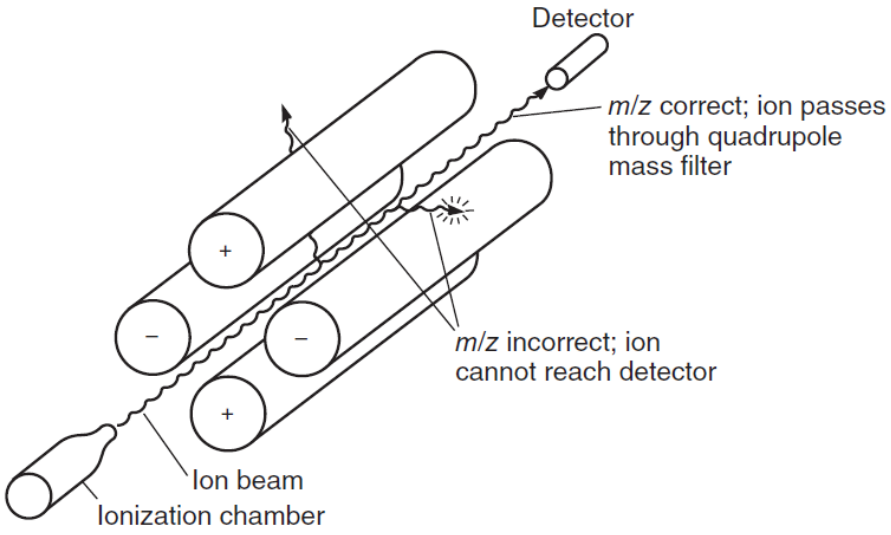


FIGURE 3.13 Quadrupole mass analyzer.

The quadrupole **ion trap** mass analyzer operates by similar principles as the linear quadrupole described above and is a common mass analyzer found in GC-MS instruments. The ion trap consists of two hyperbolic endcap electrodes and a doughnut-shaped ring electrode (the endcap electrodes are connected). An alternating current (AC) (or DC) and an RF potential is applied between the endcaps and the ring electrode (Fig. 3.14). In the linear quadrupole analyzer, ions of different *m/z* values are allowed to pass in turn through the quadrupole by adjusting the RF and DC voltages. In the ion trap, ions of all *m/z* values are in the trap simultaneously, oscillating in concentric trajectories. Sweeping the RF potential results in the removal of ions with increasing *m/z* values by putting them in unstable trajectory that causes them to be ejected from the trap in the axial direction toward the detector. This process is called **resonant ejection.** Ion trap mass analyzers are somewhat more sensitive than linear quadrupole instruments, but they have similar resolution capabilities.

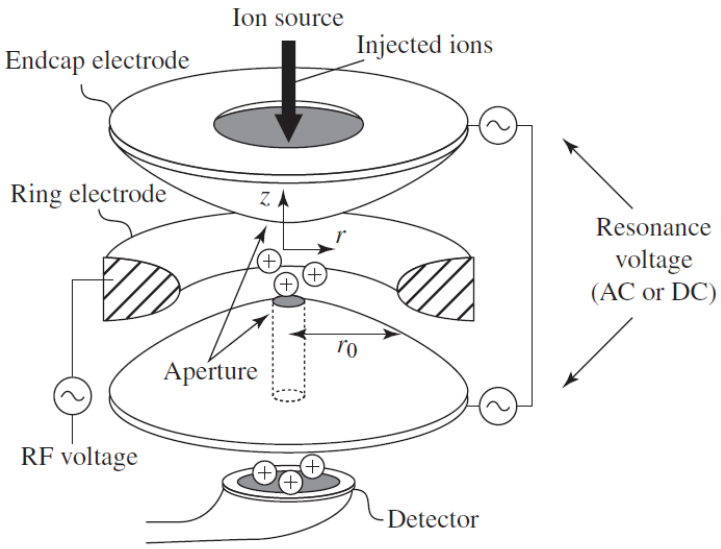


FIGURE 3.14 Quadrupole ion trap mass analyzer. (From Gross, J. H., *Mass Spectrometry: A Textbook,* Springer, Berlin, 2004. Reprinted with permission.)

Because the ion trap contains ions of all values of *m/z* at the same time (as well as neutral molecules that were not ionized prior to entering the trap), ion trap mass analyzers are also sensitive to overload and ion–molecule collisions that complicate the resulting spectrum. Recall that not all of the sample molecules get ionized—many remain uncharged. These neutral species move in a random path in the ion trap, resulting in collisions with ions as the ions oscillate in their stable trajectories. These collisions result in chemical ionization-type ionization events (Equation 3.20). This is sometimes referred to as *self-CI.*

$$\underset{\text{fragment ion}}{(R{-}H)^+} + \underset{\substack{\text{neutral}\\ \text{sample molecule}}}{M} \longrightarrow \underset{\substack{\text{neutral}\\ \text{or radical}}}{R} + \underset{\substack{\text{protonated}\\ \text{molecular ion}}}{(M+H)^+} \qquad \textbf{Equation 3.20}$$

The result is an abnormally large $(M + H)^+$ peak in the mass spectrum. This is observed in Figure 3.15, in which the base peak in the EI-MS of methyl dodecanoate under standard conditions has $m/z = 215$, representing an $(M + H)^+$ ion produced in the ion trap from ion–molecule conditions. This self-CI process can be minimized by increasing ionization efficiency, reducing the number of ions in the trap (injecting less sample), or both. The bottom spectrum in Figure 3.15 was acquired under optimized ion trap conditions with a longer ion residence time. Now, the M^+ ion is clearly visible, although the $(M + 1)$ peak is still much larger than it should be based on isotopic contributions of ^{13}C alone (see Section 3.7). Fortunately, the presence of the larger $(M + 1)$ peak rarely has an adverse effect on spectral library searches done by a computer. The visual inspection of a sample spectrum to a printed standard spectrum is quite another matter. The self-CI peak becomes quite problematic when one is attempting to characterize unknowns if one does not know the molecular formula or functional groups present ahead of time.

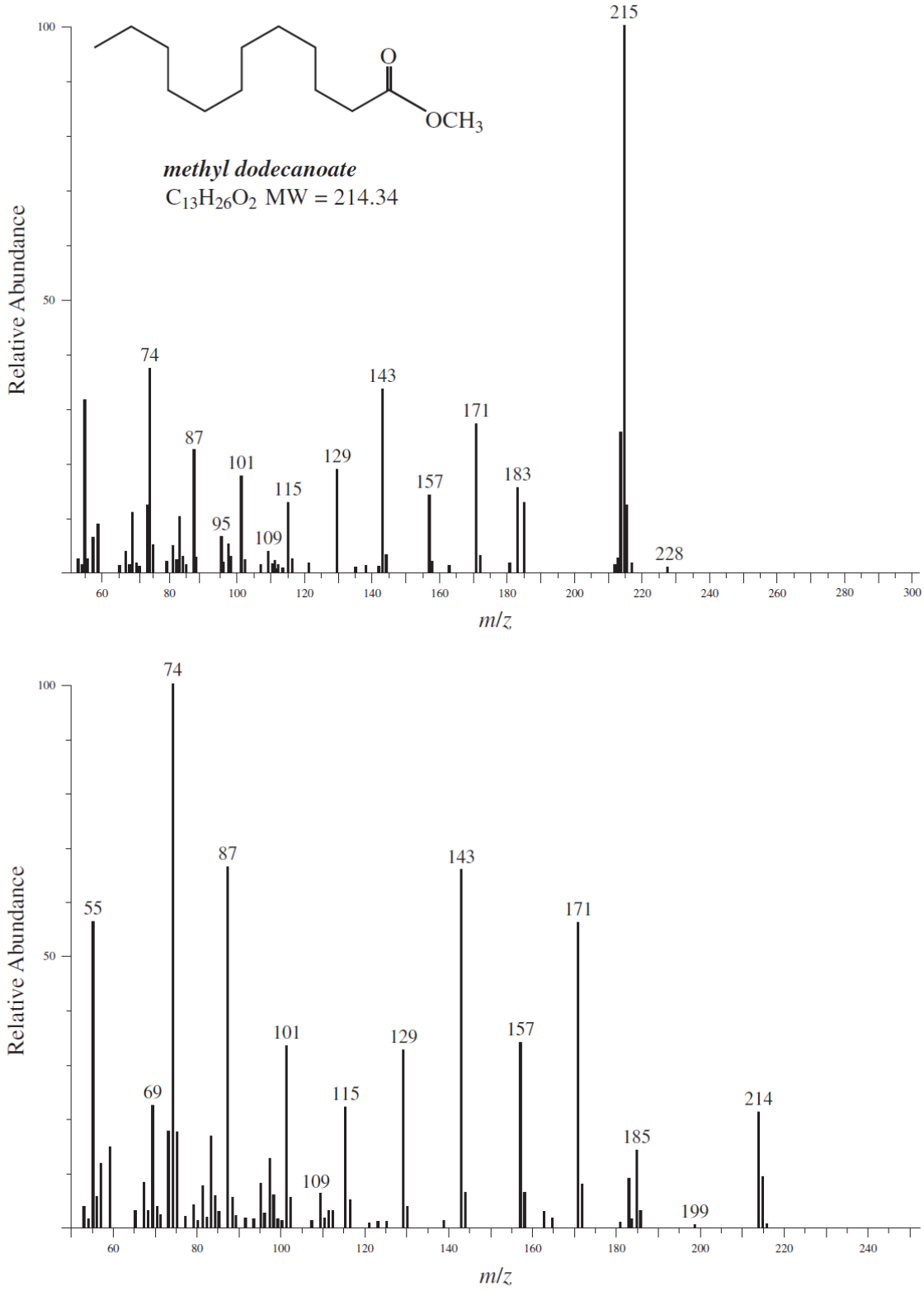


FIGURE 3.15 EI-MS of methyl dodecanoate using a quadrupole ion trap mass analyzer. Standard conditions (top) and optimized conditions to minimize ion–molecule collisions and self-CI (bottom). (Reproduced from Varian, Inc.)

D. Time-of-Flight Mass Analyzers

The **time-of-flight (TOF)** mass analyzer is based on the simple idea that the velocities of two ions, created at the same instant with the same kinetic energy, will vary depending on the mass of the ions—the lighter ion will have a higher velocity. If these ions are traveling toward the mass spectrometer's detector, the faster (lighter) ion will strike the detector first. Examining this concept further, the kinetic energy of an ion accelerated through an electrical potential V will be

$$zV = \frac{mv^2}{2} \qquad \textbf{Equation 3.21}$$

and the velocity of the ion is the length of the flight path L divided by the time t it takes the ion to travel over that distance:

$$v = \frac{L}{t} \qquad \textbf{Equation 3.22}$$

Replacing this expression for v in Equation 8.19 gives

$$zV = \frac{mL^2}{2t^2} \qquad \textbf{Equation 3.23}$$

Thus, it follows that

$$\frac{m}{z} = \frac{2Vt^2}{L^2} \qquad \textbf{Equation 3.24}$$

The TOF mass analyzer (Fig. 3.16) requires very fast electronics to accurately measure ion flight times that may be submicrosecond. Furthermore, the ions in a TOF system must be created in short, well-defined pulses so that the ions all start their journey toward the detector at the same moment. The first requirement explains why TOF instrumentation (first developed in the 1940s

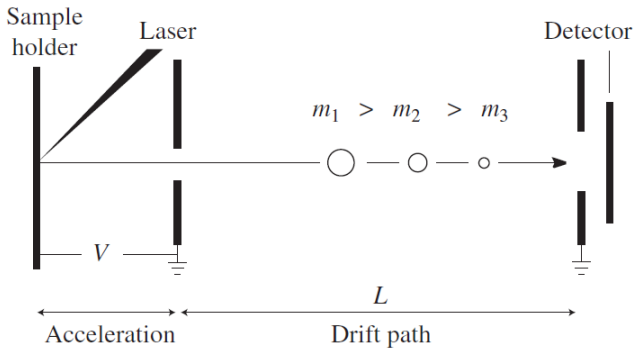


FIGURE 3.16 Schematic representation of a MALDI/TOF mass spectrometer.

and 1950s) did not become widely used until the 1980s and 1990s, when suitable circuitry became cost-effective. The last requirement is perfectly suited for the MALDI ionization technique, and MALDI/TOF mass spectrometers have found wide use in the analysis of biomolecules and synthetic polymers. In theory, TOF mass analyzers have no upper limit to their effective mass range, and these mass analyzers have high sensitivity. Unlike magnetic sector or quadrupole spectrometers, in which some of the ions are "thrown away" during the experiment, TOF instruments are able to analyze (in principle) every ion created in the initial pulse. Mass data have been obtained using MALDI/TOF from samples with molecular weights of 300,000 amu and as little as a few hundred attomoles of material.

The major disadvantage of the TOF analyzer is its inherently low resolution. The mass resolution (**R**, Eq. 3.19) of the TOF instrument is proportional to the ion's flight time, so using longer drift tubes increases resolution. Flight tubes a few meters long are commonly used in high-end instruments. With shorter drift tubes, R of only 200–500 is possible. A modification to the TOF analyzer that increases resolution is the ion reflector. The reflector is an electric field behind the free drift region of the spectrometer that behaves as an ion mirror. The reflector is able to refocus ions of slightly different kinetic energies and, if set at a small angle, sends the ions on a path back toward the original ion source. This essentially doubles the ion flight path as well. In reflector TOF instruments, a mass resolution of several thousand is possible. Combining a quadrupole with a TOF analyzer (QTOF) will provide sufficient resolution in most cases for accurate mass determination (Section 3.6).

Time-of-flight mass spectrometers are relatively simple, which makes it possible to use them in the field. During the 1991 Gulf War, concern arose that Iraqi troops might be releasing chemical warfare agents against American troops. To guard against that possibility, the U.S. Army deployed a number of tracked vehicles, each equipped with a mass spectrometer. The mass spectrometer was used to sample the air and provide advance warning should any poisonous gases be released into the air. Basic TOF mass spectrometers are also used to detect residue from explosives and illegal drugs at security screening stations in airports. Because of their value for studying short-lived species, TOF mass spectrometers are particularly useful in kinetic studies, especially with applications to very fast reactions. Very rapid reactions such as combustion and explosions can be investigated with this technique.

3.5 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.

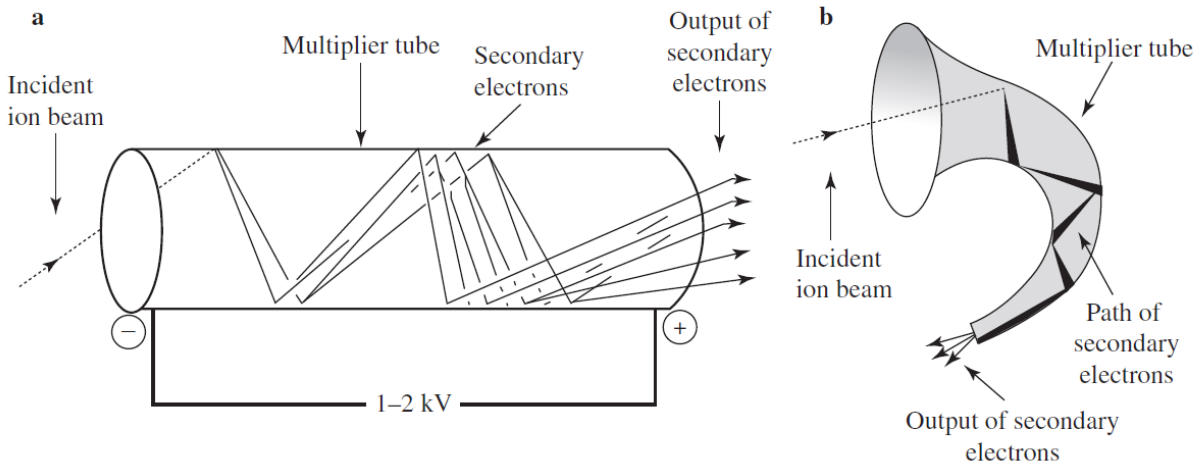


FIGURE 3.17 Schematic representation of a linear channel electron multiplier (a) and a curved channel electron multiplier (b). (Based on, J. H., *Mass Spectrometry: A Textbook,* Spinger, Berlin, 2004.)

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.

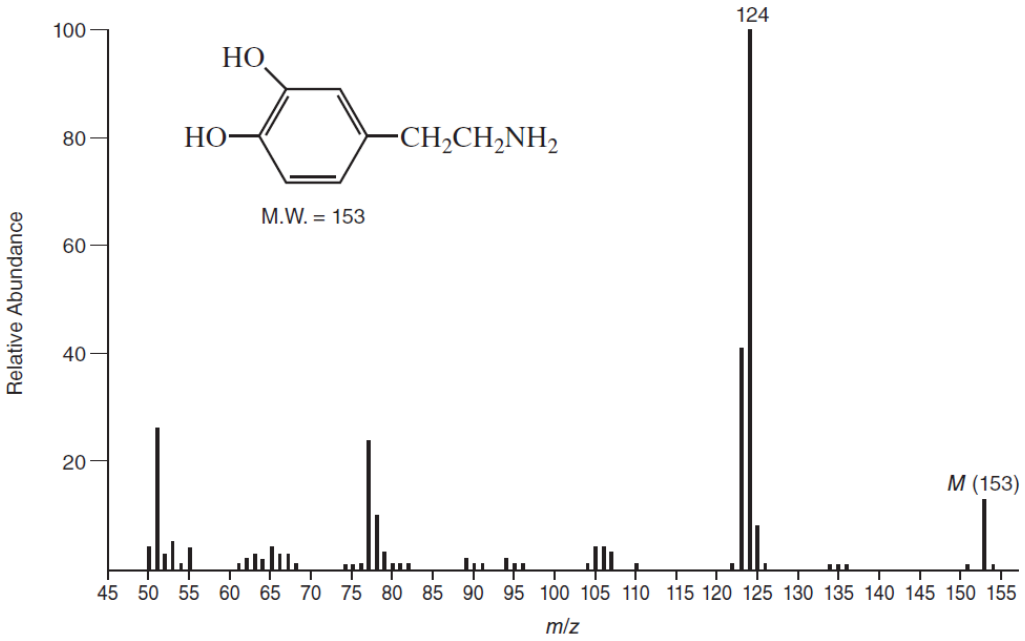


FIGURE 3.18 Partial EI-MS of dopamine.

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 $\mathbf{M^+}$ 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).

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).

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

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.

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 nonintegral 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

$$m_1^+ \longrightarrow m_2^+ \qquad \textbf{Equation 3.25}$$

and

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

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.

3.6 DETERMINATION OF MOLECULAR WEIGHT

Section 3.3 showed that when a beam of high-energy electrons impinges on a stream of sample molecules, ionization of electrons from the molecules takes place. The resulting ions, called **molecular ions,** are then accelerated, sent through a magnetic field, and detected. If these molecular ions have lifetimes of at least 10^{-5}sec, they reach the detector without breaking into fragments. The user then observes the *m/z* ratio that corresponds to the molecular ion to determine the molecular weight of the sample molecule.

In practice, molecular weight determination is not quite as easy as the preceding paragraph suggests. First, you must understand that the value of the mass of any ion accelerated in a mass spectrometer is its true mass, the sum of the masses of each atom in that single ion, and not its molecular weight calculated from chemical atomic weights. The chemical scale of atomic weights is based on weighted averages of the weights of all of the isotopes of a given element. The mass spectrometer can distinguish between masses of particles bearing the most common isotopes of the elements and particles bearing heavier isotopes. Consequently, the masses that are observed for molecular ions are the masses of the molecules in which every atom is present as its most common isotope. In the second place, molecules subjected to bombardment by electrons may break apart into fragment ions. As a result of this fragmentation, mass spectra can be quite complex, with peaks appearing at a variety of *m/z* ratios. You must be quite careful to be certain that the suspected peak is indeed that of the molecular ion and not that of a fragment ion. This distinction becomes particularly crucial when the abundance of the molecular ion is low, as when the molecular ion is rather unstable and fragments easily. The masses of the ions detected in the mass spectrum must be measured accurately. An error of only one mass unit in the assignment of mass spectral peaks can render determination of structure impossible.

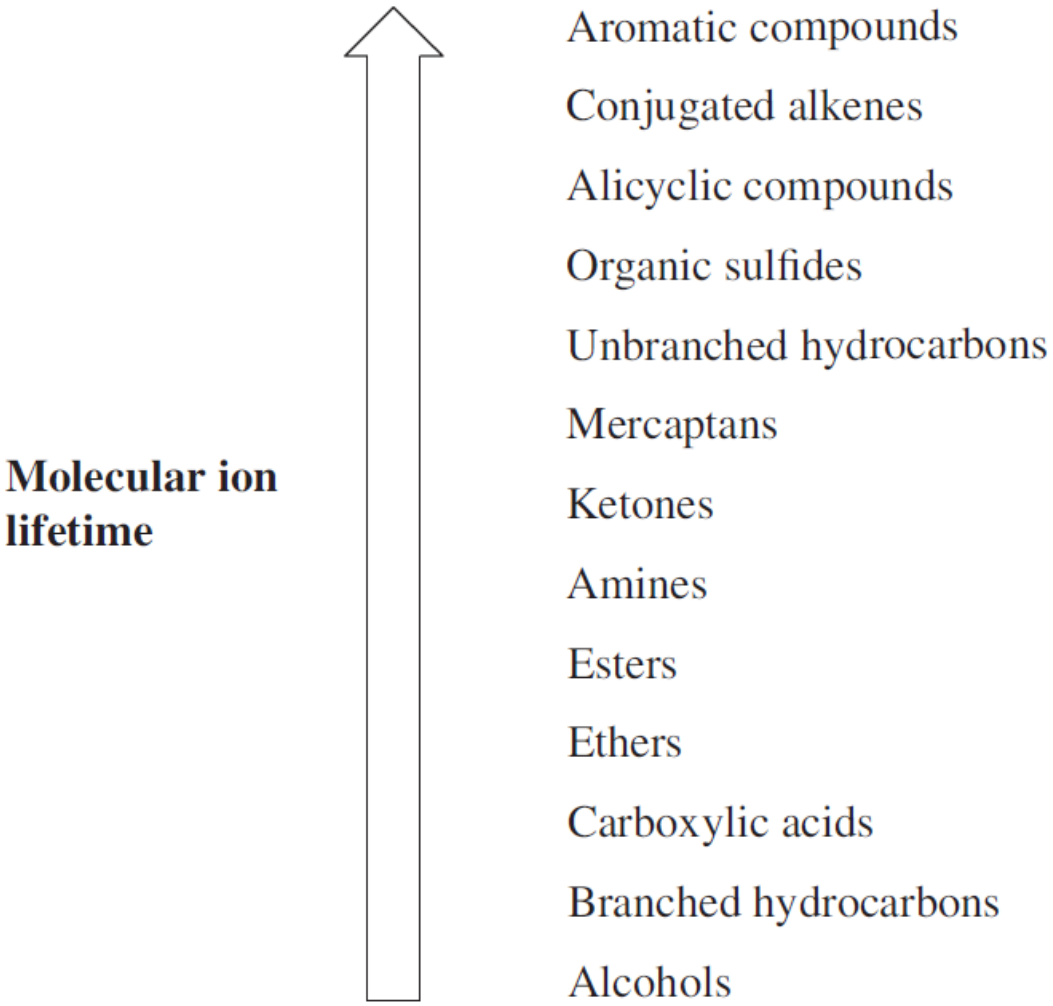
One method of confirming that a particular peak corresponds to a molecular ion is to vary the energy of the ionizing electron beam. If the energy of the beam is lowered, the tendency of the molecular ion to fragment lessens. As a result, the intensity of the molecular ion peak should increase with decreasing electron potential, while the intensities of the fragment ion peaks should decrease. Certain facts must apply to a molecular ion peak:

1. The peak must correspond to the ion of highest mass in the spectrum, excluding isotopic peaks that occur at higher masses. The isotopic peaks are usually of much lower intensity than the molecular ion peak. At the sample pressures used in most spectral studies, the probability that ions and molecules will collide to form heavier particles is quite low. Care must be taken, especially with GC-MS spectra, to recognize background ions that are a result of column bleed—small pieces of the silicone-based stationary phase of the capillary GC column.
2. The ion must have an odd number of electrons. When a molecule is ionized by an electron beam, it loses one electron to become a radical cation. The charge on such an ion is 1, thus making it an ion with an odd number of electrons.
3. The ion must be capable of forming the important fragment ions in the spectrum, particularly the fragments of relatively high mass, by loss of logical neutral fragments. Fragment ions in the range from (M – 3) to (M – 14) and (M – 21) to (M – 25) are not reasonable losses. Similarly, no fragment ion can contain a greater number of atoms of a particular element than the molecular ion. Chapter 4 will explain fragmentation processes in detail.

The observed abundance of the suspected molecular ion must correspond to expectations based on the assumed molecule structure. Highly branched substances undergo fragmentation very easily. Observation of an intense molecular ion peak for a highly branched molecule thus would be unlikely. The lifetimes of molecular ions vary according to the generalized sequence shown in below.

Another rule that is sometimes used to verify that a given peak corresponds to the molecular ion is the so-called **Nitrogen Rule.** This rule states that if a compound has an even number of nitrogen atoms (zero is an even number), its molecular ion will appear at an even mass value. On the other hand, a molecule with an odd number of nitrogen atoms will form a molecular ion with an odd mass. The Nitrogen Rule stems from the fact that nitrogen, although it has an even mass, has an odd-numbered valence. Consequently, an extra hydrogen atom is included as a part of the molecule,

giving it an odd mass. To picture this effect, consider ethylamine, $CH_3CH_2NH_2$. This substance has one nitrogen atom, and its mass is an odd number (45), whereas ethylenediamine, $H_2NCH_2CH_2NH_2$, has two nitrogen atoms, and its mass is an even number (60).



One must be careful when studying molecules containing chlorine or bromine atoms since these elements have two commonly occurring isotopes. Chlorine has isotopes of 35 (relative abundance = 75.77%) and 37 (relative abundance = 24.23%); bromine has isotopes of 79 (relative abundance = 50.5%) and 81 (relative abundance = 49.5%). When these elements are present, take special care not to confuse the molecular ion peak with a peak corresponding to the molecular ion with a heavier halogen isotope present. This is discussed further in Section 3.7B.

In many of the cases that you are likely to encounter in mass spectrometry, the molecular ion can be observed in the mass spectrum. Once you have identified that peak in the spectrum, the problem of molecular weight determination is solved. However, with molecules that form unstable molecular ions, you may not observe the molecular ion peak. Molecular ions with lifetimes less than 10^{-5} sec break up into fragments before they can be accelerated. The only peaks that are observed in such cases are those due to fragment ions. In many of these cases, using a mild CI method will allow for detection of the pseudomolecular ion $(M + H)^+$, and one can determine the molecular weight of the compound by simply subtracting one mass unit for the extra H atom present. If a molecular ion is not able to be detected by this method, then you will be obliged to deduce the molecular weight of the substance from the fragmentation pattern on the basis of known patterns of fragmentation for certain classes of compounds. For example, alcohols undergo dehydration very easily. Consequently, the initially formed molecular ion loses water (mass = 18) as a neutral fragment before it can be accelerated toward the mass analyzer. To determine the mass of an alcohol molecular ion, you must locate the heaviest fragment and keep in mind that it may be necessary to add 18 to its mass. Similarly, acetate esters undergo loss of acetic acid (mass = 60) easily. If acetic acid is lost, the weight of the molecular ion is 60 mass units higher than the mass of the heaviest fragment.

Since oxygen compounds form fairly stable oxonium ions and nitrogen compounds form ammonium ions, ion–molecule collisions form peaks in the mass spectrum that appear one mass unit *higher* than the mass of the molecular ion. This was referred to as self-CI in the discussion of the ion trap mass analyzer in Section 3.4. At times, the formation of ion–molecule products may be helpful in the determination of the molecular weight of an oxygen or nitrogen compound, but this self-CI can sometimes be confusing when one is trying to determine the true molecular ion in a spectrum of an unknown sample.

3.7 DETERMINATION OF MOLECULAR FORMULAS

A. Precise Mass Determination

Perhaps the most important application of high-resolution mass spectrometers is the determination of very precise molecular weights of substances. We are accustomed to thinking of atoms as having integral atomic masses—for example, H = 1, C = 12, and O = 16. However, if we determine atomic masses with sufficient precision, we find that this is not true. In l923, Aston discovered that every isotopic mass is characterized by a small "mass defect." The mass of each atom actually differs from a whole mass number by an amount known as the *nuclear packing fraction*. Table 3.4 gives the actual masses of some atoms.

TABLE 3.4
PRECISE MASSES OF SOME COMMON ELEMENTS

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

Depending on the atoms contained in a molecule, it is possible for particles of the same nominal mass to have slightly different measured masses when precise determinations are possible. To illustrate, a molecule with a molecular weight of 60.1 g/mole could be C_3H_8O, $C_2H_8N_2$, $C_2H_4O_2$, or CH_4N_2O (Table 3.3). Thus, a **low-resolution mass spectrum (LRMS)** will not be able to distinguish between these formulas. If one calculates the precise masses for each formula using the mass of the most common isotope for each element, however, mass differences between the formulas appear in the second and third decimal places. Observation of a molecular ion with a mass of 60.058 would establish that the unknown molecule is C_3H_8O.

TABLE 3.3
SELECTED COMPARISONS OF MOLECULAR WEIGHTS AND PRECISE MASSES

Molecular Formula (MF)	Molecular Weight (MW) (g/mole)	Precise Mass
C_3H_8O	60.1	60.05754
$C_2H_8N_2$	60.1	60.06884
$C_2H_4O_2$	60.1	60.02112
CH_4N_2O	60.1	60.03242

An instrument with a resolution of about 5320 would

be required to distinguish among these peaks. That is well within the capability of modern mass spectrometers, which can attain resolutions greater than one part in 20,000. A **high-resolution mass spectrum** (**HRMS**), then, not only determines the exact mass of the molecular ion, it allows one to know the exact molecular formula. Typical high-resolution instruments can determine an ion's *m/z* value to four or five decimal places. When the precise mass is measured to this degree of precision, only one formula (excluding isotopes) will fit the data. HRMS is extremely valuable to synthetic chemists as well as researchers doing natural product isolation/structure determination work or drug metabolism studies. It is interesting to compare the precision of molecular weight determinations by mass spectrometry with the chemical methods described in Chapter 1, Section 1.2. Chemical methods give results that are accurate to only two or three significant figures (±0.1% to 1%). Molecular weights determined by mass spectrometry have an accuracy of about ±0.005%. Clearly, mass spectrometry is much more precise than chemical methods of determining molecular weight. Precise mass values for

B. 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

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 $(0.016 \times 0.016)/100 = 0.00000256\%$, 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 3.5
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

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

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.

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

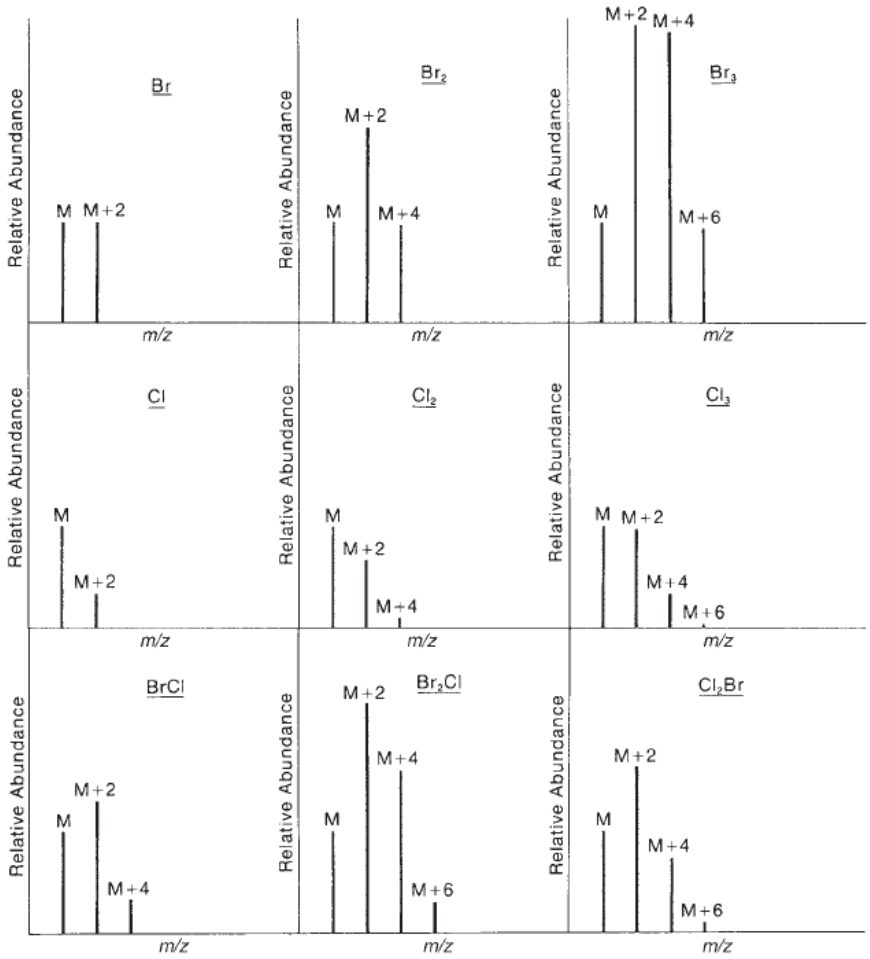


FIGURE 3.19 Mass spectra expected for various combinations of bromine and chlorine.