1

INSTRUMENTATION

1.1. INTRODUCTION

1.1.1. Overview

Mass spectrometry (MS) differs from other common forms of organic spectral analysis in that the sample does not absorb radiation such as infrared, ultraviolet, or radio waves from the electromagnetic spectrum. In contrast to infrared (IR) or nuclear magnetic resonance (NMR) spectrometry, both of which identify compounds with specificity comparable to that of mass spectrometry, MS is a destructive method of analysis—that is, the sample cannot be recovered after mass spectral analysis. On the other hand, MS is highly sensitive and requires less sample than either IR or NMR in order to provide more information about the structure of the analyte.

Mass spectrometers are typically not standalone instruments. Most often they are connected physically and electronically to a chromatograph as well as a computer. Figure 1.1 shows a typical arrangement of a chromatograph/mass spectrometer/computer system. The chromatograph separates mixtures and introduces the sample into the mass spectrometer. The mass spectrometer ionizes analyte molecules, then separates and detects the resulting ions. The computer system controls the operation of the chromatograph and the MS, and provides data manipulation and storage during and after data collection. For volatile samples, gas chromatography (GC) is

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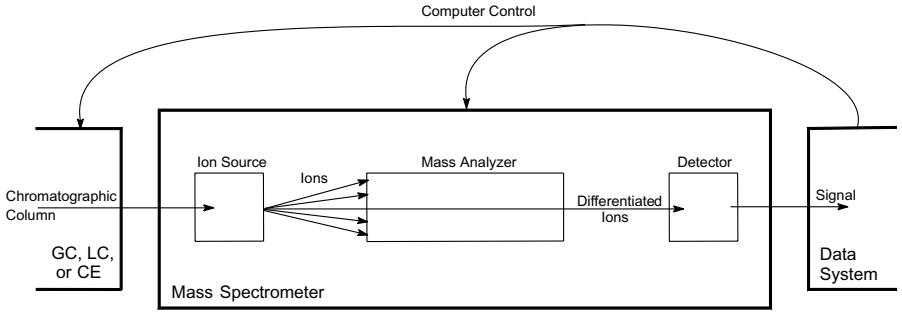


Figure 1.1. Block diagram of a chromatograph/MS/computer system.

used for mixture separation. For nonvolatile or thermally labile molecules, high pressure liquid chromatography (HPLC or just LC) is used. The abbreviated terms GC/MS and LC/MS are commonly used to describe the combination of these chromatographic techniques with MS.

In order to be analyzed by mass spectrometry, sample molecules must be ionized. In the case of electron ionization mass spectrometry (EIMS, the focus of this book), electrically neutral molecules are converted to molecular ions ($M^{+\bullet}$; see Section 3.1) by means of a beam of high-energy electrons. Ionization is followed almost immediately by fragmentation of the $M^{+\bullet}$ in which some bonds break, and in many instances new bonds form, in ways that are characteristic of the structure of the fragmenting ion. The product ions thus formed often undergo further characteristic fragmentation before leaving the ion source (Section 1.2), creating a cascade of ion-forming reactions. This is why mass spectrometry, especially when coupled with separation techniques such as GC or HPLC, is a highly specific way to identify organic compounds.

The components of the mass spectrometer that cause ion formation, separation, and detection are contained in an ultraclean housing usually kept at moderately high vacuum (10^{-3}–10^{-6} torr[1]; some exceptions will be mentioned later). High vacuum ensures that, once the ions formed in the ion source begin to move toward the detector, they will not collide with other molecules because this could result in further fragmentation or deflect them from their desired path. Nearly all fragmentation reactions occurring under these conditions are *intramolecular* (involving only the decomposition of individual ions) rather than *intermolecular* (involving the reaction of ions with other species that may be present). High vacuum also protects the metal and oxide surfaces of the ion source, analyzer, and detector from corrosion by air and water vapor, which could compromise the spectrometer's ability to form, separate, and detect ions.

[1] 1 torr = 1 mm Hg, which is equivalent to ~133 pascal (Pa).

1.1.2. Sample Introduction

High sample purity is critical for unambiguous identification by mass spectrometry. The simultaneous presence of several different compounds in the ion source creates a situation in which ions from all these compounds are analyzed at the same time. This results in a composite mass spectrum that may be impossible to interpret. When capillary column GC is used for sample separation prior to introduction into the mass spectrometer, sample molecules can be introduced directly into the ion source of the spectrometer through the end of the capillary column. Carrier gas flow through a capillary column is low enough that the carrier gas can be removed by the vacuum system of the mass spectrometer. Helium (He) and hydrogen (H_2) are good choices as carrier gases for GC/MS work because their extremely low atomic and molecular masses (4 u and 2 u, respectively; 1 u = 1 unified atomic mass unit[2]) fall below those of all the ions normally seen in organic mass spectrometry.

HPLC has become increasingly important as an option for sample separation prior to mass spectral analysis—especially for compounds that are nonvolatile, thermally labile, or otherwise not amenable to analysis by GC. Capillary electrophoresis (CE) has also been coupled with mass spectrometry to separate and identify inherently ionic molecules such as amino acids, proteins, and DNA fragments. Whereas separation of sample and carrier gas is relatively straightforward in GC/MS, separating sample molecules from HPLC or CE solvents is more complex, so that combinations of these techniques with mass spectrometry for routine use have occurred only recently.

Other methods of sample introduction must be mentioned briefly. Analysis of a pure volatile liquid can be accomplished by placing the liquid in a small, evacuated glass bulb that is connected to the ion source with narrow metal or glass tubing and isolated from the MS vacuum system by a valve. Opening the valve causes the sample vapor to flow directly into the ion source. This method is used for introduction of the calibration and tuning standard perfluorotri-*n*-butylamine (PFTBA; see Section 1.5.1).

Samples that have low volatility or that may decompose during their passage through the GC can be placed on the tip of a probe that is inserted directly into the ion source. The probe tip containing the sample is inserted into a chamber that is isolated from the main vacuum system by a valve. This chamber is evacuated using an auxiliary vacuum pump, after which the valve is opened and the probe tip is inserted all the way into the ion source. Gentle heating of the probe tip provides volatilization of the sample and, in ideal cases, rudimentary fractional distillation of the desired compound. Nonetheless, sample purification prior to introduction by direct insertion probe is desirable. The added expense, potential for ion source

[2] There is currently a lack of consistency regarding the terms used for the atomic mass unit. The single term *amu* was used at one time, but it had different definitions in physics and chemistry, both involving ^{16}O as a standard mass. This term was discontinued when a unified standard mass was adopted. The International Union of Pure and Applied Chemistry (IUPAC) suggests the *unified atomic mass unit* (abbreviated u), which is based on ^{12}C (Section 2.1.2). The *dalton* (abbreviated Da) is identical in size to u and is the term used in biological and biochemical applications as well as for stoichimetric calculations.

contamination by introduction of too large a sample, and the versatility of modern chromatographic techniques have made these devices increasingly rare.

1.2. IONIZATION SOURCE

Sample molecules must be ionized in order to be analyzed and detected in mass spectrometry. Until fairly recently, volatile compounds were ionized primarily in the electron ionization (EI) source, which is still the most common ion source used in GC/MS work. Since the focus of this book is the interpretation of EI mass spectra, most of this section will describe the EI source. As the number of larger and less volatile molecules requiring analysis by mass spectrometry has grown, sample introduction and ionization techniques have been developed that produce detectable numbers of ions of these compounds. Some of these ionization techniques are now used so routinely that a brief description of them is warranted. A list of ionization methods and their application to various sample types is given in Table 1.1.

Table 1.1. Molecular ionization methods in mass spectrometry

Type of Ionization	Ionizing Agent	Source Pressure	Uses
Electron ionization (EI)	50–70 eV electrons	10^{-4}–10^{-6} torr	Extensive fragmentation allows structure determination; GC/MS (Section 1.2.1)
Chemical ionization (CI)	Gaseous ions	~1 torr	Molecular mass determination; GC/MS (Section 1.2.2)
Desorption ionization (DI)		10^{-5}–10^{-6} torr	Molecular mass and structures of high mass, nonvolatile compounds in condensed phase
Fast atom bombardment (FAB)	Energetic Ar or other neutral atoms		
Laser desorption (LDI) and matrix-assisted LDI (MALDI)	Energetic photons		Section 1.2.3.2
Electrospray (ES) ionization	Electric field; ions in solution	Atmospheric or slightly reduced pressure	HPLC/MS and CE/MS (Section 1.2.3.1)
Atmospheric pressure chemical ionization (APCI)	Corona discharge; gaseous ions	Atmospheric	HPLC/MS

1.2.1. Electron Ionization Source

Ion sources from different instrument manufacturers (and sometimes even different models from the same manufacturer) may differ from one another both in appearance and in names assigned to the component parts. However, most have the same basic design. A typical example is shown in Figure 1.2.

The EI source is most commonly a small chamber about 1 cc in volume, in which analyte molecules interact with a beam of highly energetic electrons that have typically been accelerated through a potential difference of 50–70 volts (V) across the volume of the ion source [50–70 electron volts (eV); 1 eV = 23 kcal]. This electron beam is produced by boiling electrons off a narrow strip or coil of wire made of a tungsten-rhenium alloy. Between the filament and the center of the ion source is a metal plate with a slit called the electron aperture. This slit limits the size of the electron beam and confines ionization to a small volume within the center of the ion source. Opposite the filament is the collector, a metal plate held at a positive electrical potential ($+V$ in Figure 1.2) that attracts and intercepts the electron beam after it has passed through the source. Surrounding the entire ion source

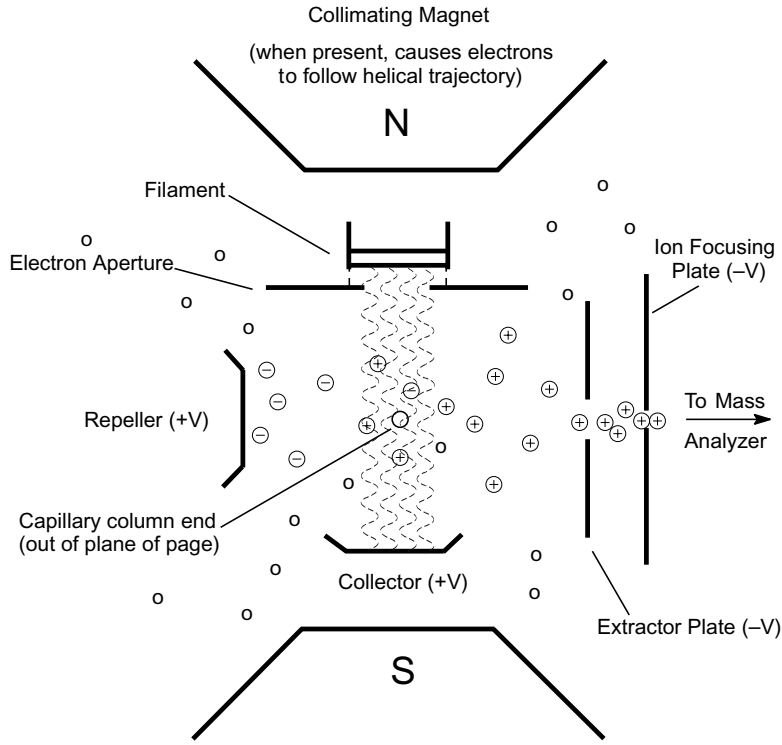


Figure 1.2. Schematic diagram of a typical electron ionization (EI) source. Samples can enter the source through a capillary GC column, a heated probe, or evacuated bulb through openings that are perpendicular to the plane of the page.

in some cases is a collimating magnet, which causes the electrons in the beam to travel in a helical path, as shown in Figure 1.2. Although this helical trajectory improves the probability that the electrons and molecules will interact, sample ionization is still very inefficient—less than one molecule in a thousand undergoes ionization.

What happens during ionization is complex. It is naïve to view electrons as literally smashing into sample molecules and knocking electrons out of orbitals. Instead, when an energetic electron approaches the electron density field of the molecule closely enough that sufficient energy is transferred quantum mechanically to overcome the ionization potential of the molecule, one electron is ejected from one of the bonding or nonbonding orbitals of the molecule (Section 3.3). Ionization energies (IE) for most organic compounds range from about 5–15 eV. Bond dissociation energies are even smaller, so this method of ionization not only causes molecules to expel one or more electrons, it also provides enough energy for substantial fragmentation of the first-formed ion (the molecular ion, $M^{+\bullet}$). Because of the excess energy present in 50–70 eV electrons, enough additional energy may be transferred to overcome the second, or even third, ionization potential of the molecule, leading to ions having +2 or +3 charges. The ionization process is discussed in more detail in Chapter 3.

Many different products form during ionization. Some of these are not positive ions. Table 1.2 lists the most important of these products. If the sample absorbs enough energy to raise an electron from the ground state to an excited state, but not enough to cause ejection of the electron, an "excited molecule" is formed (product *a* in Table 1.2). Excited molecules can return to their neutral ground state through thermal vibrations or the emission of light, and because no ions are formed in the process, they are simply pumped away from the ion source by the vacuum system.

Table 1.2. Types of ionization reactions

	a. Excited molecule (not detected)
	b. Negative ion formation (not detected by positive EIMS):
	$(A{-}B{-}C)^{-\bullet}$
	$(A{-}B)^{-} + C^{\bullet}$ and others
	c. Electron Ionization: $(\mathbf{A{-}B{-}C})^{+\bullet} + 2e^{-}$
	d. Dissociative ionization:
$e^{-} + (A{-}B{-}C) \rightarrow$	$A\cdot + (\mathbf{B{-}C})^{+} + 2e^{-}$
	$(\mathbf{A{-}B})^{+} + C^{\bullet} + 2e^{-}$
	$(A{-}B)^{\bullet} + \mathbf{C}^{+} + 2e^{-}$ and others
	e. Dissociative ionization with rearrangement:
	$(\mathbf{A{-}C})^{+\bullet} + B + 2e^{-}$
	$(A{-}C) + \mathbf{B}^{+\bullet} + 2e^{-}$
	f. Multiple ionization:
	$(\mathbf{A{-}B{-}C})^{2+} + 3e^{-}$
	$(\mathbf{A{-}B})^{+} + \mathbf{C}^{+} + 3e^{-}$ and others

Ions detected by positive ion EIMS are shown in boldface.

Sometimes the analyte molecule absorbs an electron and a negative ion is formed (Table 1.2, product *b*). In order to be absorbed by the molecule, the electron must be of very low energy (~0.1 eV), and there are few electrons of this energy in a standard EI source. By reversing the polarity of the repeller, ion focusing plate, and extractor plate in the ion source, and by altering the detector so that it will detect negative ions, a negative ion mass spectrum can be recorded. For most compounds negative ion MS offers few advantages over positive ion MS, and overall it tends to be less sensitive.[3] There are some specific applications, however, most notably with halogenated compounds. In this book only positive ion products and their fragmentations will be covered.

The remaining products listed in Table 1.2 are positive ions. The ion that is formed first results directly from ejection of a single electron from the neutral molecule (product *c*). This *molecular ion* ($M^{+\bullet}$) is very important because it has virtually the same mass as that of the analyte molecule (the small mass of the lost electron can be ignored). Indeed, mass spectrometry is one of the few analytical tools available for determining the molecular mass of a compound.

Ion products *d* and *e* in Table 1.2 are formed by unimolecular dissociation of $M^{+\bullet}$. In the first case a single bond is broken and a neutral group of atoms having an odd number of electrons (called a radical; see Section 3.1) is lost. The second process (dissociation with rearrangement) involves breaking some bonds while at the same time forming new ones. This results in expulsion of a fragment containing an even number of electrons, usually as a neutral molecule. The equations in Table 1.2 imply that such ions are formed in a concerted process in which ionization, bond making, and bond breaking all occur at about the same time. However, fragmentations that involve rearrangement of atoms usually occur in a stepwise fashion through one or more intermediates.

If more than one electron is ejected from the analyte molecule, ions having charges of +2, +3, or even +4 may be formed (Table 1.2, products *f*). Biopolymers such as peptides may have charge states of +10 or more from protonation of basic sites on the molecule. Since mass spectrometry actually measures the mass-to-charge ratio (*m*/*z*) of an ion, not its mass, an ion having a charge greater than +1 is found not at the *m*/*z* value corresponding to its mass (*m*), but rather at *m*/2, *m*/3, or *m*/4, depending on the number of charge states. Further, if *m* is not evenly divisible by the number of charges *z*, *m*/*z* will have a nonintegral value. For example, the double charged molecular ion (M^{2+}) of a compound having a molecular mass of 179 is found at *m*/*z* 179/2 = 89.5.

Most compounds do not produce multiple charge molecular ions in EI, but they may be formed in low abundance from small molecules that have few possible modes of fragmentation or from compounds with aromatic rings or large heteroatoms such as Cl, Br, or S. Mass spectrometers used for routine organic analysis

[3] A very sensitive and highly specific technique called resonance electron capture ionization (RECI) takes advantage of the low-energy electrons expelled during the EI of methane and results in the formation of negatively charged molecular ions ($M^{-\bullet}$).

often report m/z values only to the nearest integral mass, or they may report only one peak for each m/z value (Section 1.5.2). In such cases, detecting ions having nonintegral masses, even if they occur, is not always possible. Mass spectrometers with higher resolving power may be necessary to identify these ions with certainty.

The complex mixture of ionic and neutral products formed by any ionization method must be separated so that positive ion products travel in the direction of the m/z analyzer, and negative ions and neutral products are left behind. Neutral products are removed by the vacuum system, because the electric and magnetic fields present in the ion source have no effect on their motion. Positive and negative ions, on the other hand, can be separated by appropriately placed charged surfaces in the ion source (Figure 1.2). To accomplish this, the repeller is kept at a positive potential ($+V$) both to attract and neutralize negative ion products and to repel positive ions. Conversely, the extractor plate and ion focusing plate (the ion optics) are both kept at a negative electrical potential ($-V$) to attract and accelerate the positive ions toward the m/z analyzer. Slits in the extractor and ion focusing plates allow passage of the positive ions and help focus the ion beam as it approaches the analyzer.

When the filament is on and analyte molecules are flowing into the ion source, many reactive species are produced. Indeed, the intensity of the electron beam itself is sufficient to corrode metal surfaces in the ion source that are directly in its path—those on the electron aperture and collector. In addition, ion products may become electrically neutralized or undergo polymerization on the surfaces of the repeller, extractor plate, and ion focusing plate. Over time, the sensitivity of the instrument declines, as these surfaces are less able to maintain the potentials necessary for optimal ejection and focusing of positive ions from the source. Mechanical and chemical cleaning of the metal surfaces in the source is needed to restore sensitivity. The daily acquisition and evaluation of the spectrum of a standard compound whose ions' m/z values and abundances are known help determine when tuning and source cleaning are necessary (Section 1.5.1).

Keeping the filament off when high concentrations of sample are present in the ion source (especially while solvents are eluting during a GC run) allows the source to remain usable for several months without cleaning. Chemical ionization (CI) mass spectrometry (Section 1.2.2), which depends on the presence of high ion concentrations in the source, leads to the deterioration of ion source performance more rapidly than EI under normal circumstances.

1.2.2. Chemical Ionization

Unlike EIMS, in which molecules are ionized through interaction with high-energy electrons, ionization in chemical ionization mass spectrometry (CIMS) depends on collisions of ions and molecules. In positive ion CIMS the sample is ionized by reaction with ions generated within a large excess of a relatively low molecular mass reagent gas such as methane (as CH_5^+), isobutane [as $(CH_3)_3C^+$], or ammonia

(as NH_4^+), at a pressure of about 1 torr. Although some reagent gas ions are themselves formed by ion/molecule reactions

$$CH_4 + e^- \rightarrow CH_4^{+\bullet} + 2e^-$$
$$CH_4^{+\bullet} + CH_4 \rightarrow CH_5^+ + {}^{\bullet}CH_3$$

others are formed by unimolecular decomposition of the $M^{+\bullet}$, for example,

$$(CH_3)_3CH + e^- \rightarrow (CH_3)_3CH^{+\bullet} + 2e^- \rightarrow (CH_3)_3C^+ + H^{\bullet}$$

In CIMS the concentration of analyte molecules (at approximately 10^{-3} torr) is small compared to that of reagent gas molecules. Thus, the electron beam, which is more energetic than that used in EIMS ($\sim$200 eV), preferentially ionizes the reagent gas. Analyte molecules are ionized through reaction with reagent gas ions, rather than by the electron beam. Most reagent gas ions are strong proton donors and form protonated molecules (sometimes incorrectly called pseudomolecular ions) that have a mass 1 u greater than that of the molecular mass of the original compound[4]:

$$M + CH_5^+ \rightarrow MH^+ + CH_4$$

This type of ion formation (often called *soft ionization*) imparts significantly less energy to analyte molecules than do interactions with high-energy electrons, so that the resulting ions have little excess internal energy. These ions therefore fragment less than those formed by EIMS. As a result, although CIMS is useful for determining the molecular mass of compounds that do not produce a detectable $M^{+\bullet}$ by EIMS (see Figure 1.3), CI mass spectra may show an insufficient number of fragment ion peaks to yield structural information. The protonated molecules produced during CIMS can be induced to undergo fragmentation by combining CI with product-ion mass spectrometry/mass spectrometry (MS/MS; see Section 1.3.4.1). This technique yields structural information similar to that obtained by fragmentation of the $M^{+\bullet}$ in EIMS.

The interpretation of CI spectra, as well as spectra produced by electrospray and desorption ionization methods (Section 1.2.3), will not be covered in this book.

1.2.3. Other Ionization Methods

1.2.3.1. Electrospray Ionization. The conventional ion source shown in Figure 1.2 can be used for both EI and CI, provided the sample enters the ion source in the gaseous state. Although many organic compounds can be analyzed in this

[4] Some reagent gas ions may react with sample molecules by addition, rather than by proton donation. It is not unusual to observe weak intensity peaks at *m/z* values greater than that expected for the protonated molecule, corresponding to the addition of one or more reagent gas ions to the sample molecule. In some instances, CI can also result in hydride abstraction, thereby forming an $(M - H)^+$ ion, which has a mass 1 u less than the analyte molecule.

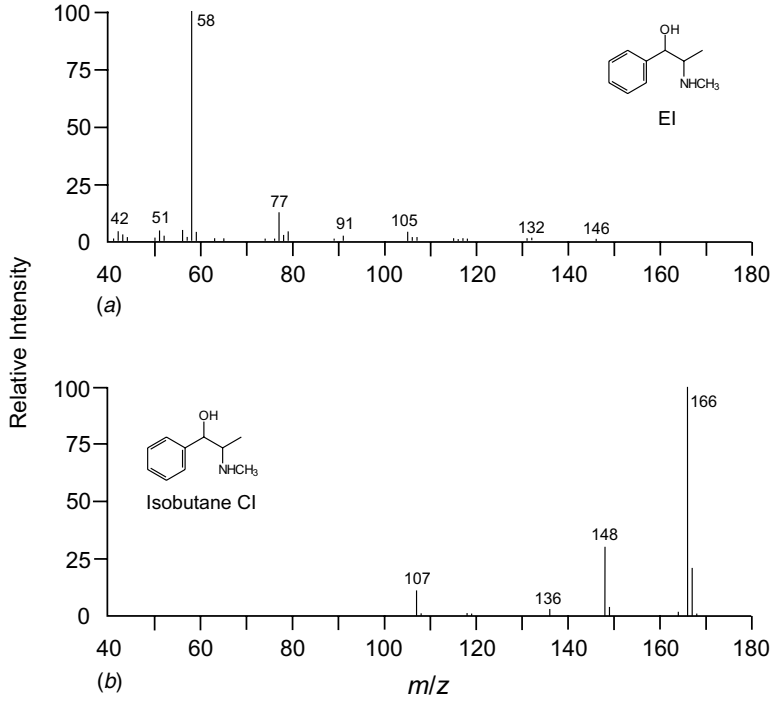


Figure 1.3. Mass spectra of ephedrine resulting from (*a*) EI and (*b*) chemical ionization (CI) using isobutane as the reagent gas (adapted with permission from Fales et al., 1975. Copyright American Chemical Society). The peak at *m*/*z* 166 in (*b*) corresponds to the protonated molecule.

manner, a large number of compounds, because of their inherent size and/or charge state, are nonvolatile or thermally labile. Many of these compounds are most easily separated by HPLC or CE, in which separation takes place in solvents that have an aqueous component. John B. Fenn and Koichi Tanaka shared the 2002 Nobel Prize in chemistry for their development of methods such as electrospray ionization (ESI) and desorption ionization in the analysis of large biological molecules.

The ESI source has allowed LC/MS and CE/MS to become routine analytical tools. Basically ESI works by converting the HPLC or CE effluent, already containing the sample in ionic form, into an aerosol and subjecting the resulting spray to high voltage in a chamber held near atmospheric pressure (Figure 1.4). This process creates a mist of charged droplets that flow toward the orifice of the capillary. In the configuration shown, the nebulizing needle, which creates the aerosol, is orthogonal (perpendicular) to the eventual direction of ion flow toward the *m*/*z* analyzer. Other geometric configurations are possible and have been used.

As the charged droplets travel toward the capillary opening, they are subjected to the counterflow of a drying gas, such as nitrogen (N_2), which causes evaporation of

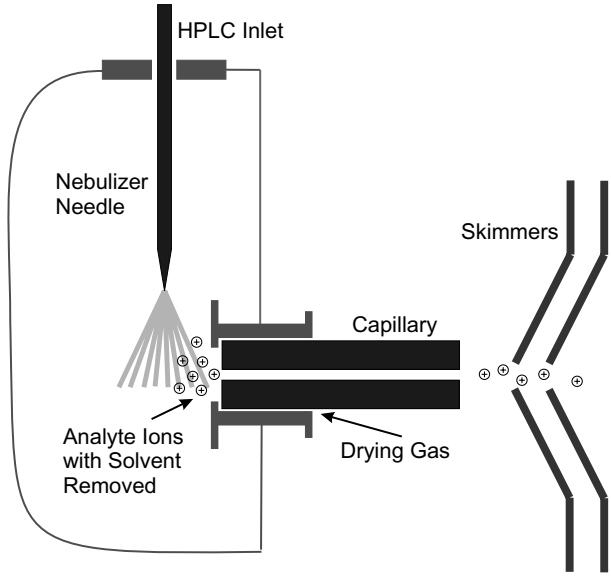


Figure 1.4. Orthogonal electrospray ionization source. Gas phase sample ions, which are desorbed from the condensed phase, exit toward the right into the *m*/*z* analyzer. (Copyright 2002 Agilent Technologies, Inc. Reproduced with permission courtesy of Agilent Technologies, Inc.)

solvent molecules from the droplets. Evaporation continues until electrostatic repulsions between the increasingly concentrated charges cause the droplet to break apart. Evaporation, charge concentration, and droplet disintegration continue until the analyte ions are finally desorbed into the vapor phase, passed into the sampling capillary, then on into the high vacuum of the *m*/*z* analyzer.

For the most part, the nature of the ions analyzed by ESI will be determined during the chromatographic run. At low pH, protonated molecules (sometimes protonated several times) will predominate. Because little additional energy is imparted to these ions in the ESI source, fragmentation is minimal (compare the EI spectrum in Figure 1.5*a* with the ESI spectrum of the same compound shown in Figure 1.5*b*). ESI thus offers an additional tool for determining the molecular mass of compounds that do not produce an $M^{+\bullet}$ by EIMS. As with CI, structural information about the analyte can be obtained by coupling ESI with MS/MS (Section 1.3.5.1).

ESI has a wide range of applications—from the analysis of low and medium molecular mass compounds (Figure 1.5) to large biomolecules such as intact proteins. High molecular mass molecules that have multiple sites for protonation will form multiple-charge ions that can be separated and detected by conventional mass spectrometers (remember that mass spectrometers measure the *m*/*z* of an ion, not its mass). Automation of HPLC equipment allows high sample throughput, which is used in the pharmaceutical industry, for example, to rapidly analyze large collections of closely related compounds that have potential as new drugs. Additional

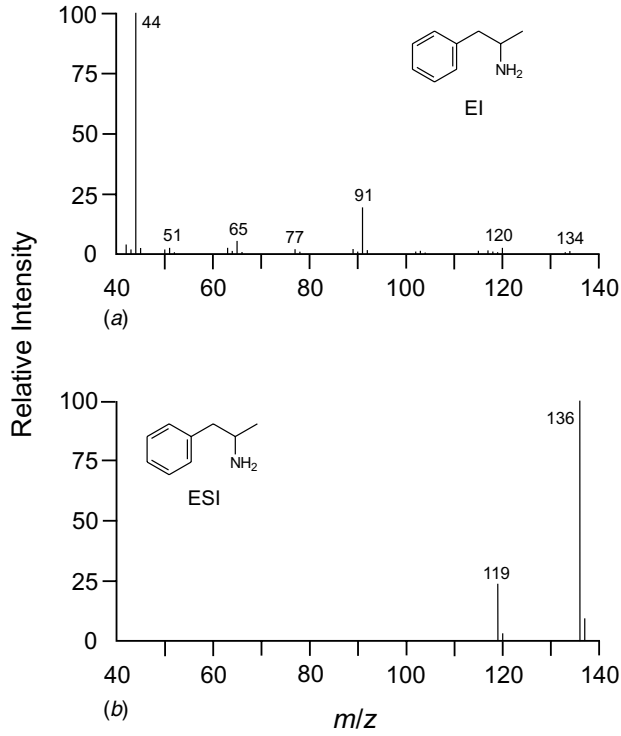


Figure 1.5. Mass spectra of amphetamine resulting from (*a*) EI and (*b*) electrospray ionization (ESI; figure adapted from Kataoka et al., 2000 by permission of Preston Publications, a division of Preston Industries, Inc.). The peak at *m*/*z* 136 in (*b*) corresponds to the protonated molecule.

information about ESI can be found in R. B. Cole's *Electrospray Ionization Mass Spectrometry: Fundamentals, Instrumentation and Applications* (see the References at the end of this chapter).

1.2.3.2. Desorption Ionization. Like ESI, matrix-assisted laser desorption/ionization (MALDI) has proven very effective for the analysis of some large biopolymers—especially when combined with a time-of-flight (TOF) mass spectrometer, which has an essentially limitless *m*/*z* range (Section 1.3.1). Laser desorption ionization occurs when the sample is irradiated with an intense beam of photons. Ionization and desorption are facilitated by mixing an aqueous solution of the analyte with an excess of a compound that enhances light absorption, then placing this mixture on a probe and evaporating the water. The exact mechanism by which ionization occurs is not fully understood. High sample throughput is possible by using as the ''probe'' a plate containing a large number of sample wells that can be sequentially irradiated and analyzed. The rapid spectral acquisition rates of TOF instruments allow analysis times of only a few seconds per sample.

Fast atom bombardment (FAB) is related to MALDI, although FAB uses a beam of energetic Ar atoms to induce ionization and desorption. MALDI has replaced FAB as a preferred ionization method for these large molecules.

1.3. m/z ANALYSIS

The mixture of molecular and fragment ions formed in the ion source contains information that would be lost were these ions not separated and identified in some meaningful way. In particular, the mass-to-charge ratio (m/z; in early literature, m/e) of each of these ions must be measured. To do this, the analyzer must be able to take advantage of some unique property of the ion that results from the imposition of an electrical or magnetic field. Although quadrupole m/z filters are commonly used in GC/MS and HPLC/MS, other types of m/z analyzers are currently used for both these and other applications. These include the time-of-flight (TOF) and magnetic sector analyzers, both of which played prominent historical roles in the development of mass spectrometry as an analytical tool, and the quadrupole ion trap (QIT). The TOF analyzer is a convenient example with which to begin because of the relative ease with which a mathematical connection can be made between physical motions of ions and their m/z values.

1.3.1. Time-of-Flight (TOF)

Ion separation in a TOF analyzer is based on the principle that ions which are given the same initial energy will have velocities that are proportional to their m/z values. In the ion source of a TOF instrument (Figure 1.6), ions of all m/z values are formed almost simultaneously using a very brief burst of energy from the filament. This method of ionization is called pulse ionization. These ions are then accelerated out of the ion source using a positive electrical potential V. From fundamental physics relationships, the potential energy given to each ion as it leaves the ion source is zV, where z is the charge on the ion ($=ne$, the number of charges times the charge on one electron). In the flight tube of the spectrometer, all of this energy is converted in the moving ion into kinetic energy ($=\frac{1}{2}mv^2$, where m is the mass of the ion and v is its velocity). The more massive the ion, the more slowly it travels.

The potential energy of each ion as it leaves the ion source must equal its kinetic energy when it reaches the detector, that is,

$$zV = \tfrac{1}{2}mv^2$$

The velocity of the ion during its journey through the flight tube is simply the length of the flight tube D divided by the time t it takes for the ion to travel this distance, so that

$$zV = \tfrac{1}{2}m(D/t)^2$$

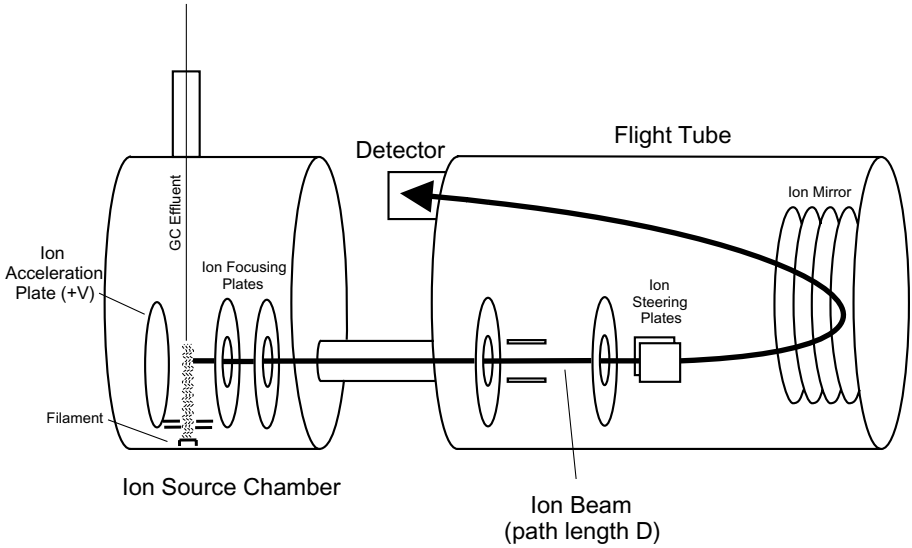


Figure 1.6. Reflectron time-of-flight (TOF) mass spectrometer. (Adapted with permission. Copyright LECO Corporation, St. Joseph, MI)

Solving for m/z leads to the mass spectrometric equation governing TOF mass spectrometry:

$$m/z = 2Vt^2/D^2 \tag{1.1}$$

In Equation 1.1, D is fixed by instrument design and V can be held constant electronically, so that m/z is proportional to the square of the travel time t. The mass spectrum is collected by plotting the signal output of the detector as a function of time, the latter of which can be converted to m/z values by the data system. Ions that differ in their flight times by as little as 1 ns can be recorded. As soon as the slowest-moving (highest m/z) ion is detected, another set of ions is formed and accelerated out of the ion source toward the detector. The range of m/z values over which the spectrum will be acquired must be selected with care, because ions having m/z values greater than the selected range will continue to drift toward the detector even though a pulse of new ions has been formed and accelerated.

TOF mass spectrometers have recently enjoyed a resurgence of popularity. Because the time between ion pulses in the source can be regulated as desired, TOF instruments can analyze ions having virtually any m/z value. Further, because the TOF mass analyzer detects essentially *all* the ions created in each ionization pulse (in contrast to many scanning analyzers; see below), it provides a very sensitive means of ion analysis and detection. When coupled with a pulsed laser ionization source such as MALDI (Section 1.2.3.2), TOF spectrometers have found widespread applications in the analysis of proteins, DNA fragments, and other

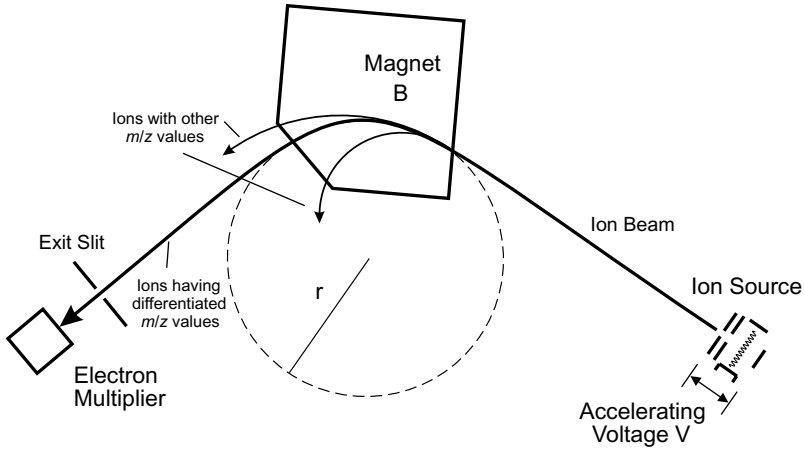


Figure 1.7. Magnetic sector mass spectrometer.

macromolecules with masses in the kilodaltons. R. J. Cotter's *Time-of-Flight Mass Spectrometry: Instrumentation and Applications in Biological Research* provides a more detailed description of TOF spectrometers.

1.3.2. Magnetic Sector

A schematic diagram of a typical magnetic sector mass spectrometer is shown in Figure 1.7. In contrast to the TOF spectrometer, ions are formed continuously in the ion source of the magnetic sector mass spectrometer and accelerated toward the detector by an electrical potential V. Once ions in a magnetic sector analyzer come under the influence of the magnetic field B, whose lines of force are perpendicular to the plane of Figure 1.7, they are constrained to travel along an arc of a circle whose radius is r. The mass spectrometric equation for this instrument can be derived from fundamental physics relationships using these variables.

In a magnetic field, an ion with mass m will experience a centripetal force (one pulling the ion toward the center of the circle) equal to Bzv, where B is a measure of the strength of the magnetic field, z is the charge on the ion, and v is the velocity of the ion. At the same time, any particle moving on a circle having a radius r experiences a centrifugal force (one pulling it away from the center of the circle) equal to mv^2/r. When these two forces are equal, the ion travels along an arc of the circle and

$$Bzv = mv^2/r \quad \text{or} \quad m/z = Br/v \tag{1.2}$$

If the velocity v were used to determine m/z, such an instrument would be essentially a TOF spectrometer, but another expression for v is available that can be substituted into Equation 1.2. Because the kinetic energy of the moving ion when it

reaches the magnet ($=\frac{1}{2}mv^2$) is equal to the potential energy ($=zV$) it had when it left the ion source (Section 1.3.1),

$$\tfrac{1}{2}mv^2 = zV \quad \text{or} \quad v^2 = 2zV/m$$

and

$$v = \sqrt{\frac{2zV}{m}}$$

When this expression for v is substituted back into Equation 1.2,

$$\frac{m}{z} = \frac{Br}{\sqrt{2zV/m}}$$

By squaring both sides and canceling like terms, the mass spectrometric equation for the magnetic sector analyzer is produced:

$$m/z = B^2r^2/2V \tag{1.3}$$

Since B, r, and V are all measurable quantities, the m/z of an ion can be determined without knowing its velocity.

In order to measure m/z and obtain a mass spectrum, two of the variables on the right-hand side of Equation 1.3 must be held constant. There are several ways of doing this in a magnetic sector instrument. In one case, both B and V are held constant. Under these conditions, ions having different m/z values pass through the magnetic field along paths with different values for r. Instead of an electron multiplier or photomultiplier detector (which only detects ions traveling along a single path of fixed radius r; see Section 1.4), a high resolution photographic plate or photodiode array is placed perpendicular to the paths of the ions after they have passed through the magnet. Under these conditions, the position at which an ion collides with the detector is related to its m/z value, and the mass spectrum is obtained by developing the photographic plate or analyzing the pattern of signals detected by the photodiode array.

A more common arrangement of the magnetic sector mass spectrometer holds V constant and requires a constant value for r by using a narrow slit and an electron multiplier or photomultiplier detector (as shown in Figure 1.7). By scanning through a range of values for B, ions of different m/z values sequentially attain the appropriate value for r, and then pass through the analyzer to the detector. At any given value of B, all other ions have different trajectories (i.e., different r) and collide with the inner surfaces of the spectrometer. This method of m/z separation, which involves continuous ion formation while scanning through a range of values for one variable, differs from the TOF. Instead of having nearly all the initially formed ions reach the detector, only a small fraction of the ions are allowed to pass through the analyzer at any given time.

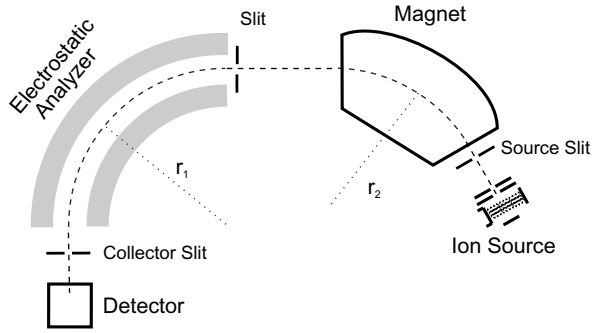


Figure 1.8. Double-focusing, high resolution mass spectrometer of the reverse Nier–Johnson geometry. The electrostatic analyzer focuses the kinetic energy of the ion beam as it emerges from the magnetic sector analyzer. This allows separation of *m*/*z* values that differ by less than 0.001. The *m*/*z* separation capability of the instrument is determined by the size of the slit openings.

Spectra can be acquired by scanning either from low to high *m*/*z* values, or vice versa. Some instruments acquire data in both directions. The rate at which a spectrum can be acquired (the scan speed) is limited by the ability of the magnet to respond to changes in field strength. Scan times of less than 0.1 s are achievable. The upper limit for the *m*/*z* range of a magnetic sector instrument is determined by the maximum field strength that can be reached.

A third way to achieve mass separation in a magnetic sector mass spectrometer is to hold *B* and *r* constant while scanning through a range of values for *V*. Although this type of instrument was commercially available at one time, problems with instrument stability, limited scan ranges, and arcing in the ion source prevented it from being successful. In fact, single sector magnetic sector mass spectrometers like that in Figure 1.7 are no longer commercially available. Magnetic sectors are now only found in double-focusing spectrometers such as the one in Figure 1.8. These instruments are capable of separating much smaller *m*/*z* differences than the magnetic sector instrument alone (Section 1.3.5.2).

1.3.3. Transmission Quadrupole

Quadrupole mass spectrometers, particularly when coupled with a GC, are the most widely used mass spectrometers in many types of organic analytical laboratories. As seen in Figure 1.9, the transmission quadrupole analyzer consists of four electrical poles (usually called rods) that are held in strict alignment with one another. Indeed, it is crucial that these poles remain parallel to and at a fixed distance from one another. Opposing poles are connected in pairs to both radio frequency (RF) and direct current (dc) generators, bathing ions in a combined electric and RF field during their passage through the analyzer. The output of the RF generator is energy in the radio frequency part of the electromagnetic spectrum. It can be pictured as a sinusoidal wave having a zero-to-peak amplitude *U* and a fixed frequency ω (the

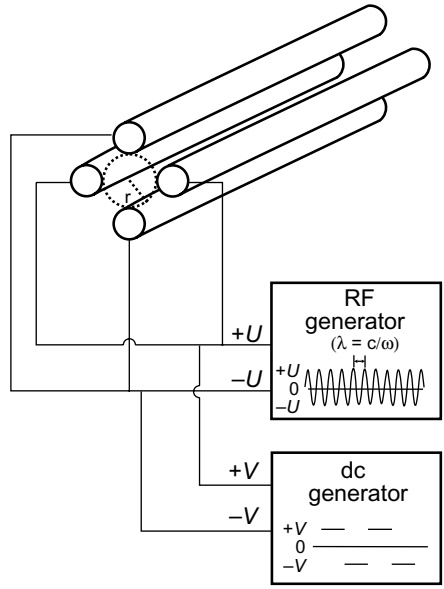


Figure 1.9. Transmission quadrupole analyzer. The RF generator produces a sinusoidal wave having a variable amplitude U and frequency ω that is held constant. The dc generator produces a voltage V that alternates in sign.

number of wave crests per second). The output of the dc generator is a pair of voltages, $+V$ and $-V$, the magnitude of which can be changed. The amplitude of the RF output can be changed even while the frequency is held constant.

In contrast to the mass spectrometric equations derived in previous sections for TOF and magnetic sector analyzers, those for quadrupoles are complex. Their complete derivation involves solution of the differential equations that describe the motions of ions in combined electromagnetic and electric fields. This derivation generates two combination variables a and q:

$$a = \frac{8zU}{mr^2\omega^2} \tag{1.4}$$

$$q = \frac{4zV}{mr^2\omega^2} \tag{1.5}$$

where

U = zero-to-peak voltage of the applied RF field
V = applied dc voltage
r = radius of the circle tangent to the inner surfaces of the quadrupole
ω = applied radio frequency
m/z = mass-to-charge ratio of the ion

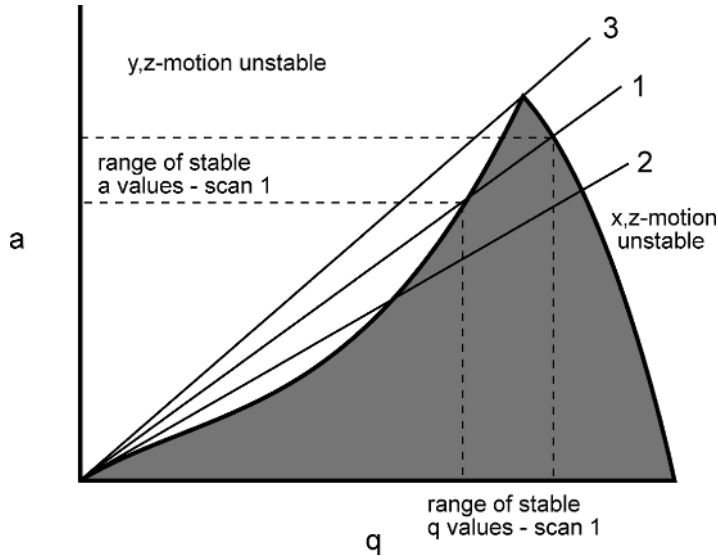


Figure 1.10. Stability diagram for quadrupole mass spectrometry. Choosing scan line 3 fixes values for *a* and *q* so that *m*/*z* varies as either of the voltages *U* or *V*.

It is important to recognize that a and q arise solely from solution of the differential equations and have no physical meaning in the instrument.

In these equations r and ω can be held constant (the former by physical design of the quadrupole and the latter by choice), but some further constraint must be added in order to determine m/z as a function of a single variable. To do this, a and q are chosen to be proportional to one another, so that

$$\frac{a}{q} = \frac{2U}{V} = \text{constant}$$

This is easy to accomplish instrumentally, because U and V can be controlled simultaneously through linked electronic circuitry. Once a, q, U, and V are interrelated in this manner, m/z varies with either U or V.

The relationship between all these variables and the determination of m/z can best be understood in terms of a plot of a vs. q, which is called a stability diagram (Figure 1.10). Actually, this figure shows only a small portion of the entire plot for all values of a and q; for reasons involving instrument design, only the part of the plot near the origin is normally used. The shaded area of the graph contains values for both a and q that define stable ion motion along the z-axis (the axis parallel to the four poles)—in other words, motion that allows ions to pass through the quadrupole to the detector. For other values of a and q, ions wander far enough from the z-axis that they collide with the poles and are removed from the ion beam.

The lines marked 1, 2, and 3 in Figure 1.10 are called scan lines. All scan lines in this plot are straight lines passing through the origin and having a slope a/q that is

fixed by the choice of $2U/V$ ($= a/q$) as a constant. The scan line that is used experimentally is determined by the smallest difference in m/z values (ΔM) that can be distinguished by the analyzer. For transmission quadrupoles ΔM is often set at about 1 m/z unit (referred to as *unit resolution*) over the instrument's entire range[5]. High resolution mass spectrometers that offer ΔM values of 0.001 or less are available (Section 1.3.5.2). Analyzers with $\Delta M > 1$ have only limited utility because individual masses are not distinguishable with these instruments.

Choosing scan line 1 in Figure 1.10 defines a range of values for a and q pairs (those lying along that line within the shaded area of the figure) that allows stable ion motion through the quadrupole at any instant. Because a and q are both related to m/z, all ions within the range of m/z values defined by that range of values for a and q pass through the poles at the same time. If the range of m/z values >1, m/z values for individual single-charge ions cannot be determined. If scan line 2 is chosen, the situation is worse because an even wider range of m/z values are allowed to pass at the same time.

The ideal choice, therefore, is scan line 3, which passes through the apex of the shaded area of Figure 1.10. Solving Equations 1.4 and 1.5 for m/z gives

$$\frac{m}{z} = \frac{8U}{ar^2\omega^2}$$

and

$$\frac{m}{z} = \frac{4V}{qr^2\omega^2}$$

By passing through a unique point of z-stable motion on this graph, scan line 3 fixes unique values for both a and q and thereby renders them constant. Because the values of r and ω were fixed during manufacture or installation, m/z then becomes dependent on only one variable—either U or V, since U and V are chosen to be dependent on one another. Thus,

$$m/z = kU \quad \text{or} \quad k'V \tag{1.6}$$

where k and k' are constants.

Equation 1.6 is convenient because m/z varies linearly with voltage, which is not only easy to control instrumentally but also can be varied rapidly over a scan with

[5] The term *resolution* is often used in MS. In general, *high resolution* spectrometers are those that can separate and detect ions having very similar m/z values. When $m/z < 500$, this usually means that ions differing by less than 0.001 unit can be distinguished. A *low resolution* spectrometer is one that can only distinguish ions differing by roughly whole m/z units. However, a *high resolution* instrument may only be able to distinguish integral m/z units when $m/z > 10{,}000$. The term $M/\Delta M$ is often defined as the resolution of the instrument, but it has also been defined as the resolving power, with the inverse term ($\Delta M/M$) defined as the resolution. In this book, the m/z separation capability of the instrument will be emphasized and the term resolution used sparingly.

little or no lag time between scans. In the Agilent Technologies Mass Selective Detector, a popular brand of benchtop quadrupole instruments designed for routine GC/MS work, the dc generator is scanned from approximately 0–200 V and the RF generator from about 0–1,200 V, producing a range of m/z values from 0 up to about 800. Like the magnetic sector analyzer, the quadrupole may be scanned from high to low values of m/z—which in fact is what happens in the Mass Selective Detector. The upper limit of the m/z range for most quadrupole GC/MS instruments is from 800–1,000, although instruments with ranges up to m/z 2,000 are used in LC/MS applications. This upper limit is determined by the RF wave, which becomes unstable after its amplitude is increased beyond a certain point.

Experimentally, the scan line that is used does not pass exactly through the apex of the stability diagram. Instead, by allowing a small range of m/z values through the analyzer at one time, the instrument is able to detect a larger number of ions and the sensitivity is improved.

Figure 1.11 illustrates how the chosen scan line applies to various m/z values during one spectral acquisition. At low values of a and q (and thus of U and V), the scan line passes through the apices of stability diagrams for low m/z ions, allowing their passage through the quadrupole. At the same values of a and q, the motions of higher m/z ions are unstable. As values for a and q increase, the scan line passes through the apices of stability diagrams for progressively higher values of m/z. These ions sequentially have stable motion through the quadrupole, and lower m/z ions do not.

A more thorough discussion of quadrupole analyzers may be found in Miller and Denton (1986), Leary and Schmidt (1996), and Henchman and Steel (1998).

1.3.3.1. Selected Ion Monitoring (SIM). One advantage of having m/z dependent on an easily controllable variable such as U or V is that the dc and RF generators

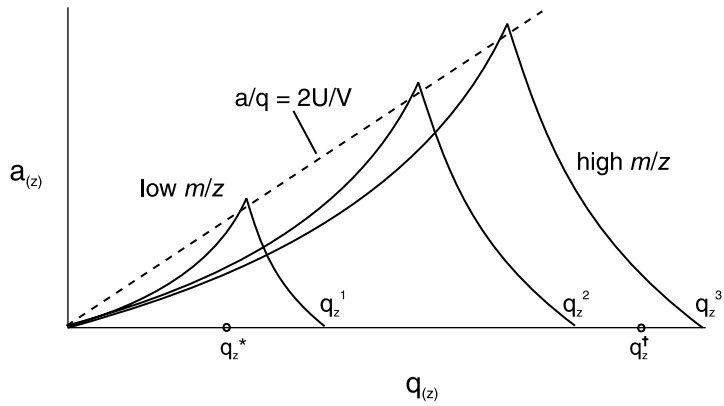


Figure 1.11. Stability diagrams for different values of m/z. The z subscripts are used when discussing the quadrupole ion trap (QIT; see Section 1.3.4). When the QIT is used for routine GC/MS analysis, $a_z = 0$, and only points along the q_z-axis are considered.

can be programmed to produce only discrete values for U or V. This process, called selected ion monitoring (SIM), allows ions having only specific m/z values to traverse the analyzer while all others are rejected. SIM offers enhanced sensitivity for detecting low concentrations of compounds in samples. Normally, when a transmission quadrupole repeatedly scans full spectra, most of the time is spent collecting information about m/z values for which few, if any, ions are formed by the compound in question. Thus, despite high concentrations in the ion source of fragment ions that are abundant in the spectrum, these ions pass through the analyzer for only very brief periods of time and, as a consequence, only a small fraction of them ever reach the detector. For example, if the analyzer scans from m/z 35 to 435, only 1/400th of the ions with each m/z value that were formed during the scan will be detected over this range.

In SIM the user selects a few ions (usually three) that are both abundant and characteristic of the compound's structure. By increasing the amount of time (called the *dwell time*) that the analyzer remains at a given value of U or V (and thus m/z), the fraction of these ions that reach the detector is increased. Whereas nanogram (10^{-9}) quantities of compounds normally can be detected using full-scan quadrupole mass spectra, SIM can sometimes lower the limit of detection into the picogram (10^{-12} g) range. SIM can also be performed using magnetic sector analyzers (Section 1.3.2) and quadrupole ion traps (see the next section). Instruments are now available that permit collection of both full-scan and SIM data during a single chromatographic run.

1.3.4. Quadrupole Ion Trap (QIT)

The quadrupole ion trap (QIT) that is shown in cross section in Figure 1.12 is related to the transmission quadrupole (March, 1997). The dome-shaped end caps and toroidal (roughly doughnut-shaped) ring electrode bathe the interior cavity with RF and/or electric fields. Although ions may be formed prior to their entering the QIT, ionization is most often provided by electrons emitted from a filament imbedded in the upper end cap and focused through an aperture in the end-cap surface. A similar opening in the lower end cap allows ions to reach the detector. As ions are formed, they do not "pass through" the analyzer as they do in the magnetic sector or quadrupole instruments, but rather are trapped in concentric, three-dimensional orbits according to their m/z values around the center of the ion trap.

The equations of motion for the QIT are almost the same as those for the transmission quadrupole, except that they relate to motion that is perpendicular to the surfaces of the end caps (here defined as the z-direction):

$$a_z = \frac{-8zV}{mr_0^2\omega^2}$$
$$q_z = \frac{4zU}{mr_0^2\omega^2} \tag{1.7}$$

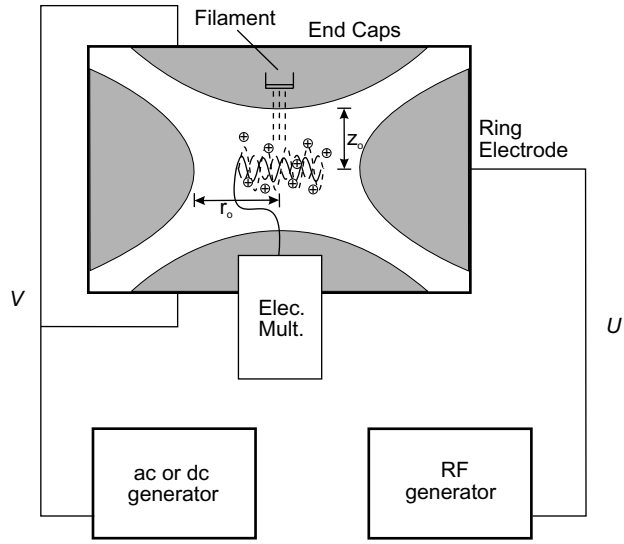


Figure 1.12. Quadrupole ion trap (QIT) mass spectrometer.

where

$V =$ dc (or ac) voltage applied to the end caps

$U =$ amplitude of the RF voltage applied to the ring electrode

$z_0 =$ distance from the closest approach of the end cap to the center of the ion trap

$r_0 = (\sqrt{2})z_0 =$ distance of closest approach of the ring electrode to the center of the ion trap

$\omega =$ applied radio frequency

As might be expected, the stability diagram for the QIT is virtually identical to that for the transmission quadrupole (Figure 1.10). For ordinary GC/MS work the dc generator in the end caps is not used, and ions are subjected only to an RF field. When $V = 0$ (i.e., the dc or ac generator is turned off), $a_z = 0$ and q_z is directly proportional to U at a given value of m/z. Because of this, only points along the q_z-axis need to be considered, and an ion having a particular m/z value will have stable motion in the QIT below some value of q_z (and thus of U) that is determined by the stability diagram for that ion (Figure 1.11). When U is set to a very low value, q_z will fall within the stable motion region for all ions except those having the smallest m/z values (q_z^* in Figure 1.11). Under these conditions most of the ions are thus "trapped" in complex motions in the center of the analyzer. At high values of U ($q_z^\dagger$ in Figure 1.11), only ions having large values of m/z will have stable motions.

For any given value of q_z, m/z is proportional to U:

$$\frac{m}{z} = \left(\frac{4}{r_0\omega^2 q_z}\right)U$$

As the instrument scans from low to high values of U, the motions of ions having progressively higher and higher m/z values develop larger and larger oscillations in the z-direction until they finally escape the trapped ion cloud and exit to the detector. To acquire a sequence of full-scan spectra, the QIT will:

1. Briefly turn on the filament and form ions (pulse ionization)
2. Trap most of the ions over a broad m/z range using a very low value for U
3. Sequentially destabilize ions by increasing U
4. Repeat steps (1) through (3)

A relatively high pressure of inert gas (usually 10^{-2}–10^{-3} torr of He) is used to dampen the motions of the ions so that they remain within their individual m/z orbits. This improves the ability to resolve adjacent m/z values.

Because a significant proportion of the ions formed in each pulse are available for detection over the scanned m/z range, the QIT is somewhat more sensitive in the scan mode than is the transmission quadrupole. Pulse ionization also keeps QIT spectra from exhibiting concentration-related spectral skewing while analytes are eluting from the GC (Section 1.3.6). Although its primary use has been for GC/MS, the QIT is in fact a versatile instrument. Its mass range can be extended to m/z 70,000 by a process called resonance ejection, which uses an ac, rather than a dc, generator attached to the end caps. In normal GC/MS use, the QIT provides m/z separation comparable to that of the transmission quadrupole, but it can also produce high resolution and MS/MS spectra (Section 1.3.5). A detailed discussion of these, and other, uses for the QIT is beyond the scope of this book.

1.1. In contrast to the transmission quadrupole and the magnetic sector analyzers, the QIT cannot be scanned from high to low m/z values. Why?

1.3.5. Other Types of Mass Analysis

1.3.5.1. Mass Spectrometry/Mass Spectrometry (MS/MS). The term MS/MS (also known as tandem mass spectrometry) refers to instruments in which two independent stages of m/z analysis are used. This type of analysis helps to establish the relationships between ions in a mass spectrum—for example, what ions are formed when the $M^{+\bullet}$ or other ions in the spectrum undergo fragmentation. This, in turn, helps to elucidate fragmentation pathways for the molecule being studied (de Hoffman, 1996) or, when used in conjunction with ionization techniques like CI and ESI that produce little fragmentation (Sections 1.2.2 and 1.2.3), to determine the structure of the analyte.

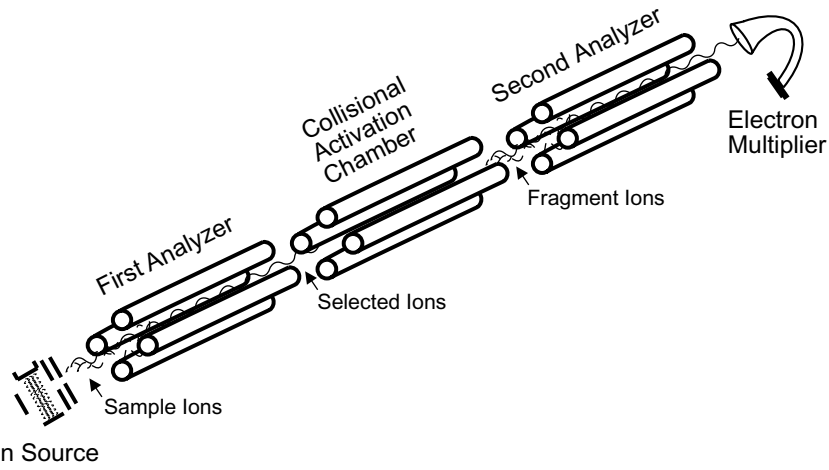


Figure 1.13. Triple-quadrupole (abbreviated QQQ) MS/MS in the mode for collecting product ion spectra. Voltages to the first analyzer are fixed to allow transmission of only a single m/z value. Collisions of these ions with an inert gas in the middle quadrupole cause fragmentation, the products of which are analyzed by scanning the second analyzer.

A typical analyzer array for doing MS/MS uses a linear arrangement of three quadrupoles between the ion source and the detector (the *triple quad*; see Figure 1.13). The first and the third quadrupoles act as independent m/z analyzers, while the second (middle) quadrupole acts as a collisional activation chamber through which ions from the first quadrupole must pass before they enter the final quadrupole. To obtain a product ion MS/MS spectrum, the first quadrupole (the ''first analyzer'' in Figure 1.13) is set to pass one or more ions of selected m/z value(s) (as in SIM, Section 1.3.3.1) into the collisional activation chamber. The ions thus selected are normally observed in the mass spectrum of the analyte and could be the $M^{+\bullet}$, a protonated molecule (MH^+), or any of the fragment ions produced by the compound. These m/z-selected ions are known as precursor ions.

The middle quadrupole is filled with an inert target gas at a pressure exceeding that normally found in either of the m/z-analyzing quadrupoles. When precursor ions pass through the middle quadrupole, collisions between these ions and gas molecules become likely. In these inelastic collisions, part of the kinetic energy of the precursor ion is converted into internal energy, which causes dissociation of the precursor ion. The middle quadrupole is also operated with only RF frequency applied to the poles, that is, $V = 0$ in Equation 1.5. As such, it acts as an ion-focusing device, conducting both precursor ions and the fragment ions formed from them into the final (third) quadrupole.

The last quadrupole (the ''second analyzer'' in Figure 1.13) is scanned over a range with its upper limit just above the m/z value of the precursor ion. The detector thus records a mass spectrum in which all the ions must have originated from the

precursor ion itself. This mass spectrum is called a product ion spectrum and provides a direct connection between a precursor ion and its fragments.

Product ion spectra have been recorded using instruments having virtually every combination of m/z analyzers, as well as with the QIT. In the case of the QIT, ion selection, collisional activation, and product ion analysis can be performed one after the other within the same chamber. Although the operational details differ from instrument to instrument, the basic description and premise of generating product ion spectra remain the same.

1.3.5.2. Accurate m/z Analysis. Mass spectrometers that measure very small differences in m/z values ($\Delta M \ll 1$) were mentioned previously. These spectrometers are often referred to as high resolution instruments. Accurate m/z measurements can be used to distinguish between ion elemental compositions that have the same nominal mass ($C_8H_5O_3^+$ vs. $C_9H_9O_2^+$ or $C_{11}H_{17}^+$, e.g., all of which have a nominal mass of 149; see Section 2.1.2). Several different types of instrumental arrangements can achieve accurate m/z analysis—a specially designed QIT (Section 1.3.4) has already been mentioned. Accurate m/z analysis is also possible with TOF and quadrupole analyzers, although in the latter case (Section 1.3.3), sensitivity may have to be sacrificed to achieve this.

More typically, accurate m/z analysis has been carried out using sector instruments such as the double-focusing instrument shown in Figure 1.8, in which the magnetic and electric sectors work in concert to correct for aberrations in ion optics. The value of ΔM achieved by the instrument is ultimately defined by the width of the slits along the ion path. Wider slits give higher ΔM values but higher ion throughput, whereas narrower slits provide lower ΔM values, but decreased ion transmission. Nonetheless, ion transmission through such double-focusing sector instruments is usually higher than through transmission quadrupole analyzers, and m/z values differing by less than 0.001 can be distinguished.

1.3.6. Spectral Skewing

With scanning m/z analyzers such as the magnetic sector or transmission quadrupole, the scan time may be slow enough that it affects the appearance of sequential spectra obtained during a chromatographic run. Because ions are being formed continuously in these instruments, the relative abundances of ions will not be reproducible from one scan to the next if the concentration of the analyte changes continuously during repetitive scans. This is especially a problem in capillary GC/MS, where analyte concentrations change rapidly with time over narrow GC peaks. Spectra obtained from different points on the GC peak may look quite different from one another, despite being from the same compound.

An example of this phenomenon is illustrated in Figure 1.14, which shows a narrow GC peak obtained during an analysis for Δ^9-tetrahydrocannabinol (THC) in marijuana. Notice that over 95% of the sample elutes within 0.06 min = 3.6 s. During this time the analyzer (in this case a transmission quadrupole) scanned through the range from m/z 35–400 eight times, a scan rate of 3.6/8 = 0.45 s

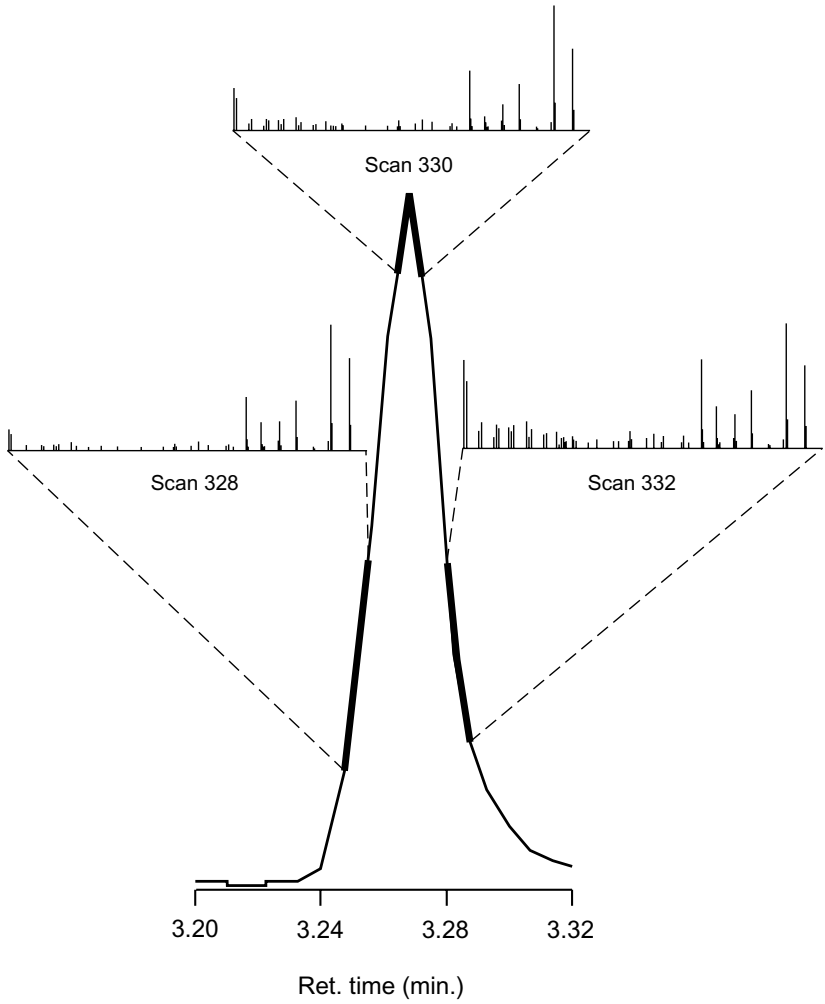


Figure 1.14. Variations in mass spectral peak intensities due to concentration differences over a narrow GC peak (spectral skewing).

scan^{-1}. As indicated by the heavy shading on the front and back sides of the peak, the concentrations of THC change by roughly a factor of 2 from the beginning to the end of the scan during scans 328 and 332. The result is that the spectra of THC obtained in scans 328 and 332 differ from one another—a phenomenon called spectral skewing. In scan 328 the high m/z peaks are intense compared to low m/z peaks, because the concentration of the analyte at the end of the scan is nearly twice what it was at the beginning of the scan. In scan 332 the low m/z peaks are more prominent because the analyte concentration was greater when those ions were detected. Scan 330, obtained at the top of the chromatographic peak, shows an intermediate situation.

The spectra shown in Figure 1.14 are obtained if the spectrometer is scanned from low to high *m*/*z* values. Under these conditions the low *m*/*z* ions in scan 328 reach the detector when the concentration of THC is relatively low, whereas the high *m*/*z* ions are detected when sample concentration is higher. In scan 332 the reverse is true. Since magnetic sector and quadrupole analyzers can also be scanned from high to low *m*/*z* values, you can convince yourself that the spectra obtained from scans 328 and 332 under these circumstances will be interchanged.

For GC peaks resulting from only a single component, the fact that the spectra change over the course of the peak is not a significant problem because the single scan over the crest of the peak, or a composite spectrum derived from averaging the spectra over the entire peak, will represent a ''normal'' spectrum for that compound. When two or more compounds coelute, however, the ability to obtain ''clean'' spectra of each of the compounds present (i.e., spectra lacking peaks due to the other coeluting substances) may be compromised unless spectra from the sides of the individual chromatographic peaks are used.

In theory, the data system should be able to eliminate contributions from unwanted background spectra, but selecting a good ''background'' spectrum for subtraction when chromatographic peaks overlap can prove problematic. For example, if the desired component elutes first, the concentration of the second component will still be increasing when that of the first has reached its maximum (Figure 1.15). The logical choice for a background spectrum, then, is the point

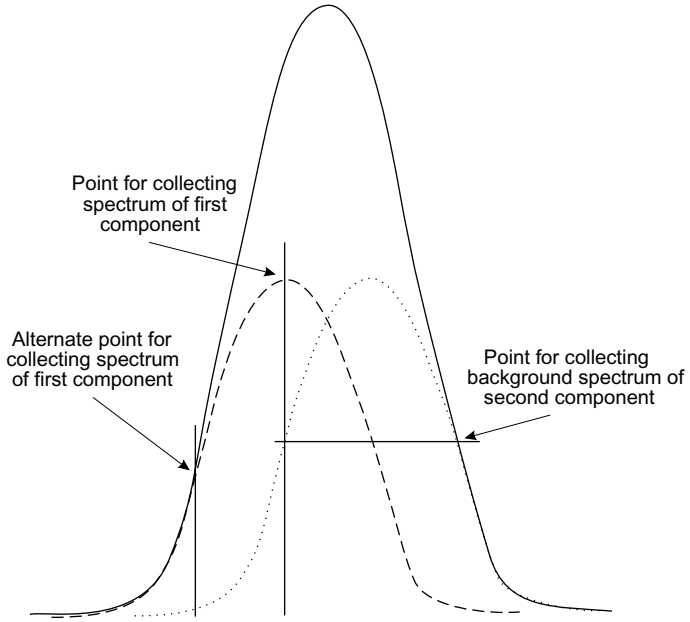


Figure 1.15. Coeluting GC peaks present a special problem for obtaining acceptable spectra of the individual components. Obtaining a ''clean'' spectrum may be limited to points on the back or front sides of the peak.

on the backside of the chromatographic peak for the second component where its concentration is approximately equal to that at the point where the spectrum of the first component was taken. However, the two spectra of the second component that are to be subtracted from one another *are not the same*, because the concentration of this compound is changing in opposite directions during the two scans. Thus, some residual peaks from the spectrum of the second component will remain in the spectrum of the first, despite background subtraction. The only way to eliminate peaks due to the second component may be to choose a spectrum of the first component on the front side on the first chromatographic peak—but in that case the relative intensities over the entire spectrum may not be representative of ''standard'' spectra for that compound.

Spectral skewing does not occur with spectrometers like TOF (Section 1.3.1) or QIT (Section 1.3.4) that use pulse ionization to produce ions, because pulse ionization forms ions in discrete packets that are subsequently analyzed for their m/z values. In the case of TOF, time intervals between spectra are also very short ($<500\ \mu s$) when compounds of low molecular mass are analyzed. When ions are not formed continuously during each spectral acquisition, or when spectra can be collected in rapid succession, the changing concentration of the analyte will not significantly alter the relative intensities of the peaks observed in the acquired spectra. As a result, background subtraction with these instruments should produce spectra that are devoid of peaks from coeluting compounds.[6]

The reproducibility of rapidly acquired GC/TOF mass spectra is good enough that chromatographic resolution can be severely reduced without sacrificing mass spectral quality. This technique, known as fast GC, allows complex mixtures to be analyzed in a fraction of the time needed on slower scanning instruments.

Figure 1.16 shows part of the chromatogram from a 4-min GC/TOFMS analysis of naphtha—an analysis that may take up to an hour or more on a more traditional GC/transmission quadrupole MS. Mass chromatographic plots for characteristic ions (see Section 1.5.4) showed that at least four components elute under this single chromatographic envelope. After data acquisition the software sequentially compared adjacent pairs of spectra to see which peaks were increasing, and which were decreasing, in intensity. The retention time for each component was identified as the time when a whole set of peak intensities uniformly reached a maximum. All peaks not at maximum intensity at that instant were assumed to be those of other components and were subtracted from the spectrum. The resulting spectra of the four components are shown in Figure 1.17.

[6] The National Institute for Standards and Technology (NIST) offers a free computer program called AMDIS (Automated Mass Spectral Deconvolution and Identification System) that examines multiple component GC/MS chromatographic peaks from most commercial instruments and provides the spectra of the individual components. AMDIS can be downloaded from http://chemdata.nist.gov/mass-spc/amdis.

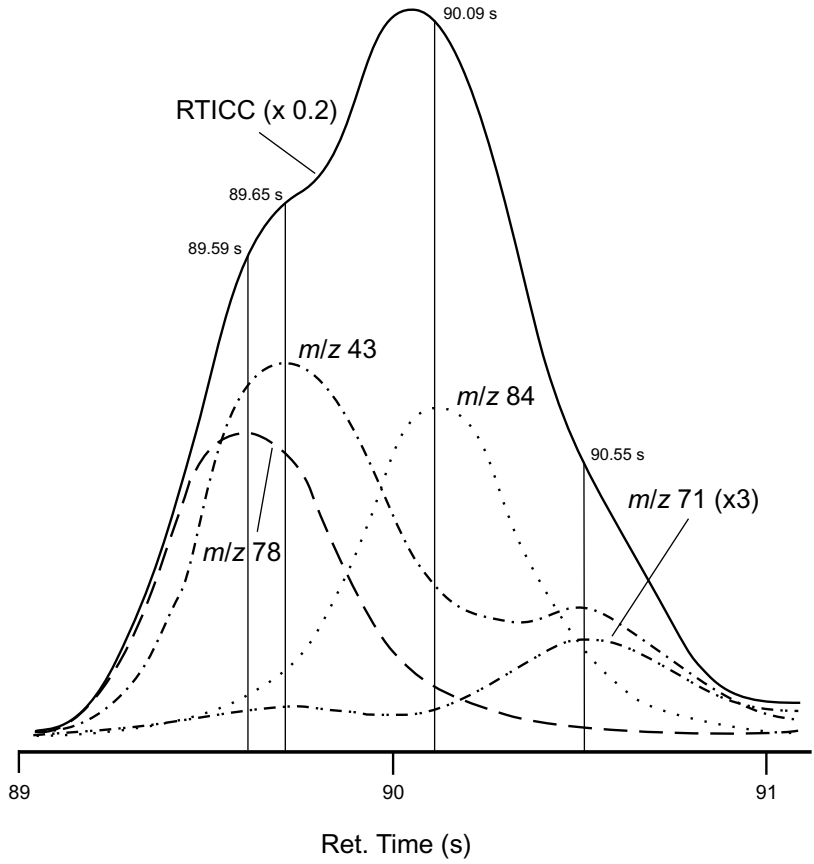


Figure 1.16. Complex chromatographic peak obtained from a 4-min fast GC analysis of naphtha. Individual ion chromatograms (mass chromatograms) indicate where four major components elute. Data were generated using Automated Unique Peak Find and Deconvolution software from a LECO Pegasus® II TOF mass spectrometer. (Data courtesy of LECO Corporation, St. Joseph, MI; adapted with permission)

1.4. ION DETECTION

Because the EI process is inefficient (Section 1.2), the number of positive ions that actually reach the detector is very small. Consider the capillary GC/MS analysis of a 100-ng sample of a compound having a molecular mass of 200 using a transmission quadrupole spectrometer. This sample, which is large for GC/MS work, contains approximately 3×10^{14} molecules. A spectrum acquired at the top of the GC peak may represent only 10–20% of the eluting material, lowering by a factor of 5–10 the amount available for ionization. Even if one molecule in 10^3 becomes ionized, only 10^{10}–10^{11} ions are produced at maximum sample concentration.

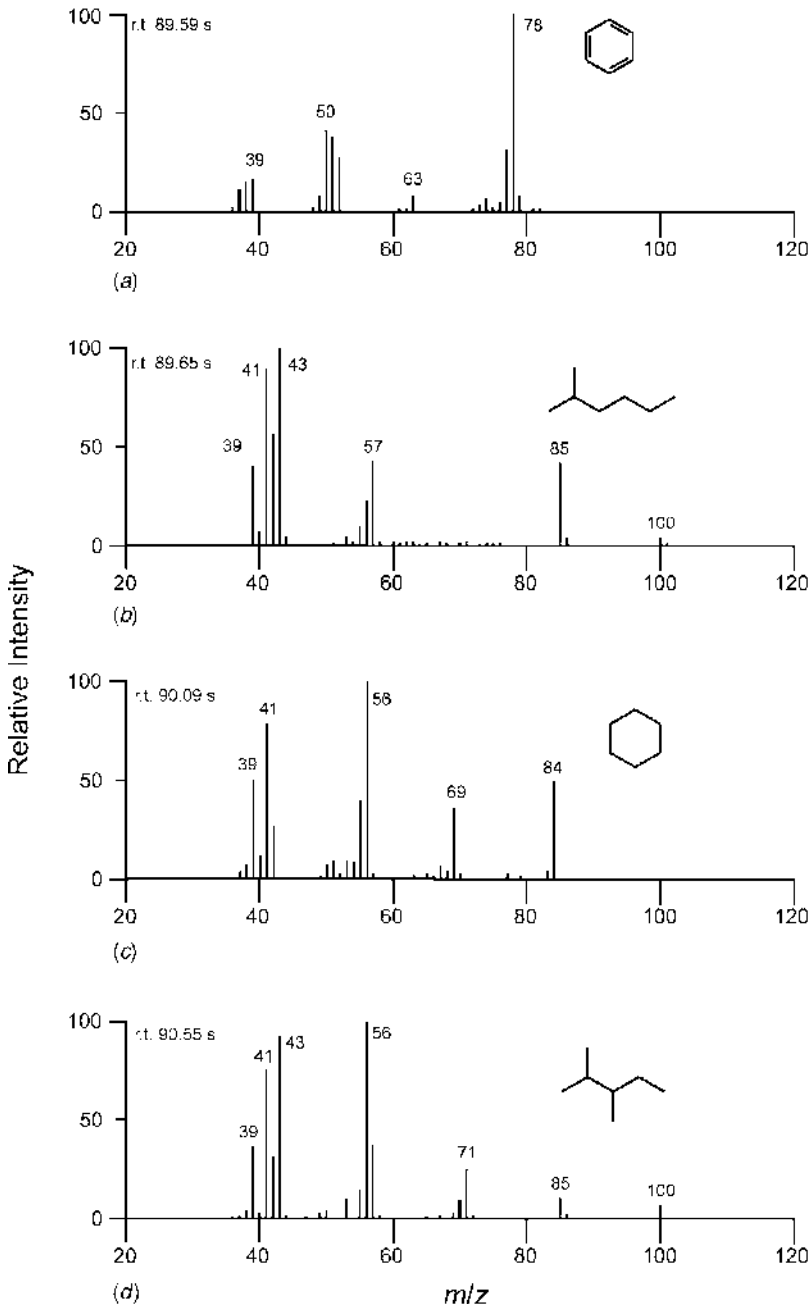


Figure 1.17. Mass spectra of four compounds eluting under the chromatographic peak shown in Figure 1.16. (*a*) Benzene, (*b*) 2-methylhexane, (*c*) cyclohexane, and (*d*) 2,3-dimethylpentane. (Data courtesy of LECO Corporation, St. Joseph, MI)

Further, in a scanning spectrometer most of these ions never make it to the detector, because the analyzer allows passage of only one m/z value at a time. If the instrument scans over a range of 300 m/z units, ions having any given m/z value pass through the analyzer only 1/300th of the time. This leaves a maximum concentration of $10^{14} \times 1/10 \times 1/10^3 \times 1/300 \approx 10^7$–$10^8$ of the most abundant ions (10^{-15}–10^{-16} moles) striking the detector during that scan. For a 100-pg sample, only about 10^4–10^5 ions will be observed. Because each ion carries a charge of only 1.6×10^{-19} coulomb (C), the current generated even under the best conditions will be very weak. Some means of amplifying the signal is clearly needed.

1.4.1. Electron Multiplier

An electron multiplier detector restores this lost sensitivity by exploiting the ability of surfaces that contain glass doped with about 10–20% lead oxide to expel more than one electron when a charged particle collides with it. Figure 1.18 shows a diagram of a continuous dynode electron multiplier, in which the entire surface of the multiplier is physically and electrically continuous. Other types of electron multipliers may have discrete dynodes or stages that are physically distinct but electrically connected to one another. Until recently, electron multiplier surfaces have been sensitive to air and especially sensitive to high concentrations of ions, so that venting the vacuum system when the multiplier is on, or leaving the filament on while the solvent is eluting during a GC run, could seriously damage the multiplier. With GC/MS, it is desirable not to measure ion current below m/z 10 because of the high concentration of He (m/z 4) or H_2 (m/z 2) in the ion source. Many users routinely do not acquire spectra below m/z 35 to avoid ions due to air and water background. Electron multipliers having less sensitive surfaces, as well as

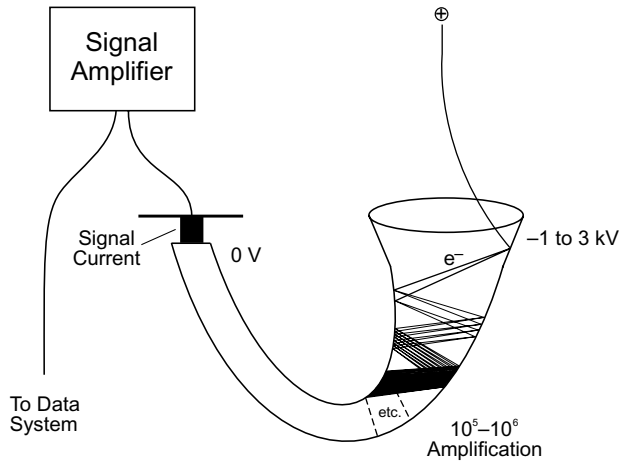


Figure 1.18. Continuous dynode electron multiplier detector.

photomultipliers (Section 1.4.2), are becoming more popular as detectors in MS because of their decreased sensitivity to such degradation.

The interior surface of the electron multiplier that is located near the entrance is held at a highly negative potential (usually -1.2 to -3 kV); the exit end is referenced to ground (0 V). As each incoming ion collides with the multiplier surface, approximately two electrons are ejected from the surface. To the ejected electrons the remaining interior of the multiplier appears more positive than the entrance does, so that they are attracted further into the multiplier where they collide with the interior surface. Each electron ejected by the second collision also results in the ejection of two electrons, and this process continues down to the exit or last dynode of the multiplier.

The total number of electrons ejected depends on the gain of the multiplier, which is roughly a function of the total potential difference between the entrance and exit to the multiplier surface. The gain can be adjusted daily during instrument tune-up so that a standard quantity of a reference sample such as PFTBA (Section 1.5.1) will produce approximately the same signal intensity. The total signal amplification is approximately 2^n, where n is the total number of collisions with the multiplier surface. Most multipliers provide about a 10^5- to 10^6-fold increase in signal—about 18–20 collisions. Electrons generated in the last collision with the multiplier surface constitute the signal current output of the multiplier. This current is sent to an external electronic signal amplification circuit and finally to the data system.

1.4.2. Photomultiplier Detector

Photomultipliers have been in use for a long time as detectors in radiation-based spectrometry such as IR and UV. Magnification of the signal in a photomultiplier is based on the same principle as that governing the electron multiplier, except that the inner surface of the photomultiplier is sensitive to photons rather than to charged particles. In order to use a photomultiplier as a detector in EIMS, the positive ions must cause the generation of photons that can be detected by the photomultiplier. This is accomplished by means of a fluorescent screen placed across the entrance to photomultiplier. As positive ions collide with the fluorescent screen, photons are produced in proportion to the number of ions present.

1.5. DATA SYSTEM

1.5.1. Instrument Tuning and Calibration

A mass spectrometer will produce no meaningful information if the analyzer does not satisfy the mass spectrometric equations given in Section 1.3. Once these equations are satisfied and a reasonable degree of m/z separation and sensitivity is achieved, each instrument also must be fine-tuned to adjust for the slight variations

that exist from unit to unit, as well as for changes in the ion source, analyzer, and electron multiplier surfaces that occur during routine use. Mass spectrometers should be tuned regularly if their performance is to remain optimal and reliable. Indeed, standards for good laboratory practice require that the instrument's tune be evaluated daily.

Tuning is accomplished by introducing a standard calibration compound into the instrument, and then adjusting variables until both the sensitivity and *m*/*z* separation are within acceptable limits. In many units this can be accomplished automatically by the data system with almost no user input. The most commonly used calibration standard for routine GC/MS work is perfluorotri-*n*-butylamine [$(CF_3CF_2CF_2\text{-}CF_2)_3N$; PFTBA], which gives fragment ions over the range from *m*/*z* 30 – 600 (see Problem 1.2). Prominent peaks at *m*/*z* 69, 219, and 502 in the spectrum of this compound can be used to adjust settings for instrument variables. Some laboratories prefer to tune manually using a compound that is frequently encountered during their analyses so that its mass spectrum is reproduced as closely as possible each day.

Usable sensitivity is achieved by adjusting both the voltage gain applied to the detector, which directly affects the intensity of the signal output, and voltages to various components in the ion source. The latter adjustments must be made because the metal surfaces inside the source change somewhat each time a sample is run. As more and more ions are formed, collisions with the source surfaces cause polymeric organic deposits to build up, leading to local variations in the electric fields present in the source. This interferes with both ionization and the movement of ions out of the source and into the analyzer. Tuning helps counteract the effects of these deposits.

After long-term use, ion source surfaces become so dirty that even tuning does not provide the necessary remedy. At this point the source has to be removed from the instrument, disassembled, and cleaned. Ironically, clean sources may need to be tuned more often than dirty ones since the amount of effective deposit on the surfaces increases dramatically at first, then tapers off after several samples have been run.

Achieving good *m*/*z* separation and peak shape is complex and cannot be done without some loss of sensitivity—the more restrictive the conditions for allowing ions through the analyzer, the fewer the number of ions that will be allowed through (Section 1.3.3). Peak shape and *m*/*z* separation are affected by variables in both the ion source and analyzer, and these must be adjusted against each other in order to obtain both acceptable *m*/*z* separation and sensitivity.

Once peaks of reasonable intensity having an acceptable degree of *m*/*z* separation are obtained, the *m*/*z* value of each peak must be determined. It is easy to forget that the MS/computer combination really is not very ''intelligent''—that is, the MS can only provide information as either voltages or electric currents that must be interpreted by the computer through its software. Conversely, the MS will produce no meaningful results without intelligent direction. The interactions between the MS and computer during one mass spectral acquisition in a transmission quadrupole are shown in Figure 1.19.

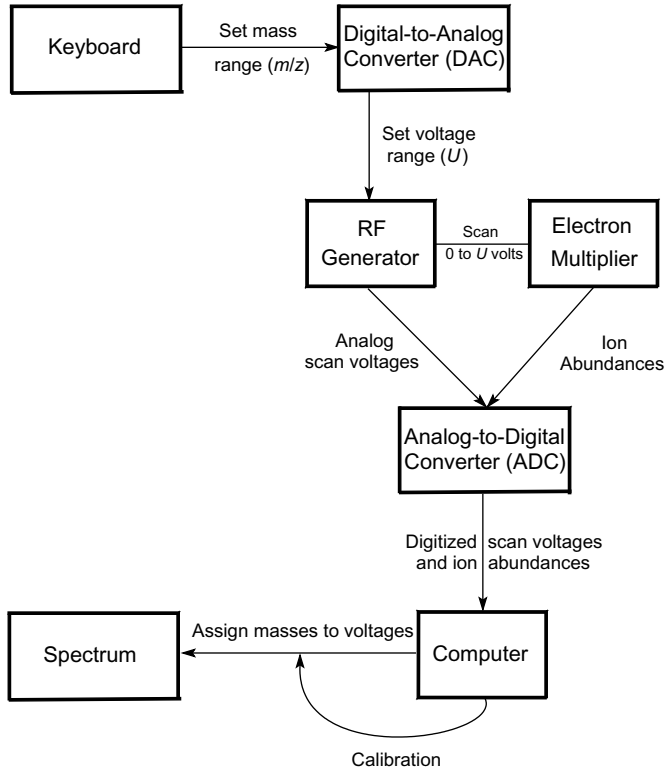


Figure 1.19. Interactions between the MS and computer during a scan.

The operator, through the computer keyboard or other input device, tells the MS the *m*/*z* range over which data are to be acquired and rate at which spectra are to be collected. This information is stored in digital form (i.e., having only distinct values) by the computer, but it must be converted to analog (continuously variable) form if it is to be used by the electronic circuits in the MS. This conversion is accomplished by means of a digital-to-analog converter (DAC)—an electronic circuit that takes the digital values provided by the computer and approximates the analog condition by causing small incremental ''steps'' to occur in the voltage circuitry.

During a typical GC/MS run, the computer, at a time determined by the operator, turns on the ion source and causes current to flow through the filament. The ions thus formed are separated repetitively by the analyzer over the assigned *m*/*z* range. Throughout this time the detector produces a variable signal current, the magnitude of which depends on how many ions of each *m*/*z* value there are at the time that ions having those *m*/*z* values are allowed to reach the detector. To be understood by the computer, the analog signal produced by the detector must first be converted to digital form by passage through an analog-to-digital converter (ADC).

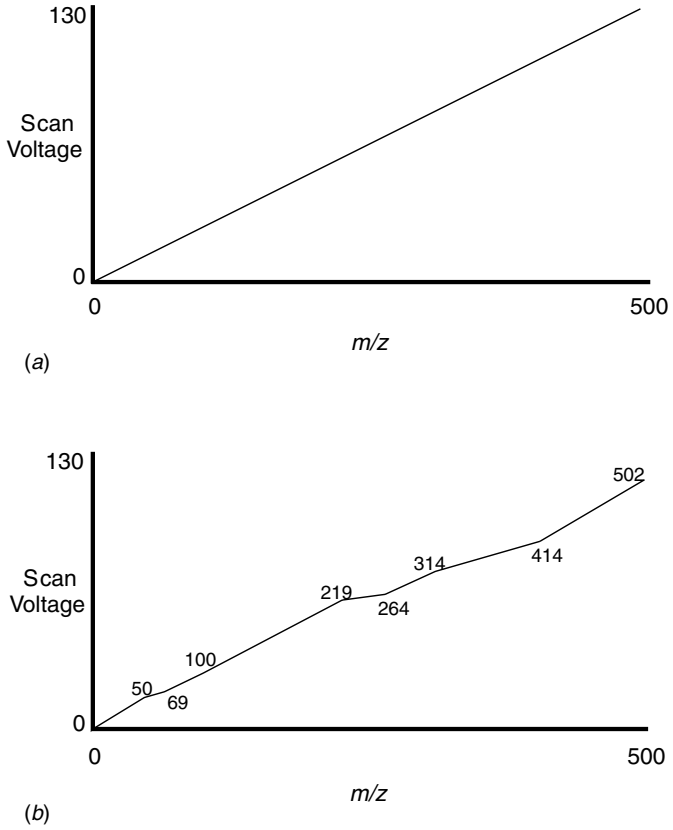
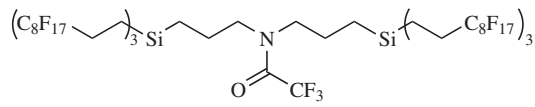


Figure 1.20. Mass spectrometers must be calibrated by direct assignment of analyzer variables to known *m*/*z* values. The situation for the quadrupole analyzer is shown here. (*a*) Theoretical scan of voltage vs. *m*/*z*. (*b*) Actual scan, in which the computer matches known *m*/*z* values for the calibration standard with observed voltages and interpolates between them.

The variable(s) used by the analyzer (time, magnetic field strength, RF voltage, etc.) to separate ions must be correlated to the output of the detector in order to produce the mass spectrum. If the *m*/*z* values of interest were related to analyzer variables in a completely reproducible manner (as they should be in theory; see Figure 1.20*a*, e.g.), the computer would have little difficulty assigning *m*/*z* values to each value for the analyzer variable and could produce the mass spectrum from those values and detector signal output alone. However, the relationship between these variables and *m*/*z* values is usually not ideal (see Figure 1.20*b*; the deviations in this figure are purposely exaggerated), so that the analyzer variables must be calibrated to correspond to known *m*/*z* values if the computer is to assign them correctly. With magnetic sector instruments, the relationship between *m*/*z* and the scanned variable is not linear (Section 1.3.2) and requires more closely chosen points in order to calibrate the *m*/*z* scale. Because time is the ''analyzer variable'' in TOFMS, these instruments can be calibrated using a single point.

Calibration is accomplished by obtaining the spectrum of a known standard. The calibration standard most commonly used for the analysis of compounds having molecular masses in the 10–700 range is PFTBA, the same compound used for tuning (see above). Other calibration standards include perfluorokerosene and homologs of PFTBA (with mass ranges up to about 900). The compound whose structure is shown below can be used up to *m/z* 3,000 (Fishman et al., 2001). Highly



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fluorinated compounds tend to make good calibration standards because they are more volatile than H-containing compounds in the same molecular mass range and produce ions having almost no mass defect (Section 2.1.2).

The known *m/z* values of the fragment ions of calibration standards can be preprogrammed into the computer, along with a range of probable analyzer variables that will produce these values. The computer then compares the observed values with those expected for the calibration standard and assigns the corresponding values for analyzer variables to the correct *m/z* values. Between these values for the observed fragment ions of the standard, the computer must interpolate. Although instruments should have their calibration evaluated on a daily basis to ensure correct *m/z* assignments, transmission quadrupole mass spectrometers tend to be very stable and may hold nearly the same calibration for weeks or months at a time.

1.5.2. The Mass Spectrum

1.5.2.1. Production of the Mass Spectrum. The output of the detector varies with time as ions of different *m/z* values are detected. The *m/z* values reaching the detector at any given time are related to the value of some instrument variable (e.g., magnetic field strength or voltage) at that moment. But what does a mass spectral ''peak'' really look like to the detector? Consider, as an example, an ion having an *m/z* value of 200.

It may seem at first as if all the *m/z* 200 ions strike the multiplier surface at the precise instant when the instrument variable allows passage of ions having *m/z* 200. However, when $\Delta M \sim 1$, there are several reasons why this does not happen. First, ions having the same *m/z* value have a small range of initial energies as they leave the ion source and thus are not expected to reach the analyzer and detector at exactly the same time. Second, at these values of ΔM, a small range of *m/z* values is permitted through the analyzer at any given time (Section 1.3.3), so that a few *m/z* 200 ions will begin to ''leak'' through the analyzer when the value of the appropriate variable corresponds to approximately *m/z* 199.5. As the value of this variable approaches that corresponding to *m/z* 200.0, the number of ions will increase, then taper off again as it approaches the value corresponding to *m/z* 200.5. If many *m/z* 200 ions pass through the analyzer at lower or higher values, they will overlap with the passage of *m/z* 199 or 201 ions, resulting in lower *m/z* separation.

In some cases, ions that have the same nominal *m*/*z* value, but differ in elemental composition, may be present at the same time. These ions will have slightly different exact *m*/*z* values (Section 2.1.2) and thus are not expected to pass through the analyzer at the same instant. For example, $C_{13}H_{27}OH$ has an exact mass of 200.2133, whereas $C_{15}H_{30}$ has an exact mass of 200.2340. This will lead to a small degree of peak broadening if $\Delta M \sim 1$. To detect both of these ions separately, ΔM must be decreased at least to 0.02 (Section 1.3.5.2). In that case, ions will not arrive at the detector until analyzer values correspond more closely to that of the exact *m*/*z* value of the ion. However, in no case will all identical ions reach the detector at precisely the same instant.

The mass spectral peak for the ion under discussion is a curve similar to a typical GC peak, having a maximum value at approximately *m*/*z* 200.0 (Figure 1.21) and a peak width that is determined by the *m*/*z* discrimination ability of the instrument. The actual *m*/*z* value of the maximum will usually differ somewhat from *m*/*z* 200.0 for reasons discussed in Section 2.1.2 (note the exact masses of the examples in the previous paragraph), but will usually not be lower than approximately 199.8 or greater than approximately 200.3 for most organic compounds.

In order for the data system to recognize this peak, it must identify a maximum signal coming from the detector during the time window when analyzer variables allow the detection of ions having *m*/*z* values from 199.5–200.5. In practice, this window of permissible *m*/*z* values is programmed to be from about 199.7–200.7 because, on average, the actual current maximum will occur at values slightly greater than *m*/*z* 200.0 (Section 2.1.2). The data system then assigns two values

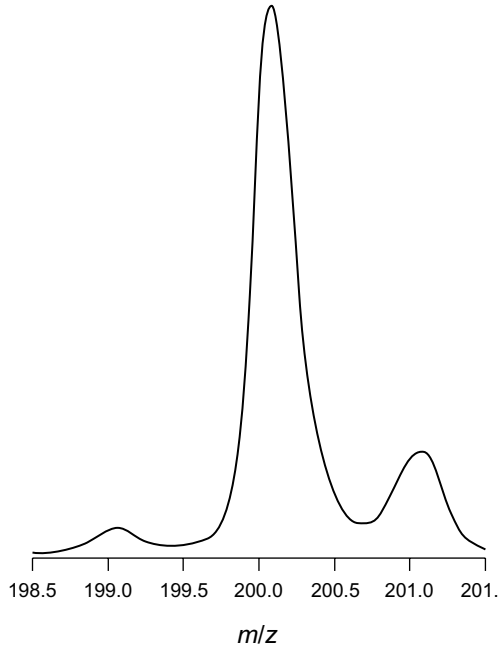

Figure 1.21. Mass spectral "peak."

to these data—one identifying the *m/z* value, the other quantifying the amplitude of the maximum signal produced by the detector during that window. Some data systems assign only the nearest integral *m/z* value to the maximum, so that any peak in the *m/z* 199.7–200.7 window would be labeled *m/z* 200. Others report the maximum more accurately, sometimes to the nearest 0.05 *m/z* value.

Most data systems used with spectrometers having $\Delta M \sim 1$ do not assign more than one signal maximum per window. In these cases, multiple charged or metastable ions (Section 3.6.2) that occur at fractional *m/z* values will not be identified separately if they are located between two more intense single charged fragment ions at sequential *m/z* values.

The mass spectrum, then, consists of the collection of *m/z* values and the corresponding values for detector signal maxima that are obtained during the windows for the analyzer variable that define those *m/z* values, over the entire range of *m/z* values for which data are acquired. It is customary to assign the value of 100% to the largest of all the ion current maxima obtained during the acquisition of an individual spectrum and to report the remaining values relative to this figure. The largest peak in the mass spectrum (100% relative intensity) is called the *base peak*. Although it is easy to think of the base peak as fixed for the mass spectrum of any given compound, it is, in fact, dependent on the displayed *m/z* range. For example, if the spectrum of an intermediate molecular mass, primary aliphatic amine is acquired (and displayed) from *m/z* 10–300, the base peak in the spectrum will nearly always occur at *m/z* 30 (Section 6.3). If the spectrum is only acquired (or displayed) from *m/z* 35–300, however, the peak at *m/z* 30 will not be reported, and some other peak in the spectrum, depending on the structure of the compound, will appear as the base peak.

Graphically, *m/z* values are plotted along the horizontal axis and relative peak intensities along the vertical axis. The mass spectra shown in the illustrations in this book are in this form. The same data may be presented in tabular form, such as that in Table 1.3 (compare this with the graphical presentation in Figure 1.22). Although the graphical presentation is more visually instructive, the tabular

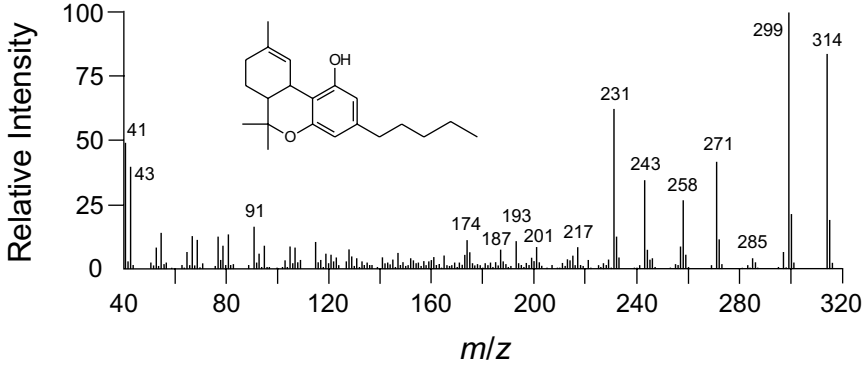

Figure 1.22. Graphic presentation of the mass spectrum of Δ^9-tetrahydrocannabinol that is given in tabular form in Table 1.3.

Table 1.3. Tabulated mass spectrum of Δ^9-THC

m/z	Rel. Int.	*m/z*	Rel. Int.	*m/z*	Rel. Int.	*m/z*	Rel. Int.
36.85	0.19	64.05	0.67	86.15	0.39	108.10	6.31
39.05	7.57	65.05	4.25	88.15	0.25	109.10	2.75
41.05	33.58	66.05	1.30	89.05	1.31	110.00	0.32
42.15	2.39	67.05	9.27	91.05	11.27	111.10	0.39
43.05	26.97	69.05	8.03	92.05	1.92	112.00	0.25
44.05	1.11	70.15	0.66	93.05	4.96	113.10	0.58
44.95	0.17	71.05	1.73	94.05	1.11	115.00	6.72
50.05	0.35	72.05	0.19	95.05	6.88	116.10	1.68
51.05	2.01	75.05	0.36	96.05	0.84	117.10	2.46
52.05	1.09	77.05	8.67	97.05	0.57	118.10	0.69
53.05	5.51	78.05	2.54	98.05	0.32	119.10	4.39
54.15	0.89	79.05	6.30	99.15	0.35	120.10	1.48
55.05	9.98	80.15	1.42	100.75	1.25	121.10	4.44
56.05	1.51	81.05	9.77	102.15	0.82	122.10	2.77
57.05	1.81	82.05	1.19	103.00	2.33	123.10	3.40
58.05	0.27	83.05	1.37	104.10	0.89	124.10	1.32
59.05	0.67	84.15	0.49	105.00	5.65	125.00	0.26
62.05	0.23	85.15	0.29	106.10	1.61	126.10	0.40
63.05	1.03	86.15	0.25	107.10	6.31	127.10	2.24
128.10	5.62	148.10	1.03	167.10	0.95	186.05	1.16
129.00	3.63	149.10	1.82	168.10	0.98	187.05	5.57
130.00	1.00	150.00	0.81	169.10	1.54	188.05	2.34
131.10	2.73	151.10	0.92	170.10	0.75	189.15	1.65
132.10	0.91	152.10	2.92	171.05	1.91	190.15	1.21
133.10	2.09	153.10	2.44	172.05	1.02	191.15	1.35
134.00	1.43	154.10	1.12	173.05	3.62	193.05	8.60
135.10	1.97	155.10	1.47	174.05	8.21	194.15	1.40
136.10	1.22	156.10	0.83	175.05	4.60	195.05	1.33
137.00	1.40	157.10	2.23	176.05	1.89	196.15	0.73
138.10	0.43	158.10	1.36	177.05	1.07	197.05	1.90
139.00	0.57	159.10	2.27	178.05	1.29	198.05	1.09
141.10	3.38	160.10	2.36	179.05	0.76	199.05	3.23
142.10	1.47	161.10	3.51	180.05	0.53	200.05	2.08
143.10	1.51	162.10	0.90	181.05	1.88	201.05	6.42
144.00	1.41	163.10	1.56	182.05	1.09	202.15	2.19
145.10	2.76	164.10	0.63	183.05	1.78	203.15	1.07
146.10	0.82	165.10	3.60	184.05	0.79	204.15	0.30
147.10	4.39	166.10	1.29	185.05	1.95	205.05	0.55
206.25	0.32	224.15	0.45	246.10	3.41	274.20	0.21
207.15	1.70	225.15	1.03	247.10	0.67	281.20	0.52
208.05	0.43	226.15	0.59	253.10	0.31	282.20	0.20
209.05	0.55	227.15	1.57	254.20	0.23	283.20	0.72
210.15	0.54	228.15	1.34	255.10	1.09	284.20	0.43
211.05	1.42	229.15	2.59	256.20	1.08	285.20	3.03

Table 1.3 (*Continued*)

m/z	Rel. Int.	*m/z*	Rel. Int.	*m/z*	Rel. Int.	*m/z*	Rel. Int.
212.15	0.95	231.15	46.22	257.20	7.02	286.20	2.16
213.05	2.78	232.15	10.54	258.20	21.89	287.20	0.49
214.15	1.93	233.15	4.27	259.10	4.62	295.20	0.30
215.15	3.88	234.15	0.55	260.10	0.71	297.20	5.96
216.15	1.15	239.15	0.36	267.10	0.25	299.20	100.00
217.15	6.79	240.10	0.50	268.20	0.19	300.20	22.01
218.15	1.47	241.10	1.16	269.10	0.96	301.20	2.46
219.15	0.88	243.10	27.41	271.20	38.45	314.15	70.52
221.15	3.39	244.10	6.00	272.20	9.38	315.25	16.83
222.15	0.69	245.10	3.21	273.20	1.52	316.15	2.46
223.25	0.30						

presentation often contains additional information that is not readily apparent from the graphic display.

Not every *m/z* value is represented in Table 1.3 or in any other mass spectrum. In fact, no organic compound can produce fragment ions at every *m/z* value. In addition, to minimize the number of spurious "noise" peaks that occur in all spectra, the data system often applies a threshold value for detector output below which peaks will not be reported. In Table 1.3 the threshold appears to be approximately 0.1% of the size of the most intense peak.

1.5.2.2. Terminology: Ions vs. Peaks. It is important to distinguish between the terms *ions* and *peaks* in mass spectrometry. Ions are particles that have both mass and charge, and they can fragment to form other ions. There can be large or small numbers of ions, so that it is appropriate to speak of their relative *abundance*. On the other hand, peaks in a mass spectrum correspond to localized maximum signals produced by the detector and have only *m/z* values associated with them. These signals are either weak or strong (depending on the numbers of ions produced) and therefore are best described as having *intensity*. The abundance of peaks implies that there are many peaks, not that a given peak is big or little.

The previous section described how the ions that are produced inside the spectrometer are recorded as spectral peaks. In that sense, the peaks in the spectrum represent the ions formed by the compound in question. However, the correspondence of ions to peaks is often not one to one. When $\Delta M \sim 1$, two or more ions that have nearly the same *m/z* ratios cannot be distinguished by the detector and thereby give rise to a single peak that represents all these ions. Throughout this book an attempt will be made to distinguish between the peaks that are observed in the mass spectra and the ions that those peaks represent.

1.5.3. Library Searches

Although the computer can perform many data reduction and manipulation routines, the identification of an unknown mass spectrum by comparison against

a collection of spectra stored in the computer is one of the most useful. This library search is a very powerful tool because it accomplishes in a few seconds what might take the operator many hours or more to perform manually. It is important to remember, however, that library searches are not foolproof.

Computer libraries may contain either full or condensed spectra—the latter being spectra that contain only the most meaningful (characteristic) peaks for each compound. The number of peaks retained in condensed spectra can vary from 10–50, depending on the search program. In practice, very little is sacrificed by using condensed spectra (McLafferty et al., 1999).

Spectra used in library searches may be weighted in order to give increased importance to peaks at higher m/z values. These peaks are usually more characteristic of the compound in question than those at lower values. A common weighting factor is the square root of the m/z value, so that

$$\text{Weighted intensity} = \text{observed intensity} \times \sqrt{(m/z)}$$

In the probability-based matching (PBM) algorithm, developed by Fred McLafferty of Cornell University, individual uniqueness and abundance factors are assigned to various peaks in each spectrum (McLafferty et al., 1974). Like the weighting factor above, the uniqueness of the peak increases logarithmically with its m/z value.

The library search can be performed either as a forward search or as a reverse search. The forward search algorithm compares the weighted unknown spectrum with similarly weighted library spectra. This comparison is often made by treating each m/z value/weighted intensity pair as a vector originating at the origin. The vectors for all the peaks in the unknown spectrum are added, and the result is compared with a similarly summed vector generated from the peaks in each library spectrum. The relative degree of ''match'' between these vectors reflects the relative similarity between the spectra (Stein and Scott, 1994). In a reverse search, each spectrum in the library is compared with the unknown spectrum to allow for the possibility that the unknown mass spectrum might actually be a mixture of spectra. If the data system has access to more than one spectral library, the algorithm may be set up to search all the libraries sequentially and provide a composite list of search results.

Library search programs have obvious strengths: rapid comparison of an unknown spectrum with up to several hundred thousand standards; the possibility of compound identification even when the spectrum is not that of a pure compound; and relative insensitivity to the types of instruments (but *not* to the type of ionization) on which the spectra were obtained.[7]

However, a high correlation (high *match index* or *probability of match*) between an unknown spectrum and a library spectrum does not necessarily mean that the unknown has been identified unequivocally. This point cannot be overstated. *The*

[7] Although most search algorithms will correctly identify spectra from different instruments or obtained under different conditions, the best matches will be obtained from spectra run on your instrument under your conditions.

criteria needed to identify an unknown by mass spectrometry must include a visual comparison of the unknown and library spectra by the analyst and may also demand additional information such as a comparison of GC retention times.

There are several reasons why this is so. First, similar structures often give spectra that are not easily distinguished by library search (or even visually, for that matter). Optical isomers cannot be distinguished at all, and other stereoisomers may be distinguishable only if enough spectra are acquired to determine that minor differences are repeatable. Even positional isomers (see Figure 1.23, e.g.) may not be identifiable with complete certainty by mass spectrometry alone.

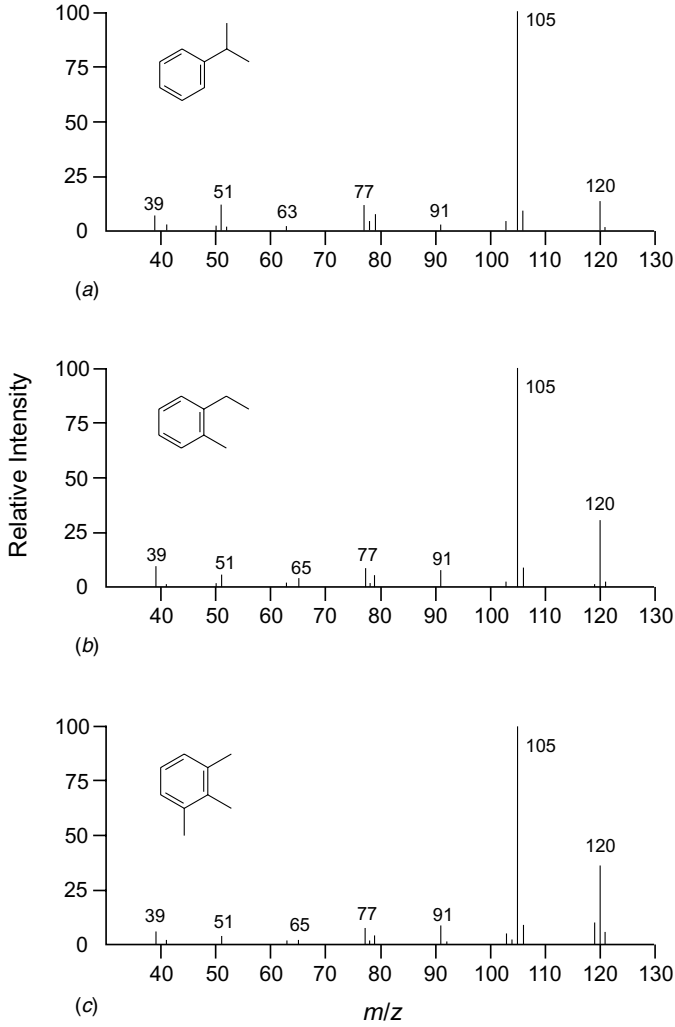

Figure 1.23. Mass spectra of these positional isomers are so similar that they may not be distinguishable at all. (*a*) Isopropylbenzene, (*b*) 2-methylethylbenzene, and (*c*) 1,2,3-trimethylbenzene.

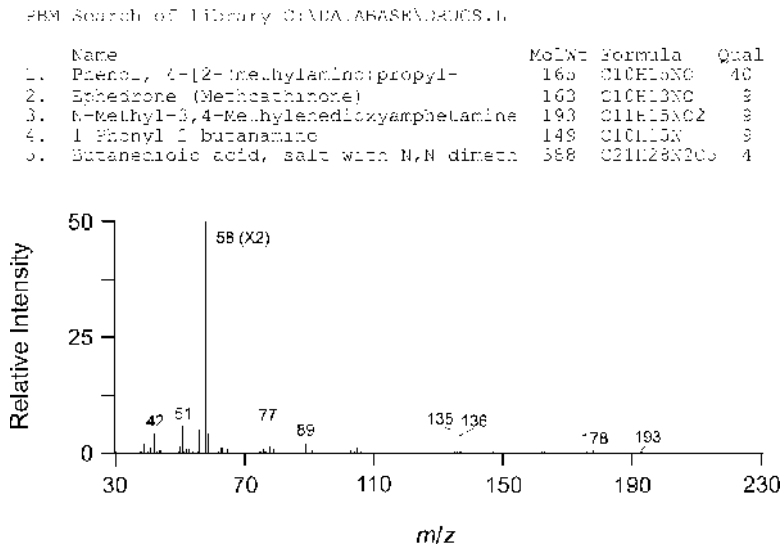

Figure 1.24. Mass spectrum of *N*-methyl-3,4-methylenedioxyamphetamine (MDMA) obtained during analysis of an illicit drug sample. The correct match is not listed first in the search results, in spite of the fact that the spectrum matches well with the standard (Figure 9.2*b*). All the compounds listed in the search results produce intense *m*/*z* 58 peaks.

Second, some search algorithms are more discriminating among certain types of compounds than others. The PBM algorithm, in fact, has such difficulty discriminating among certain aliphatic amines that the ''correct match'' may not appear at the top of match list, if it appears at all (Figure 1.24). Even with spectra containing several characteristic high *m*/*z* peaks, this search may fail to produce a viable match candidate under some circumstances (Figures 1.25*a* and *b*).

Third, search algorithms may fail to match spectra that are run on different types of instruments, particularly if ionization of the compound is achieved in different ways. Spectra that are produced by ''soft'' ionization methods such as CI (Section 1.2.2) or ESI (Section 1.2.3.1) cannot be compared directly with EI spectra.

Even though the search algorithm may show strong correlations between the unknown and one or more library spectra, the library may not contain the correct match for the unknown spectrum. Further, if the spectrum is not ''clean'' (i.e., numerous peaks due to extraneous materials are present), search results may identify the impurities rather than the unknown. Finally, although editors of mass spectral databases have become more and more critical of the spectra that are included in their libraries (Ausloos et.al., 1999), errors occur just often enough that it should give any user pause. *Never just assume that the library spectrum is correct!* Indeed, one of the purposes of this book is to give you tools that will

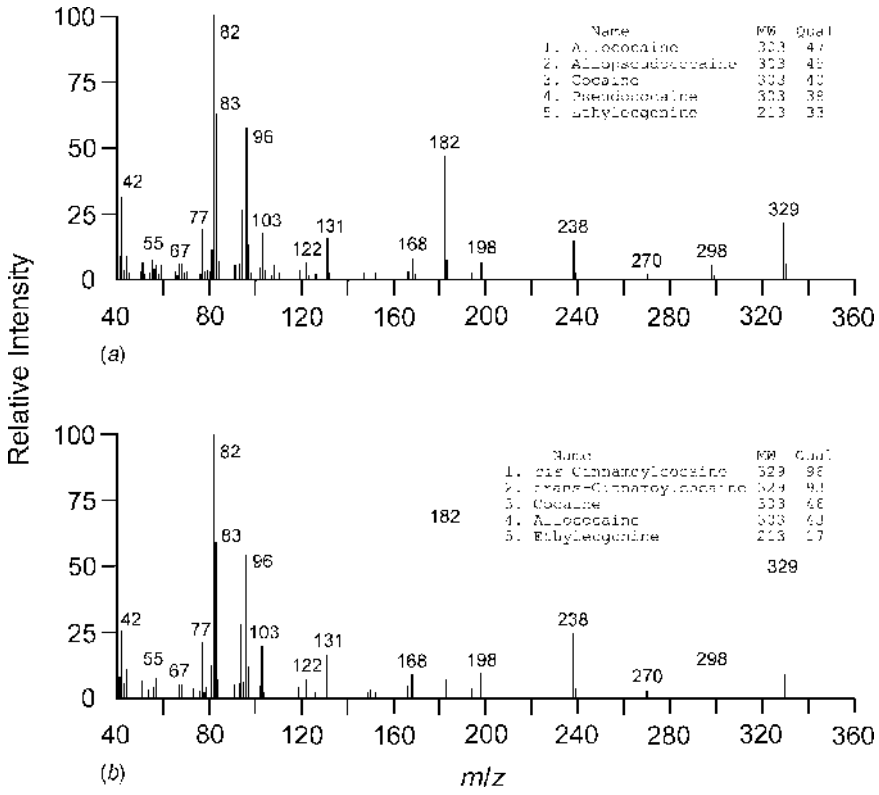

Figure 1.25. Mass spectra of *cis*-cinnamoylcocaine obtained from two different, but otherwise similar, samples. The correct match does not even appear in the search results for the upper spectrum (*a*), while the lower spectrum (*b*) is correctly identified using the same search algorithm.

help you evaluate whether a given spectrum reflects the assigned chemical structure or not.

If the library search fails to identify an unknown spectrum by direct match, software is available to help the user develop structural characteristics about the compound. Probably the best known of these programs is the Self-Training Interpretive and Retrieval System (STIRS) developed by McLafferty et al. (Kwok et al., 1973; McLafferty and Stauffer, 1985). This interactive program compares spectral features of the unknown, such as losses from the $M^{+\bullet}$, characteristic peaks, and series of peaks at low *m/z* values (Section 4.2), to those of compounds contained in a large library. If the unknown spectrum shares several features in common with a class of compounds in the library, the program indicates that the unknown probably contains a certain structural feature which is found in this class of compounds. If

STIRS identifies several probable structural features in the unknown, the analyst may be able to determine its overall structure.

More widely available is MS interpretation software from the National Institute of Standards and Technology (NIST) in association with the NIST/EPA/NIH Mass Spectral Library. This program identifies peaks that may result from simple cleavage of the $M^{+\bullet}$ and generates isotopic intensity patterns for ions whose elemental compositions are consistent with the proposed structure. A demonstration of this software is available at http://www.nist.gov/srd/nist1a.htm.

1.5.4. Using the Data System to Analyze GC/MS Data

Even before sophisticated data collection and manipulation software began to appear commercially, the combination of GC and MS revolutionized the analysis of complex mixtures. Because many of the readers of this book will (or already do) work in laboratories where GC/MS is an important method of sample analysis, it is worthwhile to explore how the data system can be used in the analysis of sample mixtures. Alternate data handling methods for other highly complex mixtures are given in Sections 1.3.6 and 4.2.5.

As an example, consider an unknown solid brought to a forensic laboratory for illicit drug analysis. To minimize sample workup, a small portion of the solid was dissolved directly in methanol, and 1 μL of this solution was injected onto a 10-m phenylmethylsilicone (HP-1) capillary column. The GC oven was programmed (via the data system) to begin the analysis at 160°C, increase the temperature from 160–280°C over a 6-min period, and then remain at 280°C for an additional minute. During the same time interval, the MS was programmed to (1) keep the filament and electron multiplier *off* for 1 min while the methanol was passing through the GC column (Sections 1.2 and 1.4); (2) acquire spectra repeatedly from *m*/*z* 35–350 at a rate of about 0.5 s $scan^{-1}$ for the next 3 min; and (3) scan repeatedly from *m*/*z* 35–400 for the remainder of the analysis.

By acquiring data continuously in this way, the MS acts as an elaborate GC detector. In fact, one way to report GC/MS data is as a reconstructed total ion current chromatogram or RTICC, which is a plot of the total ion current output of the detector for each mass spectral acquisition over the time required by the analysis. These plots are sometimes also referred to simply as total ion current chromatograms (TICC) or just total ion chromatograms (TIC). In the RTICC for this sample (Figure 1.26), the ion current intensity reflects the total number of ions produced in each spectrum (Section 1.4). The RTICC looks like chromatograms produced by other GC detectors, but because MS measures a different property of the analytes than other detectors do, the same sample mixture can produce somewhat different chromatograms by MS and by other common detectors such as flame ionization (FID).

The advantage of using MS as a GC detector is that the computer stores the data from the analysis not as total ion current, but rather as a collection of sequentially acquired mass spectra. Therefore, these spectra can be retrieved one at a time, and the makeup of the GC effluent at any given time during the run can be identified.

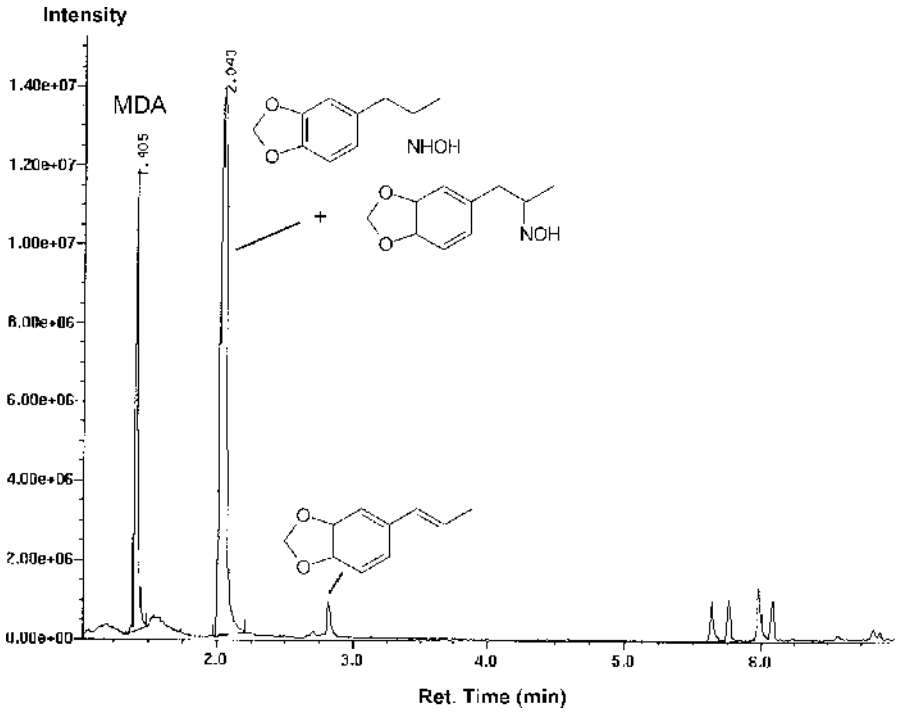

Figure 1.26. Reconstructed total ion current chromatogram (RTICC) generated from mass spectral data collected during analysis of an illicit drug sample.

Using the software available with most GC/MS units, spectra can be retrieved simply by having the operator move the cursor on the monitor to the point on the RTICC that requires investigation, then press "Enter" on the keyboard, or click a mouse button. For single-component GC peaks, an acceptable spectrum can be obtained either by placing the cursor at the top of the chromatographic peak or by asking the data system to average several spectra from the top of the peak (Section 1.3.6). The data system can subtract from this spectrum background that is reasonably constant over the peak by choosing a point near the front (or back) base of the chromatographic peak or by averaging background spectra from the same area(s). Background subtraction may not be necessary if the GC peak is intense and background from substances bleeding off the GC column or septum (commonly called *column bleed*) is minimal.

Such a process produced the spectra in Figures 1.27*a* and *d*, which were identified by library search and visual confirmation by the analyst as 3,4-methylenedioxyamphetamine [with a retention time (r.t.) of 1.405 min] and 1-(3,4-methylenedioxyphenyl)propene (with a r.t. of 2.8 min). 3,4-Methylenedioxyamphetamine (MDA) is a hallucinogenic drug (Section 9.2) and 1-(3,4-methylenedioxyphenyl)

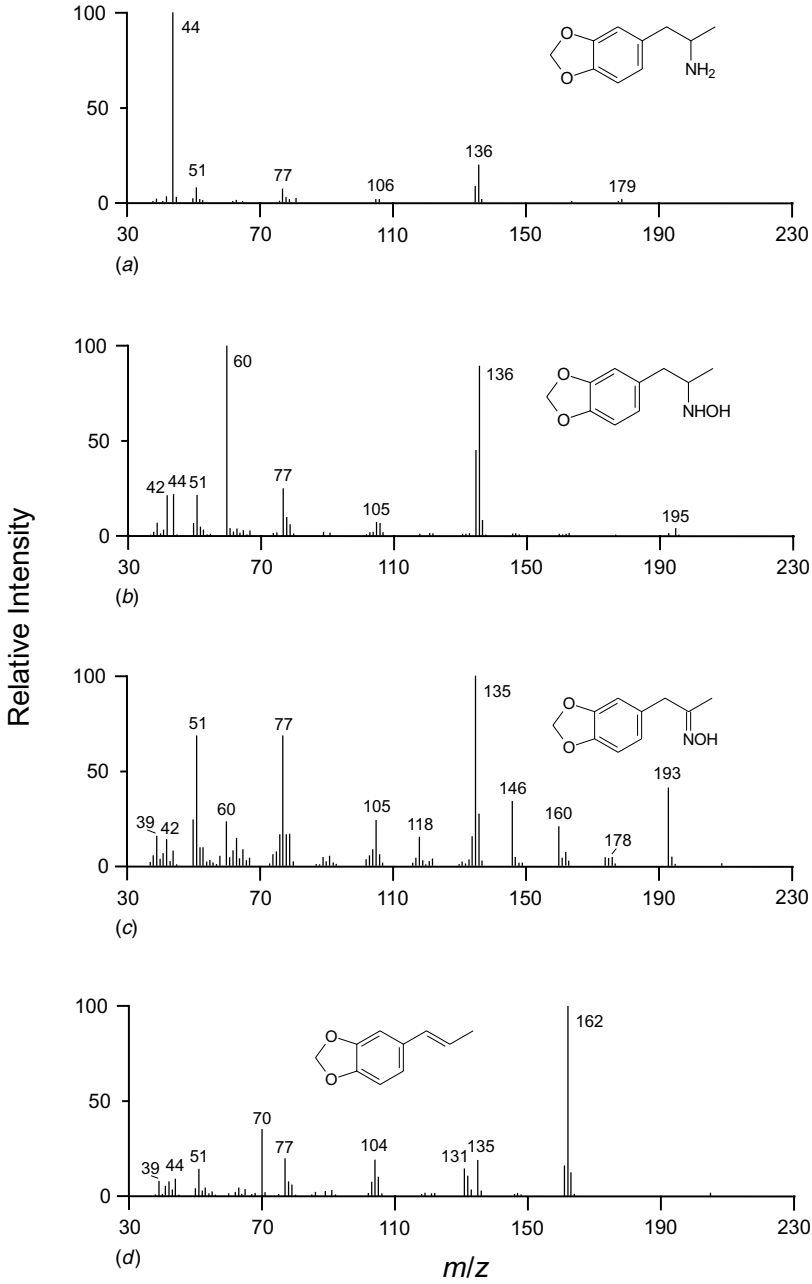

Figure 1.27. Mass spectra of four components found in the illicit drug sample whose RTICC is shown in Figure 1.26. (*a*) 3,4-Methylenedioxyamphetamine (MDA; r.t. 1.40 min), (*b*) *N*-hydroxy-MDA (r.t. 2.02 min), (*c*) 1-(3,4-methylenedioxyphenyl)-2-propanone oxime (r.t. 2.05 min), and (*d*) 1-(3,4-methylenedioxyphenyl)propene (r.t. 2.81 min).

propene is an intermediate produced during one of the common syntheses of MDA and its analogs.

Similar treatment of the large, broad chromatographic peak at r.t. 2.04 min led to a spectrum that was much like the one shown in Figure 1.27*c*, except for the addition of a few small peaks—most notably that at *m*/*z* 195. This component was identified by library search as 1-(3,4-methylenedioxyphenyl)-2-propanone oxime, which was an unexpected result because IR analysis of the solid identified only the presence of *N*-hydroxy-MDA. Further examinations of individual spectra indicated that two different compounds were coeluting under this chromatographic peak.

In order to produce an acceptable spectrum for each of these compounds (one not contaminated by peaks from the other), the elution time of each component had to be determined (see Section 1.3.6 and Figures 1.15 and 1.16). Although mathematical deconvolution can be done even in the absence of MS data (Stein, 1999), the data already collected in this case contained enough information to generate the desired result. The mass spectra of the two coeluting compounds (Figures 1.27*b* and *c*) each contained intense, characteristic peaks not present in the other—in particular, the base peak (*m*/*z* 60) in the spectrum of the first-eluting compound and the relatively intense $M^{+\bullet}$ peak (*m*/*z* 193) in the spectrum of the second.

Plots of the intensities of only these two characteristic peaks—a process known by a variety of names including mass chromatography and reconstructed ion chromatography (RIC)—are shown in Figure 1.28 for the small window of time between r.t. 1.75 and 2.27 min. Mass chromatography differs from selected ion monitoring (SIM; see Section 1.3.3.1) in that the mass chromatogram is generated *after collecting continuously scanned mass spectral data* for the entire chromatogram. In SIM, ions must be selected prior to sample injection. A mass chromatogram can be generated for any *m*/*z* value in the acquired range, but no data are available in SIM for any *m*/*z* values other than those monitored during the analysis.

Figure 1.28 makes clear that, although the two compounds elute less than 2 s apart and with a considerable amount of overlap, it is possible to obtain spectra of both compounds that have little contamination due to peaks from the other. The spectrum of *N*-hydroxy-3,4-methylenedioxyamphetamine (*N*-hydroxy-MDA; see Figure 1.27*b*) was obtained from the upper front side of the peak in the *m*/*z* 60 mass chromatogram, at a point corresponding to the base of the peak in the *m*/*z* 193 chromatogram. The spectrum of the oxime (Figure 1.27*c*) was obtained at a point just beyond the top of the *m*/*z* 193 mass chromatogram where the first compound had stopped eluting (Figure 1.28).

These results indicate that *N*-hydroxy-MDA had undergone partial disproportionation to MDA and the oxime in the injection port of the GC. This is an important point to consider: Even when the mass spectra obtained from the sample are of high quality, there is no guarantee that they represent the actual composition of the original sample. Instead, they may arise as an artifact of some aspect of the analysis.

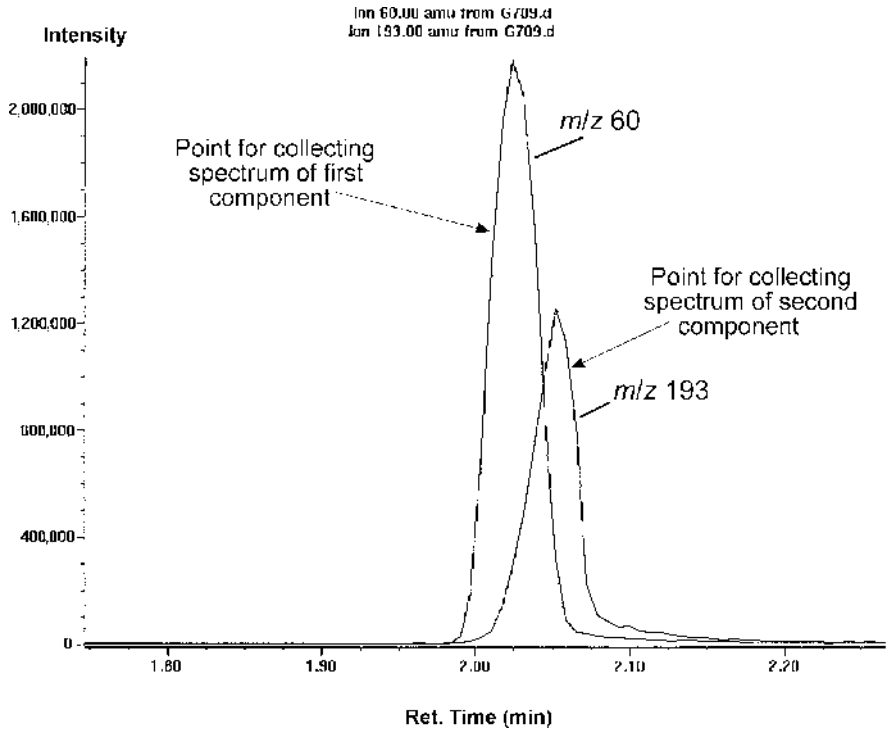

Figure 1.28. Mass chromatograms for *m*/*z* 60 and 193 generated from the data collected during the run shown in Figure 1.26. These chromatograms were used to generate acceptable spectra for each of the coeluting components (shown in Figures 1.27*b* and *c*).

1.6. CRITERIA FOR GOOD-QUALITY SPECTRA

Mass spectra cannot be interpreted if they contain misinformation. Unfortunately, even refered journals and carefully edited collections of standard spectra sometimes contain spectra that fail to meet this criterion. Judging whether or not a mass spectrum is credible is sometimes the most critical step in its interpretation.

Several criteria are useful for evaluating the quality of spectra. These apply not only to spectra generated in your own laboratory but also to those in the literature or under consideration for placement in user-generated libraries or for publication. Good-quality spectra:

1. Should show appropriate isotope peaks (e.g., should not be missing expected peaks due to ^{13}C), especially at high *m*/*z* values (see Chapter 2);
2. Should not exhibit excessive background noise (e.g., spurious peaks of instrument origin throughout the mass range) or the obvious presence of extraneous materials (column bleed, coeluting GC peaks, contaminants in the ion source); and

3. Must be consistent with the known or proposed structure—that is,
   a. all *m*/*z* values must be correct;
   b. the $M^{+\bullet}$ peak, if present, must correspond to the molecular mass;
   c. important peaks at high *m*/*z* values that reflect the loss of neutral fragments must correspond to functional groups or structural arrangements present in the molecule (Chapter 4); and
   d. the base peak must be consistent with the structure (Chapters 6–8).

After reading the next several chapters of this book, it should become evident why these criteria are important. You may want to review this section after solving some mass spectral unknowns and studying the material contained in later chapters.

ADDITIONAL PROBLEMS

Although answers to all the problems are given in the final chapter of this book, you are strongly encouraged to try to solve each problem before proceeding further. Experience has shown that there is no substitute for solving unknowns if you want to become proficient at interpreting mass spectra. Because most of the topics in this book build on preceding material, beginning students who do not spend time working problems soon get lost. Very few readers, unless they are already adept at mass spectral interpretation, will be able to absorb the concepts illustrated in the problems if they depend entirely on the explanations provided.

1.2. The compound commonly used for tuning and calibration of mass spectrometers is PFTBA, a trade name for perfluorotri-*n*-butylamine, $(CF_3CF_2CF_2CF_2)_3N$. This compound exhibits peaks of at least moderate intensity over the entire mass range normally used in GC/MS work (Figure 1.29). The most prominent

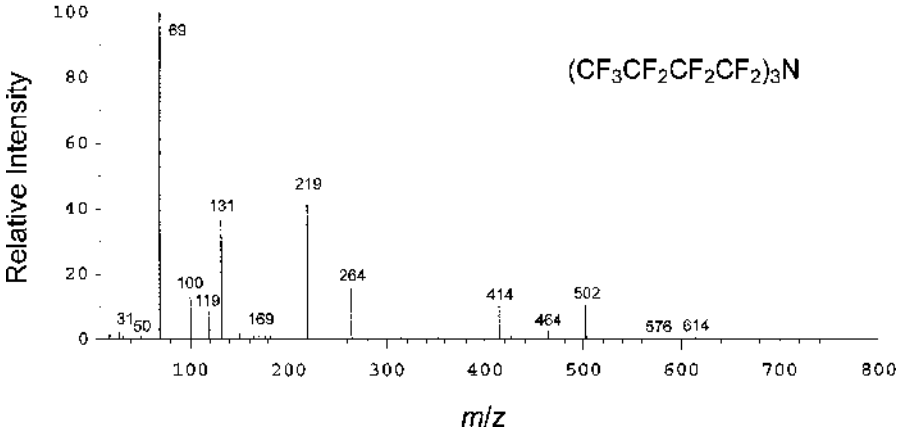

Figure 1.29. Mass spectrum of perfluorotributylamine (PFTBA; Problem 1.2).

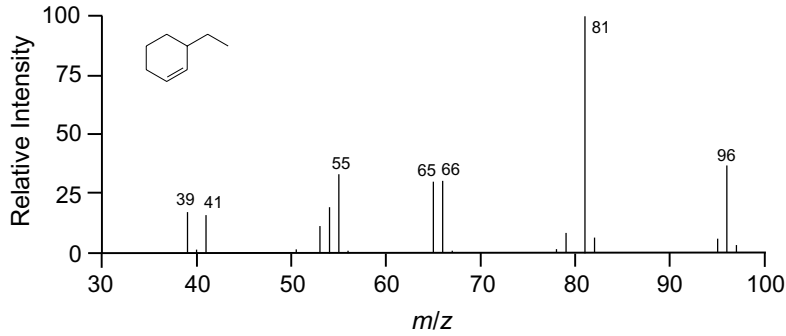

Figure 1.30. Library spectrum for 3-ethylcyclohexene (Problem 1.3).

peaks in the spectrum of PFTBA occur at *m/z* 31, 69, 100, 114, 119, 131, 219, 264, 414, 464, 502, 576, and 614. Devise elemental compositions (and, if possible, hypothesize structures) for the ions that correspond to each of these peaks.

1.3. The spectrum for 3-ethylcyclohexene shown in Figure 1.30 is from a published mass spectral library collection. This spectrum exhibits a $M^{+\bullet}$ peak at *m/z* 96 and a base peak at *m/z* 81. What is wrong with this spectrum? (*Hint*: Organic compounds do not lose fragments of 14 u from the $M^{+\bullet}$—see Section 4.1.1.)

MASS SPECTROMETRIC RESOURCES ON THE INTERNET

Anyone interested in mass spectrometry will find a number of resources available on the Internet. These include access to publications, professional societies, instrument vendors, employment, mass calculators, and tutorials—even tips on troubleshooting instruments. A list of websites for specific information can be found by using your Internet provider's search engine. The following websites provide examples of the types of information that are available.

General

- *http://base-peak.wiley.com* Part of a European-based website that includes all areas of spectroscopy. It contains links to articles, groups, books, conferences, employment, and MS software.
- *http://www.i-mass.com* Also a European-based site similar to base-peak. The isotope pattern calculator (see Chapter 2) is versatile and easy to use.
- *http://www.sisweb.com/mslinks.htm* A U.S.-based site sponsored by Scientific Instrument Services, Inc. It includes links to spectra for MS calibration compounds, as well as a table of exact masses and isotopic abundances for all the elements.

- *http://jeol.com/ms/ms.html* Sponsored by one of the manufacturers of mass spectrometers. The link to ''Essays and Tutorials'' contains brief descriptions and diagrams of instruments and other aspects of MS theory. The site also includes a discussion of elemental composition calculations.

Calculators

- *http://www.cem.msu.edu/~reusch/OrgPage/mass.htm* (*Note*: The URL is case-sensitive.) Calculators for exact masses and isotopic peak intensity ratios (see Chapter 2). Maintained by Michigan State University.
- *http://www.shef.ac.uk/~chem/chemputer/isotopes.html* Isotope peak intensity ratio calculator (see Chapter 2). Maintained by the University of Sheffield.
- *http://www.chem.uni-potsdam.de/tools/index.html* Calculator for losses from the $M^{+\bullet}$ (see Chapter 4). Maintained by the University of Potsdam.
- *http://www.nist.gov/srd/nist1a.htm*. This website, maintained by the National Institute for Standards and Technology (NIST), contains an aid to mass spectral interpretation, as well as isotope and formula calculators associated with a demonstration version of NIST's MS Search program. This version also contains about 1,000 EI spectra.

Standard Spectra

The following two sites provide free access to a reasonable, but not extensive, number of standard spectra that can be searched by molecular mass, name, or empirical formula. The spectra can be printed.

- *http://webbook.nist.gov/chemistry* Maintained by NIST.
- *http://www.aist.go.jp/RIODB/SDBS/menu-e.html* Maintained by the Japanese National Institute of Advanced Industrial Science and Technology.

REFERENCES AND SUGGESTED READING

The field of mass spectrometry is very large and growing at a rapid pace in different directions. Even the interpretation of mass spectra is a broad topic. Thus, any list of suggested sources for additional information must necessarily be incomplete. Rather than duplicate here lists that have already been compiled by others, the reader is referred to other books that provide additional references and resources that are not found in this book.

F. W. McLafferty and F. Tureček's *Interpretation of Mass Spectra*, 4th edition (University Science Books, Mill Valley, CA, 1993) presents a more detailed approach to spectral interpretation than is offered in this book. The appendices contain an extensive collection of useful data. McLafferty's texts remain classics in the field.

O. D. Sparkman's *Mass Spec Desk Reference* (Global View Publishing, Pittsburgh, PA, 2000) could serve as a large appendix to any book about mass spectral theory and interpretation. Two important assets of this book are the carefully constructed glossary of MS terms, with discussions of which terms are currently in use and why, and a list of original references that should suffice to introduce the beginning student to the most important mass spectral literature up to 2000. Both of these books are relatively inexpensive.

Two other books that serve as general references are J. Throck Watson's *Introduction to Mass Spectrometry*, 3rd edition (Lippincott Raven, Philadelphia-New York, 1997) and W. L. Budde's *Analytical Mass Spectrometry: Strategies for Environmental and Related Applications* (Oxford University Press for the American Chemical Society, New York, 2001). Watson's book expands on most of the topics covered in this chapter, whereas Budde's book deals primarily with GC/MS applications.

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