Chapter 1 Introduction

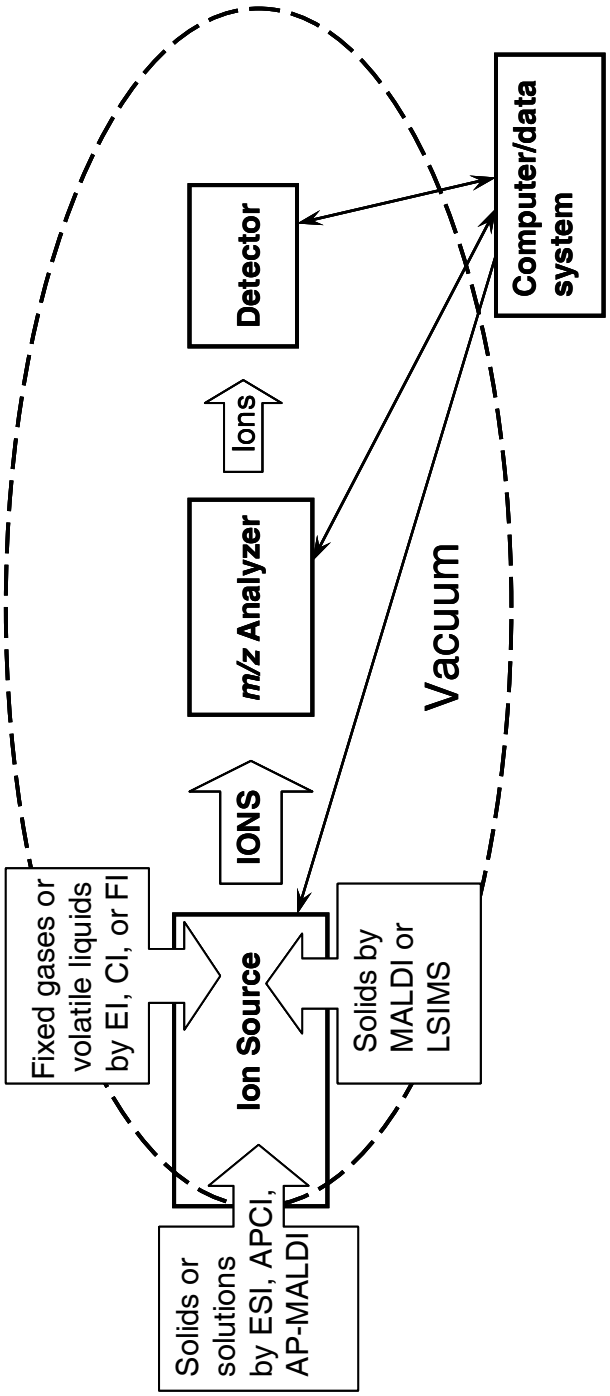

Figure 1–1. ***This conceptual illustration of the mass spectrometer shows the major components of mass spectrometer; i.e., sample inlets (dependent on sample and ionization technique; ion source (origin of gas-phase ions); m/z analyzer (portion of instrument responsible for separation of ions according to their individual m/z values); detector (generates the signals that are a recording of the m/z values and abundances of the ions); vacuum system (the components that remove molecules, thereby providing a collision-free path for the ions from the ion source to the detector); and the computer (coordinates the functions of the individual components and records and stores the data).***

I. Introduction

Mass spectrometry is a microanalytical technique that can be used selectively to detect and determine the amount of a given analyte. Mass spectrometry is also used to determine the elemental composition and some aspects of the molecular structure of an analyte. These tasks are accomplished through the experimental measurement of the mass of gas-phase ions produced from molecules of an analyte. Unique features of mass spectrometry include its capacity for direct determination of the nominal mass (and in some cases, the molar mass) of an analyte, and to produce and detect fragments of the molecule that correspond to discrete groups of atoms of different elements that reveal structural features. Mass spectrometry has the capacity to generate more structural information per unit quantity of an analyte than can be determined by any other analytical technique.

Much of mass spectrometry concerns itself with the mass of the isotopes of the elements, not the *atomic mass*[1] of the elements. The *atomic mass* of an element is the weighted average of the naturally occurring stable isotopes that comprise the element. Mass spectrometry does not directly determine mass; it determines the *mass-to-charge ratio* (*m/z*) of ions. More detailed explanations of *atomic mass* and *mass-to-charge ratios* follow in this chapter.

It is a fundamental requirement of mass spectrometry that the ions be in the gas phase before they can be separated according to their individual *m/z* values and detected. Prior to 1970, only analytes having significant vapor pressure were amenable to mass spectrometry because gas-phase ions could only be produced from gas-phase molecules by the techniques of electron ionization (EI) or chemical ionization (CI). Nonvolatile and thermally labile molecules were not amenable to these otherwise still-valuable gas-phase ionization techniques. EI (Chapter 6) and CI (Chapter 7) continue to play very important roles in the combined techniques of gas chromatography/mass spectrometry (GC/MS, Chapter 10) and liquid chromatography/mass spectrometry (LC/MS, Chapter 11). After 1970, the capabilities of mass spectrometry were expanded by the development of desorption/ionization (D/I) techniques, the generic process of generating gas-phase ions directly from a sample in the condensed phase. The first viable and widely accepted technique[2] for D/I was fast atom bombardment (FAB), which required nanomoles of analyte to produce an interpretable mass spectrum. During the 1980s, electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) eclipsed FAB, in part because they required only picomoles of analyte for analysis. ESI and MALDI are mainly responsible for the dominant role of mass spectrometry in the biological sciences today because they are suitable for analysis of femtomole quantities of thermally labile and nonvolatile analytes; therefore, a chapter is devoted to each of these techniques (Chapters 8 and 9).

Mass spectrometry is not limited to analyses of organic molecules; it can be used for the detection of any element that can be ionized. For example, mass spectrometry can analyze silicon wafers to determine the presence of lead and iron, either of which can

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[1] In the United States, the term *atomic weight* is used for the relative mass of the elements. In the rest of the world, which is based on the metric system, the term *atomic mass* is used. This book uses the term *atomic mass* instead of the more widely accepted term in the U.S., *atomic weight*.

[2] It should be mentioned that the techniques of ^{252}Cf (Ron MacFarlane) and Laser Microprobe Mass Analysis (LAMMA) (Franz Hillenkamp and Michael Karas) were less popular D/I techniques that were developed in the same temporal arena as FAB, but they were not commercially viable. More information on these two techniques can be found in Chapter 9.

cause failure of a semiconductor for microprocessors; similarly, drinking water can be analyzed for arsenic, which may have health ramifications. Mass spectrometry is extensively used in geology and material sciences. Each of these two disciplines has developed unique analytical capabilities for the mass spectrometer: isotope ratio mass spectrometry (IRMS) in geology and secondary ion mass spectrometry (SIMS) in material sciences. Both of these techniques, along with the analysis of inorganic ions, are beyond the scope of this present book, which concentrates on the mass spectrometry of organic substances.

1. The Tools and Data of Mass Spectrometry

The tools of mass spectrometry are *mass spectrometers*, and the data are *mass spectra*. Figure 1-1 is a conceptual representation of a mass spectrometer. Each of the individual components of the instrument will be covered at logical stages throughout this book. Figure 1-2 depicts the three ways of displaying the data recorded by the mass spectrometer. The acquired mass spectra can be displayed in many different ways, which allow the desired information about the analyte to be easily extracted. These various techniques for data display and their utility are covered later in this chapter.

2. The Concept of Mass Spectrometry

Ions are charged particles and, as such, their position in space can be manipulated with the use of electric and magnetic fields. When only individual ions are present, they can be grouped according to their unique properties (mass and the number of charges) and moved from one point to another. In order to have individual ions free from any other forms of matter, it is necessary to analyze them in a vacuum. This means that the ions must be in the gas phase. Mass spectrometry takes advantage of ions in the gas phase at low pressures to separate and detect them according to their mass-to-charge ratio (*m/z*) – the mass of the ion on the atomic scale divided by the number of charges that the ion possesses. This definition of the term *m/z* is important to an understanding of mass spectrometry. It should be noted that the *m/z* value is a dimensionless number. The *m/z* term is always used as an adjective; e.g., the ions with *m/z* 256, or the ion has an *m/z* value of 256. A recording of the number of ions (abundance) of a given *m/z* value as a function of the *m/z* value is a mass spectrum. Only ions are detected in mass spectrometry. Any particles that are not ionic (molecules or radicals[3]) are removed from the mass spectrometer by the continuous pumping that maintains the vacuum.

The mass component that makes up the dimensionless *m/z* unit is based on an atomic scale rather than the physical scale normally considered as mass. Whereas the mass physical scale is defined as one kilogram being the mass of one liter of water at a specific temperature and pressure, the atomic mass scale is defined based on a fraction of a specific isotope of carbon; i.e., 1 mass unit on an atomic scale is equal to 1/12 the mass of the most abundant naturally occurring stable isotope of carbon, ^{12}C. This definition of mass, as represented by the symbol u, which is synonymous with dalton (Da), will be used throughout this book [1].

A previous standard for the atomic mass unit was established in chemistry in 1905 (based on the earlier suggestion of the Belgium chemist, Jean Servais

[3] Molecules and radicals are particles that have no charge. Molecules are characterized by an even number of electrons; radicals, by an odd number of electrons.

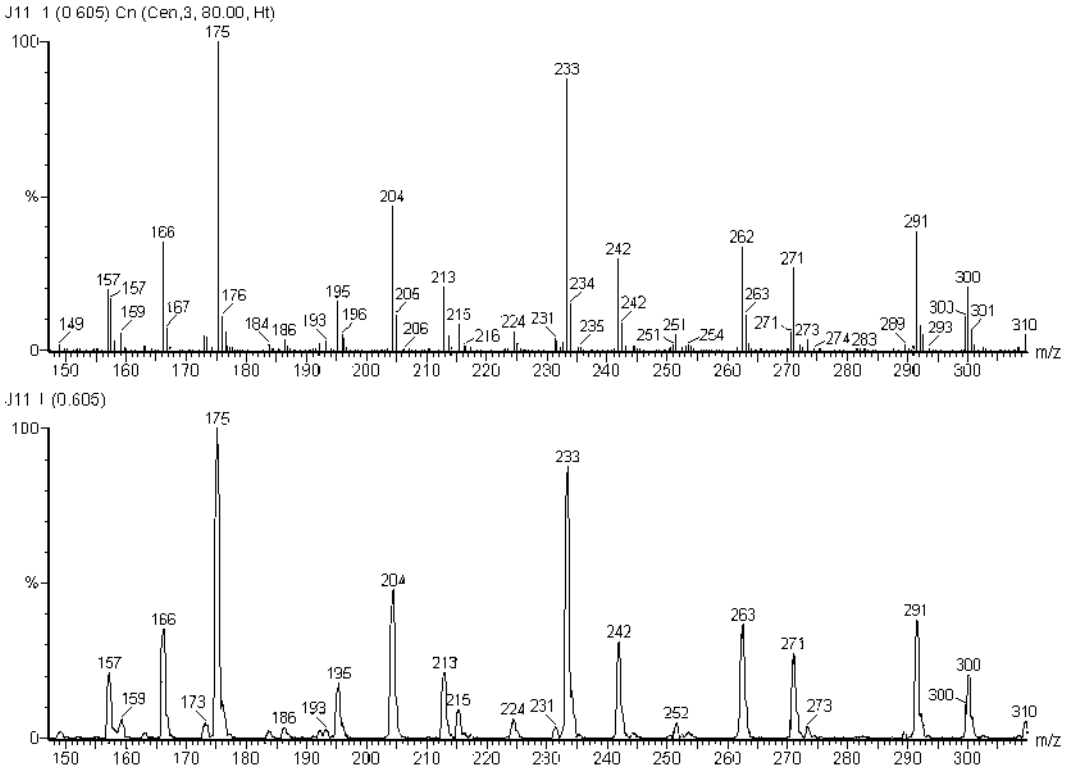

Figure 1-2. The top part of this figure is a bar-graph presentation of a mass spectrum; this is the presentation most often used for data acquired by GC/MS. The middle display is the same mass spectrum presented in profile mode; this type of display is often used with LC/MS data because the mass spectral peaks represent ions of different m/z values that may not be well resolved by the mass spectrometer, such as is sometimes the case with multiple-charge data. The third way spectra are displayed is in a tabular format (not shown). The tabular format is a listing of pairs of m/z values and intensities. Often mass spectral peaks of significant intensity are observed in the tabular display, but not in a graphical display because of its limited resolution. The graphical displays provide the general mass spectral image of the analyte; the tabular display provides the mass spectral details.

Stas, 1813–1891) when it was agreed that all masses would be relative to the atomic mass of oxygen. This later became known as the "chemistry mass scale". By setting the atomic mass of oxygen to an absolute value of 16, it was relatively easy to determine the atomic mass of new elements (in the form of their oxides) as they were discovered. Francis William Aston (British physicist and 1922 chemistry Nobel laureate for the development of the mass spectrograph and the measurement of the nuclides of the elements, 1877–1945) realized that the "chemistry mass scale" was not usable with his mass spectrograph (a device used to determine the existence of individual isotopes of the elements) because, *rather than dealing with the atomic mass of elements*, he *was measuring the mass of individual isotopes*, and oxygen had three naturally occurring stable isotopes, the most abundant of which accounted for only 99.76%. Therefore, ca. 1920, Aston established the "physics mass scale" by declaring the exact mass of the most abundant stable isotope of oxygen, ^{16}O, to be 16. This meant that there were now two different definitions for the *atomic mass unit* (amu). In one case, 1 amu was equal to 1/16 the mass of ^{16}O (the physics mass scale) and, in the second case, 1 amu was 1/16 the weighted average of the three naturally occurring isotopes of oxygen [1]. The amu on the physics mass scale was a factor of 1.000275 greater than that on the chemistry mass scale. This created confusion. Based on the 1957 independent recommendations of D. A. Ölander and A. O. Nier, the International Union of Physicists at Ottawa in 1960 and the International Union of Chemists at Montreal in 1961 adopted the *carbon-12 standard*, which, as stated above, establishes a single *unified atomic mass unit* (u) as 1/12 the most abundant naturally occurring stable isotope of carbon (^{12}C). At the same time, to keep from having three different values associated with the amu term, the symbol for the *unified atomic mass unit* was established as u [1]. Unfortunately, an atomic mass unit based on carbon-12 is incorrectly assigned the amu symbol in many textbooks with current copyright dates.

Another symbol used for the unified atomic mass unit is Da, dalton. Although not officially recognized by any standard governing boards, the dalton has become an accepted standard term for the unified atomic mass unit. This arbitrary scale for the atomic mass unit is closely related to that established ca. 1805 by John Dalton (1766–1844), which assigned a value of 1 for the lightest element, hydrogen [2]. In 1815, the Swedish scientist, Jöns Jacob Berzelius (1779–1848), set the atomic mass of oxygen to 100 in his table of atomic masses [3]; however, the Berzelius standard of mass was never adopted by others.

In the study of mass spectrometry, it is important to always keep in mind that the entity measured in the mass spectrometer is the mass-to-charge ratio of an ion, not the mass of the ion. In the case where there is only a single charge on the ion, the *m/z* value and the mass are the same. This statement is not true in the case where ions have multiple charges. It is inappropriate to use a unit of mass when describing the mass-to-charge ratio of an ion. Ions have both mass and an *m/z* value.

The mass spectrometer first must produce a collection of ions in the gas phase. These ions are separated according to their *m/z* values in a vacuum where the ions cannot collide with any other forms of matter during the separation process. The functionality of the mass spectrometer's vacuum system and its components are described in Chapter 2. Ions of individual *m/z* values are separated and detected in order to obtain the mass spectrum. Separation of ions in an evacuated environment is mandatory. If an ion collides with neutrals in an elastic collision during the ion separation process, the ion's direction of travel could be altered and the ion might not reach the detector. If an ion's collision with a neutral is inelastic, sufficient energy transfer may

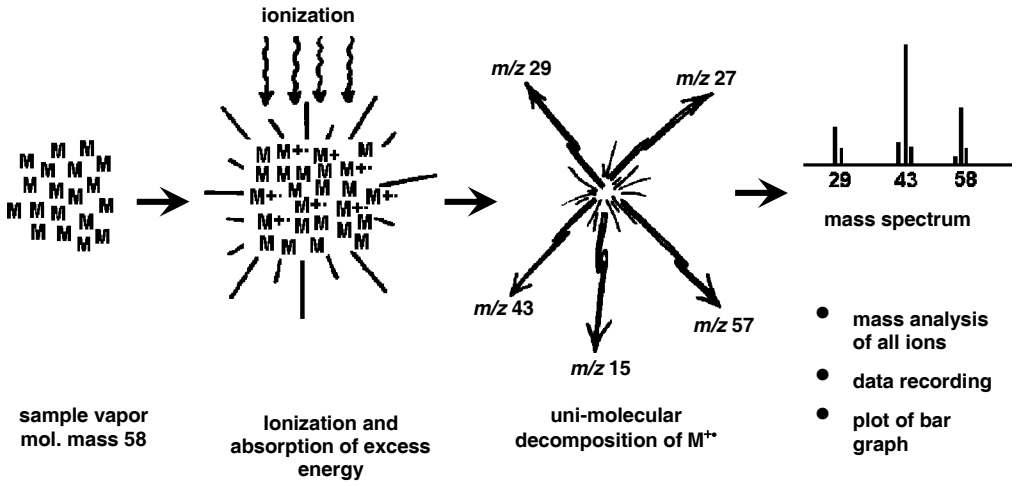

Figure 1-3. Conceptual illustration of gas-phase ionization of analytes followed by ion separation according to the m/z value.

cause it to decompose, meaning that the original ion will not be detected. Close encounters between ions of the same charge can cause deflection in the path of each. Contact between ions of opposite charge sign will result in neutralization.

Figure 1-3 is a conceptual illustration of the entire process of a mass spectral analysis by electron ionization (EI), culminating in a bar-graph mass spectrum that is often seen in published literature. In this illustration, M represents molecules of a pure compound in the gas phase. For ionization to occur in the gas phase, the sample must have a vapor pressure greater than 10 Pa because molecules of the sample must migrate by diffusion from the inlet system into the ionization chamber. For EI, samples may be introduced into the mass spectrometer using a direct probe or a batch inlet for pure solids or volatile liquids. Analytes purified by separation techniques (GC, LC, CE, etc.) can enter the mass spectrometer as the separation takes place in an on-line process. As the neutral molecules randomly diffuse throughout the ion source, only a few hundredths to a few thousandths of a percent of them are ionized.

The most common ionization process for gas-phase analysis, EI, transfers energy to the neutral molecule (a species characterized as having an even number of electrons) in the vapor state, giving it sufficient energy to eject one of its own electrons, thereby leaving a residual positive charge on the now ionic species. This process produces a molecular ion with a positive charge and odd number of electrons, as represented by the $M^{+\bullet}$ in Figure 1-3. This $M^{+\bullet}$ may have considerable excess energy that can be dissipated through fragmentation of certain chemical bonds. Cleavage of various chemical bonds leads to the production of positive-charge fragment ions whose mass is equal to the sum of the atomic masses of the constituent atoms. Not all of the molecular ions necessarily decompose into fragment ions. For compounds producing a relatively stable $M^{+\bullet}$, such as those stabilized through resonance, like aromatic compounds, an intense molecular-ion peak will be recorded because the $M^{+\bullet}$ tends to survive or resist fragmentation. For compounds that do not produce stable molecular ions, like aliphatic alcohols, nearly all of them decompose into fragment ions. In these cases, the mass spectrum contains only a small peak representing the $M^{+\bullet}$. Various combinations of the above-described processes are the basis of the chemical “fingerprint” in the form of a mass spectrum for a given compound.

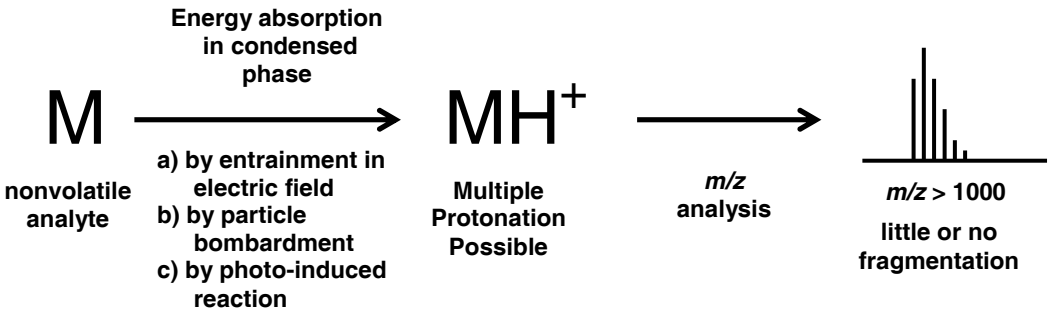

Figure 1-4. Conceptual illustration of generic condensed-phase analysis (desorption/ionization) by mass spectrometry.

Although not manipulable or directly detected in the mass spectrometer, radicals and molecules (neutral species) formed during the fragmentation of ions are represented in the mass spectrum. This is the *dark matter* of the mass spectrum. The difference between two *m/z* values (that of the precursor and that of the product) indicates that an ion of lesser mass was formed by the loss of a radical (e.g., a $^{\bullet}CH_3$ which has a mass of 15 Da) or the loss of a molecule (e.g., H_2O (mass 18 Da) or NH_3 (mass 17 Da)). The exact mass of the *dark matter* is as important as the exact *m/z* value of an ion. This exact mass allows for differentiation between the loss of a $H_3C^{\bullet}CH_2$ radical and a $H^{\bullet}C{=}O$ radical. In this way, the *dark matter* is an important part of the chemical fingerprint of an analyte.

For nonvolatile analytes, ions of the intact molecule are produced during entrainment of a solution into an electric field (see Chapter 8 on Electrospray) or through interaction with a photon-energized matrix compound (see Chapter 9 on Matrix-Assisted Laser Desorption/Ionization). The nonvolatile molecules are ionized by adduct formation usually involving a proton through a wide variety of processes, as summarized in an oversimplified fashion in Figure 1-4.

Mass spectrometry involves many different techniques for producing gas-phase ions. Ionization can take place in either the gas or condensed phase; however, the end result is ions in the gas phase. Many of these different ionization techniques are discussed in this book with respect to when to use them and the details of how they work. Today, ions are separated according to their *m/z* values by six specific types of *m/z* analyzers: magnetic sectors; transmission quadrupoles; quadrupole ion traps (both linear and three-dimensional); time-of-flight (TOF) analyzers (both linear and reflectrons); ion cyclotron resonance mass spectrometers (magnetic ion traps) that Fourier transform oscillating image currents to record the mass spectrum (FTICR); and most recently, by orbitraps, which store ions using electrostatic fields and, like the ICR, detect them through Fourier transformation of oscillating image currents. The operation of all these types of instruments is described in Chapter 2.

Mass spectrometers do not measure a physical property on an absolute scale such as is measured in various spectroscopies. For example, in infrared or ultraviolet spectroscopy, the absorbance or transmittance at a specific wavelength of electromagnetic energy is measured, and appropriate instrument parameters are calibrated and set during construction and final testing of the hardware. In mass spectrometry, ions are analyzed under control of the *m/z* analyzers that require day-to-day verification or calibration. The *m/z* value of each ion as a function of

instrument settings can only be determined by calibrating the entire *m/z* scale of the instrument using substances that produce ions of known *m/z* values. The requirement of calibration and instrument suitability is more important in the mass spectrometer than in other spectra-producing instruments. Methods of calibrations of different types of *m/z* analyzers and different types of ion sources are detailed in Chapter 2. Descriptions of different methods of ion detection and various vacuum systems are found in Chapter 2. However, discussions of different methods of obtaining gas-phase ions are covered in several chapters.

II. History

Most chronicles of science are somewhat divided on whom to give credit for the development of the mass spectrometer. Many credit Sir John Joseph Thomson (British physicist and 1906 physics Nobel laureate for the discovery of the electron, 1856–1940) (Figure 1-5, upper right). Others credit Francis William Aston (British physicist and 1922 chemistry Nobel laureate for the development of the mass spectrograph and the measurement of the isotopes of the elements, 1877–1945) (Figure 1-5, upper left); however, Thomson's curiosity about the behavior of electrical discharges under reduced pressure has its origin in the work of the German physicist, Eugene Goldstein (1850–1930) (Figure 1-5, lower right). While at the Berlin Observatory, Goldstein reported that luminous rays in a discharge tube containing gases at low pressure traveled in straight lines from holes in a perforated metal disk used as a cathode. He called the rays *Kanalstrahlen* (canal rays) [4]. In his book, *Introduction to Mass Spectrometry Instrumentation and Techniques*, John Roboz [5] calls the Goldstein paper the first mass spectrometry publication, even though the terms *mass spectra* and *mass spectrometer* were coined much later by Aston and Josef Heinvich Elizabeth Mattauch (Austrian physicist and designer of mass spectrometers) (Figure 1-5, lower left) ca. 1920 and 1926, respectively [6].

Goldstein's work was expanded upon in 1895 by Jean-Baptiste Perrin's (French physicist, 1870–1942) report that the *Kanalstrahlen* were associated with a positive charge [7]. Perrin's suggestion was confirmed by Wilhelm Carl Werner Otto Fritz Franz Wien (German physicist and 1911 physics Nobel laureate for discoveries regarding the laws of heat radiation, 1862–1928) [8, 9]. Wien's publications demonstrated that these rays were deflected in a magnetic field and that their behavior could be studied by the combined effects of magnetic and electric fields. The device used by Wien in this study of Goldstein's *Kanalstrahlens*, the *Wien Filter*, has endured longer than any of the other devices used for ion separation developed in the same era. The Wien filter is an integral part of many ion sources on modern secondary ion mass spectrometers and is a significant component of the accelerator mass spectrometer used in ^{14}C dating and other isotope studies, as well as in other low-pressure analyzers. At the same time that Wien was exploring the works of Goldstein and Perrin, Thomson was developing a device that allowed for the determination of the difference in the *e/m* of an electron and a hydrogen atom (nucleus) [10]. It was a later refinement of this apparatus (the parabola machine) that Thomson used to observe two distinct signals when looking at the *positive rays* of neon, although it took Aston some 20 years later to realize that these data represented two of the three naturally occurring stable isotopes of neon.

Wien did not pursue the possibilities of the Wien filter. Thomson's changes to his original apparatus were reportedly based in part on Wien's work [11]. Thomson's student, Aston, refined the previous two apparatuses developed by Thomson and produced the *mass spectrograph*, which he used in *mass spectroscopy*. Aston's use of the term *mass spectroscopy* was in part due to the fact that his instrument used an

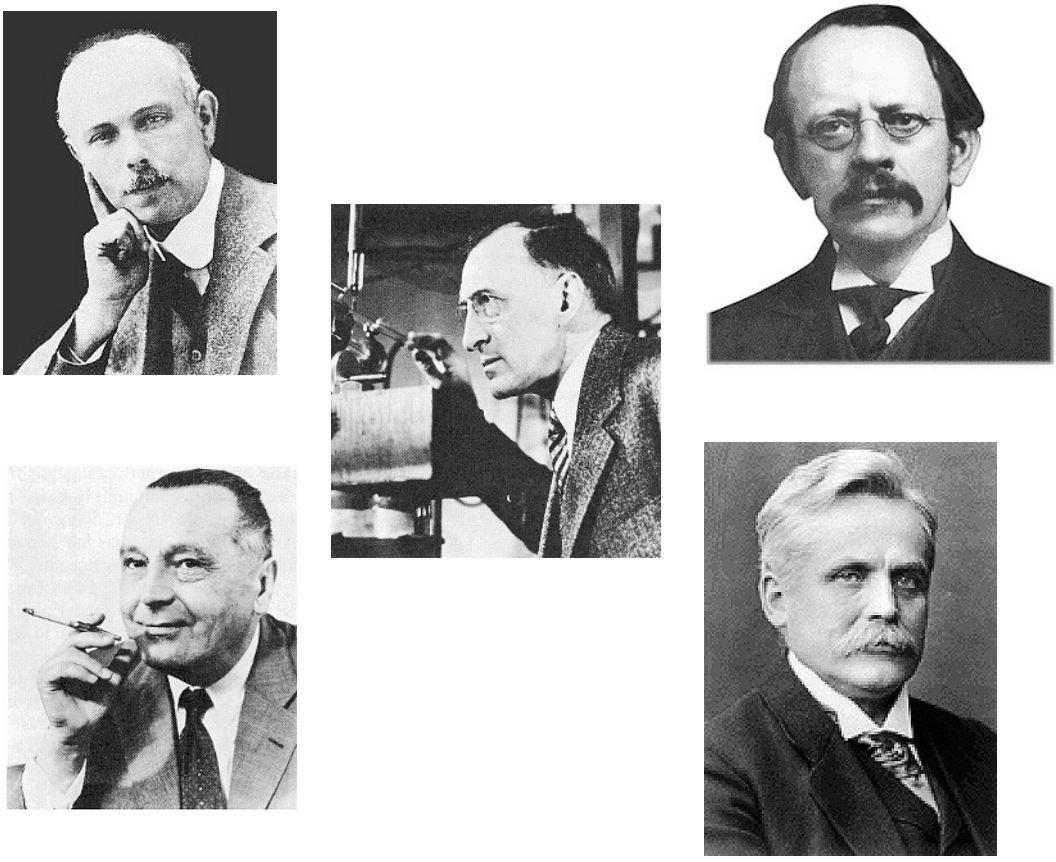

Figure 1-5. Five people who may be considered the founding fathers of mass spectrometry. Clockwise from upper left: Francis William Aston (British physicist and 1922 chemistry Nobel laureate for the development of the mass spectrograph and the measurement of the isotopes of the elements, 1877–1945); Sir John Joseph Thomson (British physicist and 1906 physics Nobel laureate for the discovery of the electron, 1856–1940); German physicist, Eugene Goldstein (1850–1930); Josef Heinrich Elizabeth Mattauch (Austrian physicist and designer of mass spectrometers); and (center) Canadian-American physicist, Arthur Jeffery Dempster (1886–1950).

arrangement of the electric and magnetic fields for ion separation that was analogous to that of an achromatic set of prisms without lenses, which produced a spectrum of lines such as an optical spectrograph. The term *mass spectroscopy* grew to encompass many different types of studies involving ions and, as such, was too broad and is no longer recommended for what is currently referred to as *mass spectrometry*. The preferred term for techniques involved with the measurement (electrically metered output) of ions according to their *m/z* values and their abundances is mass spectrometry.

Aston's instrumentation was ideally suited for the accurate measurement of mass of an ion relative to the mass of a standard, ^{16}O. While Aston was perfecting the mass spectrograph using electric and magnetic fields to perform velocity focusing of an ion beam, the Canadian-American physicist, Arthur Jeffery Dempster (1886–1950) (Figure 1-5, center) independently developed a single magnetic-sector instrument that employed direction focusing of constant-energy ions at the University of Chicago. Dempster's instrument provided accurate ion abundances as opposed to accurate mass measurements. This distinction between velocity-focusing and direction-focusing was the difference between the mass spectrograph and the mass spectrometer. It is interesting to note that the term *mass spectrography* was coined by Aston along with the term used to describe the data recording of such instruments, the *mass spectrum*, ca. 1920; *mass spectrometer* was a term first used by two well-known early pioneers of mass spectrometry, William R. Smythe (U.S. scientist) and Josef Heinrich Elizabeth Mattauch (Austrian physicist) ca. 1926.

Thomson, Aston, and Dempster, and to a lesser extent Wien, are considered to be the founders of the field of mass spectrometry. Many others have followed in the succeeding years up to World War II. During this time, Thomson published two editions of his popular book *Rays of Positive Electricity* [12], and Aston published two editions each of two books: *Isotopes* and *Mass Spectra and Isotopes* [13]. Other than these six volumes, three French-language books published in 1937 and 1938 [14–16], and books on negative ions by Sir Harrie Massey [17, 18] and electrical discharge in gases by Leonard Loeb [19], no other books were published regarding the field until the 1950s. The most notable of these books was published by The Institute of Physics entitled *Modern Mass Spectrometry* by G. P. Barnard. Barnard's book, one by Henry E. Duckworth, a small monograph by A. J. B. Robertson (all in the UK), a book by Heinz Ewald and Heinrich Hintenberger (translated from German into English in 1965 by the U.S. Atomic Energy Commission), a Russian-language book by G. Rich published in 1953 with a German-language version published in 1956, and the 1954 publication by Mark G. Inghram and Richard J. Hayden published by the U.S. National Academy of Science–National Research Council, Committee on Nuclear Science, Division of Physical Science, Subcommittee on Instruments and Techniques were the only books published during the 1950s other than the proceedings of three different meetings held in the United Kingdom and one held in the United States. As the privy of mass spectrometry turned from physics to organic chemistry, the data became more complex; and the 1960s saw the publication of several books dealing with data as much as, if not more than, the operating principles of the instrumentation, some of which are still relevant in dealing with today's data (Biemann, Beynon, McLafferty, Budzikiewicz, etc.). A complete list of the books published between Thomson's 1902 book and 1970 can be found at the end of this chapter.

In 1949, two years after the American Chemical Society changed the name of the *Analytical Edition of Industrial and Engineering Chemistry*, then in its 19th year, to *Analytical Chemistry*, the first issue of the year carried the first *Analytical Chemistry* Review of mass spectrometry [20]. Then the youngest division in the ACS, the Analytical and Micro Chemistry Division, in its third year was aiming for a membership of 1000 by the end of the year. The five-page review by John A. Hipple and Martin Shepherd at the National Bureau of Standards in Washington DC began with a rather interesting statistic, "*Chemical Abstracts* reported 11 references to mass spectrometry in 1943, 15 in 1944, 17 in 1945, 26 in 1946, and 40 in 1947". This review had 176 citations. Even taking into account the effect World War II had on the number of publications, as Hipple and Shepherd pointed out, there was an unprecedented expanding interest in the field at that

time; it continues at a similar rate today. When the last of these reviews appeared in *Analytical Chemistry* in 1998, it had grown to 70 pages in length. To some extent, the Review has been replaced by *CA Selects Plus: Mass Spectrometry*.

Mass spectrometry's primary role was in the study of small molecules and isotopes from Thomson's first parabola machine until just before World War II. The only instruments that were available during these formative years of mass spectrometry were those that were designed by individual researchers or those that were custom built to order by craftsman such as Mattauch and his German colleague, Richard Herzog. These instruments were employed by physicists who used them in the determination of isotopes and the study of ion formation. Thomson had talked of the potential of the mass spectrometer due to the fact that ions were not only formed during the initial absorption of external energy, but some secondary ions also were produced by decomposition of these initially formed ions. Physicists were annoyed by the presence of peaks in their mass spectra that could be attributed to the instrument's background. As the organic chemists looked at these peaks more closely, they realized that they represented ions of various hydrocarbon substances, and they predicted that the mass spectrometer would have broader applications.

Based on the possibility of using such an instrument in hydrocarbon analysis, in 1937, Herbert Hoover Jr, son of the 31st President of the United States (1929–1933) and the first scientific person (chemical engineer) to become a U.S. President, formed the Consolidated Engineering Company (CEC) as the engineering and manufacturing subsidiary of the United Geophysical Company. This company, which had close ties with the California Institute of Technology and the petroleum industry, was founded to develop instrumentation to locate petroleum deposits by detecting hydrocarbon gases emanating from the ground. However, due to the ubiquitous nature of methane, such a device was not possible. This business venture would have died at this point except for the growing interest in using mass spectrometry to increase the speed of analyzing aviation gasoline, which was becoming increasingly important because of the nearing possibility of World War II [21].

During World War II, mass spectrometry played a pivotal role in the preparation of weapons-grade plutonium [22]. Preparative mass spectrometers such as the Calutron described by Yergy were used to produce weapons-grade fissionable materials. Another important consideration of mass spectrometry was its need in research for the development of synthetic rubber. Because of the Japanese occupation of Malaysia in 1941, there was no longer a supply of natural rubber for the U.S. [23]. The mass spectrometer saw an ever-increasing role. CEC's newly developed instrument, the CEC 21-101, looked like the answer, with a single exception. CEC mandated that purchasers of its instruments disclose to them all data and information made with purchased instruments so that the technology could be shared. This requirement did not sit well with the U.S. petroleum industry, which consisted of very competitive, and just as arrogant, companies. Another company, Westinghouse Electric, had also announced a commercial mass spectrometer, but had not started delivery. Westinghouse was pressured by the U.S. Government, acting on behalf of the petroleum industry, to produce instruments similar to the one being offered by CEC to aid the war effort.

After World War II, mass spectrometry began to have a broad number of applications in organic chemistry. The recovering economies of Germany, France, England, and Japan all saw developments of mass spectrometry instrumentation. There was also work on new instruments occurring in the then Soviet Union, but because of the closed nature of that country, not much is known of the details. By the very early 1950s,

there were three companies building magnetic-sector mass spectrometers in the U.S. (CEC, General Electric, and Westinghouse). Soon, first Westinghouse and later GE left the market. A new technology was introduced by the aircraft manufacturer, the Bendix Corporation, with the publication of a seminal paper on time-of-flight mass spectrometry in December of 1955 by two of their researchers (William C. Wiley and Ian H. McLaren) working in Bendix Aviation Corporation Research Laboratories in Detroit, MI [24]. This instrument was the first incarnation of the time-of-flight mass spectrometer, which eluded earlier researchers such as Smythe and Mattauch in 1932 [25], W. E. Stephens [26, 27], while at the University of Pennsylvania (Philadelphia, PA) in 1946, and Henry S. Katzenstein and Stephen S. Friedland [28] in the Physics Department at the University of Connecticut in April 1955.

By the mid-1960s, there were significant offerings from England's Associated Electronics Industry (AEI), Germany's Mes und Analysen-Technik (MAT), Japan's Hitachi and JEOL, and, to a lesser extent, France's Thompson's Electronics, as well as those from the U.S. that had been known for pioneering developments in other fields of analytical instrumentation such as Varian, Beckman, and Perkin-Elmer. Another factor that had a profound effect on the development of mass spectrometry in the analysis of organic compounds was the revelation of gas chromatography (GC). The gas chromatograph separated mixtures of volatile nonthermally labile compounds into individual purified components and delivered them in the gas phase (a requirement at the time for mass spectrometry) to the mass spectrometer. GC did present one formidable problem for the mass spectrometer. Early gas chromatographs delivered the individual analytes to the mass spectrometer in a very dilute concentration in a large volume of the GC's mobile phase, helium, hydrogen, or, in rare cases, nitrogen. In order to detect the analyte, the mass spectrometer had to "digest" this large volume of superfluous carrier of the compound of interest. After this problem was overcome by the development of analyte-enrichment devices such as the jet, effusion, and membrane separators, invented, and later patented, by Einar Stenhagen (Swedish medical scientist) and perfected by Ragnar Ryhage [29], J. Throck Watson (while a PhD student of Klaus Biemann at the Massachusetts Institute of Technology in Cambridge, MA) [30, 31] and Duane Littlejohn and Peter Llewellyn at the Varian Research Center in Palo Alto, CA [32], respectively, gas chromatography/mass spectrometry (GC/MS) became the instrument that produced more information for amenable analytes from less sample than any other analytical technique. This made GC/MS an indispensable tool in the environmental, medical, and other biological sciences as well as in forensics, the food and flavor industry, and so forth (see Chapter 10).

During this era, analytes were converted to ions (the principal requirement of mass spectrometry) using the technique of electron ionization (EI). EI used a beam of high-energy electrons (50–70 eV) to produce molecular ions ($M^{+\bullet}$). Some of these $M^{+\bullet}$ then reproducibly fragment to produce a spectrum of ions that have various masses and usually a single-charge state; i.e., ions of various mass-to-charge ratios (*m/z*). These fragmentation patterns in the form of a mass spectrum are what allow for the unambiguous identification of a compound by GC/MS. Unfortunately, some compounds have such energetic $M^{+\bullet}$ produced by EI that almost none of the $M^{+\bullet}$ remains intact; therefore, it is not possible to determine the analyte's molecular mass. Without the nominal mass of an intact analyte, no matter how much information was available from the fragments, it is not possible to identify the analyte. This lack of molecular-ion current led to a way to have less energy imparted to the analyte during a different type of ionization process. The resulting technique, developed by Burnaby Munson and Frank Field, is known as chemical ionization (CI) [33–37]. CI is an ion/molecule reaction that

usually produces protonated molecules (MH^+) of the analyte. These MH^+ are much lower in energy than the $M^{+\bullet}$ and are much more likely to remain intact for detection in the mass spectrometer. Together, EI and CI GC/MS have advanced many areas of science and have resulted in a much better quality of life through a better understanding of the chemistry of organic compounds.

GC/MS was taken to the next higher plane of advancement by the commercialization of the transmission quadrupole, invented by Wolfgang Paul and colleagues [38] at the University of Bonn (Bonn, Germany) in the early 1950s.[4] In the mid-1960s, Robert A. Finnigan and associates [39, 40] produced a quadrupole GC-MS at Electronic Associates Inc. (EAI) (Long Branch, NJ), an analog computer company. This instrument became the basis for the first instrument (the Finnigan 1015) produced by the subsequent company formed by Finnigan and T. Z. Chu (Finnigan Corporation, founded in Sunnyvale, CA, now known as Thermo Finnigan, a subsidiary of Thermo Electron). In addition to Finnigan Corporation's development, Hewlett-Packard of Palo Alto, CA, and, to a lesser extent, Extranuclear Corporation of Pittsburgh, PA, also contributed to this emerging technology. Today, the transmission quadrupole is the most ubiquitous of all mass spectrometers. Its contributions to GC/MS were due to the speed at which the analyzer could be scanned for fast data acquisition, the linear nature of the *m/z* scale, simple operation, its lower acceleration potential, and smaller size compared to that of the sector-based instruments, and the ease with which it could be operated and controlled by the then emerging minicomputer technology. As gas chromatography evolved into the use of capillary columns resulting in higher concentrations of analyte in the eluant and narrower peaks, the transmission quadrupole resulted in fast, easy, and reliable instruments for GC/MS. See Chapter 10 for GC/MS and Chapter 6 for strategies in dealing with EI data.

Another significant development in mass spectrometry came about with the introduction of resonance electron capture negative ionization (ECNI) [41, 42]. Gas-phase analytes with high electron affinities are ionized by capturing thermal energy (0.1 eV) electrons resulting in the formation of negative-charge molecular ions ($M^{-\bullet}$). This technique, developed by George Stafford, while working on his doctorate with Don Hunt at the University of Virginia in the mid-1970s, allows for the analysis, at unprecedented detection levels (fg μL^{-1} injected into the instrument), of halogenated pesticides in very complex matrices such as those produced by extraction from the skins of fruits and vegetables. ECNI also had a significant impact on analytical methods for drug metabolism. Using fluorinated reagents, derivatives of nonvolatile drugs and their metabolites could be formed through reactions with the polar sites on these drugs and metabolites, thus making them volatile as well as electrophilic. These electrophilic derivatives of drugs and metabolites extracted from blood and urine could be analyzed by GC/MS based on ECNI without interference from endogenous substances. Before the LC/MS became a practical technique in the late 1990s, ECNI GC/MS was a major technique in studies of drug metabolism.

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[4] An important note about the transmission quadrupole might have disappeared from recorded history if Bob Finnigan had not published an interesting article in the A-pages of *Analytical Chemistry* on the history of transmission quadrupole; namely, while Paul pursued the quadrupole technology, Richard Post at the University of California Lawrence Berkeley Laboratory carried out independent research during the 1950s on the same technology. Post did not publish or apply for patents on his findings. Other than information contained in his personal notebooks, the only record of his work is a University of California Radiation Laboratory report (UCRL 2209) published in 1953.

EI and CI GC/MS continued to be the mainstay of mass spectrometry until the development of desorption/ionization (DI) techniques. These latter techniques allowed for the determination and fragmentation of nonvolatile thermally labile analytes such as peptides. DI techniques not only expanded the types of analytes that were amenable to mass spectrometry, but also opened the door to use of liquid chromatography as a technique to separate and purify components of a mixture. The first of these techniques to have a major impact on mass spectrometry was fast atom bombardment (FAB), a variation of secondary ion mass spectrometry (SIMS) carried out using a liquid matrix. SIMS is a process of producing ions from a solid surface by bombarding it with a beam of high-energy ions. SIMS is used in the characterization of organic and inorganic surfaces as well as metal or composite materials, such as those in the wings of aircraft. FAB, developed by Mickey (Michael) Barber [43] in the Department of Chemistry at the University of Manchester Institute of Science and Technology in the mid-1970s, employs a beam of high-energy (5–10 keV) atoms of a nonreactive element such as xenon to bombard the surface of a glycerol solution of analyte molecules; e.g., a peptide. In this way, FAB causes the desorption of protonated molecules from the condensed phase into the gas phase. FAB revolutionized the study of biopolymers such as DNA and proteins known for their nonvolatility and thermal lability.

The next phase of development in DI was ^{252}Cf desorption/ionization (Cf DI) (also known as plasma desorption, PD) as pioneered by Ronald D. MacFarlane at Texas A&M [44]. This technique remained a laboratory curiosity throughout its useful life. The dependence of PD on time-of-flight mass spectrometry was not sufficient to prevent the last U.S. manufacturer of these instruments (CVC Corporation) from discontinuing their manufacture in the late 1970s. There was a single commercial attempt at a PD instrument in the 1980s by the Uppsala, Sweden, company, Bio-Ion. The instrument was later marketed by Kratos Analytical, UK. Bio-Ion was later acquired by Applied Biosystems (now PE Biosystems). The primary reason for the lack of popularity of the ^{252}Cf-DI technique was the radioactive nature of the ionization source, which presented a significant safety and disposal problem for a number of laboratories. However, PD was what Frans Hillenkamp and Michael Karas credit with inspiring them to look at other possible desorption techniques as they developed matrix-assisted laser desorption/ionization (MALDI) [45], one of the two most significant DI tools (the other being John B. Fenn's electrospray ionization [46], ESI), which has continued as a primary factor in the development of proteomics and areas of biopolymer analysis.

Although the 2002 Nobel Prize in chemistry was not shared by Hillenkamp and Karas (the prize was awarded to John Fenn and Koichi Tanaka, who was the first to use laser desorption in a matrix (glycerol/metal filings in a one-time, never reproduced experiment) for the mass spectrometry part and Kurt Wüthrich for the NMR part of "… methods in chemical analysis applied to biomacromolecules"), their contributions to the technique have spawned numerous commercial instrument designs, the resurgence of the time-of-flight mass spectrometer, and technology used in thousands of analyses performed each day in analyses of protein, DNA, and synthetic polymer samples. The use of MALDI involves mixing the analyte with a thousand-fold excess of a solid matrix of small organic molecules that absorb the energy of a laser to explosively discharge protonated molecules of the analyte in to the gas phase. When used with extended-range mass spectrometers such as the time-of-flight instrument, the mass of intact heavy (up to tens of thousands of daltons) proteins can be measured with an accuracy of 0.01% as compared to 1% for electrophoretic techniques (see Chapter 9 for details on MALDI).

Unlike MALDI, which primarily produces single-charge protonated molecules, ESI can produces multiple-charge ions, provided that the analyte molecule has multiple sites that can be protonated; e.g., a peptide with multiple basic amino acid residues. In ESI, ions are produced in solution through acid/base chemistry. The ions are then desorbed into the gas phase as the analyte-containing solution is sprayed from a charged needle into an electric field; this facilitates ion evaporation or coulombic ejection of gas-phase ions. One significant advantage of ESI is that instruments such as the transmission quadrupole with a normal *m/z* range to ~1000–4000 can be used to analyze ions that have a mass of several tens of thousands of daltons because of the high charge state of the ions. This is the very reason that it is important to remember that the mass spectrometer detects ions based on their mass-to-charge ratio, not their mass alone. Another advantage of ESI is that the sample is analyzed as a solution, which can be the eluant from an HPLC, which allows mixtures of compounds in solution to be separated on-line while performing an analysis by LC/MS. The combination with MS can produce challenges such as having to rethink the use of traditional buffer systems for LC mobile phases (see Chapter 11).

LC/MS did not have as seamless a start as did GC/MS. Like GC/MS, soon after the development of high-performance liquid chromatography (HPLC), efforts began to connect this purification technique to the mass spectrometer. These efforts were frustrated by the fact that many of the analytes were thermally labile and/or nonvolatile. In the transformation of the HPLC mobile phase from liquid to gas, the mass spectrometer's vacuum system was often overwhelmed by the gas load. Several attempts were made to develop an interface, such as Patrick Arpino's direct inlet system [47] and Bill McFadden's moving belt [48] of the mid-1970s, both of which relied on conventional gas-phase EI and CI. Other attempts included Marvin Vestal's thermospray (a new method of ion formation) and Ross Willoughby's particle interface [49] of the 1980s (based on conventional gas-phase EI and CI), and, of course, the one enduring technique of the 1970s, Evan Horning's atmospheric pressure chemical ionization (APCI) [50], another new method of ionization. Another factor that frustrated the development of LC/MS was the mass spectrometry paradigm that dominated the 1960s, 1970s, and 1980s, as well as the early part of the 1990s, which was that for the technique to be useful, ions representing the intact molecule had to fragment so that structural information would be forthcoming. This paradigm is why APCI remained a research laboratory curiosity for many years. LC/MS came into an era of practicality when ESI was developed, and at the same time MS/MS became much more user friendly. For more on LC/MS, the history of development of its interfaces, and its applications, see Chapter 11.

The development of MALDI and ESI prompted improvements in the TOF mass spectrometer. One of the major factors involved with advancement of ESI and a companion technique that has become very popular in LC/MS, atmospheric pressure chemical ionization (APCI), was the development of the triple-quadrupole mass spectrometer by Rick Yost and Christie Enke at Michigan State University in the late 1970s [51]. This development made the technique of mass spectrometry/mass spectrometry (MS/MS) practical. MS/MS is a tandem process by which ions of a specific *m/z* value formed by an initial ionization are isolated in the first stage of mass spectrometry, caused to fragment through some process such as inelastic collisions with an inert gas particle, and then the resulting fragment ions are separated according to their *m/z* values and abundances by a second stage of mass spectrometry. MS/MS is sometimes referred to as tandem mass spectrometry (one iteration of mass spectrometry (ionization and ion separation) followed by a second iteration of mass spectrometry). ESI

and APCI primarily form ions containing the intact molecule; therefore, they yield little structure information such as that obtained through fragmentation of molecular ions in EI. The MS/MS process allows for the additional structural information following ionization by ESI or MALDI, and in many cases, a higher degree of specificity for target-compound analysis. Today, MS/MS is carried out in triple-quadrupole mass spectrometers and in hybrid *tandem-in-space* instruments such as a transmission quadrupole analyzer used for the first iteration of mass spectrometry followed by a time-of-flight analyzer used for the second iteration (as in a Q-TOF mass spectrometer). See Chapter 3 for more on the technique of MS/MS.

At the same time ESI was being advanced by tandem mass spectrometry and MALDI was finding increased success with new developments in TOF mass spectrometry, new *m/z* analyzers such as the three-dimensional quadrupole ion (3D QIT), the linear QIT, and, most recently, the orbitrap (only commercialized in mid-2005) were developed. Also, a more dated technique of separating ions according to their *m/z* values, the ion cyclotron resonance (ICR) mass spectrometer (developed in the mid-1960s), was given new life through the detection of ions via image currents with data processing by Fourier transformation [52, 53]. All of these mass spectrometers have the advantage that they are tandem-in-time instruments, meaning that the different iterations of MS/MS take place using a single piece of hardware with the essential actions (precursor-ion selection, precursor-ion dissociation, product-ion analysis) performed as a function of time.

The 3D QIT was also a result of Wolfgang Paul's research in the 1950s; it was commercialized by the same company that is primarily responsible for the introduction of the transmission quadrupole mass spectrometer, Finnigan Corp. The 3D QIT, again like the transmission quadrupole, began its life as a GC/MS system. Both of these analyzers have now seen even bigger roles in LC/MS. Finnigan Corp.'s successor, Thermo Fisher, is responsible for the development of the linear QIT as a standalone mass spectrometer (2003) and the orbitrap, which is used as the second stage of a tandem-in-space instrument (2005). Details of functionality and more on the history of these and other *m/z* analyzers can be found in Chapter 2.

The latest chapter in ionization is currently being written with the developments of techniques for the formation and desorption of ions on the surface of various substances. All mass spectral analyses (except maybe the early analyses of petroleum) of organic compounds have involved some type of sample preparation; i.e., extraction, concentration, derivatization to stabilize nonvolatile, thermally labile substances, separation though a GC or an LC (connected to the mass spectrometer or not), etc. This *sample-preparation/extraction* requirement is no longer an issue. In late 2004 through late 2005, several new ionization techniques were developed. These techniques allow for ionization and desorption of ions of the analyte directly into the gas phase from the surface containing the analyte; the sampling, analysis and detection of ions in accomplished in seconds. This means that pesticides on the skin of a piece of fruit can be detected without the need to remove the skin from the fruit, extract the pesticide, and then submit the concentrated extract to analysis by GC/MS or LC/MS. In cases of analyzing the urine of subjects suspected of driving while impaired for illicit drugs, there is no need to extract the urine and wait for a chromatographic process to complete the analysis. In forensic analyses such as determining whether a piece of porous concrete block might contain traces of a substance like VX nerve agent, there is no risk of having the analyte decompose as it might during analysis by GC/MS or LC/MS, etc. These techniques are DART (direct analysis in real time) [54], DESI (desorption electrospray

ionization) [55], and ASAP (atmospheric pressure solid analysis probe) [56]. As of mid-2006, only DART is provided as a technique with a mass spectrometer. The DESI interface is an add-on for existing mass spectrometers and most reports have been associated with unit-resolution MS/MS instruments such as the 3D QIT mass spectrometers. At this time, the ASAP technique is still a research curiosity. ASAP involves modifying an existing APCI interface on a Q-TOF instrument, exposing the operator to a rather high voltage. DART is part of a JEOL AccuTOF atmospheric pressure ionization time-of-flight mass spectrometer with a resolving power of >7000. This high resolving power allows for accurate mass measurement, resulting in unambiguous elemental compositions for analytes of >500 Da, which is a major part of the reason that the DART technique has proven so successful. More about all three of these new-era ionization techniques can be found in Chapter 4.

Two of the real challenges in mass spectrometry from its beginnings through the later 1960s and early 1970s were involved with the vacuum system and the data. Vacuum became less of a problem with the development of the turbomolecular pump, and dealing with the huge quantities of data (especially those generated during GC/MS) went from being a nightmare to being reasonable and straightforward with the development of the minicomputer.

As can be imagined while viewing Figure 1-6, when Francis William Aston designed his instruments to determine the masses of various nuclides, implementation was a monumental challenge. Crude mechanical devices and water aspirators were

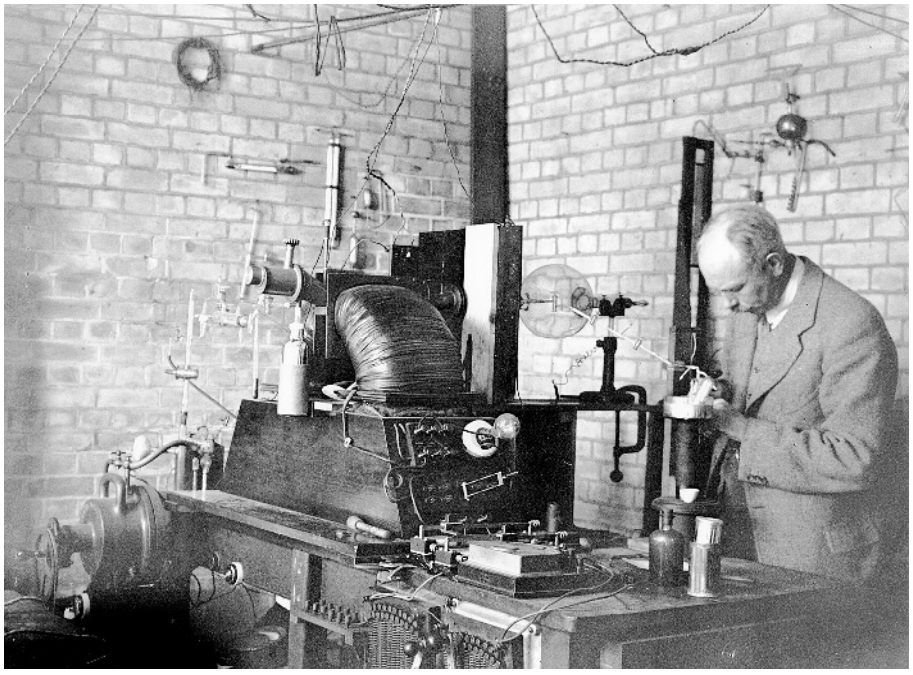


Figure 1-6. Francis William Aston in his workshop at the Cavendish Laboratory at Cambridge ca. 1921. In addition to his many other accomplishments, he was a very talented glass blower which was important in these early days of mass spectrometry.
From the American Institute of Physics archive, with permission.

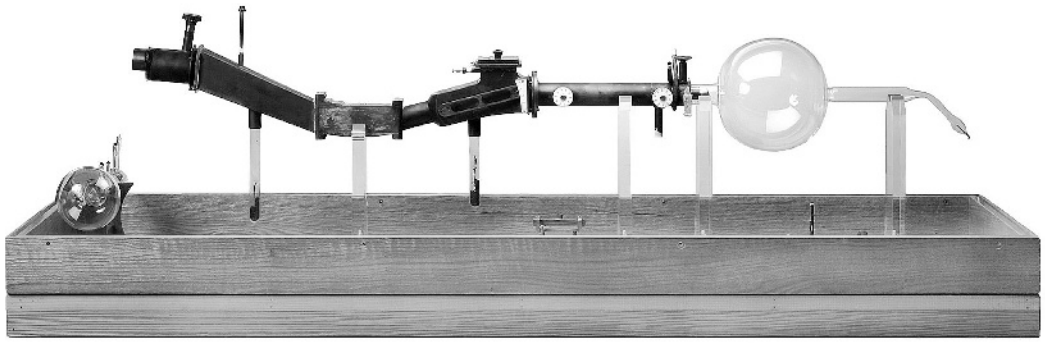


Figure 1-7. Replica of Aston's third design commissioned by the American Society for Mass Spectrometry after the restored instrument housed in the Thomson Museum at Cambridge.

used for the primary vacuum, but a clean high vacuum could only be achieved through the use of a mercury diffusion pump. Like a good portion of the mass spectrometer, except for the electromagnet (Figure 1-7), the mercury diffusion pump was constructed of glass similar to the device shown in Figure 1-8. The mercury diffusion pump was invented simultaneously by Wolfgang Max Paul Gaede (German physicist, 1878–1945) in Germany and Irving Langmuir (American chemist, 1881–1957) in the United States in 1915–1916. Operation of the mercury diffusion pump involves heating the liquid to 110 °C to force a stream of mercury vapor through the volume to be evacuated; collisions between the atoms of Hg and gas molecules force the fixed gas toward a fore pump as the Hg atoms condense back to the liquid state on the relatively cool walls of the chamber being evacuated. These pumps presented many challenges, including dealing with the toxic effects of breathing the mercury vapors. Oil was also used in diffusion pumps; although the background from these pumps interfered with the measurements of the various nuclides, these background spectra gave the organic chemist the idea of using the mass spectrometer for the identification of organic molecules. The oil and mercury were replaced first with silicon-based oils, but these led to the accumulation of silicon oxides on the slits of the instrument, causing a reduction in resolving power. The silicone oils have been largely replaced with synthetic organic polymers, usually polyphenyl ethers.

Figure 1-8. A modern glass mercury diffusion pump. *Permission of Yves-Marie Savoret, Chemistry Department, University of Guelph, Ontario, Canada.*

Recording data from these early mass spectrometers evolved from the hand-tracing of the phosphorescent patterns on willemite screens to the use of photographic plates. Because partial pressures of the analytes were relatively constant, mass spectra could

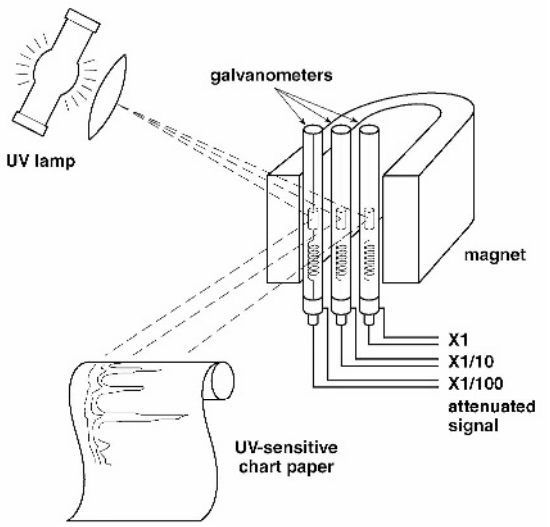


Figure 1-9. Schematic illustration of the light-beam oscillographic recorder. Mirrors attached to galvanometers having different torques reflect light beams to provide correspondingly different amplitudes of the instrument signal, thereby expanding the dynamic range of this analog recording device.
From McFadden WH, Ed. Techniques of Combined Gas Chromatography/Mass Spectrometry: Application in Organic Analysis, Wiley-Interscience, New York, 1973, with permission.

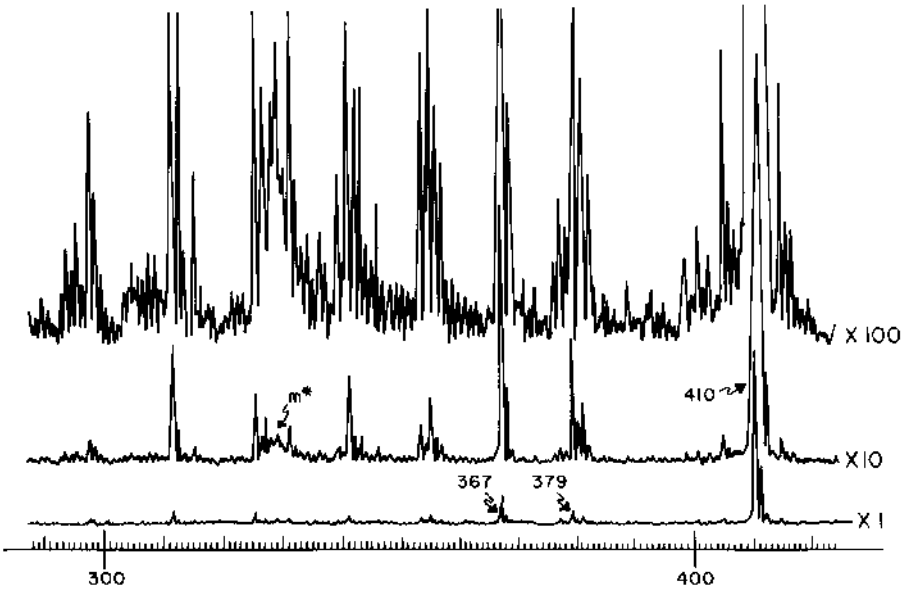


Figure 1-10. Artist's tracing of light-beam oscillographic data from photosensitive recording paper. The numeric m/z scale shown at the bottom was not present on the original data presentation

be recorded over long periods of time, and these recording methods were satisfactory. In the early days of organic mass spectrometry, strip-chart recorders could be used to make a permanent record of the mass spectrum because there were no transient changes in sample concentration associated with chromatography. As the need developed for rapid acquisition of spectra as dictated by GC/MS, Polaroid® photographs were taken of the displays of mass spectra on oscilloscopic screens. The last device to be used to create an analog recording of the mass spectrum before the age of digitization and the use of the minicomputer was the light-beam oscillographic (LBO) recorder. This device reflected discrete light beams onto photosensitive paper from tiny mirrors attached to galvanometers as illustrated in Figure 1-9. These devices provided a good dynamic range for measuring the ion current through the use of several galvanometers, each having a different spring-loaded torque resistance, which produced different deflection amplifications as illustrated in Figure 1-9.

Such a device does allow for a rapid acquisition of a spectrum, but does not alleviate the cumbersome problem of dealing with huge numbers of analog mass spectral recordings. As seen in the illustration of the data obtained using the LBO recorder in Figure 1-10, this data record must be manually processed to produce a bar-graph mass spectrum that can be used for interpretation. Not only is there no *m/z* scale on the original data output, but the scale is not linear. Often the *m/z* scale must be created from the presence of peaks representing ions of known *m/z* values produced by substances that are present in the ion source with the analyte. Spectra in the published literature on mass spectrometry such as the one seen in Figure 1-11 are manual presentations prepared from LBO data. Another problem with the LBO output is that it is based on photosensitive paper, which when exposed to sunlight or fluorescent lighting will darken to the point that the multiple data profiles cannot be seen.

Spectra were acquired individually during GC/MS by pressing a button on the mass spectrometer that initiated a scan of the *m/z* range of the instrument. The analyst would initiate a scan based on observing the magnitude of the total ion current from the ionization source (a recording of this signal resembled a chromatogram). The operator would label the resulting spectrum to coordinate with a mark on the total ion current

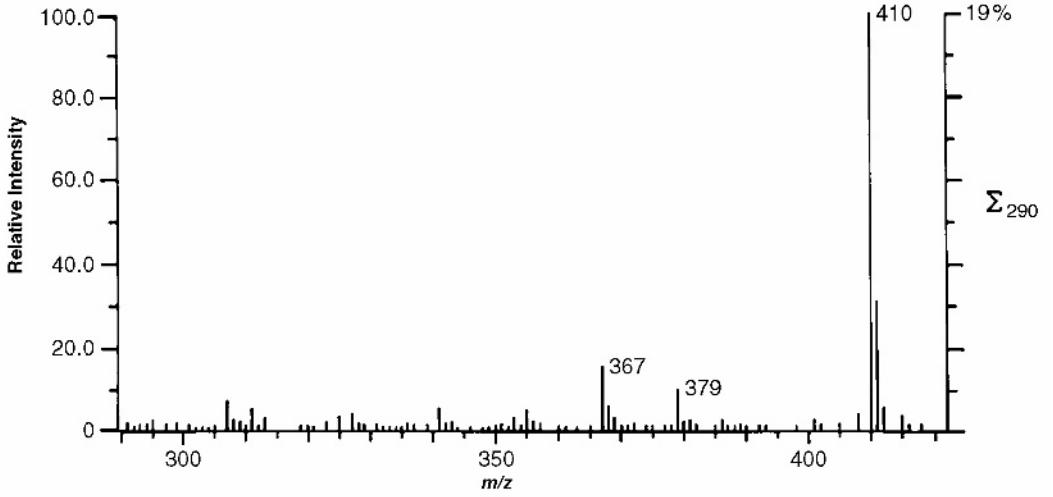


Figure 1-11. A bar-graph mass spectrum resulting from laborious manual processing of the analog data shown in Figure 1-10.

chromatogram so that during data analysis, the acquisition time of the spectrum could be correlated with the chromatogram. Usually no more than two or three spectra could be recorded for a single chromatographic peak. If a spectrum was to be used as a background spectrum, it was often acquired on the back side of the chromatographic peak because of the difficulty of anticipating when to acquire the spectrum on the front side of the peak. There was no real way to record mass chromatograms (defined later in this chapter); the concept of the mass chromatogram was introduced with computerized data systems.

As can be appreciated from this description of the manual effort involved in obtaining interpretable data, organic mass spectrometry in the years before the minicomputer was very challenging.

III. Some Important Terminology Used in Mass Spectrometry

1. Introduction

The definitions of the terms mass-to-charge ratio (*m/z*), dalton (Da), and unified atomic mass unit (u) and their significances have already been stated in the beginning of this chapter. Other important terms used in mass spectrometry (isotope, nominal mass, monoisotopic mass, and mass defect) are found under the heading "IV. Elemental Composition of an Ion and the Ratios of Its Isotope Peaks" in Chapter 5. In addition, there are some other very important terms that are necessary to the understanding of the literature and discussions of mass spectrometry. These definitions follow.

2. Ions

A **molecular ion** is a charged species that has an odd number of electrons and is formed from a molecule (an even-electron neutral species) through the addition or removal of an electron. A molecular ion is not a charged species that results from the addition of a charged species that has a significant mass such as a proton, sodium ion, chloride ion, etc. A molecular ion is not a species that represents an intact molecule that has had a proton or hydride (a proton with two electrons) removed from it or resulted from it. Under no circumstances should a molecular ion ever be called a *parent ion*. At one time, the precursor ion involved in collisional activation dissociation (CAD) analyses were inappropriately referred to as *parent ions*, prompting the discontinuances of the use of this anthropomorphic term as a synonym for molecular ion. The term parent ion has no place in mass spectrometry to describe a molecular ion, a precursor ion, or any other type of ion.

A **fragment ion** results from the decomposition of another ion. This term usually refers to an ion that is produced by the fragmentation of a molecular ion or a species that represents the intact molecule such as a protonated molecule, a deprotonated molecule, an ion produced by hydride abstraction, a sodiated molecule, etc. Fragment ions can be the results of the fragmentation of a fragment ion formed from a molecule ion: a secondary fragmentation. Fragment ions are formed through the breakage of chemical bonds. They do not result from the loss of nuclear matter from one of the atoms comprising the ion. No fragment ion can result from the loss of 12 Da from a molecular ion. Fragment ions always have a mass that is less than that of their precursor. This statement illustrates the importance of separating the terms related to mass and the mass-to-charge ratio of an ion. The fragment ion will have a mass less than the mass of its precursor, but it may have an *m/z* value greater than that of the precursor ion if the precursor ion has multiple charges and the fragment ion has fewer charges. In most

forms of mass spectrometry, the products of fragmentation are an ion of lesser mass and the same charge sign as the percussor ion and a neutral species that is a radical (an odd-electron species) or molecule (an even-electron species). In some cases involving multiple-charge ions, the fragmentation involves the formation of two or more ions with the same charge sign, both of which can be detected in the mass spectrometer. In some very rare cases, mass spectrometry fragmentation results in an ion-pair formation (two ions of opposite charge sign).

3. Peaks

In mass spectrometry **peaks** appear in the recording of the mass spectrum and these peaks represent the **ions** that are formed in the mass spectrometer. Ions are not found on mass spectra, and peaks do not occur in mass spectrometers. Another important characteristic of peaks and ions is that *peaks* have **intensities** and *ions* have **abundances**. The peak with the highest intensity in the mass spectrum is called the **base peak**. The peak at *m/z* 175 in the two graphical presentations of the mass spectrum in Figure 1-2 is the base peak. The presentation of the mass spectrum can be absolute with the peak intensity representing the actual signal strength of the ion current for an ion with that *m/z* value, or the peaks can have a *relative* intensity which presents the data in such a way that the intensity of the base peak is 100%. This latter presentation has the disadvantage that the signal strength of the mass spectrum is not known. Unless the mass spectrum has something in the header of its presentation to indicate signal strength, the relevance of missing mass spectral peaks may remain a mystery. Commercial instruments vary in the way that they display graphical mass spectra. Some have an absolute intensity display and others have a relative intensity display. Those that display mass spectra with relative intensities usually have an indication of the base peak's signal strength in the header. All mass spectral databases (mass spectral libraries) use a relative intensity display.

When using the word "peak" in a discussion of GC/MS or LC/MS data it is important to make sure that there is a distinction between a *mass spectral peak* and a *chromatographic peak*. In today's modern mass spectrometry, analyses are carried out by acquiring one spectrum after another at a constant rate. These individual spectra can be displayed as a bar graph, in a profile graphic presentation, or in a tabular format as described in Figure 1-2. It may be convenient to display a group of spectra in a contiguous presentation of all the spectra acquired during a given time period. This type of display is often created by summing the ion current at each *m/z* value that exhibits any ion current in each spectrum. The sum of the ion current is then presented graphically as a plot of the total ion current for each spectrum vs the spectrum number. This plot is referred to as a **reconstructed total ion current** (**RTIC**) **profile**. The term "reconstructed" is appropriate because the profile displayed is created from the only data that are acquired, mass spectral data; this profile is not created directly by monitoring the total ion current as a function of time. When spectra are acquired of the eluate from a chromatographic process, the RTIC profile is called an **RTIC chromatogram**. The *RTIC profile/chromatogram* is a fourth way of displaying or viewing mass spectral data.

Another way of viewing mass spectra, where data are acquired over a range of *m/z* values, is to prepare a plot of the ion current at a single *m/z* value, a subset of the acquisition range (a range of *m/z* values), or the sum of the ion current of selected *m/z* values in the acquisition range. Such a display is called an **extracted ion current** (**EIC**) **profile** or, more appropriately for data acquired during a chromatographic analysis, a

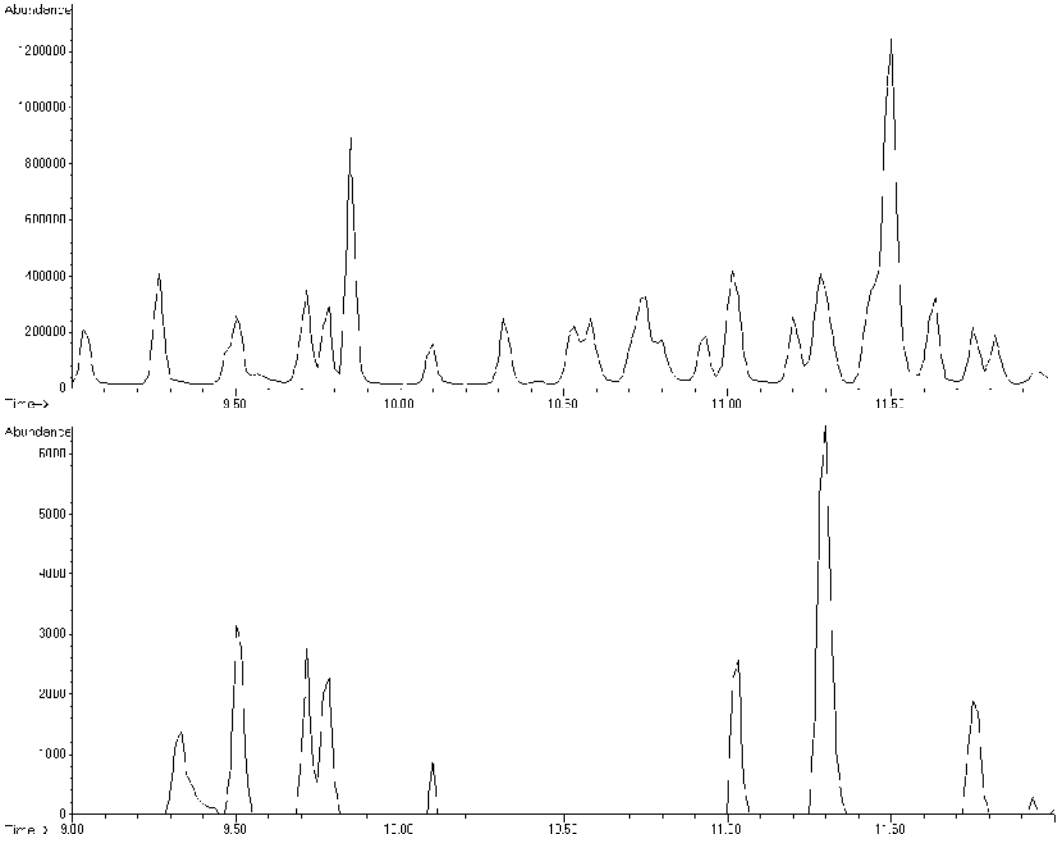


Figure 1-12. (Top) Reconstructed total ion current chromatogram of mass spectral data acquired at the rate of 1 spectrum sec^{-1} over an m/z range of 40–450. (Bottom) Mass chromatogram/extracted ion current chromatogram for m/z 232 from same data set.

mass chromatogram [57]. Because data are acquired during infusion or flow injection in LC/MS and when probes are used for sample inlets in GC/MS, the profile term is as appropriate as the chromatogram term. However, the chromatography terms (RTIC chromatogram or mass chromatograms) should be used when the data are a result of chromatography. If the data are a result of capillary electrophoresis, the term should be **RTIC pherogram** or **EIC pherogram**. These EIC profiles/mass chromatograms should not be confused with SIM profiles and SIM chromatograms, which result from data acquired during selected ion monitoring experiments. There can also be SRM profiles and SRM chromatograms, which are displays of data acquired during experiments using selected reaction monitoring. Figure 1-12 is an example of an RTIC chromatogram (top) and a mass (or an EIC) chromatogram (bottom).

The importance of employing a distinguishing adjective to differentiate between a reconstructed chromatographic peak and a mass spectral peak cannot be overemphasized. Both have height and width, which is significant for each. The peaks representing the chromatographic processes in chromatography/mass spectrometry are different from the chromatographic peaks observed when ultraviolet (UV) and refractive index (RI) detectors are used with LC or when flame ionization (FI) and electron capture

(EC) detectors are used with GC. These latter detection systems are analog devices and produce the chromatographic profile directly. In chromatography/mass spectrometry, the process of recreating (reconstructing) the chromatographic data profile from mass spectral data is a digital process.

4. Resolution and Resolving Power

There are two terms that are inappropriately and, more often than not, incorrectly used in an interchangeable way in mass spectrometry: **resolution** and **resolving power**. A venerable mass spectrometrist, Keith Jennings, said, "Resolution pertains to the data of mass spectrometry, whereas resolving power is a function of the mass spectrometer." The term *resolution* is always relevant to the separation of ions of two different *m/z* values. In mass spectrometers that separate ions using quadrupole fields (quadrupole ion traps and transmission quadrupoles), the resolution is constant throughout the *m/z* scale. That is to say, peaks representing two pairs of ions that differ by 1 *m/z* unit will have the same separation at *m/z* 100 and 101 as they do at *m/z* 2000 and 2001.

The term *resolving power* is defined as the difference in *m/z* values of ions that can be separated (according to some definition) from one another (Δm) divided into a specific *m/z* value (M) (i.e., R = M / Δm). This means that the resolution at this particular *m/z* value can be considered to be Δm [58]. Therefore, for an instrument that operates at constant unit resolution (Δm) throughout the *m/z* scale, the values for R would be 100 (R = 100 / 1) at *m/z* 100 and 2000 (R = 2000 / 1) at *m/z* 2000. There is no single resolving power for these types of instruments, and the value for R will be different at every *m/z* value. However, by saying that the instrument will function at a *constant resolution of X m/z units*, the differences in *m/z* values of ions that can be separated is clearly understood. Instruments that do not operate at a constant resolution throughout the *m/z* scale, such as the TOF and double-focusing mass spectrometers and instruments that use Fourier transforms for the detection of ions, do operate at constant resolving power. An instrument that has a resolving power (R) of 1000 will have a resolution of 1 at *m/z* 1000. This instrument will separate an ion with *m/z* 1001 from an ion with *m/z* 1000. This same instrument will produce data with a resolution of 0.1 at *m/z* 100; the mass spectrometer will be able to separate ions with *m/z* 100.1 from ions with *m/z* 100.0.

When the term *high-resolution mass spectrometry* is used, it usually means that the instrument is capable of *high resolving power*. Unfortunately, the terms *high resolution* and **accurate mass measurement** are also often used interchangeably. The term accurate mass measurement means mass measurement performed to a sufficient number of significant figures to allow for an unambiguous determination of an elemental composition. The number of decimal places needed will be a function of the ion's actual mass. For ions with a mass of less than 500 Da, an accuracy of 0.0025 Da should allow for an unambiguous assignment of an elemental composition. This type of mass accuracy can be obtained with any mass spectrometer provided that the ion is monoisotopic (there is a single elemental composition and all the atoms of all the elements present represent only the most abundant naturally occurring stable isotopes of those elements). The need for a high-resolving power mass spectrometer occurs when the *m/z* value represents two different ions (a **doublet** and a **multiplet**) as would be the case for a single-integer *m/z* value representing both an $[M - OH]^+$ and an $[M - NH_3]^+$ ion. A separation of these two ions at *m/z* 220 would require an R value of >7000 using a 10% valley definition to describe the overlap of the corresponding peaks.

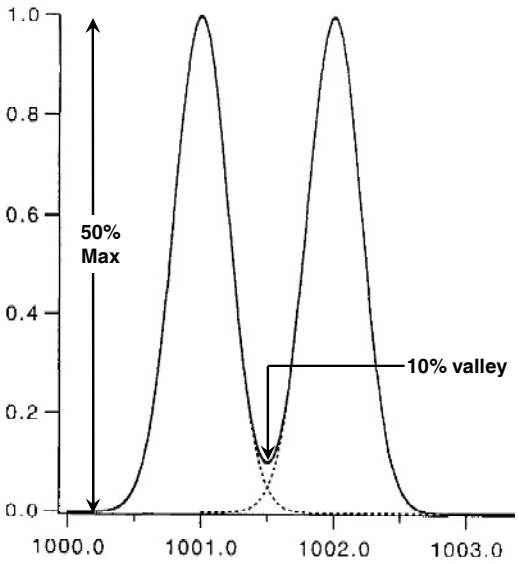


Figure 1-13. Illustration of 10% valley definition of resolving power.

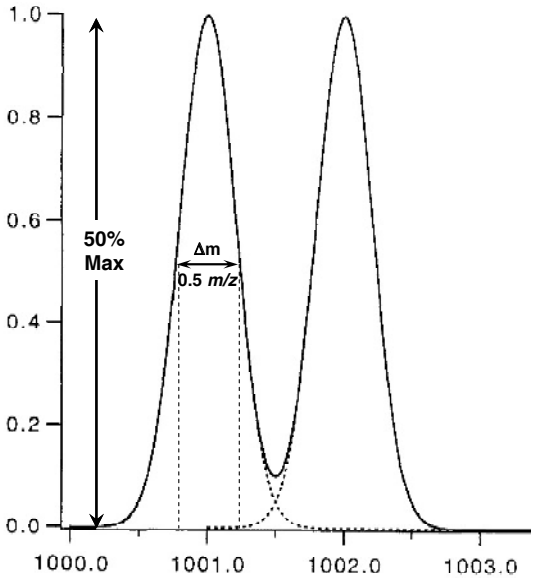


Figure 1-14. Illustration of FWHM definition of resolving power.

When *resolving power* was defined above, the definition included a qualifier. This qualifier has to do with the determination of ∆m. Resolving power qualifier definitions are **% valley** or **full width at half maximum** (**FWHM**). Two mass spectral peaks of equal intensity will separate with an overlap sometimes referred to as the crosstalk between two ions of adjacent *m/z* values. The difference in these two *m/z* values is ∆m. The distance from the baseline to the point of overlap (see Figure 1-13) expressed as a percentage of the mass spectral peak height is the percent valley. An acceptable separation (or percent valley) is 10–20%. Some instrument manufacturers (usually of double-focusing instruments) have used resolving power definitions of 50% valley. Such an instrument would be incapable of resolving ^{13}C-isotope peaks above *m/z* 150.

It is very difficult to obtain data resulting in two mass spectral peaks that have the same intensity and that are separated to the limit achievable by the particular instrument. When adjacent mass spectral peaks of equal intensity overlapping to the designated extent are not available, the value for ∆m is taken as the FWHM of a mass spectral peak. From an examination of the FWHM of the peak on the left side of Figure 1-14, it is clear that the value for ∆m is half that obtained using the 10% valley definition. This means that values for R obtained using the FWHM definition are twice those obtained with the 10% definition.

The use of the full width at 5% maximum of a single mass spectral peak can be used to define the resolving power; however, accurate measurements at this position on the mass spectral peak often prove to be very difficult.

When using the FWHM definition, it is best to determine the number of *m/z* units represented by the physical distance between two non-overlapping mass spectral peaks. The difference in two mass spectra, both with the same resolving power but calculated using two different definitions, FWHM (left) and 10% valley (right), is illustrated in Figure 1-15.

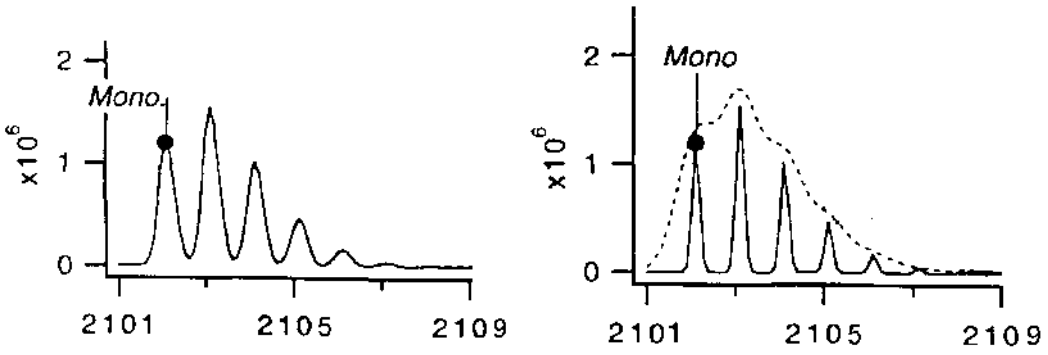


Figure 1-15. Data recorded at a resolving power value of 5000. R for the spectrum on the left was calculated using the FWHM definition, whereas R for the spectrum on the right was calculated using the 10% valley definition.

If the numerical value of the mass of an ion exceeds the resolving power (R) at which a measurement is made, the reported *m/z* value is the weighted average mass-to-charge ratio of all of the ions (likely of several different isotopic variants) represented by the mass spectral peak. The accuracy of the assignment of that *m/z* value may be within a range of ±1 *m/z* units, but the mass may be what is referred to as the *average mass* (M_r), which is the mass that corresponds to the weighted average of the masses of the naturally occurring stable isotopic variants of the ion. When measured using a resolving power of 2000, a single-charge ion with a nominal mass of 1000 will be represented by a series of peaks one *m/z* unit apart. The peak at the lowest *m/z* unit represents ions that are composed of only the lightest isotope of each element (the monoisotopic ion). The peaks at consecutively higher *m/z* units represent ions that have different isotopic compositions and are called the isotope peaks; i.e., for an ion containing C, H, N, and O, the peak at one *m/z* unit higher than the monoisotopic peak represents mostly an ion that has one atom of ^{13}C rather than all constituent carbon atoms being ^{12}C. This same peak also represents ions in which one atom of nitrogen is ^{15}N, or one atom of hydrogen is ^{2}H, or one atom of oxygen is ^{17}O with all carbon atoms being ^{12}C. All of these ions represented by the X+1 peak have different *m/z* values on the millimass scale; however, the resolution obtainable at a resolving power of 2000 is insufficient to separate the four isotopic variants of the ion represented by this single peak, which would be reported to the nearest integer *m/z* value. If an accurate *m/z* value were determined for this X+1 ion at R = 2000, the value would be the average of the accurate masses of the four species described above.

When a linear TOF mass spectrometer with a resolving power (R) of 2000 is used to measure the *m/z* value of an intact protein that has a mass of 200,000 Da, the peak representing this ion will be the result of the abundances of ions over a range of 100 *m/z* units. The so-called isotope peaks for these ions cannot be resolved with R = 2000. The accurate mass determined from such a measurement will be a weighted average of the masses of all the isotopic variants of the ion; therefore, it will be the average mass (M_r) of the ion or the mass based on the atomic masses of all the atoms of the elements that comprise the ion.

The same is true for a multiple-charge ion. If an ion with 20 charges is reported with an integer *m/z* value of 1000 as measured at a resolving power of 2000, the *m/z*

value of the peak representing this ion will be influenced by a variety of ions including the monoisotopic ion and all of its isotopic variants. The charge-transformed mass corresponding to this peak will be a weighted average or the M_r.

Normally, it is said that mass spectrometry deals with the nominal mass (monoisotopic mass) of the elements, not their atomic masses; however, when measuring *m/z* values that are higher than the achievable resolving power, mass spectrometry also measures average mass based on the atomic mass of the constituent elements.

IV. Applications

From an instrumentation standpoint, mass spectrometry has had an exciting history of development of ionization techniques and in the way that ions have been separated according to their *m/z* value. In some cases, an analyzer type was developed and ways of applying that analyzer to specific analytical challenges came about. In other cases, an ionization method resulted from trying to find a way to determine the mass of a heavy compound such as an intact protein. Some instrument developments, like MALDI, were application driven. Some applications like the analysis of the skins of fruits and vegetables for pesticides were developed because someone designed an instrument that could ionize electrophilic compounds through resonance electron capture. DART and DESI were developed to form ions of analytes on surfaces and desorb these ions into the gas phase. The applications for such an instrumental technique are developing with each passing day. Refinements in these specific analyses will eventually result in routine analytical techniques such as those performed today by GC/MS and LC/MS. Some applications have resulted because certain kinds of instruments have been available for a required analysis. In other cases, instruments had to be developed for a required analysis. This has been the story of developments in mass spectrometry ever since its utility was first observed.

The following representative examples are designed to provide a brief exposure to some types of qualitative and quantitative applications of mass spectrometry. Some of the examples also provide an illustrative introduction to some of the operating principles or fundamental aspects of the technology and provide hints to the strategy of data interpretation.

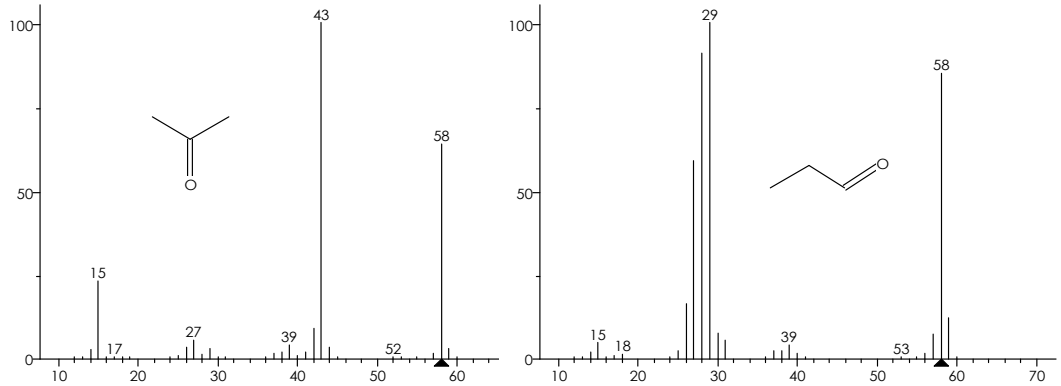


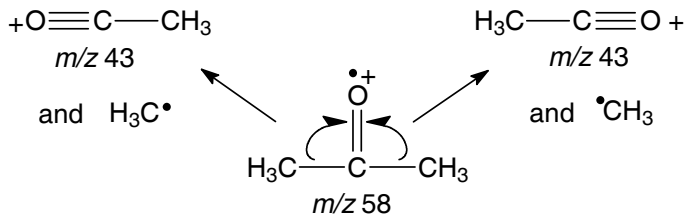
Figure 1-16. EI mass spectrum of acetone (left) and propionaldehyde (right). Both compounds have an elemental composition of C_3H_6O and a nominal mass of 58.

1. Example 1-1: Interpretation of Fragmentation Patterns (Mass Spectra) to Distinguish Positional Isomers

One way of analyzing an organic compound by mass spectrometry involves conversion of the analyte molecule to a charged species (the molecular ion, $M^{+\bullet}$) by electron ionization (described in detail in Chapter 6). In the case of positional isomers such as acetone and propionaldehyde (both of which has the elemental composition C_3H_6O), a peak at *m/z* 58, which represents the $M^{+\bullet}$, does not distinguish the two compounds. However, in this case, the fragmentation pattern can be used to distinguish and identify the two compounds.

Compare the bar-graph spectra of acetone and propionaldehyde in Figure 1-16. The differences in the fragmentation patterns are related to structural differences between these two molecules. The base peak (the most intense peak in the spectrum) appears at *m/z* 43 in the mass spectrum of acetone (left side of Figure 1-16); in the mass spectrum of propionaldehyde (right side of Figure 1-16) there is no significant ion current at *m/z* 43. Conversely, the mass spectrum of propionaldehyde shows a substantial amount of ion current at *m/z* 29 and 28; the spectrum of acetone shows no appreciable ion current in this region. In addition, the propionaldehyde spectrum has a significant peak at *m/z* 57, the $[M - 1]^+$ or $[M - H]^+$ peak.

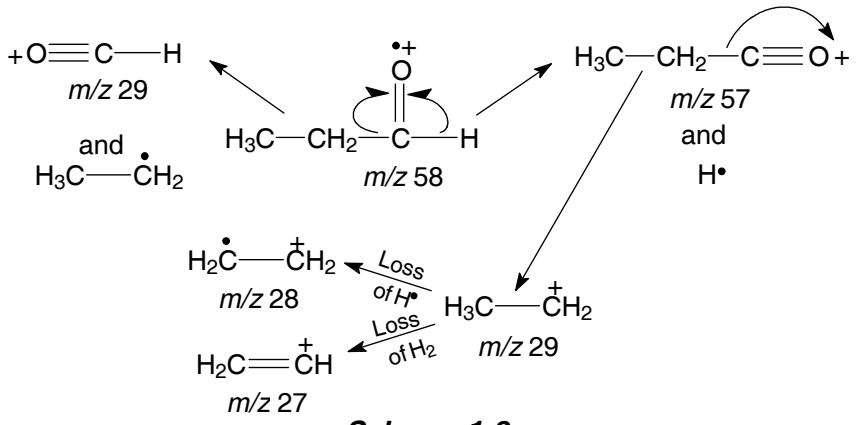
Many of the driving forces that led to cleavage of certain chemical bonds in the $M^{+\bullet}$ are described in detail in Chapters 5 and 6. In this preliminary example, let it suffice that the bond on either side of the carbonyl carbon will break, as illustrated in the



Scheme 1-1

following schemes. Scheme 1-1 shows the $M^{+\bullet}$ of acetone with a positive charge and an odd electron on the oxygen atom. The carbon–carbon bond is cleaved as one of the electrons moves to "pair up" with the odd electron on the oxygen. Cleavage of either carbon–carbon bond therefore results in expulsion of a methyl radical (15 Da) from the $M^{+\bullet}$ of *m/z* 58 to yield a fragment ion of *m/z* 43. The ion of *m/z* 43 does not have an odd electron; i.e., all of its electrons have been paired. The high abundance of this stable acylium ion is reflected by the intense peak at *m/z* 43 (represented by the $[M - 15]^+$ peak) in the left side of Figure 1-16.

Scheme 1-2 shows the $M^{+\bullet}$ of propionaldehyde, also with an odd electron on the oxygen atom. Again, the bonds adjacent to the carbonyl can transfer a single electron to pair with the odd electron on the oxygen. Cleavage of the carbon–carbon bond results in expulsion of an ethyl radical (29 Da) from the $M^{+\bullet}$, which has a nominal mass of 58 Da to yield a fragment ion of *m/z* 29. Again, this type of resonance-stabilized fragment ion (an acylium ion) is represented by an intense peak, but at *m/z* 29 in this case (see right side of Figure 1-16). Cleavage of the carbon–hydrogen bond (on the other side of the carbonyl group) results in expulsion of a hydrogen radical (1 Da) from the $M^{+\bullet}$ to produce



Scheme 1-2

the fragment ion of *m/z* 57; this ion is represented by the peak at *m/z* 57 (representing the $[M-1]^+$ ion) in the right side of Figure 1-16. The acylium ion with *m/z* 57 can undergo a fragmentation to lose a molecule of CO. The resulting ethyl ion can then fragment by either the loss of a hydrogen radical or a molecule of hydrogen to account for the peaks at *m/z* 28 and *m/z* 27, respectively.

Certain structural features in a molecule can manifest themselves in a particular mode of fragmentation. The phenomenon of structure-related fragmentation is the basis by which each compound that can be ionized by the EI process has its own unique and characteristic fragmentation pattern, a chemical fingerprint.

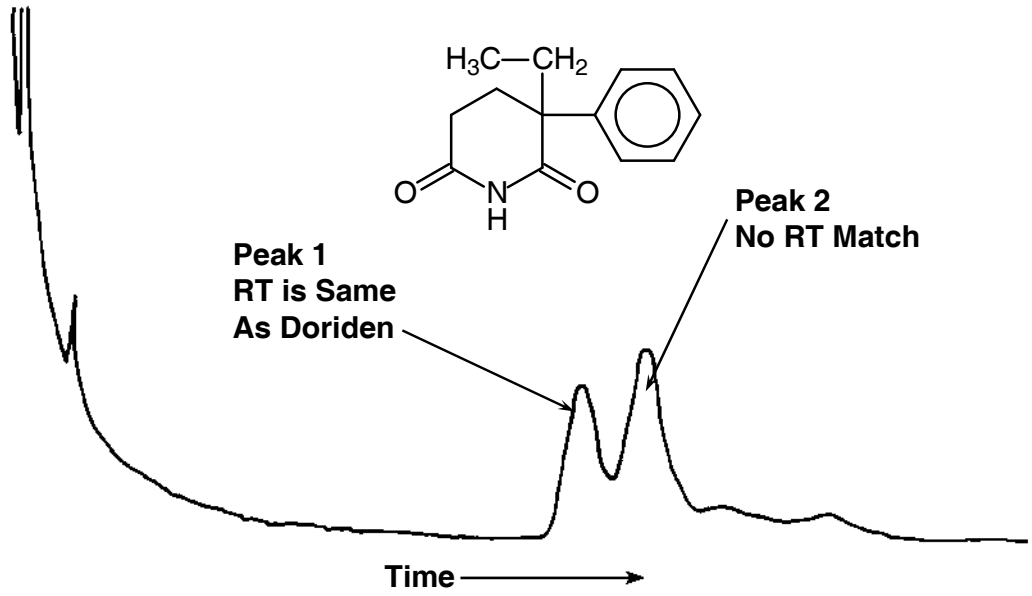


Figure 1-17. Gas chromatogram resulting from analysis of blood sample extract from a comatose patient following drug overdose.

2. Example 1-2: Drug Overdose: Use of GC/MS to Identify a Drug Metabolite

In an effort to recommend rational therapy for a comatose drug-overdose victim, physicians and toxicologists prefer to analyze the patient's blood for suspected drugs. Figure 1-17 is a gas chromatogram showing separation of two major components from other constituents of a plasma extract of such a patient [59]. Peak 1 has the same retention time as that of authentic glutethimide (Doriden, a Schedule II hypnotic sedative), which was an expected finding because an empty prescription bottle labeled for that drug had been found near the victim. What did Peak 2 represent? Had the victim ingested yet another drug?

Analysis of successive blood samples indicated that whereas Peak 1 (glutethimide) represented a decreasing amount with time, Peak 2 remained constant in intensity. Because the patient's condition was deteriorating, the possibility of hemodialysis was being considered, pending identification of the substance represented by Peak 2 in the chromatogram (Figure 1-17). The retention time of Peak 2 under the analytical conditions did not match that for any drug known to be available to the victim or those for other drugs commonly abused.

Analysis of the plasma extract by GC/MS confirmed that Peak 1 does represent glutethimide. The mass spectrum (Figure 1-18, top) obtained during the elution of Peak 1 was identical with that from authentic glutethimide [59]. The peak at *m/z* 217, labeled A in the top panel of Figure 1-18, represents the $M^{+\bullet}$. The mass spectrum (Figure 1-18, bottom) obtained during gas chromatographic elution that accounted for Peak 2 (Figure 1-17) indicates a $M^{+\bullet}$ peak at *m/z* 233 (also labeled A). Note that both $M^{+\bullet}$ peaks have an odd *m/z* value (217 and 233), which means that each of the corresponding molecular ions contains an odd number of nitrogen atoms (see Chapter 5 for explanation of the *Nitrogen Rule*). Furthermore, the *m/z* values of the two $M^{+\bullet}$ peaks differ by 16, and several other prominent peaks (B, C, D) in the mass spectra differ by 16 *m/z* units. This shift of 16 *m/z* units suggests incorporation of oxygen into the molecule of glutethimide; i.e., Peak 2 in the gas chromatogram (Figure 1-17) could represent a hydroxylated metabolite of glutethimide. To confirm this possibility, the material represented by Peak 2 in the gas chromatogram was treated with acetic anhydride and pyridine, and was reanalyzed by GC/MS. The mass spectrum of the derivatized (chemically modified) material (data not shown) was identical with that of the acetate derivative of 4-hydroxy-2-ethyl-2-phenylglutarimide, an active metabolite of glutethimide that has demonstrated toxicity in animals [60].

Hemodialysis was then considered to be of reasonable risk because the 4-hydroxy metabolite was sufficiently polar to be removed by this technique. During hemodialysis, the plasma level of the 4-hydroxy metabolite dropped more rapidly than that of the less polar parent drug and the patient regained consciousness [59], possibly living happily ever after.

This example illustrates that several clues to the possible identity of the compound can be obtained from even cursory examination of a mass spectrum. In this case, the molecular ions of odd mass immediately suggested nitrogen-containing compounds (via the *Nitrogen Rule*), and the shift of 16 *m/z* units for major mass spectral peaks for the two compounds suggested that the heavier one (II) was an oxygenated variant of the other (I). A more detailed examination of the mass spectra than described here was required for complete verification. A similar result has been reported for the metabolism of aminoglutethimide [61].

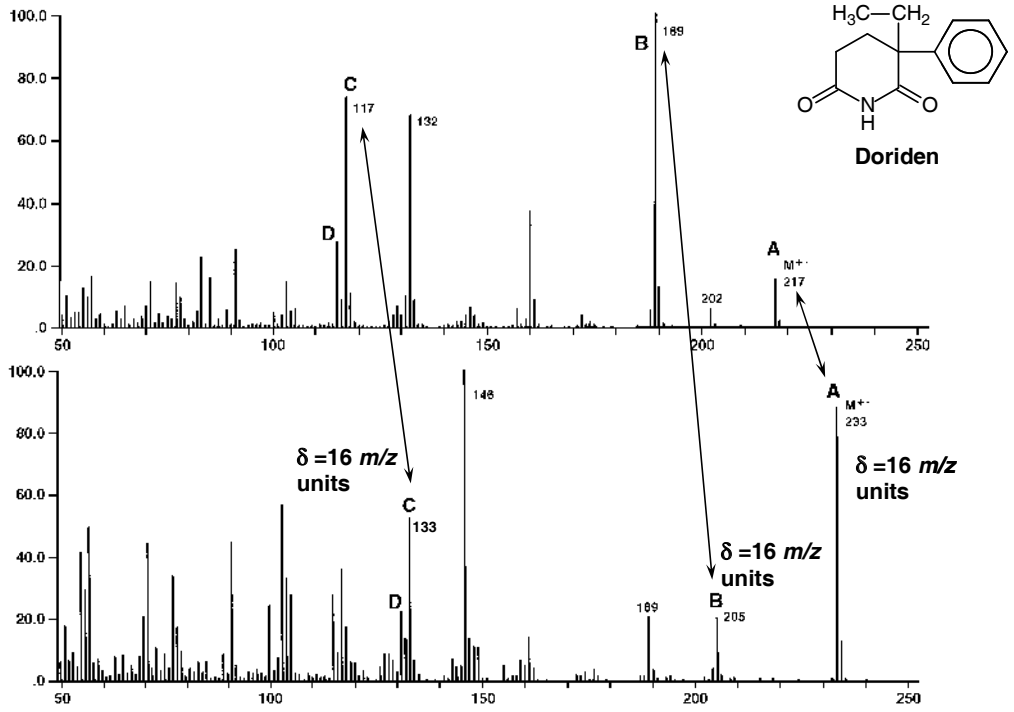


Figure 1-18. (Top) EI mass spectrum obtained during elution of Peak 1 in Figure 1-17. This spectrum is identical to that of glutethimide (Doriden®). (Bottom) EI mass spectrum obtained during the elution of Peak 2 in Figure 1-17. Note the $M^{+\bullet}$ peak in both spectra is at an odd m/z value and that major peaks in the bottom spectrum occur 16 m/z units higher than the corresponding peaks in the top spectrum.

3. Example 1-3: Verification that the Proper Derivative of the Compound of Interest Has Been Prepared

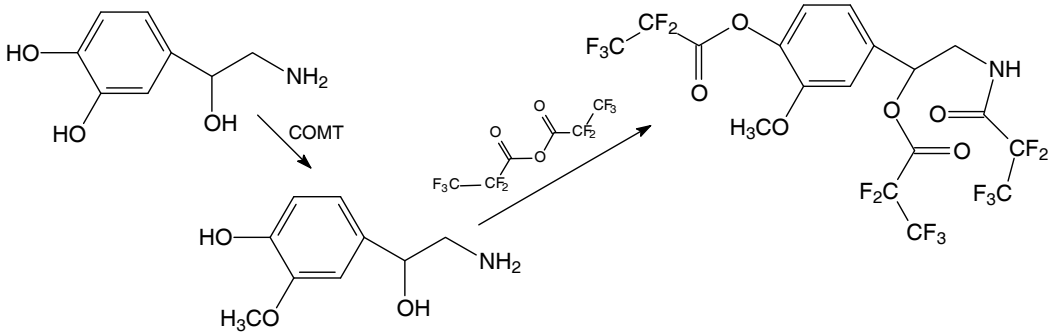


Figure 1-19. Norepinephrine is metabolized to normetanephrine by the enzyme catechol-O-methyltransferase (COMT). Normetanephrine is reacted with pentafluoropropionyl (PFP) anhydride to form a thermally stable and volatile derivative.

Many compounds have polar functional groups that do not permit direct analysis by the vapor-phase techniques of GC or GC/MS. Figure 1-19 shows a chemical scheme or modifying the structure of normetanephrine (NMN), a metabolite of norepinephrine via catechol-O-methyltransferase (COMT), which has free hydroxyl and amino groups that are unstable at temperatures of 150 to 250°C, temperatures usually employed in GC. The functional groups on these molecules tend to undergo hydrogen bonding, which is partially responsible for the low vapor pressures of these compounds. The chemical reaction on the right of Figure 1-19 shows the treatment of NMN with pentafluoropropionic (PFP) anhydride to produce a (*O,O′,N*-tris-pentafluoropropionyl) derivative, which greatly increases the volatility and thermal stability of the molecule.

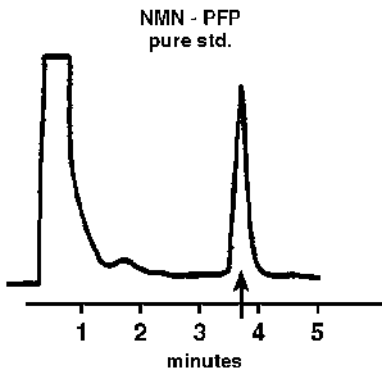


Figure 1-20. Gas chromatogram resulting from injection NMN-PFP reaction mixture.

The PFP derivatives of biogenic amines, such as NMN, have good vapor-phase properties. During the development of vapor-phase methods for such compounds it is good practice to verify that the proper derivative has been formed as anticipated under realistic analytical conditions, such as in low-level samples and in the presence of biological residue. During such a process of verification, analysis of an aliquot of the reaction mixture by GC produced the chromatogram illustrated in Figure 1-20. It is encouraging that Figure 1-20 indicates only a single GC peak, but does this chromatographic peak represent the structure at the bottom of Figure 1-19? That is, has the NMN molecule taken on three PFP groups, or has it taken on four? Four is a reasonable possibility because there is another replaceable hydrogen on the nitrogen atom of NMN, as seen in the structure at the bottom of Figure 1-19. Obtaining the mass spectrum should resolve the question.

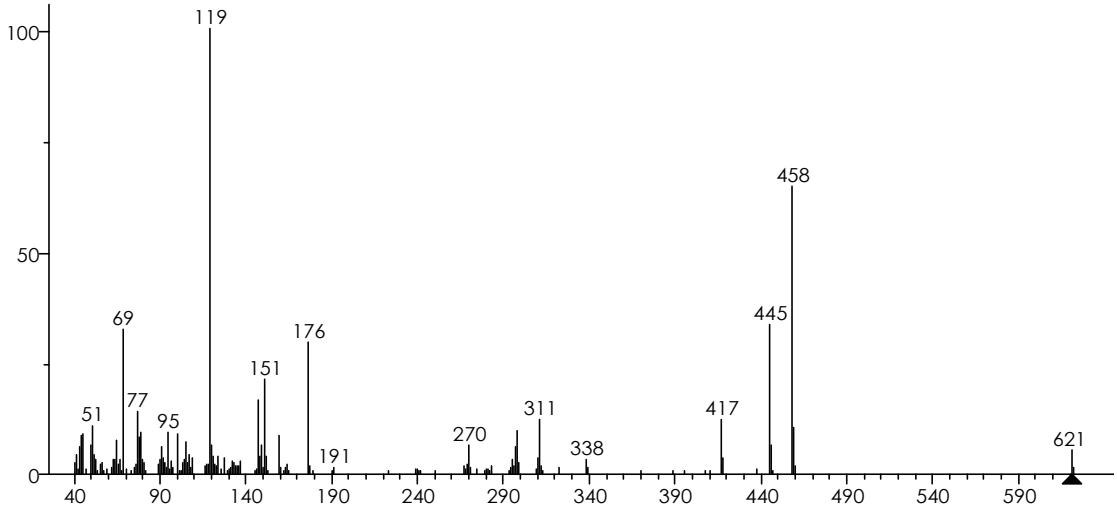
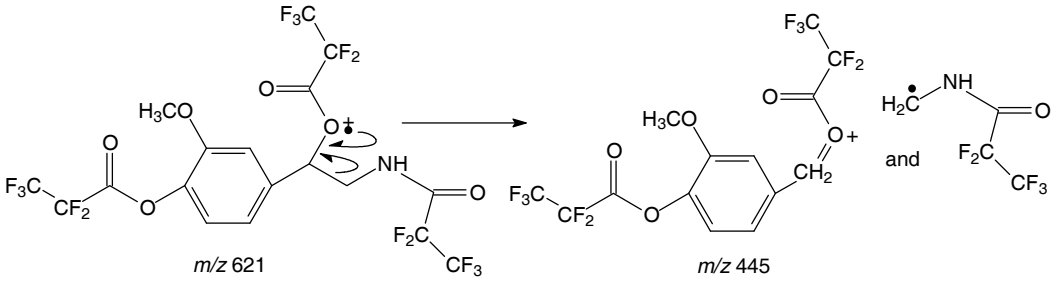


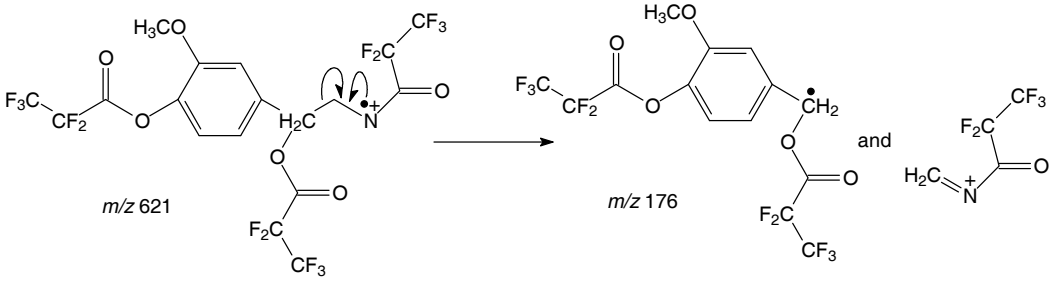
Figure 1-21. EI mass spectrum of NMN as the PFP derivative.

The nominal mass of the derivative is substantially increased (a net increase of 146 Da for each PFP moiety (COC_2F_5)) over that of the underivatized compound. The mass spectrum (Figure 1-21) of the product of derivatization exhibits an apparent $M^{+\bullet}$ peak at *m/z* 621. A nominal mass of 621 daltons is consistent with the structure at the bottom of Figure 1-19. Had there been four PFP groups on the molecule, its nominal mass would have been 767 Da; however, no peak was detected at *m/z* 767 during analysis by GC/MS. Note that the $M^{+\bullet}$ peak at *m/z* 621 is consistent with the *Nitrogen Rule*, which states that a compound containing an odd number of nitrogen atoms will have an odd nominal mass.

The $M^{+\bullet}$ (an odd-electron species) is positively charged because it loses an electron during the process of ionization. Reasonable assumptions (described in detail in Chapters 5 and 6) concerning the site of electron deficiency in the $M^{+\bullet}$ can be proposed as an aid in rationalizing the formation of fragment ions. For example, formation of the ion of *m/z* 445 from NMN-PFP could be rationalized by the fragmentation shown in Scheme 1-3. Observing the peak at *m/z* 445 (an odd value) is consistent with a corollary to the *Nitrogen Rule*, which states that a fragment ion (as an even-electron species) having an even number of nitrogen atoms (including zero) will have an odd nominal mass.

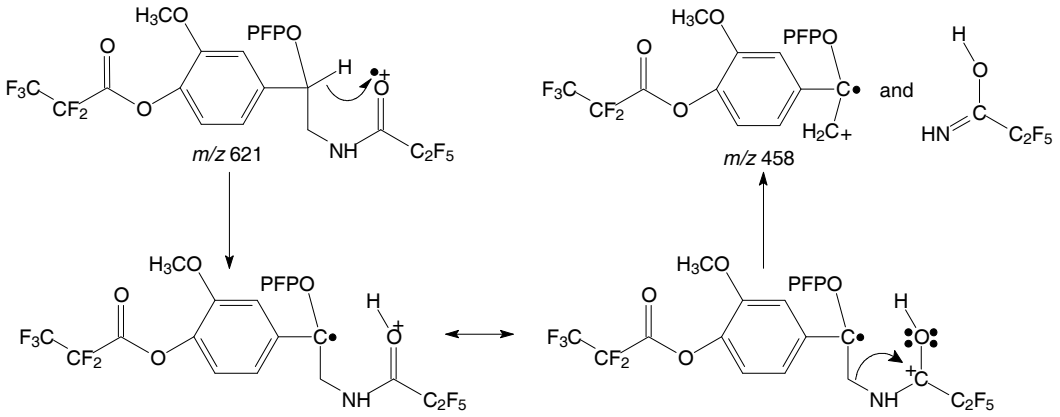


Scheme 1-3



Scheme 1-4

The fragmentation pathway leading to formation of the ion of *m/z* 176 may involve a $M^{+\bullet}$ species that has an electron-deficient site on the amide nitrogen, as shown in Scheme 1-4. Observing the peak at *m/z* 176, an even value, is consistent with another corollary to the *Nitrogen Rule*, which states that a *fragment ion* that retains an odd number of nitrogen atoms will have an even nominal mass.



Scheme 1-5

The predominant ion of *m/z* 458 is apparently formed by a so-called gamma-hydrogen rearrangement [62] (explained in detail in Chapter 6), which in this case also involves charge migration as indicated in Scheme 1-5. Observing the peak at *m/z* 458, an even value, for the designated species in Scheme 1-5 is consistent with the same corollary to the *Nitrogen Rule* as described above for the peak at *m/z* 445. The fragment ion of *m/z* 458 is an odd-electron species; the fragment ion of *m/z* 445 is an even-electron species.

The ion with *m/z* 417 is formed by the expulsion of CO (28 Da) from the fragment ion with *m/z* 445 [63]. This process is supported by the observation of a "metastable" peak at *m/z* 391 (not shown in Figure 1-21), which is consistent with the transition *m/z* 445 → *m/z* 417 (417^2 / 445 = 390.8). An explanation of metastable peaks [64] and their utility in interpretation of mass spectra is rarely used these days because they are not revealed with the use of modern data systems; instead, the technique of MS/MS is commonplace, as explained in Chapter 5. Further details on rationalization of fragmentation can be found in the strategy for interpretation of EI mass spectra in Chapter 6.

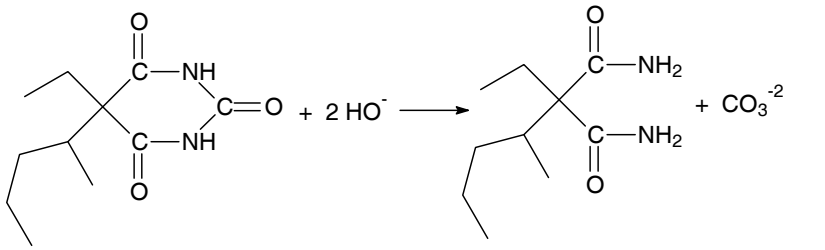
The major peaks in the mass spectrum (Figure 1-21) can be explained by reasonable cleavage processes for the proposed structure, thereby verifying the presence of such a structure. This means, the molecule whose structure is illustrated on the right of Figure 1-19 and is represented by the GC peak in Figure 1-20 is the product of the derivatization reaction.

4. Example 1-4: Use of a CI Mass Spectrum to Complement an EI Mass Spectrum

The barbituric acid derivative pentobarbital can be saponified by treatment with sodium hydroxide (Scheme 1-6). Because alkali can cleave one or more of the C–N bonds in the barbituric acid ring [65], it was of interest to verify that only C-2 had been removed from pentobarbital (III) to form the desired product, the malonamide (IV), which has a nominal mass of 200 Da. The purified reaction product was introduced into a mass spectrometer via the direct inlet probe to obtain the EI mass spectrum in Figure 1-21. No peak at *m/z* 200 was discernible in this mass spectrum or in others obtained at lower electron energies. Hence, there was considerable uncertainty concerning the sample

and the EI mass spectra obtained from it. The peak at *m/z* 183 could represent a $M^{+\bullet}$, but not of the expected compound. Furthermore, if the peak at *m/z* 183 does, in fact, represent a $M^{+\bullet}$, the peaks at *m/z* 157 and *m/z* 130 would indicate losses of 26 and 53 Da, respectively, from an ion of *m/z* 183; these fragmentations are not easily rationalized. A preliminary explanation might be that the spectrum in Figure 1-22 is possibly the combined EI mass spectra of two or more impurities in the sample that distilled off the probe before the expected compound. However, increasing the temperature of the probe to temperatures much higher than required for barbituric acid derivatives produced no additional mass spectra. Furthermore, as the putative reaction product had been recrystallized, it seemed unlikely that the sample would be impure.

It is also possible that the malonamide of pentobarbital (IV) does not produce a stable $M^{+\bullet}$ under EI; such a $M^{+\bullet}$ could readily lose ammonia (17 daltons) to produce an ion of *m/z* 183. However, for purposes of identification, it would be desirable to observe an ion that consists of the intact molecule.



Pentobarbital (III) Malonamide of Pentobarbital (IV)

Scheme 1-6

Chemical ionization (CI) using methane produced the mass spectrum in panel A of Figure 1-23. The series of peaks at *m/z* 201, 229, and 241 is consistent with the expected adduct ions of the series $[M + 1]^+$, $[M + 29]^+$, and $[M + 41]^+$, respectively, for CI by methane (see Chapter 7). Thus, the series of high-mass peaks appears to include a molecular species having a nominal mass of 200 Da. On the other hand, it is somewhat unusual to observe such intense low-mass peaks at *m/z* 156 and 184, although CI by methane can effect a significant amount of fragmentation of the intact molecule.

The ammonia CI spectrum shown in panel B of Figure 1-23 gives a more distinct indication of the intact molecule of the malonamide (IV). Because ammonia has a higher proton affinity than methane, its reagent ions effect protonation of the sample with less energy transfer; therefore, CI with ammonia results in less fragmentation of the ion representing the intact molecule than CI by methane (see Chapter 7). The predominant peak for the protonated molecule, MH^+, in panel B Figure 1-23 is a clear indication that the nominal mass of the analyte is 200 Da.

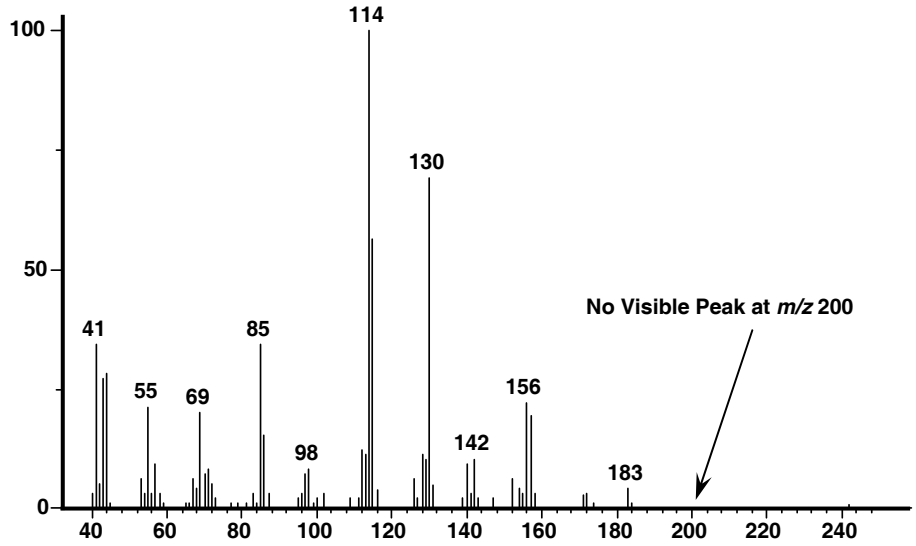


Figure 1-22. EI mass spectrum of the malonamide (IV) of pentobarbital.

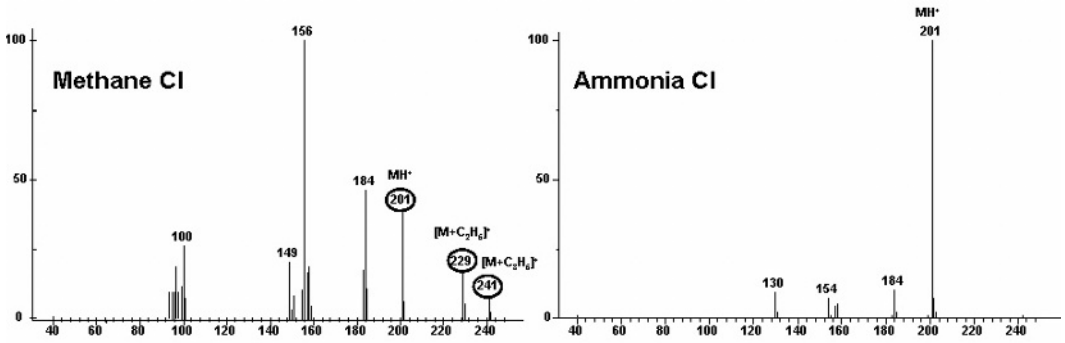


Figure 1-23. CI spectra of the malonamide of pentobarbital using methane (panel A) or ammonia (panel B) as reagent gas.

The CI spectra in panels A and B of Figure 1-23 confirm that the malonamide of pentobarbital is present and that an $[M + 1]^+$ of nominal mass 200 Da should be assumed when interpreting the EI mass spectrum in Figure 1-22. The peak at *m/z* 183 does represent a fragment ion $[MH - 17]^+$ formed by loss of ammonia from the $[M + 1]^+$, ($[M - NH_3]^+$). The ion of *m/z* 156 results from loss of one of the amide groups from the $[M + 1]^+$. The ion of *m/z* 130 is formed by elimination of pentene via a gamma-hydrogen rearrangement (explained in Chapter 6). Two possible routes of formation for an ion of *m/z* 114 could be seriously considered; the use of accurate mass data obtained from the EI of this malonamide would clarify which pathway is extant.

The important point illustrated by this example is the complementary information available from EI and CI. Structural information is available from the fragmentation pattern in EI, whereas CI provides nominal mass information of the intact molecule.

5. Example 1-5: Use of Exact Mass Measurements to Identify Analytes According to Elemental Composition

Several different types of modern mass spectrometers have sufficient resolving power and mass accuracy that two or more ionic species having the same nominal mass can be separated and analyzed accurately. For example, two ionic species of *m/z* 114.0555 and 114.0918 can be resolved easily into two separate peaks by a high-resolving power mass spectrometer, whereas a low-resolving power instrument (R = 500) would produce only a single mass spectral peak representing both species. A resolving power of 10,000 and mass accuracy of a few parts per million (ppm) is usually sufficient to define the elemental composition of an unknown ion having a nominal mass <500 Da. The availability of such data strengthens the basis for proper interpretation of a mass spectrum for purposes of structure identification [66, 67]. For example, measuring the mass of oligonucleotides to 0.01% restricts the possible compositions that must be considered for an unknown [68].

An appreciation of the value of accurate mass measurements can be gained by considering problems in the interpretation of a low-resolution unknown mass spectrum such as that in Figure 1-24. A reasonable start for interpreting such a mass spectrum might be to guess that the $M^{+\bullet}$ peak is at *m/z* 308. If the peak at *m/z* 308 is a $M^{+\bullet}$ peak, then the unknown does not contain an odd number of nitrogen atoms. It might even be reasonable to assume that the unknown does not contain any atoms of nitrogen because zero is an even number; further, the peak at *m/z* 279 is an odd number, also indicating that the corresponding ion does not contain an odd number of nitrogen atoms. The loss of 29 Da could represent the loss of an ethyl radical or the loss of an $HC^{\bullet}O$ radical from the $M^{+\bullet}$. The peak at *m/z* 238 might represent the loss of a C_5H_{10} (70 Da) molecule from the $M^{+\bullet}$ although this would be rather unusual. Unfortunately, as will become evident from data in Tables 1-1 and 1-2, some of these preliminary interpretations are off-base even though they do not violate the *Nitrogen Rule* or involve illogical losses.

If Figure 1-24 represented the only information available for this unknown, several different compounds having a nominal of 308 Da, including those that contained an even number of nitrogen atoms, would have to be considered. In fact, there are over 200 different combinations of carbon, hydrogen, nitrogen, and oxygen that have a

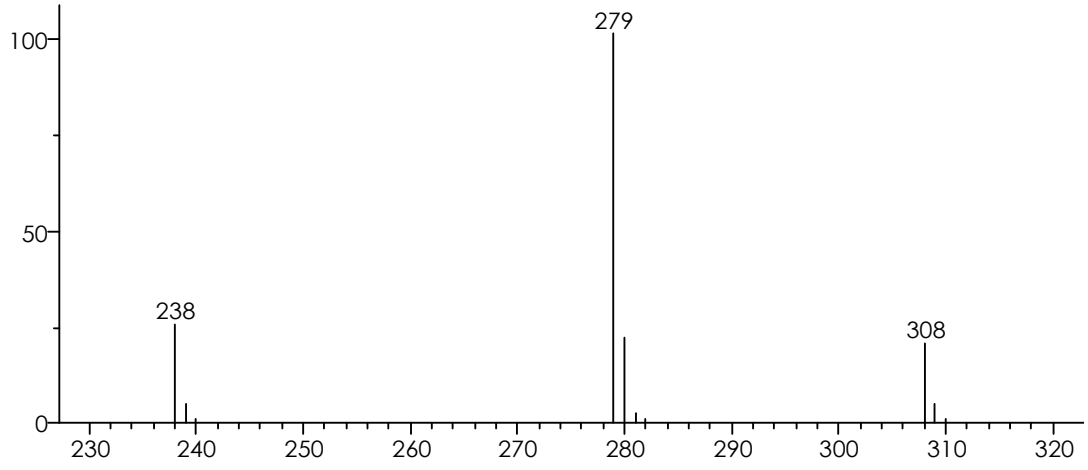


Figure 1-24. An abbreviated EI mass spectrum of an unknown compound.

nominal mass of 308 Da. Table 1-1 contains some representative listings of possible elemental compositions that have a nominal molecular weight of 308 Da, but different exact masses [69]. Some of these possibilities are implausible, such as the third entry, which indicates that 18 carbon atoms are substituted with only 4 hydrogen, 4 nitrogen, and 2 oxygen atoms. However, the vast majority are reasonable elemental compositions that are worthy of serious consideration unless specifically eliminated by other data that may be available for the unknown compound.

Table 1-1. Abbreviated listing of C, H, N, and O combinations that have a nominal mass of 308.

Elemental Composition				
C	**H**	**N**	**O**	**Exact Mass**
18	20	4	1	308.1637
18	12	–	5	308.0685
18	4	4	2	308.0334
19	2	1	4	307.9984
19	20	2	2	308.1525
19	36	2	1	308.2827
20	12	4	–	308.1062
20	24	2	1	308.1888

Table 1-2 shows experimentally determined accurate mass measurements for the unknown compound obtained during analysis with a double-focusing instrument. The second column lists the elemental composition that most closely agrees with the experimentally determined mass. The third column shows the calculated exact mass of the elemental composition in column two; this value was calculated by summing the exact monoisotopic masses of the designated elements and is provided to show the accuracy of the experimental value. As a further indication of the high probability that the elemental composition corresponds to the measured accurate mass, the fourth and fifth columns show the deviation in exact mass and in ppm between the measured accurate mass and the calculated exact mass, respectively.

The data in Table 1-2 indicate that there were some incorrect assumptions in the preliminary interpretation described above of the unknown low-resolution mass spectrum (Figure 1-24). For example, the exact mass of the molecular ion corresponds to an elemental composition of $C_{20}H_{24}N_2O$. There was no way to tell from the low-resolution data that the peak at *m/z* 308 represented an ion containing two atoms of nitrogen; a compound containing two atoms of nitrogen has an even nominal mass, just like compounds containing no nitrogen according to the *Nitrogen Rule*. The assumption that the $M^{+\bullet}$ lost an ethyl radical to give an $[M - 29]^+$ ion represented by the peak at *m/z* 279 was correct. The data in Table 1-2 indicate that peaks at *m/z* 308 and *m/z* 279 both represent ions containing two atoms of nitrogen and one of oxygen. The other incorrect assumption (besides the fact that the $M^{+\bullet}$ contained no atoms of nitrogen) was that the peak at *m/z* 238 (loss of 70 Da) represented the loss of C_5H_{10} from the $M^{+\bullet}$; in fact, it

Table 1-2. High-resolution mass spectral data on selected peaks of unknown mass spectrum.

Accurate Mass Determination	Most likely Corresponding Elemental Composition	Calculated Exact Mass	Δ mmu	Δ ppm
308.1878	$C_{20}H_{24}N_2O$	308.1888	−1.0	3.2
279.1510	$C_{18}H_{19}N_2O$	279.1497	1.3	4.6
238.1249	$C_{16}H_{16}NO$	238.1232	1.7	7.1

represents the loss of a C_4H_8N radical. Note that the last entry in column two of Table 1-2 indicates that the ion of nominal mass 238 contains only one atom of nitrogen and one atom of oxygen; therefore, one of the two nitrogen atoms in the $M^{+\bullet}$ is lost in forming this ion.

For many analyses, low-resolution mass spectra are adequate to solve the problem, because reference spectra or other data are available to help clarify any ambiguities that may arise from the data. In a few cases in which the sample is truly an unknown, and especially if no ancillary data are available, accurate mass measurements are essential to guide correct interpretation of the mass spectrum.

6. Example 1-6: Is This Protein Phosphorylated? If So, Where?

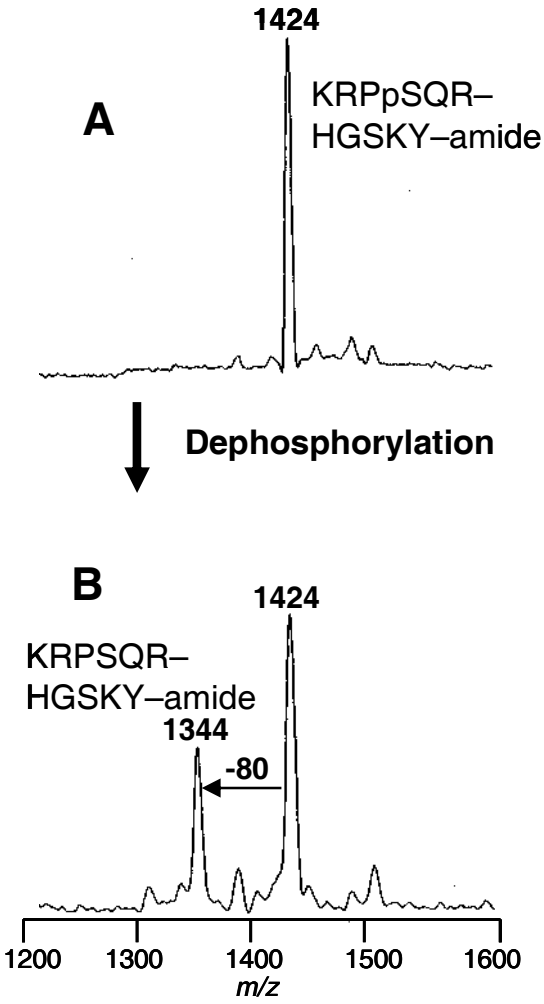


Figure 1-25. Matrix-assisted laser desorption/ionization (MALDI) mass spectrum of a phosphopeptide before (A) its treatment with phosphatase; (B) shift of 80 from m/z 1424 to m/z 1344 indicates loss of the phosphate group.

Determination of whether a given protein or peptide is phosphorylated involves analyzing the protein before and after treatment with phosphatase (an enzyme that cleaves a phosphate ester). If comparison of the data before and after treatment with phosphatase shows a shift in the molecular weight of the protein by multiples of 80 Da (the additional mass associated with attaching a phosphate group), then the original peptide or protein contains a number of phosphate groups equal to the multiples of 80 Da corresponding to the difference in molecular weights of the treated and untreated protein. Matrix-assisted laser desorption/ionization (MALDI) as described in detail in Chapter 9 can be used to analyze the peptide.

The MALDI mass spectrum of the phosphorylated model peptide amide is shown in the top panel of Figure 1-25; this spectrum has a peak at *m/z* 1424 corresponding to the protonated molecule. Treatment of the model peptide with alkaline phosphatase yields a peptide which, when analyzed by MALDI, presents the mass spectrum shown in panel B of Figure 1-25, which exhibits the appearance of a peak at *m/z* 1344, 80 *m/z* units lower than the peak at *m/z* 1424, corresponding to the removal of a phosphate group [70].

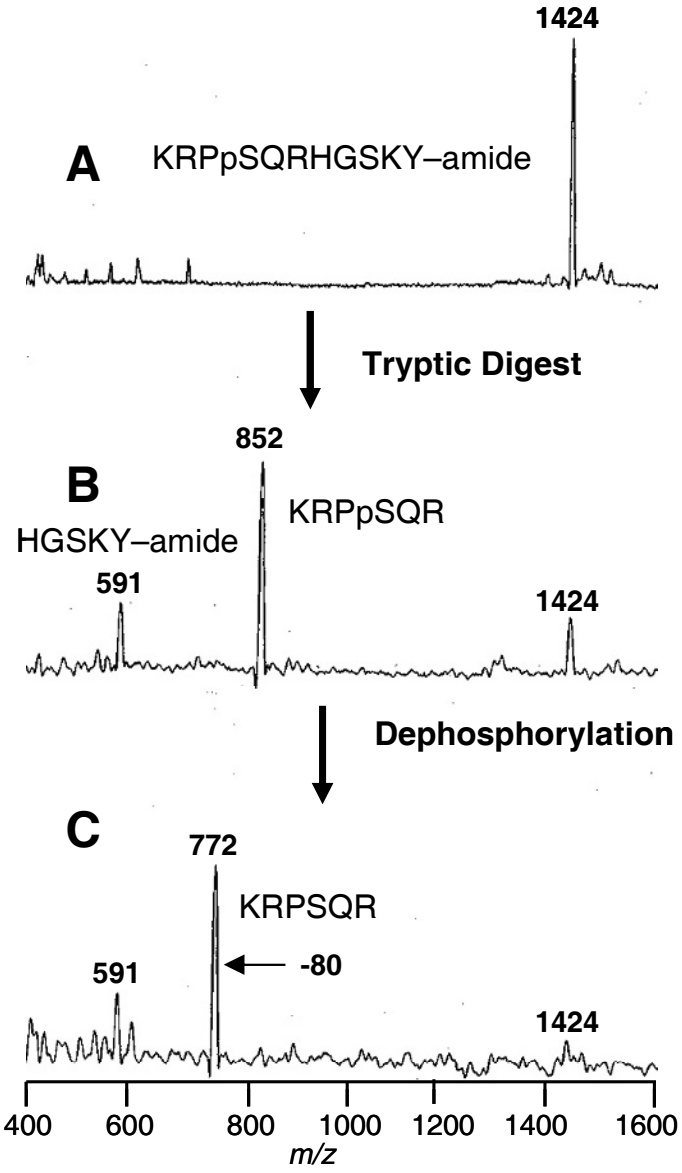


Figure 1-26. MALDI mass spectrum of phosphopeptide before (A) and after (B) treatment with trypsin in an effort to map the location of the phosphate group. (C): MALDI spectrum of tryptic digest after treatment with phosphatase. *Reprinted from Liao P-C, Leykam J, Andrews PV, Gage DA, and Allison J "An approach to locate phosphorylation sites in proteins by MALDI" Anal. Biochem. 1994, 219, 9-20, with permission from Academic Press.*

Because the location of the phosphate is of interest, all that is needed is to know the sequence of the peptide. If there were more than one potential site for phosphorylation (e.g., at serine, threonine, or tyrosine), the peptide could be treated enzymatically and then the mixture of degradation products would be analyzed by MALDI MS. The analytical process of peptide mass mapping to recognize the site of phosphorylation is demonstrated in the example illustrated in Figure 1-26 for a peptide having the following sequence: p-KRPSQRHGSKY-amide. Upon treatment of the peptide with trypsin, it would be expected that cleavage at the C-terminal side of arginine (R) would occur to yield two peptide fragments as represented in the middle panel of Figure 1-26. The peak at *m/z* 591 corresponds to HGSKY-amide, which computes to a protonated mass of 591 Da without the phosphate on either of the two possible phosphorylation sites at S9 or Y11. The peak at *m/z* 852 corresponds to KRPpSQR as a protonated molecule containing the phosphate group. Whereas the spectrum in panel B shows, by deduction, that the phosphate group must be on S4, treatment of the peptide mixture with alkaline phosphatase with subsequent analysis of that mixture by MALDI produces the data in panel C to confirm this conclusion; i.e., the peak at *m/z* 591 remains in the MALDI mass spectrum in panel C, indicating that the peptide HGSKY-amide did not contain the phosphate as the mass of this peptide fragment did not shift. On the other hand, there is no peak at *m/z* 852 in panel C, whereas there is a peak at *m/z* 772, 80 *m/z* units lower than the peak at *m/z* 852. This gives direct evidence that treatment of this peptide fragment with alkaline phosphatase removed a phosphate group, which had to be at S4 as this is the only phosphorylatable site present in this peptide fragment [70].

7. Example 1-7: Clinical Diagnostic Tests Based on Quantitation of Stable Isotopes by Mass Spectrometry in Lieu of Radioactivity

Many diagnostic methods for assessment of metabolic disorders are not available to a large segment of the population (pregnant or pediatric patients) because the tests involve oral or intravenous administration of radioactivity. However, with the increasing availability of stable isotope-labeled compounds, many existing methods can be converted to a protocol utilizing nonradioactive biochemical probes to obtain the same diagnostic information without the hazard of radiation [71–75].

A breath test for malabsorption is a noninvasive means of evaluating or recognizing this disorder. The absorption of lipid nutrients is essential for the growth and development of newborn infants [76]. Malabsorption in adults can be evaluated by administering 1-^{14}C-fatty esters and measuring the rate of production of radioactive CO_2 in the breath as the radioactive lipids are absorbed by the gut and metabolized by the liver [77]. A similar method involving administration of a ^{13}C-labeled lipid and measurement of expired $^{13}CO_2$ by mass spectrometry can be used to assess malabsorption in children, because the test imposes no radiation hazard [78].

The breath test for malabsorption consists of administering a test meal containing a dose (10 mg/kg) of 1-^{13}C-trioctanoin, a medium-chain triglyceride [78]. Octanoic acid, liberated in the small intestine by lipase, is absorbed and rapidly transported in portal blood to the liver, where it is quantitatively oxidized to CO_2. Expired air is collected by means of a face mask for a 5-min period every 30 min for 2 hr. The CO_2 is trapped by bubbling the expired air through 10 ml of carbonate-free sodium hydroxide. This solution can be stored or transported conveniently prior to analysis, at which time it is treated with acid to release quantitatively the carbon dioxide, which is transferred to an inlet reservoir on the mass spectrometer.

A typical plot of analytical results of the breath test is presented in Figure 1-27. At 1 hr after ingesting the dose of 1-^{13}C-trioctanoin, the abundance of $^{13}CO_2$ in the respiratory carbon dioxide of normal children is approximately five times greater than that in the breath of children suffering from cystic fibrosis (see dashed line in Figure 1-27) [71]. This means, this breath test readily differentiates patients with normal fat absorption from those with significant fat malabsorption.

Other tests involving biochemical probes with appropriate ^{13}C- and ^{2}H-labeled biochemicals play an important role in the diagnosis of metabolic disorders because the tests are simple, noninvasive, and sensitive and involve no radiation hazard. However, it must be emphasized that reliable quantitation of the slight changes in the relative abundances of ^{2}H and ^{13}C requires an isotope ratio mass spectrometer [79, 80].

V. The Need for Chromatography

In the evolution of mass spectrometry, there have been efforts to analyze samples that are complex mixtures without preliminary separation. In some cases, LC/MS instrumentation is used to analyze a sample by direct infusion without ever trying for a chromatographic separation. MS/MS has changed mass spectrometry. At one point, shortly after the development of the triple-quadrupole mass spectrometer, Graham Cooks, another venerable mass spectrometrist said, "There is no longer a need for GC/MS. All necessary separations of analytes of interest can be accomplished using MS/MS." Interestingly, some thirty years later, chromatography plays an even bigger role than it did at the time the triple quadrupole was being developed.

Mass spectrometry can provide useful qualifying information for a pure substance; however, mass spectral data obtained from a mixture are often not useful. This is especially true of EI mass spectra, which result from extensive fragmentation. In

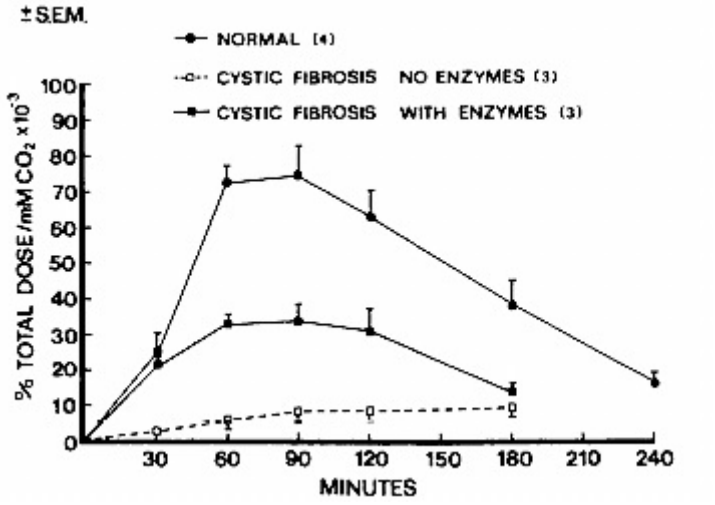


Figure 1-27. Plot of excess $^{13}CO_2$ beyond natural abundance in the breath of normal vs cystic fibrosis patients following an oral dose (10 mg kg^{-1}) of 1-^{13}C-trioctanoin. *Reprinted from Barr RG, Perman JA, Schoeller DA, and Watkins JB "Breath tests in pediatric gastrointestinal disorders: new diagnostic opportunities" Pediatrics 1978, 62(3), 393–401, with permission from the American Academy of Pediatrics.*

the case of analyzing a mixture, the fragmentation patterns of the various components overlap, and much of the interpretative value is lost by confounding. The mass spectrometer is more reliable as a separation device when soft ionization techniques like ESI, APCI, and APPI are employed. Even in these situations, analytes of different structures and elemental composition can form ions representing the intact molecule that have the same nominal *m/z* value. These analyses require chromatography for separation before they ever "see" the mass spectrometer. Simplified mass spectra, consisting principally of ions representing the intact molecules, can be acquired from simple mixtures of two to five components. The complexity of other mixtures may need to be simplified by some form of coarse separation prior to analysis by the above-referenced soft ionization techniques or MALDI.

Chromatography, with its capacity to separate similar compounds based on subtle structural features, can be used to isolate components so that they can be analyzed individually. In many cases, it is possible to use chromatography as an inlet system for MS to provide for on-line separations and acquisitions of mass spectra of individual components as described in detail in Chapters 10 (GC/MS) and 11 (LC/MS). Although it is easy to view a chromatography system as an inlet for a mass spectrometer, it is important to keep in mind that a chromatograph/mass spectrometer system is as different from either a chromatograph or a mass spectrometer as a chromatograph and a mass spectrometer are from one another.

VI. Closing Remarks

The field of mass spectrometry has grown beyond imagination since the beginning of commercial instruments in the 1940s. This is exemplified by the six current journals dedicated to mass spectrometry and the fact that two of these journals have been published since1968: *Journal of Mass Spectrometry* (formed in 1995 by the merger of *Organic Mass Spectrometry* (1968) and *Biological Mass Spectrometry* (1974) by John Wiley and Sons, Inc.) and the *International Journal of Mass Spectrometry* (originated in 1968 as the *International Journal of Mass Spectrometry and Ion Physics* by Elsevier). The other mass spectrometry journals are *Mass Spectrometry Reviews* (John Wiley & Sons, Inc., 1982), *Rapid Communications in Mass Spectrometry* (John Wiley & Sons, Inc., 1987), the *Journal of the American Society for Mass Spectrometry* (American Society for Mass Spectrometry, 1990), and *Journal of European Mass Spectrometry* (IM Publications, 1995). In addition to these six dedicated publications, many articles dealing primarily with mass spectrometry appear in countless other journals such as the *Journal of Chromatography A* and *B*, *Analytical Chemistry*, *Journal of Chromatographic Science*, *Journal of Toxicology*, etc., to name just a few.

The American Society for Mass Spectrometry's Annual Meeting on Mass Spectrometry and Allied Topics, held for the past fifty-five years in various locations in North America, now attracts over six thousand people. The triennial International Mass Spectrometry Conference (held in various locations in Europe to this point), having its official beginning in 1958 but tracing its origins back to a meeting held in Manchester, England, April 20–21, 1950, and organized by The Institute of Petroleum, is seeing the same types of increases in attendance as being seen by ASMS. All of this is occurring while other meetings, such as the annual Pittsburgh Conference held in various cities in the United States, are seeing significant declines in attendance. As mass spectrometry went from the accurate mass determination of nuclides to the determination of the nominal mass and the elucidation of the structure of volatile organic compounds to the ability to provide mass and structure information of macromolecules in the condensed phase, it has proven to be the technique that provides more information from less sample than any other spectrometric method or other analytical technique, to paraphrase the statement at the end of the first paragraph of this chapter.

VII. Monographs on Mass Spectrometry Published Before 1970.

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1920–1930 (2)

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2. Aston, FW *Isotopes*, 2nd ed.; Edward Arnold: London, 1924; 1st ed., 1922.

1930–1940 (7)

1. Aston, FW *Mass Spectrometry and Isotopes*, 2nd ed.; Edward Arnold: London, 1st ed., 1933.
2. Mott, NF; Massey, HSW *The Theory of Atomic Collisions*, 1st ed., 1933; Oxford University Press: London.
3. Bauer, SH *A Mass Spectrograph: Products and Processes of Ionization in Methyl Chloride*; 1935; iv, 51 pp; illustrations.
4. Cartan, L *Spectrographie de Masse: Les Isotopes et Leurs Masses*; No. 550 in the series *Actualités Scientifiques et Industrielles*; No. VI in the subseries *Exposés de Physique Atomique Expérimentale*; Preface by Maurice de Broglie; Hermann & Cie: Paris, 1937; pp 3–90, pbk.
5. Massey, HSW *Negative Ions*, 3 rd ed. (741 pages); Cambridge at the University: London, 1976; 1950; 2nd ed. (136 pages); 1st ed. (105 pages), 1938.
6. Cartan, L *L'optique des Rayons Positifs et ses Applications à la Spectrographie de Masse*; Hermann & Cie: Paris, 1938; p 80.
7. Thibaud, J; Cartan, L; Comparat, P *Quelques Techniques Actuelles en Physique Nucléaire*, subtitle that appears to be the contents *Méthode de la Trochoïde: Électrons Positifs: Spectrographie de Masse: Isotopes: Compteurs de Particules à Amplification Linéaire Compteurs de Geiger et Müller*; Hermann & Cie: Paris, 1938; p 276.
8. Loeb, LB *Fundamental Processes of Electrical Discharge in Gases*; Wiley: New York, 1939.

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1950–1960 (17, 6 Proceedings)

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2. *Mass Spectrometry,* a report of a conference organized by The Mass Spectrometry Panel of The Institute of Petroleum, London, April 20, 21, 1950; The Institute of Petroleum: London, 1952. (a **Proceedings**)
3. Ewald, H; Hintenberger, H *Methoden und Anwendunyngen der Massenspektroskopie*; Verlag Chemie: Weinheim, Germany, 1952 (English translation by USAEC, Translation Series AEC-tr-5080; Office of Technical Service: Washington, DC, 1962).
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5. Bainbridge, KT "Part V: Charged Particle Dynamics and Optics, Relative Isotopic Abundances of the Elements, Atomic Masses" in *Experimental Nuclear Physics*, Vol. 1; Sergè, E, Ed.; Wiley: New York, 1953; pp 559–767.
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The following is a listing of the proceedings published for the two major regularly held mass spectrometry meetings during the period of 1960 to 1970. These meetings continue to be held and should be continuing.

1. *Proceedings of the nth Annual ASMS Conference on Mass Spectrometry and Allied Topics* published annually; American Society for Mass Spectrometry: Santa Fe, NM (ASTM E14 Committee Meetings began in 1952; published proceedings annually from 1961 through 1969 by the ASTM E14 Committee as the *Proceedings of the nth Annual Conference on Mass Spectrometry and Allied Topics*; ASMS began in 1970 and published the Proceedings of the 18th Conference). Beginning with the 1999 meeting, the Proceedings are only available on CD-ROM.

2. *Advances in Mass Spectrometry,* Proceedings of the Triennial International Mass Spectrometry Conference. Vols. 1 (1958, UK), 2 (1961, UK), 3 (Paris, France, 1964), 4 (Berlin, Germany, 1967, were published during the 1960–1970 period. Over the past 50+ years, this conference has been held. Different publishers have produced different ones of the proceedings of these conferences.

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