11 chapter

Mass Spectrometry

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11.1 INTRODUCTION

Over the past decade, **mass spectrometry** (MS) techniques have become indispensable for the identification, characterization, verification, and quantitation of small molecules (e.g., caffeine, 194 Da) to large complex biomolecules (e.g., immunoglobulin, 144,000 Da). Two important developments led to the rapid rise in popularity of MS as an analytical technique. First was the development of **hyphenated** MS techniques, which coupled the separation techniques of gas chromatography (GC) (Chap. 14) or liquid chromatography (LC) (Chap. 13) to MS. This coupling of chromatography and MS dramatically lowered the detection limits for quantitative analysis while simultaneously increasing the confidence of measurement through high specificity. Second was the development of **hybrid**, benchtop, MS instruments that made high-resolution, accurate mass, LC-MS analysis routine. Hyphenated, hybrid MS techniques deliver robust, highly sensitive, precise measurements that withstand the rigor of statistical analysis for the purposes of quantitative analysis, while significantly reducing sample preparation time and effort. These advantages made MS a "must-have" technique when faced with complex bioanalytical challenges such as pesticide screening in foods, trace analysis of environmental pollutants, characterization of natural products, or rapid identification of food-borne bacteria.

The power of the MS technique is due to its ability to place a charge on a molecule, thereby converting it to an **ion** in a process called **ionization**. The generated ions are then separated according to their **mass-to-charge ratio** (m/z) by subjecting them to a combination of radio-frequency (RF) and electrostatic fields in a **mass analyzer** and finally detected by highly sensitive **detectors**. The resulting signals from the detectors are digitized and processed by software to display the information as a mass spectrum, which reveals its molecular mass and its structural composition, leading to identification. An additional stage of **ion fragmentation** may be included before detection to elicit structural information in a technique known as **tandem MS**.

The most common MS technique remains GC-MS which was first used in the late 1960s, followed by the rapidly growing LC-MS technique which made ionization from liquids possible and started to gain adoption in the late 1980s, and matrix-assisted laser desorption ionization (MALDI) or MALDI time-of-flight (TOF) techniques which offers ionization from solid crystals discovered in 1988.

11.2 INSTRUMENTATION: THE MASS SPECTROMETER

11.2.1 Overview

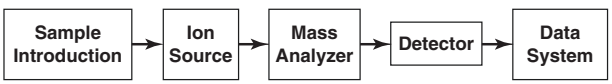
Because there are so many acronyms associated with the MS instrumentation, a listing of the acronyms used in this chapter is given. Many of those acronyms are first used in Table 11.1, which summarizes mass spectrometer components and types of instruments. This table also helps introduce the three basic functions a MS performs. (1) There must be a way to **ionize** the molecules, which occurs in the ion source by a variety of techniques. (2) The charged molecular ion and its fragments must be **separated** according to their m/z, and this occurs in the **mass analyzer** section. (3) The separated, charged ions must be detected (electron multipliers, photomultipliers). The block diagram in Fig. 11.1 represents the various components of a mass spectrometer.

Sample introduction can be **static** (or **dynamic**, the latter of which involves interfacing with GC or LC instruments. Since all mass spectrometers work in high vacuum, regardless of the state of the sample (gas, liquid, or solid), all ions are introduced into the MS as a gas. The MS **interface** converts the samples into a form that is acceptable to introduction into the vacuum chamber. Common MS interfaces will be discussed in more detail in the sections on GC-MS and LC-MS.

Figure 11.2 depicts the interior of a typical GC-MS instrument that uses quadrupole mass analyzers. The region between ion generation and detection is maintained by different vacuum pumps. Each successive region from the ion source is kept at lower vacuum than the preceding region, with the mass analyzer/detector being in the region of strongest vacuum ($\approx 10^{-6}$–10^{-8} torr). A vacuum is necessary for two critical reasons: (1) to avoid ion-molecule reactions between the charged ions and other gaseous molecules before they reach the detector, and (2) for proper operation of ion lenses, mass analyzer electrodes, and ion-detectors that require the use of high voltages. Vacuum performance determines the sensitivity and resolution of mass spectrometers.

11.1 table Summary of mass spectrometer components and types

	Types	*Applications*
Sample introduction		
Static method	Direct injection	Gas or volatile liquid
	Direct insertion probe	Solids
Dynamic	GC	Gas or volatile liquids
	LC	Nonvolatile solids or liquid
Ion source	Electron impact ionization (EI)	Primarily for GC-MS, for volatile compounds
	Electrospray ionization (ESI)	Most popular method for LC-MS, normally for polar or slightly polar compounds
	Atmospheric pressure chemical ionization (APCI)	Primarily for LC-MS, normally for compounds of low polarity and some volatility
	Atmospheric pressure photoionization (APPI)	Same uses as APCI but has advantages in signal-to-noise ratio and detection limit
	Matrix-assisted laser desorption ionization (MALDI)	A "soft ionization," ideal for large biopolymers and other fragile molecules
	Chemical ionization (CI)	A "soft ionization," ideal for large biopolymers and other fragile molecules
Mass analyzers	Quadrupoles mass analyzer/filter (Q)	Used in many types of instruments. Compact. Used in benchtop instruments
	Ion trap (IT)	LC-MS for MS/MS
	Time of flight (TOF)	Useful to analyze biopolymers and large molecules
	Fourier transform-based mass analyzer (FT-ion cyclotrons, FT-ICR; FS-orbitrap)	Allows for easy-to-use benchtop LC-MS
	Magnetic sector	Specialized applications requiring ultrahigh resolution, e.g., dioxin analysis
	Isotope ratio MS	Useful in geochemistry and nutrition science. Extreme specificity
	Accelerator mass spectrometer	Useful in geochemistry and nutrition science. Extreme specificity
Hybrid MS: common combinations of mass analyzers	Quadrupole TOF (e.g., Q-TOF, triple TOF)	Most LC-MS. Provides for MS/MS, benchtop instruments
	Triple quadrupole (e.g., TQ; tandem MS)	Common for LC-MS. Provides for MS/MS, benchtop instruments
	Ion trap (e.g., IT-FTMS, IT-orbitrap, Q-Trap)	Most LC-MS. Provides for MS/MS. Very high mass accuracy
Common MS instruments	Quadrupole MS (single quadrupole or TQ)	Quantitative and qualitative analysis
	ITMS	Qualitative analysis. Advantage of multistages of MS (MS^n)
	TOF/Q-TOF	High-resolution accurate mass needs
	FTMS	High-resolution accurate mass needs

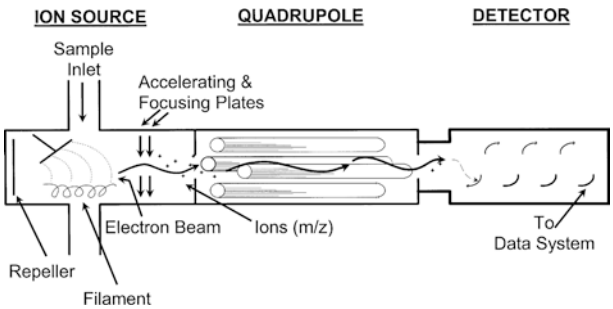


11.1 figure A block diagram of the major components of a mass spectrometer

11.2.2 Sample Introduction

11.2.2.1 *Static Method*

The initial step in operating the MS is to get the sample into the ion source chamber. Pure compounds or sample extracts that are a gas or a volatile liquid are injected directly into the source region. This requires no special



11.2 figure Schematic of a typical mass spectrometer. The sample inlets (interfaces) at the *top* and *bottom* can be used for direct injection or interfacing to a GC

equipment or apparatus and is much the same as injecting a sample into a GC. Thus, this **static method** of introducing the sample to the source is called **direct injection**. With solids that are at least somewhat volatile, the **direct insertion probe method** is used, in which the sample is placed in a small cup at the end of a stainless steel rod or probe. The probe is inserted into the ion source through one of the sample inlets, and the source is heated until the solid vaporizes. The mass spectrum is then obtained on the vaporized solid material as with the direct injection method. Both direct injection and direct insertion probe methods work well with pure samples, but their use is very limited when analyzing complex mixtures of several compounds.

Direct analysis in real time (DART) is an example of a static sampling technique, where metastable He ions (19.8 eV energy) are used to initiate ionization of the analyses of interest via the Penning process (much like EI) resulting in radical cations ($M^{+\cdot}$). A mixture of heated He and nitrogen is used to initiate the metastable ionization process, essentially creating a plasma-rich environment, wherein the metastable He reacts with ambient water, creating protonated water clusters, resulting ultimately in a charge transfer to the analyte of interest. The process has been well described by Hajslova et al. [3] for food QC and safety analysis.

11.2.2.2 *Dynamic Method*

For mixtures, sample introduction is a **dynamic method** in which the sample must be separated into the individual compounds and then analyzed by the MS. This is done typically by GC or HPLC units connected to an MS by an **interface** (see Sects. 11.4 and 11.5). The interface removes excess GC carrier gas or HPLC solvent that would otherwise overwhelm the vacuum pumps of the MS.

11.2.3 Ionization

There are many methods used to produce ions for the compounds, depending on the type of chromatographic interface and nature of the compounds (Table 11.1). The major types of ion sources are briefly described in subsections that follow.

11.2.3.1 *Electron Impact Ionization (EI)*

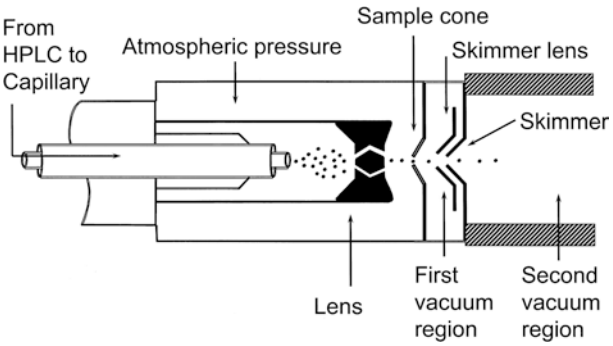
In GC-MS techniques, once the compound(s) coming from the GC enters the ion source, it is exposed to a beam of electrons emitted from a filament composed of rhenium or tungsten metal. When a direct current is applied to the filament (usually 70 electron volts, eV), it heats and emits electrons that move across the ion chamber toward a positive electrode. As the electrons pass through the source region, they come in close proximity to the sample molecule and extract an electron, forming an ionized molecule. Once ionized, the molecules contain such high internal energies they can further fragment into smaller molecular fragments. This entire process is called **electron impact** (EI) **ionization**, although the emitted electrons rarely hit a molecule.

11.2.3.2 *Electrospray Ionization (ESI)*

Electrospray ionization, the most popular LC-MS technique in use today, functions at atmospheric pressure and is a highly sensitive technique. Normally, polar compounds are amenable to ESI analysis, with the type of ion produced depending on the initial charge. That is, positively charged compounds yield positive ions, while negatively charged compounds such as those containing free carboxylic acid functional groups will produce negative ions.

The ESI source as depicted in Fig. 11.3 consists of a nozzle that contains a fused-silica capillary sample tube (serves to transfer the LC effluent) coaxially positioned within a metal capillary tube to which a variable electrical potential can be applied against a counter-electrode, which is usually the entrance to the MS. Compressed nitrogen gas at high velocity is coaxially introduced to aid in the nebulization of the LC effluent as it exits the tip of the metal capillary tube. The relative velocity difference between the streams of nitrogen gas (fast moving) and LC effluent (slow moving) at the ESI tip results in producing a fine spray of highly charged droplets. At nanoflow rates (<1 μL/min), the force of the electrical field is strong enough to break up the LC effluent into fine droplets without the use of nebulizing gas, in a process known as nanospray. For conventional HPLC flow rates (1–1000 μL/min), the sheer volume of liquid requires an initial droplet size reduction through the use of nebulizing nitrogen gas, creating the required microdroplets, which can now be influenced by the prevailing electrical field.

At this point, the repulsive forces due to the accumulation of "like" charges inside the rapidly reducing microdroplet volume create an imbalance with the forces of surface tension that are trying to conserve the



11.3 figure Schematic of an electrospray LC-MS interface

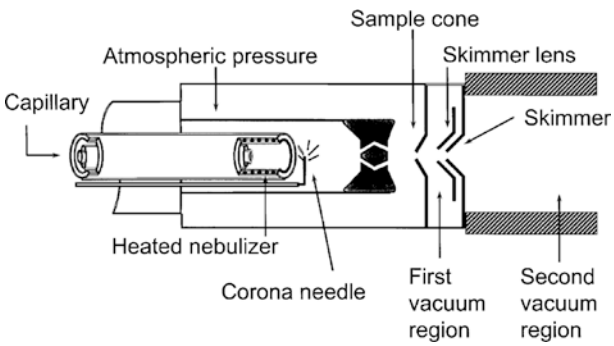
spherical structure of the microdroplet. The positive charge is drawn out, but cannot escape the surface of the liquid, and forms what is known as a **Taylor cone**. Further reduction of the diameter of the droplets causes the Taylor cone to stretch to a critical point, at which the charge escapes the liquid surface and is emitted as a gas-phase ion in a process known as a **coulombic explosion**.

One of the many advantages of the ESI process is its ability to generate multiple-charged ions and tolerate conventional HPLC flow rates. Proteins and other large polymers (e.g., between 2000 and 70,000 Da) can be easily analyzed on LC–MS systems having a mass limit of m/z 2000, due to this multiple charging phenomenon. Powerful software can process in excess of +50 charge states, to yield the molecular ion information for larger proteins. A limitation of the ESI process is the phenomenon of ion suppression/enhancement or matrix effects, which usually causes a variation in response for the analyte signal intensity in presence of matrix components. Matrix factor corrections are used to account for ion suppression/enhancement effects, including the use of stable-labeled internal standards or matrix-assisted calibration curves for quantitative analysis.

11.2.3.3 *Atmospheric Pressure Chemical Ionization (APCI)*

The **APCI interface**, which like ESI operates at atmospheric pressure, is normally used for compounds of low polarity and some volatility. It is harsher than ESI and is a gas-phase ionization technique. Therefore gas-phase chemistries of the analyte and solvent vapor play an important part in the APCI process.

Figure 11.4 shows the schematic diagram of an APCI interface. The LC effluent-carrying fused-silica capillary tube protrudes about halfway inside a silicon-carbide (ceramic) vaporizer tube. The vaporizer tube is maintained at approximately 400–500 °C and serves to vaporize the LC effluent. High voltage is applied to a corona needle positioned near the exit of the vaporizer tube. The high voltage creates a **corona discharge** that forms reagent ions from the mobile phase and nitrogen nebulizing gas. These ions react with the sample molecules (M) and convert them to ions. A common cascade of reactions occurring in the presence of water, nitrogen gas, and the high-voltage corona discharge is as follows:



11.4 figure Schematic of an atmospheric pressure chemical ionization LC–MS interface

$$e^- + N_2 \rightarrow N_2^{+\bullet} + 2e^- \quad (11.1)$$

$$N_2^{+\bullet} + H_2O \rightarrow N_2 + H_2O^{+\bullet} \quad (11.2)$$

$$H_2O^{+\bullet} + H_2O \rightarrow H_3O^+ + OH^\bullet \quad (11.3)$$

$$M + H_3O^+ \rightarrow (M+H)^+ + H_2O \quad (11.4)$$

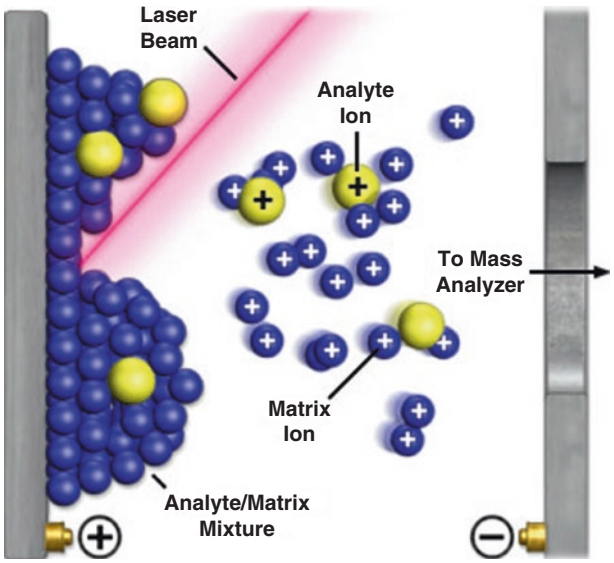
The APCI interface is a robust interface and can handle high flow rates of up to 2 mL min. It is unaffected by minor changes in buffer strength or composition and is typically used to analyze molecules less than 2000 Da. It does not facilitate multiple charges and hence cannot be used to analyze large biomolecules/polymers. In terms of matrix effects, APCI usually shows "ion enhancement" rather than "ion suppression." This is due to the matrix components enriching the plasma generation process, thereby enhancing the efficiency of the ionization process. As a result, there is an increase in response for the analyte signal in the presence of matrix components, requiring matrix factor correction through the appropriate use of stable-labeled internal standards or matrix-assisted calibration curves for quantitative analysis.

11.2.3.4 *Atmospheric Pressure Photoionization (APPI)*

APPI is an ionization technique that improves on the interface possible with APCI. The APPI interface, which uses a krypton or xenon light source to generate a beam of photons instead of a corona discharge-generated plasma as in APCI. Compounds having ionization potentials lower than the wavelength of the light source will be ionized. Since most HPLC solvents do not ionize at the wavelengths generated by the commonly used photon sources, APPI improves in the signal-to-noise ratio and hence detection limits.

11.2.3.5 *Matrix-Associated Laser Desorption Ionization (MALDI)*

In MALDI, the sample is dissolved in a matrix and ionized using an UV laser. The matrix plays an important role in ionization, acting both as the absorber of the laser energy, which causes it to



11.5 figure

Diagram of the MALDI desorption and ionization process used in some TOF instruments (From Chughtai and Heeren [2], used with permission of National High Magnetic Field Laboratory, www.magnet.fsu.edu)

vaporize, and as a proton donor and acceptor to initiate charge transfer to the analyte (Fig. 11.5). Since the sample is not directly ionized, MALDI is considered a "soft ionization" technique and amenable to ionization of large biopolymers and other fragile molecules such as nucleic acids or carbohydrates [2]. The matrix used in MALDI is usually a weak organic acid with UV-absorbing properties [e.g., 2,5-dihydroxy benzoic acid (DHB), sinapinic acid (3,5-dimethoxy-4-hyroxycinnamic acid), gentisic acid (DHDA, 2,5-dihydroxybenzoic acid), or α-cyano-4-hydroxycinnamic acid (CHCA)]. Sensitivity is usually dependent on critical pairing of the chemistries of matrix with the sample, especially for samples that are inherently nonvolatile or insoluble in most aqueous solvents.

The typical laser used for MALDI applications is neodymium-doped yttrium aluminum garnet (Nd-YAG) nitrogen laser operating at 337 or 355 nm (3.7–3.5 eV photon energies) in vacuum and pulsed at a repetition rate between 1 and 10 KHz. The laser beam size can be attenuated between 5 and 100 um, which allows hundreds of laser shots to raster through a single sample spot. While the ionization mechanism is not fully understood, it is believed that a two-step process occurs; in step one the matrix absorbs the UV energy from the laser and is consequently ionized $(M+H)^+$; in step two a charge transfer to the sample $(S+H)^+$ is completed, allowing the charged sample to be focused into the mass analyzer. Infrared lasers also are used but are less popular, as is the case with atmospheric pressure-based MALDI ion sources.

11.2.3.6 *Matrix Effects on Ionization*

One key issue with all types of ionization is a phenomenon called matrix effect. This is when ionization of a molecule is either suppressed or enhanced by coeluting endogenous interferences contained in the matrix after sample cleanup. This effect has a direct impact on sensitivity; for the same level (e.g., 1 ng/mL), the ion intensity of the compound of interest will change in response to the coeluting matrix interferences such as salts, fatty acids, phospholipids, etc. For high-sensitivity quantitative MS analysis, a study of matrix effects is essential before quantitation can be performed. For example, if the matrix is spinach extract versus corn extract, each matrix will have to be individually studied for matrix effects. A set of test pesticide standards at known levels are spiked in a clean matrix (spinach or corn not exposed to pesticides) and their peak intensities compared to the same level spiked in pure solvent. The difference in peak intensities between the pure standard and matrix-spiked standard will determine the matrix effect during the final analysis of the samples to be tested. While all modes of ionization are susceptible to matrix effects, ESI seems to be most prone to ion suppression, while techniques such as APCI can be prone to ion enhancement, wherein the peak intensity increased in the presence of matrix as compared to the pure standard.

11.2.3.7 *Transition from Ion Source to Mass Analyzer*

The eventual outcome of the ionization process, by any of the methods described in sections above, is both negatively and positively charged molecules of various sizes unique to each compound. When the repeller plate at the back of the ion source is positively charged, it repels the positive fragments toward the quadrupole mass analyzer. Thus, we look only at the **positive fragments**, although negative fragments are sometimes analyzed. As the positively charged fragments leave the ion source, they pass through holes in the accelerating and focusing plates. These plates serve to increase the energy of the charged molecules and to focus the beam of ions, so that a maximum amount reaches the mass analyzer.

11.2.4 Mass Analyzers

11.2.4.1 *Overview*

The heart of an MS is the **mass analyzer**. It performs the fundamental task of separating the charged molecules or their fragments based on their m/z, and it dictates the mass range, accuracy, resolution, and sensitivity. Listed in Table 11.1 are the basic types of mass analyzers, the common combinations of basic mass analyzers (call **hybrid MS**), and the most common types of MS instruments, along with their typical applications. Described in the subsections below

are only the four types of mass analyzers most commonly applied to food analysis.

11.2.4.2 *Quadrupole Mass Analyzers (Q)*

The word "quadrupole" of the quadrupole mass analyzer is derived from the Latin words for "fourfold" (quadruplus), and "pole," to describe the array of four rods that are used (Fig. 11.2). The four rods are used to generate two equal but out-of-phase electric potentials: one is alternating current (AC) frequency of applied voltage that falls in radio-frequency (RF) range, and one is direct current (DC). The potential difference can be varied to create an oscillating electrical field between two of the opposite rods, resulting in their having equal but opposite charges.

When, for example, a positive-charged ion enters the quadrupole field, it will be instantly attracted toward a rod maintained at a negative potential, and if the potential of that rod changes before the ion impacts, it will be deflected (i.e., change direction). Thus, every stable ion (i.e., ion with stable flight path) entering the quadrupolar region traces a sine wave-type pattern on its way to the detector. By adjusting the potentials on the rods, selected ions, a mass range, or only a single ion can be made stable and detected. The unstable ions impact one of the four rods, releasing them from the influence of the oscillating field, and they are pumped away by the vacuum pumps. A quadrupole mass analyzer is commonly referred to as a **mass filter**, because, in principle, the device filters ions that achieve stability from those that do not.

11.2.4.3 *Ion Trap (IT) Mass Analyzers*

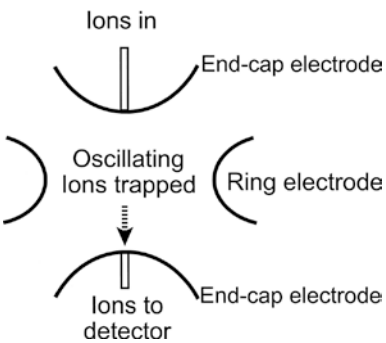
Ion traps are essentially multidimensional quadrupole mass analyzers that store ions (trap) and then eject these trapped ions according to their m/z ratios. Once the ions are trapped, multiple stages of MS (MS^n) can be achieved, mass resolution can be increased, and sensitivity can be improved. The major difference between an ion trap and a quadrupole mass analyzer is that in an ion trap, the unstable ions are ejected and detected while the stable ions are trapped (referred to as MS in time); whereas in a quadrupole, the ions with a stable flight path reach the detector, and the unstable ions hit the rods and are pumped away (MS in space).

Figure 11.6 shows the cross-sectional view of a 3-D ion trap mass analyzer. It consists of a ring electrode sandwiched between a perforated entrance, end-cap electrode, and a perforated exit, end-cap electrode. An AC (RF) voltage and variable amplitude is applied to the ring electrode, producing a 3-D quadrupole field within the mass analyzer cavity.

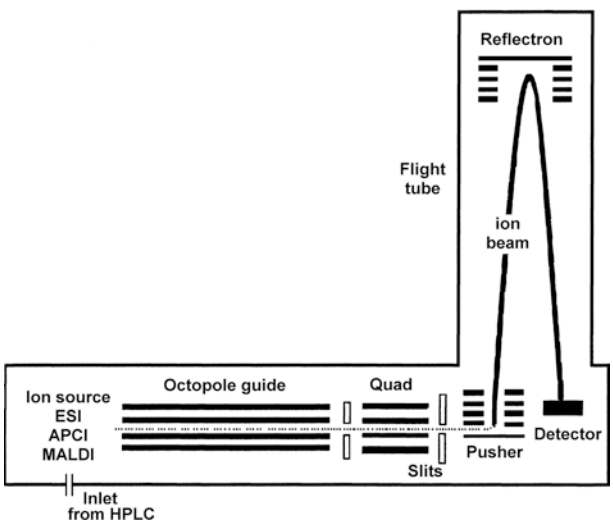
Ions formed in the source are electronically injected into the ion trap, where they come under the influence of a time-varying RF field. The ions are trapped within the mass analyzer cavity, and the applied RF voltage drives ion motion in a wure eight toward the end-caps. Thus, for an ion to be trapped, it must have a stable trajectory in both the axial and radial directions. To detect the ions, the frequency applied to the ring electrode is changed, and the ion trajectories are made unstable. Helium is continuously infused into the ion trap cavity and primarily serves as a dampening gas. Recent developments in ion trap technology have resulted in **2-D ion traps**, which substantially increase ion trapping volume by spreading the ion cloud in a quadrupole-like assembly [1].

11.2.4.4 *Time-of-Flight Mass Analyzer (TOF)*

Time-of-flight mass analyzers separate ions according to the time required to reach the detector while traveling over a known distance (Fig. 11.7). Ions are pulsed from the source with the same kinetic energy, which causes ions of different m/z ratios to acquire different velocities (lighter ions travel faster while heavier ions travel slower). The difference in velocities translates to



11.6 figure Diagram of an ion trap mass analyzer

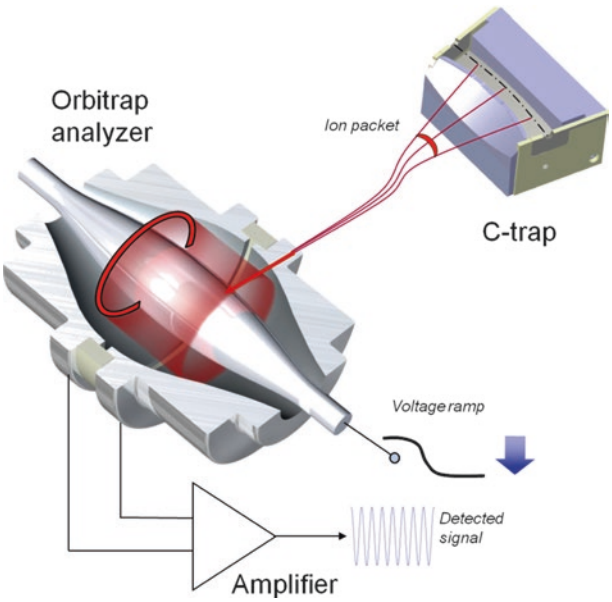


11.7 figure Diagram of a single-stage time-of-flight instrument

difference in time reaching the detector, upon which the mass spectrum is generated. Theoretically, TOF instruments have no upper mass range, which makes them useful for the analysis of biopolymers and large molecules, and have fast cycles since they technically transmit all m/z ions (full scan mode). The use of reflectrons (ion mirrors) can quickly increase mass resolution of TOF instruments by increasing ion drift path length by bouncing ions in a V or W pattern without drastically increasing the instrument footprint.

11.2.4.5 *Fourier Transform-Based Mass Spectrometry (FTMS)*

Fourier transform-based mass spectrometers deconvolute image currents produced by ion motion (harmonic oscillations or cyclotron motion) into mass spectra. A Fourier transform ion cyclotron resonance mass analyzer traps ions in a magnetic field (Penning traps), while a Fourier transform orbitrap mass analyzer traps ions in an electric field. Both analyzer types are unique from the previously listed mass analyzers because the ions themselves are not detected by impinging upon a detector, but rather the frequency (cyclotron motion) is measured as a function of the applied electric (orbitrap) or magnetic field (ICR). Commercially launched in 2005, the orbitrap brought high resolution (400 K resolution @ m/z 200) to the benchtop by using electrostatic fields, resulting in a simpler-to-operate, LC-MS instrument (Fig. 11.8). While traditional FTMS delivers significantly higher resolution (7 M resolution@ m/z 600), they require liquid helium-cooled superconducting magnets making them large in size, significantly more expensive, and require specialist operators, which limit their widespread adoption. This results in sub-part-per-million (ppm) mass accuracy measurements allowing determination of elemental composition. Extremely high resolution can be achieved with this type of mass analyzer which gives the ability to determine fine isotope structure.



11.8 figure

Diagram of the orbitrap analyzer. Ions are captured in the C-trap after ionization during typical GC or LC-MS. They are then sent to the trap analyzer where the detected signal is then converted to mass (Used with permission of Thermo Fisher Scientific (Bremen), Waltham, MA USA – Artwork by Thermo Fisher Scientific, CC BY-SA 3.0)

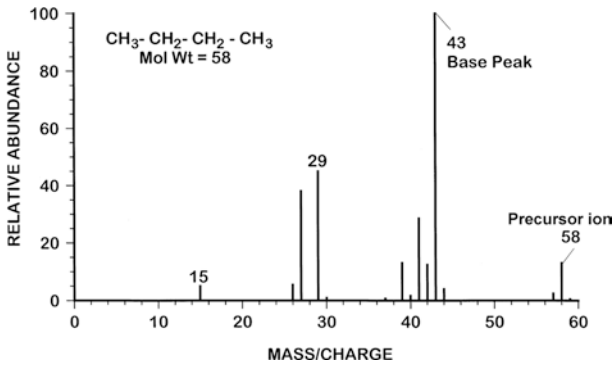
11.3 INTERPRETATION OF MASS SPECTRA

As previously indicated, a **mass spectrum** is a plot (or table) of the intensity of various mass fragments (m/z) produced when a molecule is subjected to one of the many types of ionization techniques. In classis GC-MS, the electron beam generated by a heated filament (used to ionize the molecules) is usually kept at a constant potential of 70 eV because this produces sufficient ions without too much fragmentation, which would result in a loss of the higher-molecular-weight ions. Another advantage of using 70 eV for ionization is that the resulting mass spectra are usually very similar regardless of the make and model of the instrument. This allows for computer-assisted mass spectral matching to database libraries that help in unknown compound identification. In fact, most MSs now come with a MS spectral database and the required matching software.

Typical mass spectra include only positive fragments that usually have a charge of +1. Thus, the **mass-to-charge ratio** is the molecular mass of the fragment divided by +1, which equals the mass of the fragment. As yet, the mass-to-charge ratio unit has no name and is currently abbreviated by the symbol m/z (older books use m/e).

A mass spectrum for butane is illustrated in Fig. 11.9. The relative abundance is plotted on the y-axis and the m/z is plotted on the x-axis. Each line on the bar graph represents a m/z fragment with the abundance unique to a specific compound. The spectrum always contains what is called the **base peak** or **base ion**. This is the fragment (m/z) that has the highest abundance or intensity. When the signal detector is processed by the computer, the m/z with the highest intensity is taken to be 100 %, and the abundance of all the other m/z ions is adjusted relative to the base peak. The base peak always will be presented as 100 % relative abundance. Butane has the base peak at a m/z of 43.

Another important fragment is the **precursor ion** (often called the **molecular ion** or **parent ion**), designated by the symbol $M^{+\bullet}$. This peak has the highest



11.9 figure Mass spectrum of butane obtained by electron impact ionization

mass number and represents the positively charged intact molecule with a m/z equal to the molecular mass. The harsher ionizing techniques such as the EI shown here (Fig. 11.9) produces an ion (radical cation) at m/z 58 by stripping an electron. Because the mass of a single electron can be considered insignificant, the molecular ion produced by EI-type ionization is indicative of the molecular weight of that compound. All other molecular fragments originate from this charged species, so it is easy to see why it is called the precursor (molecular) ion. It is not always present because, sometimes, the precursor ion decomposes before it has a chance to traverse the mass analyzer. However, a mass spectrum is still obtained, and this becomes a problem only when determining the molecular mass of an unknown. The remainder of the mass spectrum is a consequence of the stepwise cleavage of large fragments to yield smaller ones termed **product ions** (**daughter ions**). The process is relatively straightforward for alkanes, such as butane, making possible identification of many of the fragments.

As indicated previously, the initial step in EI ionization is the abstraction of an electron from the molecule as electrons from the beam pass in close proximity. The equation below illustrates the first reaction that produces the positively charged product ion.

$$\begin{aligned} &\text{M} + \text{e (from electron beam)} \Rightarrow \\ &\qquad \text{M}^{+\bullet}\,(\text{molecular ion}) + \\ &\qquad 2\text{e (one electron from the electron beam and one from the molecular ion, M)} \end{aligned} \tag{11.5}$$

The M symbolizes the unionized molecule as it reacts with the electron beam and forms a radical cation. The cation will have a m/z equal to the molecular weight. The precursor (molecular) ion then sequentially fragments in a unimolecular fashion. (Note that the ion is often written as M^+, for which the free electron, symbolized by the dot, is assumed. Regardless, the molecule has lost one electron and still retains all the protons; thus, the net charge must be positive.) The reactions of butane as it forms several of the predominant product ion (daughter) fragments are shown below:

$$\text{CH}_3-\text{CH}_2-\text{CH}_2-\text{CH}_3 + \text{e} \Rightarrow \text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_3^{+\bullet}\ (m/z = 58) + 2\text{e} \tag{11.6}$$

$$\text{CH}_3-\text{CH}_2-\text{CH}_2-\text{CH}_3^{+\bullet} \Rightarrow \text{CH}_3-\text{CH}_2-\text{CH}_2-\text{CH}_2^{+}\ (m/z = 57) + {}^{\bullet}\text{H} \tag{11.7}$$

$$\text{CH}_3-\text{CH}_2-\text{CH}_2-\text{CH}_3^{+\bullet} \Rightarrow \text{CH}_3-\text{CH}_2-\text{CH}_2^{+}\ (m/z = 43) + \bullet\text{CH}_3 \tag{11.8}$$

$$\text{CH}_3-\text{CH}_2-\text{CH}_2-\text{CH}_3^{+\bullet} \Rightarrow \text{CH}_3-\text{CH}_2^{+\bullet}\ (m/z = 29) + \bullet\text{CH}_3-\text{CH}_3 \tag{11.9}$$

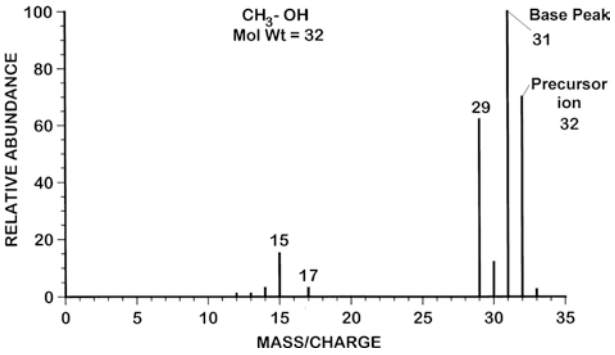
$$\text{CH}_3-\text{CH}_2 \Rightarrow \text{CH}_3^{+\bullet}\,(m/z = 15) + \bullet\text{CH}_2 \tag{11.10}$$

Many of the fragments for butane result from direct cleavage of the methylene groups. With alkanes, you will always see fragments in the mass spectrum that are produced by the sequential loss of CH_2 or CH_3 groups.

Close examination of the butane mass spectrum in Fig. 11.9 reveals a peak that is 1 m/z unit larger than the molecular ion at $m/z = 58$. This peak is designated by the symbol $M + 1$ and is due to the naturally occurring isotopes. The most abundant isotope of carbon has a mass of 12; however, a small amount of ^{13}C is also present (1.11 %). Any ions that contained a ^{13}C or a deuterium isotope would be 1 m/z unit larger, although the relative abundance would be low.

Another example of MS fragmentation patterns is shown for methanol in Fig. 11.10. Again, the fragmentation pattern is straightforward. The precursor ion (CH_3–$OH^{+\bullet}$) is at a m/z of 32, which is the molecular weight. Other fragments include the base peak at a m/z of 31 due to CH_2–OH^+, the CHO^+ fragment at a m/z of 29, and the CH_3^+ fragment at a m/z of 15.

The ionization method of **chemical ionization** (CI) (see Table 11.1) is classified as a **soft ionization** because only a few fragments are produced. In this technique, a gas is ionized, such as methane (CH_4), which then directly ionizes the molecule. The most important use of CI is in the determination of the molecular ion since there is usually a fragment that is



11.10 figure Mass spectrum of methanol obtained by electron impact ionization

1 m/z unit larger than that obtained with EI. Thus, a mass spectrum of butane taken by the CI method would have a quasimolecular (parent) ion at $m/z = 59$ (M + H). Many LC-MS interfaces use CI or electrospray ionization methods so it is common to see the $(M + H)^+$ precursor ion. As can be seen in Eqs. 11.6, 11.7, 11.8, 11.9, and 11.10, the reactions of the cleavage process can be quite involved. Many of the reactions are covered in detail in the book by McLafferty and Turecek listed in resource materials.

11.4 GAS CHROMATOGRAPHY-MASS SPECTROMETRY

Although samples can be introduced directly into the MS ion source, many applications require chromatographic separation before analysis. The rapid development of **gas chromatography-mass spectrometry** (GC-MS) has allowed for the coupling of the two methods for routine separation problems (see Chap. 14). A MS coupled to GC allows the peaks to be identified or confirmed, and, if an unknown is present, it can be identified using a computer-assisted search of a library containing known MS spectra. Another critical function of GC-MS is to ascertain the purity of each peak as it elutes from the column. Does the material eluting in a peak contain one compound, or is it a mixture of several that just happen to coelute with the same retention time?

In most cases a **capillary GC column** is connected directly to the MS source via a **heated capillary transfer line**. The transfer line is kept hot enough so as to avoid condensation of the volatile component eluting from the GC column on its way into the low-pressure MS source. The sample flows through the GC column into the interface and then on to be processed by the MS. A computer is used to store and process the data from the MS.

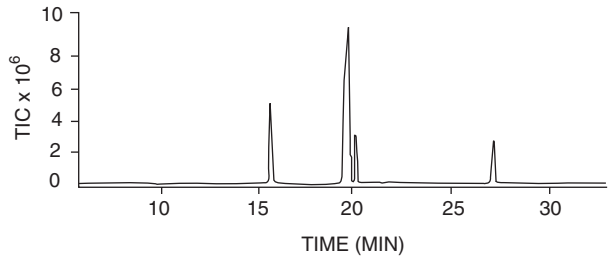
An example of the power of GC-MS is shown below in the separation of the methyl esters of several long-chain fatty acids (Fig. 11.11). Long-chain fatty acids must have the carboxylic acid group converted or blocked with a methyl group to make them volatile. Methyl esters of palmitic (16:0), oleic (18:1), linoleic (18:2), linolenic (18:3), stearic (18:0), and arachidic (20:0) acids were injected onto a column that was supposed to be able to separate all the naturally occurring fatty acids. However, the GC tracing showed only four peaks, when it was known that six different methyl esters were in the sample. The logical explanation is that one or two of the peaks contain a mixture of methyl esters resulting from poor resolution on the GC column.

The purity of the peaks is determined by running the GC-MS and taking mass spectra at very short increments of time (1 s or less). If a peak is pure, then the mass spectra taken throughout the peak should be the same. In addition, the mass spectrum can be compared with the library of spectra stored in the computer.

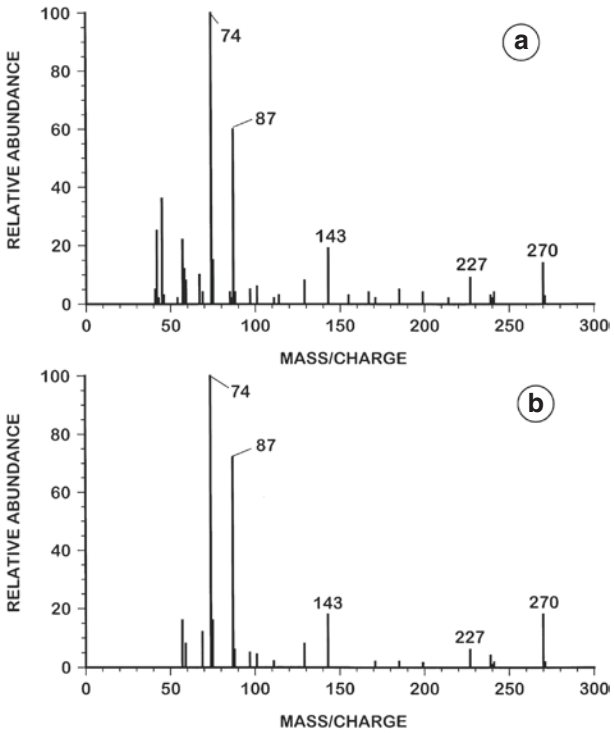
The **total ion current** (TIC) **chromatogram** of the separation of the fatty acid methyl esters is shown in Fig. 11.11. There are four peaks eluting off the column between 15.5 and 28 min. The first peak at 15.5 min has the same mass spectrum throughout, indicating that only one compound is eluting. A computer search of the MS library gives an identification of the peak to the methyl ester of palmitic acid. The mass spectra shown in Fig. 11.12 compare the material eluting from the column to the library mass spectrum.

Most of the fragments match, although the GC-MS scan does have many small fragments not present on the library mass spectrum. This is a common background noise and usually does not present a problem. The data from the rest of the chromatogram indicate that the peaks at both 20 and 27 min contain only one component. The computer match identifies the peak at 20 min as stearic acid, methyl ester, and the peak at 27 min as arachidic acid, methyl ester. However, the peak located at 19.5 min is shown to have several different mass spectra, indicating impurity or coeluting compounds.

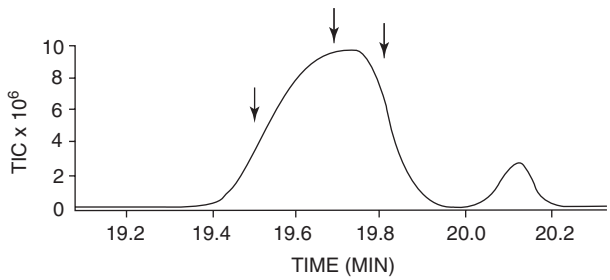
In Fig. 11.13, the region around 19 min has been enlarged. The arrows indicate where different mass spectra were obtained. The computer identified the material in the peak at 19.5 min as linoleic acid, methyl ester; the material at 19.7 min as oleic acid, methyl ester; and the material at 19.8 min as linolenic acid, methyl ester. Thus, as we originally suspected, several of the methyl esters were coeluting off the GC column. This example illustrates the tremendous power of GC-MS used in both a quantitative and a qualitative manner.



11.11 figure Total ion current GC chromatogram of the separation of the methyl esters of six fatty acids. Detection is by electron impact ionization using a direct capillary interface



11.12 figure Mass spectra of (**a**) the peak at 15.5 min in the TIC chromatogram shown in Fig. 11.11 and (**b**) the methyl ester of palmitic acid from a computerized MS library



11.13 figure Enlargement of the region 19.2–20.2 min from the TIC chromatogram shown in Fig. 11.11. *Arrows* indicate where mass spectra were obtained

11.5 LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY

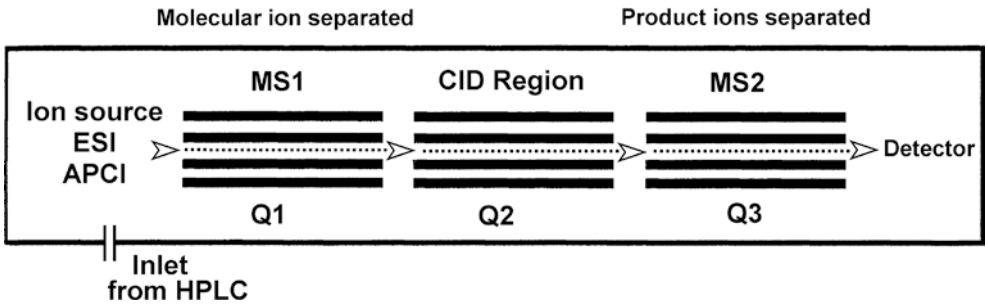
For a **high-performance liquid chromatography-mass spectrometry** (LC-MS) interface, the same overall requirements must be met as for GC-MS. There must be a way to remove the excess solvent, while converting a fraction of the liquid effluent into the gas phase, making it amenable for MS analysis. Furthermore most compounds analyzed by HPLC are either nonvolatile or thermally labile, making the task of liquid-to-gas phase transition even more challenging, especially while maintaining compound integrity.

How does LC-MS work? A modern LC-MS ionization interface converts liquid (LC eluent) into gas-phase ions (sampled by the MS) by a process of desolvation in the presence of a highly charged electrical field at atmospheric pressure. The energy applied to evaporate the solvent (thermal and electrical) is almost completely used in the desolvation process, and it does not contribute to degradation (usually thermal) of any labile species present in the LC eluant. Of the many different types of LC-MS ionization interfaces developed over the years, it was the development of the atmosphere pressure-based ionization interfaces, ESI (Sect. 11.2.3.2) and APCI (Sect. 11.2.3.3) that made LC-MS a routine technique. More recently, an APPI (Sect. 11.2.3.4) has been developed as a complementary technique to APCI.

11.6 TANDEM MASS SPECTROMETRY

Tandem MS (MS/MS, MS^n) is used in both GC-MS and LC-MS but is especially helpful in LC-MS since it allows for characterization, verification, and quantitation at ultrahigh sensitivity. There are two basic types of tandem MS, one which is a result of **collision-induced dissociation** (CID) typically observed on beam-type instruments (triple quadrupoles, TQ), and the other is a result of **collision-activated dissociation** (CAD) or MS^n, typically observed in ion trap MS (MS in time type instruments). Other fragmentation modes such as electron transfer dissociation (ETD), infrared multiphoton dissociation (IRMPD), and electron capture dissociation (ECD) are used for the analysis of compounds difficult to fragment.

Tandem MS using LC **triple quadrupoles** (TQ) (Fig. 11.14) operated in the selected reaction monitoring (SRM) or multiple reaction monitoring (MRM) scan mode provides a factor of 100–1000× more sensitivity than ultraviolet (UV) or diode array detectors, making them indispensable for high-sensitivity quantitative LC-MS analysis. Using the mechanism of CID, the precursor molecule filtered by Q1 energetically collides with argon or nitrogen gas, buffered in the collision cell (Q2), and a specific production is



11.14 figure

Diagram of a triple quadrupole mass spectrometer capable of doing MS/MS. Q1 and Q2 are used to separate ions, and Q3 is the collision-induced dissociation area (CID). Once the compounds are ionized, they can be allowed to pass through without the CID activated, or the molecular ion can be further fragmented in the Q2 area via CID to yield product ions (fragments)

transmitted through the third quadrupole (Q3). This mode offers the highest sensitivity critical for quantitative analysis that has been the mainstay of bioanalytical quantitative analysis for the past two decades. The high sensitivity of the SRM/MRM modes are due to two factors: (1) a dramatic improvement in signal-to-noise ratio by eliminating noise, and (2) TQ operation at nearly 100 %, allowing seven scans or more to ensure capturing the top of the eluting chromatographic peak to accurately determine the area under the peak. The other advantages are simplicity of data analysis and the high specificity resulting from the MRM mode. The triple quadrupole remains the instrument of choice for high-sensitive, routine LC-MS quantitative analysis, even though it was first introduced in the early 1990s.

Multiple MS or "MS-to-the-nth" made ion traps popular for structural analysis and verification of molecules. Subtle changes can be identified by piecing together the various fragments, which result in the different stages of the MS/MS process. The difference between tandem MS on a TQ and the MS^n mode on the IT is how the ion undergoes fragmentation. In the CID process on a TQ or Q-TOF, the precursor ion selected in Q1 is accelerated into the collision cell (Q2) filled with argon or nitrogen and smashed into its component pieces in one highly energetic step. In contrast, the CAD process occurring in an ion trap is energetically much gentler involving a gradual ramping of voltages so that the internal energy of the specified trapped ion increases as it collides with the helium buffer gas, until the most fragile chemical bond breaks. As soon as this occurs, the m/z changes, and the product ions no longer experience the gradual ramping of the energy but remain trapped. This process allows for multiple steps of MS/MS analysis at high sensitivity. The most abundant fragment (typically) is then isolated and re-trapped, and the CAD process is repeated. In most cases, CAD can easily perform MS^4, but there have been cases where MS^{10} could be accomplished. Such a method of MS^n analysis makes it significantly easier to piece together the intact structure from its key fragments and determine or verify changes. Since ITMS is tandem MS in time, it has a significant advantage over TQ's operating Q3 in the "full scan MS/MS" mode. The main advantage of MS^n on ITMS is the high sensitivity of the MS^n scan mode, especially when connected to ultra-HPLC (UHPLC) (Chap. 13, Sect. 13.2.3.1).

11.7 HIGH-RESOLUTION MASS SPECTROMETRY (HRMS)

The widespread adoption of orbitrap and Q-TOF MS instruments has made high-resolution, accurate mass applications a growing trend. Resolution in a mass spectrometer is defined in terms of full width half maxima (FWHM), which is the mass spectrum peak width at half height for a known m/z [4]. At unit mass resolution (nominal mass instruments such as quadrupoles and ion traps), FWHM is typically around 0.6 Da, so at m/z 300, the resolution would be 500 (300 ÷ 0.6). An orbitrap or a Q-TOF, for example, can deliver FWHM of 0.01 Da, so it would deliver a resolution of 30,000 (300 ÷ 0.01) for m/z 300. An FTMS can deliver FWHM of 0.0001 Da, enabling it to deliver a resolution of 3,000,000 (300 ÷ 0.0001). High-resolution analysis has significant advantages in terms of S/N, fine isotopic structure analysis, and determining elemental composition from highly precise accurate mass assignment.

Accurate mass is important in mass spectrometry because it can give you elemental composition and enable the identification of unknowns. With accurate mass and MS/MS fragmentation high-resolution data, uncertainty is significantly reduced in identification of "known" unknowns (compounds that are in a database, but not known to the analyst) and invaluable for screening type qualitative analysis. For mass accuracy below 5 ppm, elemental composition determination becomes straightforward through the application of sophisticated software algorithms.

The listing below for the compound caffeine ($C_8H_{10}N_4O_2$) illustrates the value of accurate mass:

- **Nominal mass:** 194 (used synonymously with molecular mass and is the sum of the integer mass of the most abundant isotope for each element, C = 12, H = 1, N = 14, O = 16)
- **Monoisotopic mass:** 194.0804 (sum of the most abundant isotopic mass for each of the constituent elements, C = 12.0000, H = 1.007825, N = 14.003074, O = 15.994915)
- **Average mass:** 194.1906 (sum of the average atomic masses or sum of isotopes taking into account the relative abundances for the constitute elements, e.g., O_{16} = 15.994915, but the average mass is 15.999405, which takes into account the O_{17} and O_{18} isotopes and their relative abundances)

Accurate mass is measured in terms of parts per million (ppm) and calculated by dividing the mass error by the theoretical mass. In the example above, assume that the measured mass on the high-resolution MS was 194.0811. Given the theoretical monoisotopic mass of 194.0804, this results in a mass error of 0.0007 (194.0811–194.0804), which would result in a mass accuracy of 3.6 ppm for that measurement ([194.0804/0.0007]∗10^6). For mass accuracy below 5 ppm, elemental composition determination becomes straightforward through the application of sophisticated software algorithms.

The advancement of high-resolution MS coupled with availability of more databases has enhanced the determination of unknown compounds, a process termed nontargeted or "scouting" analysis. With accurate mass determinations, an unknown can be compared against LC-MS libraries for identification. The elemental composition can be determined and further verified by observing the fragment ions for definitive identification. There are several databases of compounds and their ionization patterns (i.e., ions produced). Most notably is the METLIN metabolite database by the Scripps Center for Metabolomics where the ion spectra are produced by ESI Q-TOF. In the case of caffeine (mass 194.080376), the spectrum contains a total of seven major ions, more than enough for positive identification. Another database that contains some mass spectra is ChemSpider. Unfortunately the databases only cover a limited amount of molecules, and when present, the mass spectrum depends on the type of ionization interface/method (e.g., ESI, IT, TOF), which is not the case with EI GC-MS libraries. However, the limitations of these databases will only improve as more scientists provide additional data. Both Milman [5] and Lehotay et al. [6] discuss MS libraries, screening of molecules, and nontarget identification.

11.8 APPLICATIONS

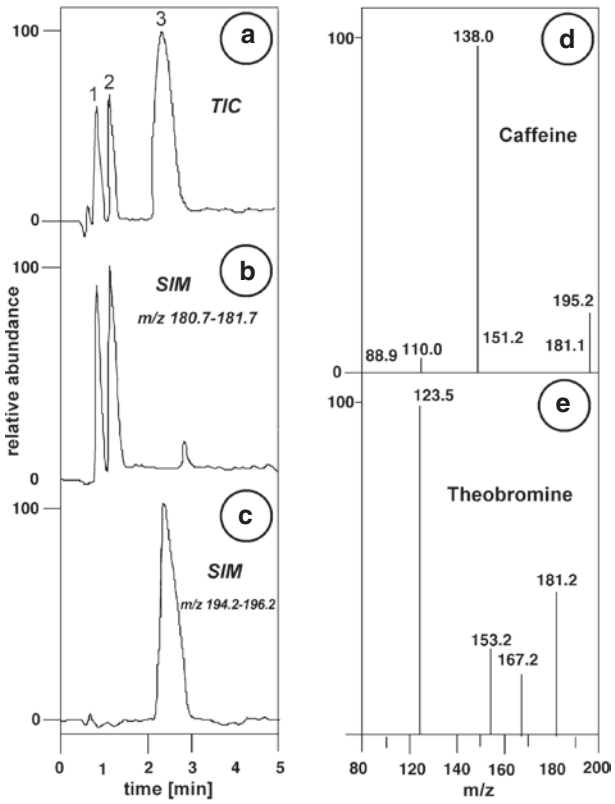
The use of MS in the field of food science is well established and growing rapidly as food exports from Asia increase yearly to the USA and Europe. While GC-MS has been used for years, LC-MS/MS instrumentation has become indispensible for the analysis of compounds such as chloramphenicol, nitrofurans, sulfonamides, tetracyclines, melamine, acrylamide, and malachite green in foods such as honey, fish, shrimp, and milk (see Chap. 33). Agencies such as the Food and Drug Administration (FDA), Center for Food Safety and Applied Nutrition (CFSAN), European Food Safety Authority (EFSA), Health Canada, and Japan Food Safety Commission use MS-based techniques to drive regulatory standards for banned substances, safeguarding the food supply for human consumption.

To give an appreciation of the usefulness of LC-MS, several examples are provided below. It is important to remember that there are a wide variety of methods now available to analyze just about any type of sample in a variety of matrices.

Due to the prevalence of consumption of caffeine-containing drinks throughout the world, the analysis of this small bioactive compound has been of interest for many years. Over 20 years ago HPLC methods were published showing that caffeine and other alkaloids, theobromine and theophylline, could be analyzed by HPLC using an ultraviolet detector. While HPLC-UV analysis is quite acceptable, the use of LC-MS can verify and enhance identification in a variety of complex food systems.

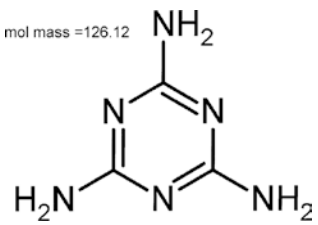
Figure 11.15 illustrates a reversed-phase HPLC column separation and MS spectrum obtained using the ESI interface coupled with MS/MS [7]. An aqueous coffee extract was filtered and separated by HPLC using an acetic acid-acetonitrile mobile phase. For comparative purposes a separate HPLC separation was achieved with the same HPLC column except detection was with a UV detector. The HPLC chromatogram in Fig. 11.15a shows the TIC, an indicator of total ions and thus compounds eluting, and matches the HPLC-UV chromatogram (not shown). Figure 11.15b is the selected ion trace of ions m/z = 180.7–181.7, which would correspond to the protonated molecular ions $(M+H)^+$ of theobromine and theophylline at 181.2 (both compounds are isomers and have identical masses of 180.2). The chromatogram in Fig. 11.15c is the selected ion trace of m/z = 194.2–196.2, which corresponds to the protonated molecular ion of caffeine (195.2). The MS/MS of caffeine and theobromine is presented in Fig. 11.15d, e and shows the protonated molecular ions for both caffeine and theobromine, and several ion fragment m/z.

The **melamine** food contamination issue of 2007–2008 is another excellent example of the use of LC-MS and MS/MS in both a detective role and later as an official analytical method. Melamine is a six-membered cyclic nitrogenous ring compound with three amines attached to the carbons in the ring and contains 67 % nitrogen (Fig. 11.16). Since a common test for protein measures nitrogen (Chap. 18, Sect. 18.2), the compound was used as an economic



11.15 figure

Reversed-phase LC-MS (parts **a**–**c**) and MS/MS separation of an aqueous coffee extract: (**a**) TIC, total ion current of extract; (1) theobromine, (2) theophylline, and (3) caffeine; (**b**) selected ion trace, $m/z = 80.7–181.7$; (**c**) selected ion trace, $m/z = 194.2–196.2$; (**d**) MS/MS ionization of caffeine; (**e**) MS/MS of theobromine (From Huck et al. [7], used with permission of Elsevier, New York)



11.16 figure

Chemical structure of melamine

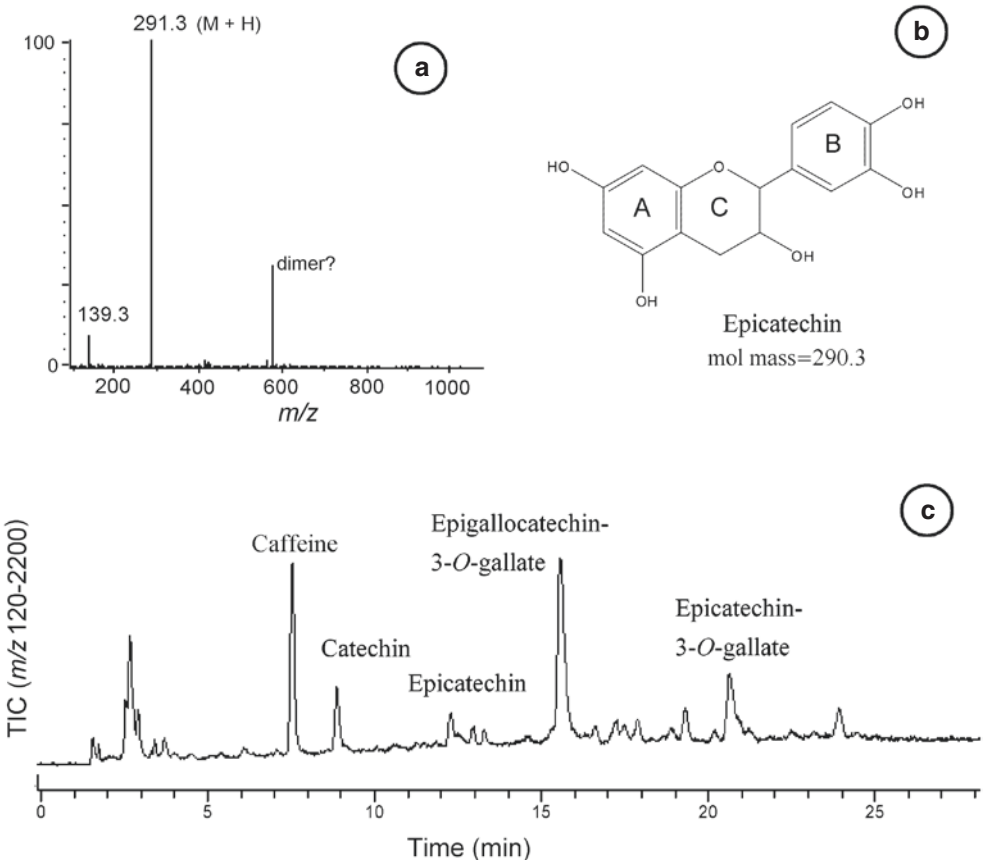
adulterant in wheat gluten and dried milk powder to artificially increase the apparent protein content. Due to the polar nature of the amine groups, melamine is not volatile and thus cannot be analyzed with GC unless a derivative is synthesized, thus LC-MS is a preferred method. Methods have been presented that entail the separation of melamine by HPLC with detection by ESI-MS/MS. ESI produces a strong protonated molecular ion at $m/z = 127.1$ with a MS/MS precursor ion (molecular) of 127.1 and a product ion of 85.1. One of the analytical methods suggested by the FDA entails monitoring of all these ions, which produces very good specificity and sensitivity.

The area of bioactive food components has grown dramatically over the last 10 years in part due to the availability of LC-MS. Many bioactive compounds in fruits, vegetables, and spices are polar and are not volatile. Thus, identification and evaluations were very difficult without HPLC methods coupled with MS. A good example of the use of LC-MS is in the measurement of the polyphenolic flavonoids called the catechins. These antioxidant compounds present in catechu, green tea, cocoas, and chocolates appear to have several beneficial biological effects including enhanced heart and blood vessel health.

Figure 11.17 shows a separation of the major green tea catechins by HPLC with ESI-MS detection [8]. At the bottom of the figure depicted is the chromatogram showing the separation of the catechins by HPLC. The ESI-MS detector was used to monitor all ions from m/z 120–2200 (TIC mode). The top left panel shows the MS obtained for the epicatechin peak eluting at about 12.1 min. As typical with ESI, there are few fragments, though the M + H molecular ion at 291.3 m/z is predominant. Further fragmentation (MS/MS) of the epicatechin yields two major protonated fragments, m/z 273.3 (loss of OH from the C ring) and 139.3 (oxidation and cleavage of the A ring). With these data it is possible to elucidate isomers and also possible degradation pathways.

High-resolution analysis on orbitrap technology has found wide application in food analysis, with LC, GC, and supercritical fluid chromatography (SCF) as hyphenated front-end separation techniques before the mass analysis is performed. Rajski et al. [9] performed a large multi-residue screening study (>250 pesticides) in fruits and vegetables using three different resolution settings on the orbitrap ($R = 17.5$ K, 35 K, and 70 K). The study revealed that using a ±0.2 min retention time window, $R = 70{,}000$, and mass tolerance of 5 ppm gave less than 5 % false-positive results at the 10 ug/kg level. Ishibashi et al. [10] used supercritical fluid (SFC) coupled to an orbitrap to simultaneously analyze 373 pesticides (10 ug/kg level which is the provisional MRL in Japan) in a QuEChERS spinach extract using an $R = 70{,}000$ and a mass tolerance of 5 ppm. The high-throughput advantage of SFC separation allowed for 72 samples in approximately 45 min using SFC-OT technology, for compounds whose molecular weights ranged from m/z 99 to m/z 900.

Recently, a GC-capable version of the orbitrap was launched [11], making high-resolution GC-MS analysis possible at resolutions exceeding $R > 50{,}000$. In contrast to GC-TOF instruments currently available, which focused mainly on MS acquisition speed at resolutions around 5000 (higher than quadrupole based instruments), the



11.17 figure

LC-ESI-MS of green tea leaf extracts. (**a**) ESI mass spectra of epicatechin. (**b**) Chemical structure of epicatechin. (**c**) TIC scanned from *m*/*z* 120–2,200 (From Shen et al. [8], used with permission of American Chemical Society, Washington, DC)

GC coupling to the orbitrap truly allows for high-resolution, accurate mass analysis in both MS and MS/MS modes. The superior chromatographic separation power of GC, coupled to high resolution ($R = 60{,}000$ @ m/z 200), allows for unambiguous analysis of 132 pesticides in even the most complex matrices at the 10 ng/g level, approaching sensitivity performance (IDL 10 ng/g) of routine GC-triple quadrupoles. These orbitrap-based techniques are currently used extensively for screening of pesticide residues and highly sensitive quantitative analysis of banned substances.

FTMS provides ultrahigh-resolution MS at the isotope fine-structure level, i.e., resolution of isobaric species of different elemental composition, which can yield elemental composition analysis. Essentially, isotope fine structure is the fingerprint of a small molecule because the m/z value and their intensities in the fingerprint for a specific molecule exactly reflect the atomic composition of the molecule. Because FTMS delivers resolutions in excess of R >1,000.000 (million), this allows for isotopologue analysis of the A+1 and A+2 ion signals. Using this capability, bioactive sulfur-containing compounds were identified in asparagus, purported to have angiotensin-converting enzyme (ACE) inhibitory function [12]. One such compound identified was aspartame (no UV-chromophores) detected at m/z 307.0893 (and confirmed by high-resolution MS/MS) that required acquisition at $R = 1{,}000{,}000$ to determine the elemental composition of $C_{10}\ H_{18}\ N_4O_3S_2$. Such discoveries are only possible with FTMS where isotope fine-structure analysis is possible.

Recently, MALDI-TOF has found key applications in food microbiology, with rapid identification of bacteria and fungi through their protein signatures. In this technique, bacteria or fungi from the culture plates are directly spotted onto the MALDI target plate, sprayed with matrix, and then directly analyzed on the MALDI-TOF instrument. The resulting spectrum representative of the microorganisms proteomic fingerprint is matched against a known, verified spectrum in the library, and, if there is positive hit, the bacteria or fungi is rapidly identified. Wieme et al. [13] used MALDI-TOF to catalogue 4200 mass spectra from 273 acetic acid bacteria and lactic acid bacteria covering 52 species responsible for beer spoilage and then used the resulting library for routine quality control in the brewing industry. MALDI-TOF has been used for the rapid confirmatory identification (within 24 h) of more than 120 strains for *S. aureus* in milk, a pathogen that causes toxic shock syndrome

toxin-1, a deadly form of food poisoning. This food-borne pathogen is usually a result of subclinical and clinical mastitis-affected dairy cattle [14].

11.9 SUMMARY

MS is a powerful analytical technique that can solve most complex problems faced by the food analytical chemist, both in a qualitative and quantitative manner. Its principles are fairly simple when examined closely. The basic requirements are to: (1) get the sample into an ionizing chamber where ions are produced; (2) separate the ions formed by magnets, quadrupoles, drift tubes, and electric fields; (3) detect the m/z of the precursor ion; (4) fragment the m/z selectively to derive more information if required; and (5) output the data to a computer for software evaluation.

Since the qualitative and quantitative aspects of MSs are so powerful, they are routinely coupled to a GC or HPLC, and find growing use with static sample introduction techniques. The interface for GCs is versatile and easy to use; however, the extensive sample preparation required for GC-MS analysis makes its utility cumbersome. The adoption of LC-MS as an analytical technique has greatly increased because of far simpler sample preparation procedures, wider ionization ranges for different classes of compounds, faster analysis times, routine high sensitivity, access to accurate mass capability, and the advent of UHPLC.

Acronyms

AMS	Accelerator mass spectrometer
APCI	Atmospheric pressure chemical ionization
API	Atmospheric pressure ionization
APPI	Atmospheric pressure photoionization
CAD	Collision-activated dissociation
CI	Chemical ionization
CID	Collision-induced dissociation
DART	Direct analysis in real time
ECD	Electron capture dissociation
EI	Electron impact ionization
ES	Electrospray
ESI	Electrospray ionization
ETD	Electron transfer dissociation
FT	Fourier transform
FT-ICR	Fourier transform-based ion cyclotrons
FTMS	Fourier transform mass spectrometry
GC	Gas chromatography
GC-MS	Gas chromatography-mass spectrometry
HRMS	High-resolution mass spectrometry
ICP-MS	Inductively coupled plasma-mass spectrometry
IMS	Ion mobility mass spectrometry
IT	Ion trap
ITMS	Ion trap mass spectrometry
LC	Liquid chromatography
LC-MS	Liquid chromatography-mass spectrometry
m/z	Mass-to-charge ratio
MALDI	Matrix-assisted laser desorption ionization
MALDI-TOF	Matrix-assisted laser desorption ionization time of flight
MRM	Multiple reaction monitoring
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
MS^n	Multiple stages of MS (tandem mass spectrometry)
OT	Orbitrap
Q	Quadrupoles mass filters
Q-TOF	Quadrupole time of flight
SFC	Supercritical fluid chromatography
SRM	Selected reaction monitoring
TIC	Total ion current
TOF	Time of flight
TQ	Triple quadrupole
TWIM	Traveling wave
UHPLC	Ultrahigh-performance liquid chromatography

11.10 STUDY QUESTIONS

1. What are the basic components of a MS?
2. What are the unique aspects of data that a MSs provide? How is this useful in the analysis of foods?
3. What is EI ionization? What is CI ionization?
4. What is the base peak on a mass spectrum? What is the precursor ion peak?
5. What is the difference between nominal mass and monoisotopic mass?
6. What are the major ions (fragments) expected in the EI mass spectrum of ethanol (CH_3-CH_2-OH)?
7. What are the major differences in how ionization occurs in the electrospray versus the APCI interface? What is ion suppression?
8. What does MALDI stand for and how does it differ from ESI?
9. What are the major differences between the quadrupole, ion trap, time of flight, and Fourier transform mass analyzer? What are the advantages of using each analyzer? What is especially unique about a Fourier transform-based mass analyzer?
10. What is the working principle behind the MALDI-TOF-based microbiology identification?
11. Which MS type is popular for quantitative analysis
12. What is the difference between CAD and CID?

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