

CHAPTER 1

INTRODUCTION AND BASIC DEFINITIONS

1.1 DEFINITION AND DESCRIPTION OF MASS SPECTROMETRY

During the past decade mass spectrometry has experienced a tremendously large growth in its uses for extensive applications involved with complex biological sample analysis. Mass spectrometry is basically the science of the measurement of the mass-to-charge ratio (m/z) of ions in the gas phase. Mass spectrometers are generally comprised of three components: (1) an ionization source that ionizes the analyte of interest and effectively transfers it into the gas phase, (2) a mass analyzer that separates positively or negatively charged ionic species according to their mass-to-charge ratio (m/z), and (3) a detector used to measure the subsequently separated gas-phase ions. Mass spectrometers are computer controlled, which allows the collection of large amounts of data and the ability to perform various and complex experiments with the mass spectral instruments. Applications of mass spectrometry include unknown compound identification, known compound quantitation, structural determination of molecules, gas-phase thermochemistry studies, ion-ion and ion-molecule studies, and molecule-chemical property studies. Mass spectrometry is routinely used to determine elements such as Li^+ , Na^+ , Cl^- , Mg^{2+} , inorganic compounds such as $\text{Li}^+(\text{H}_2\text{O})_x$ or $(\text{TiO}_2)_x^+$, and organic compounds including lipids, proteins, peptides, carbohydrates, polymers, and oligonucleotides [deoxyribonucleic acid (DNA) and ribonucleic acid (RNA)].

2 INTRODUCTION AND BASIC DEFINITIONS

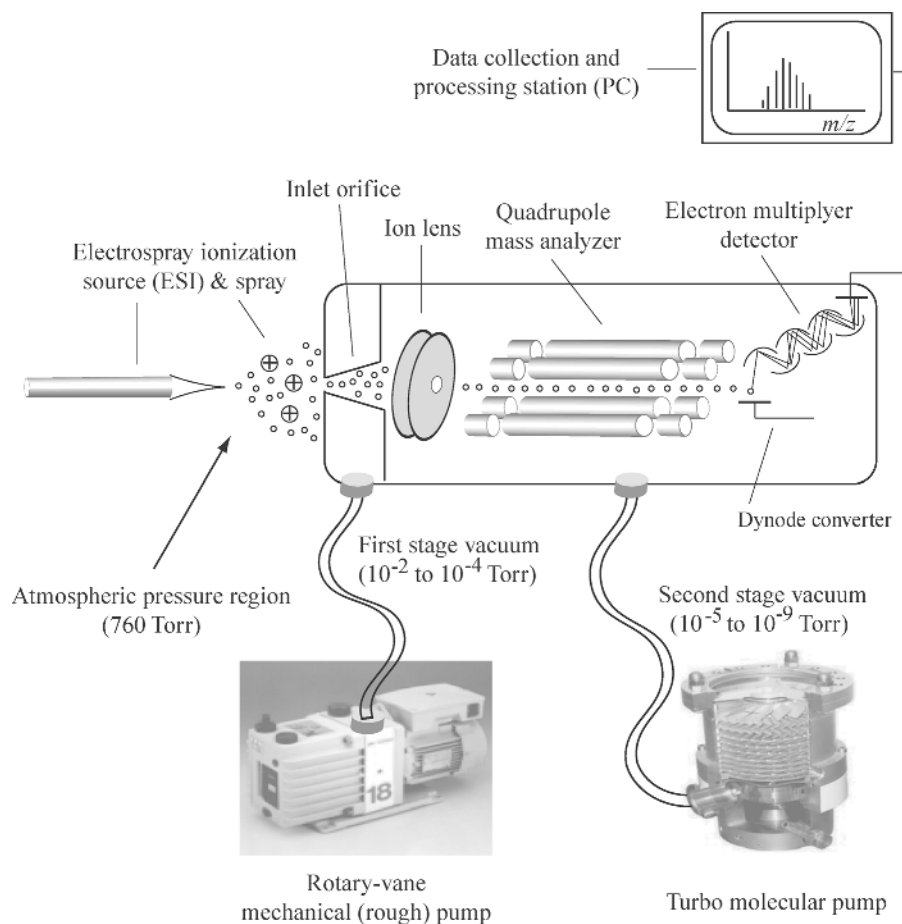


FIGURE 1.1 The six components that make up the fundamental configuration of mass spectrometric instrumentation consisting of: (1) inlet and ionization system, (2) inlet orifice (source), (3) mass analyzer, (4) detector, (5) vacuum system, and (6) data collection and processing station (PC). [See Wikipedia, Turbomolecular pump, <http://en.wikipedia.org/w/index.php?title=Turbomolecular+pump&oldid=71160479> (as of Aug. 24, 2006, 17:45 GMT).]

1.2 BASIC DESIGN OF MASS ANALYZER INSTRUMENTATION

Typical mass spectrometric instrumentation that are used in laboratories and research institutions are comprised of six components: (1) an inlet, (2) an ionization source, (3) a mass analyzer, (4) a detector, (5) a data processing system, and (6) a vacuum system. Figure 1.1 illustrates the interrelationship of the six components that make up the fundamental construction of a mass spectrometer. The *inlet* is used to introduce

a sample into the mass spectrometer and can be a solid probe, a manual syringe or syringe pump system, a gas chromatograph, or a liquid chromatograph. The inlet system can be either at atmospheric pressure, as shown in Figure 1.1, or it can be at a reduced pressure under vacuum. The *ionization source* functions to convert neutral molecules into charged analyte ions, thus enabling their mass analysis. The ionization source can also be part of the inlet system. A typical inlet system and ionization source that is used with high-performance liquid chromatography (HPLC) is electrospray ionization (ESI). In an HPLC/ESI inlet system and ionization source, the effluent coming from the HPLC column is transferred into the ESI capillary, which has a high voltage applied to it, inducing the electrospray ionization process. In this configuration the inlet system and ionization source are located at atmospheric pressure outside of the mass spectrometric instrumentation, which is under vacuum. The spray that is produced passes through a tiny orifice that separates the internal portion of the mass spectrometer, which is under vacuum from its ambient surroundings, which are at atmospheric pressure. This orifice is also often called the *inlet* and/or the *source*. In the case of the coupling of a gas chromatograph to the mass spectrometer, the capillary column of the gas chromatograph is inserted through a heated transfer capillary directly into the internal portion of the mass spectrometer, which is under vacuum. This is possible because the species eluting from the capillary column are already in the gas phase, making their introduction into the mass spectrometer more straightforward as compared to the liquid eluant from an HPLC where analytes must be transferred from the solution phase to the gas phase. An example of an ionization process that takes place under vacuum in the front end of the mass spectrometer is a process called matrix-assisted laser desorption ionization, or MALDI. In this ionization technique a laser pulse is directed toward a MALDI target that contains a mixture of the neutral analytes and a strongly ultraviolet (UV) absorbing molecule, oftentimes a low-molecular weight organic acid such as dihydroxy benzoic (DHB) acid. The analytes are lifted off of the MALDI target plate directly into the gas phase in an ionized state. This is due to transference of the laser energy to the matrix and then to the analyte. The MALDI technique takes place within a compartment that is at the beginning of the mass spectrometer instrument and is under vacuum. The compartment in which this takes place is often called the ionization source, thus combining the *inlet system* and the *ionization source* together into one compartment. As illustrated in Figure 1.1 the analyte molecules (small circles), in an ionized state pass from atmospheric conditions to the first stage of vacuum in the mass spectrometer through an inlet orifice that separates the mass spectrometer, which is under vacuum from ambient conditions. The analytes are guided through a series of ion lenses into the mass analyzer. The *mass analyzer* is the heart of the system, which is a separation device that separates positively or negatively charged ionic species in the gas phase according to their respective mass-to-charge ratios. The mass analyzer gas-phase ionic species separation can be performed by an external field such as an electric field or a magnetic field, or by a field-free region such as within a drift tube. For the detection of the gas-phase separated ionic species, electron multipliers are often used as the *detector*. Electron multipliers are mass impact detectors that convert the

4 INTRODUCTION AND BASIC DEFINITIONS

impact of the gas-phase separated ionic species into a cascade of electrons, thereby multiplying the signal of the impacted ion many times.

The *vacuum system* ties into the inlet, the source, the mass analyzer, and the detector of the mass spectrometer at different stages of increasing vacuum as movement goes from the inlet to the detector (left to right in Fig. 1.1). It is very important for the mass analyzer and detector to be under high vacuum as this removes ambient gas, thereby reducing the amount of unwanted collisions between the mass-separated ionic species and gas molecules present. As illustrated in Figure 1.1, ambient, atmospheric conditions are generally at a pressure of 760 Torr. The first-stage vacuum is typically at or near 10^{-3} Torr, immediately following the inlet orifice and around the first ion transfer lenses. This stage of vacuum is obtained using two-stage rotary vane mechanical pumps that are able to handle high pressures such as atmospheric and large variation in pressures but are not able to obtain the lower pressures required further into the mass spectrometer instrument. The two-stage rotary vane mechanical pump has an internal configuration that utilizes a rotating cylinder that is off-axis within the pump's hollow body. The off-axis positioned rotor contains two vanes that are opposed and directed radially and are spring controlled to make pump body contact. As the cylinder rotates, the volume between the pump's body and the vanes changes, the volume increases behind each vane, which passes a specially placed gas inlet port. This will cause the gas to expand behind the passing vane while the trapped volume between the exhaust port and the forward portion of the vane will decrease. The exhaust gas is forced into a second stage and then is released by passing through the oil that is contained within the pump's rear oil reservoir. This configuration is conducive for starting up at atmospheric pressure and working toward pressures usually in the range of 10^{-3} – 10^{-4} Torr.

The lower stages of vacuum are obtained most often using turbomolecular pumps as illustrated in Figure 1.1. Turbomolecular pumps are not as rugged as the mechanical pumps described previously and need to be started in a reduced-pressure environment. Typically, a mechanical pump will perform the initial evacuation of an area. When a certain level of vacuum is obtained, the turbomolecular pumps will then turn on and bring the pressure to higher vacuum. Using a mechanical vane pump to provide a suitable forepump pressure for the turbomolecular pump is known as roughing or "rough out" the chamber. Therefore, two-stage rotary vane mechanical pumps are often referred to as rough pumps. As illustrated in Figure 1.1 the turbomolecular pump contains a series of rotor–stator pairs that are mounted in multiple stages. The principle of turbomolecular pumps is to transfer energy from the fast rotating rotor (turbomolecular pumps operate at very high speeds) to the molecules that make up the gas. After colliding with the blades of the rotor, the gas molecules gain momentum and move to the next lower stage of the pump and repeat the process with the next rotor. Eventually, the gas molecules enter the bottom of the pump and exit through an exhaust port. As gas molecules are removed from the head or beginning of the pump, the pressure before the pump is continually reduced as the gas is removed through the pump, thus achieving higher and higher levels of vacuum. Turbomolecular pumps can obtain much higher levels of vacuum (up to 10^{-9} Torr) as compared to the rotary vane mechanical pumps (up to 10^{-4} Torr).

The final component of the mass spectrometer is a data processing system. This is typically a personal computer (PC) allowing the mass spectrometric instrumentation to be software controlled enabling precise measurements of carefully designed experiments and the collection of large amounts of data. Commercially bought mass spectrometers will come with their own software that is used to set the operating parameters of the mass spectrometer and to collect and interpret the data, which is in the form of mass spectra.

1.3 MASS SPECTROMETRY OF PROTEIN, METABOLITE, AND LIPID BIOMOLECULES

Proteins and lipids are two important classes of biological compounds that are found in all living species. Proteomics (the identification and characterization of proteins in cells, organisms, etc.) has now become a very important field of research in mass spectrometry, which is often used to describe a cell's or organism's proteome or to investigate a response to a stress upon a system through a change in protein expression. Global metabolic profiles (metabolomics) have also become a quickly advancing area of study utilizing mass spectrometric techniques. Lipidomics is a subclass of metabolomics that is also experiencing an increase in mass spectral applications. Lipids are often associated with proteins and act as physiological activators. Lipids constitute the bilayer components of biological membranes and directly participate in membrane protein regulation and function. In the past decade mass spectrometry has experienced increasing applications to the characterization and identification of these two important biological compound classes. Using proteomics, metabolomics, and lipidomics as examples of the application of mass spectrometry to biomolecular analysis we will briefly look at these areas in the following three sections.

1.3.1 Proteomics

The area of proteomics has been applied to a wide spectrum of physiological samples often based upon comparative studies where a specific biological system's protein expression is compared to either another system or the same system under stress. Often in the past the comparison is made using two-dimensional (2D) electrophoresis where the gel maps for the two systems are compared looking for changes such as the presence or absence of proteins and the up or down regulation of proteins. Proteins of interest are cut from the gel and identified by mass spectrometry. Figure 1.2 illustrates a one-dimensional (1D) sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel (12% acrylamide) of rabbit tear from a normal versus a dry eye diseased state model and a protein molecular weight marker.¹ The technique of SDS-PAGE accomplishes the linear separation of proteins according to their molecular weights. In this process the proteins are first denatured and sulfide bonds are cleaved, effectively unraveling the tertiary and secondary structure of the protein. Sodium dodecylsulfate, which is negatively charged, is then used to

6 INTRODUCTION AND BASIC DEFINITIONS

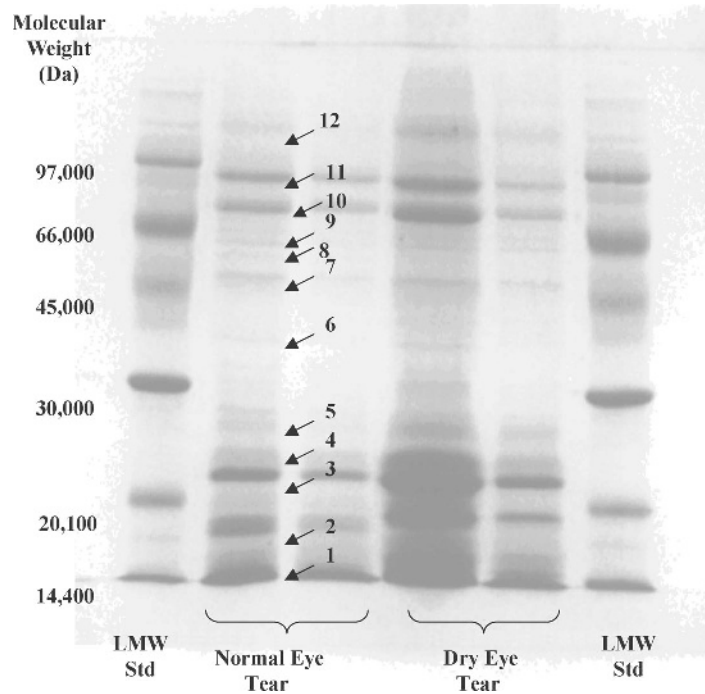


FIGURE 1.2 One-dimensional SDS-PAGE gel of rabbit tear proteins from a study of normal eye versus a dry eye model. Arrows and numbers represent bands of separated proteins that are used for protein identifications. (Reprinted with kind permission of Springer Science and Business Media from Ham, B. M., Jacob, J. T., and Cole, R. B. Single eye analysis and contralateral eye comparison of tear proteins in normal and dry eye model rabbits by MALDI-TOF mass spectrometry using wax-coated target plates. *Anal. Bioanal. Chem.* 2007, 3, Figure 1, p. 891.)

coat the protein in a fashion that is proportional to the proteins' molecular weight. The proteins are then separated within a polyacrylamide gel by placing a potential difference across the gel. Due to the potential difference across the gel, the proteins will experience an electrophoretic movement through the gel, thus separating them according to their molecular weight with the lower molecular weight proteins having a greater mobility through the gel and the higher molecular weight proteins having a lower mobility through the gel. There are 12 clear bands observed and designated in Figure 1.2, as indicated by the numbering and associated arrows. These bands represent proteins of different molecular weights that have been separated electrophoretically. Often bands such as these are cut from the gel and the proteins are digested with an enzyme such as trypsin, which is an endopeptidase that cleaves within the polypeptide chain of the protein at the carboxyl side of the basic amino acids arginine and lysine (the trypsin enzyme has optimal activity at a pH range of 7–10 and requires the presence of Ca^{2+}). The enzyme-cleaved

1.3 MASS SPECTROMETRY OF PROTEIN, METABOLITE, AND LIPID BIOMOLECULES 7

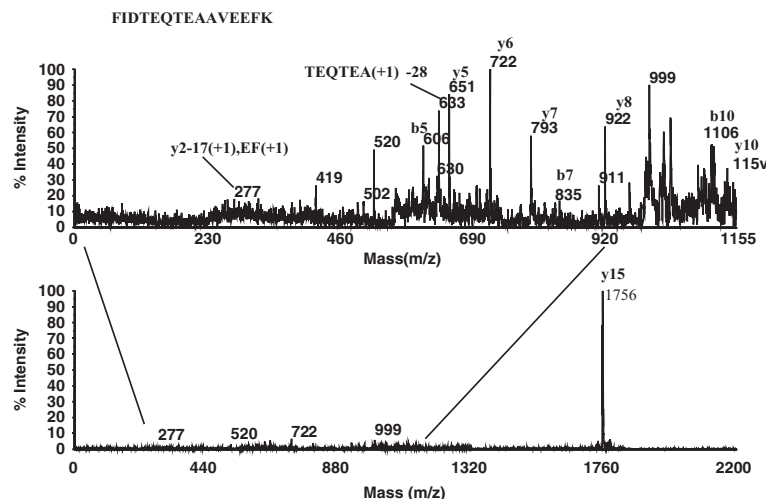


FIGURE 1.3 Matrix-assisted laser desorption ionization (MALDI) time-of-flight (TOF) postsource decay (PSD) mass spectrum illustrating the identification of the protein lipophilin CL2 recovered from the band 1 in Figure 1.2. (Reprinted with kind permission of Springer Science and Business Media from Ham, B. M., Jacob, J. T., and Cole, R. B. Single eye analysis and contralateral eye comparison of tear proteins in normal and dry eye model rabbits by MALDI-TOF mass spectrometry using wax-coated target plates. *Anal. Bioanal. Chem.* 2007, 3, Figure 5, p. 895.)

peptides are then extracted from the gel and analyzed by mass spectrometry for their identification.

Figure 1.3 shows the identification of one of the proteins as lipophilin CL2¹ recovered from band 1 in Figure 1.2 using the mass spectrometric technique of MALDI time-of-flight (TOF) postsource decay (PSD) mass spectrometry. A recent example of a 2D SDS-PAGE approach was reported by Nabetani et al.² in a study of patients with primary hepatolithiasis, an intractable liver disease, where the authors reported the up-regulation of 12 proteins and the down-regulation of 21 proteins. Another recent approach for proteomic studies by mass spectrometry uses a technique known as multidimensional protein identification technology (MudPIT).³ This is a gel-free approach that utilizes multiple HPLC–mass spectrometry analysis of in-solution digestions of protein fractions. Kislinger et al.⁴ demonstrated the effectiveness of this approach in a study of biochemical (mal)-adaptations involving heart tissue that was both healthy and diseased. Other examples include the spatial profiling of proteins and peptides on brain tissue sections,⁵ the analysis of viruses' capsid proteins for viral identification and posttranslational modifications,⁶ and structural immunology studies including antibody and antigen structures, immune complexes, and epitope sequencing and identification.⁷ The discipline of proteomics and the applications of mass spectrometry to protein analysis will be extensively covered in Chapter 7.

8 INTRODUCTION AND BASIC DEFINITIONS

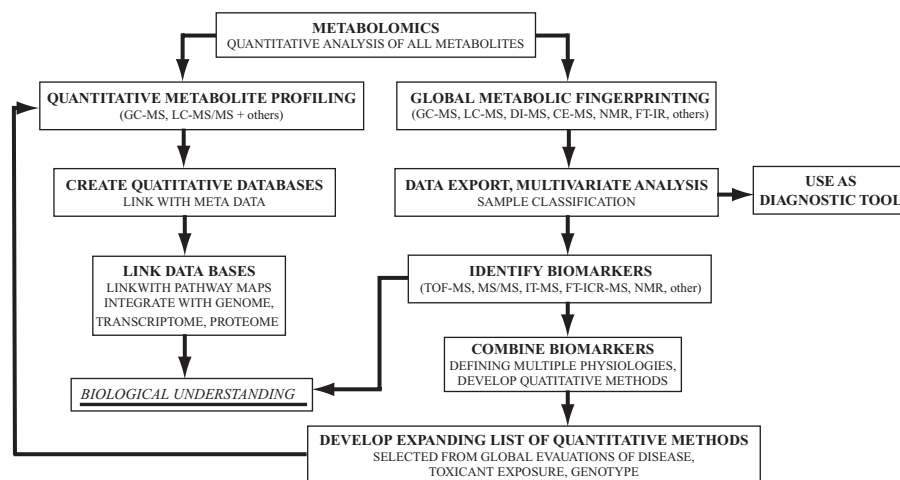


FIGURE 1.4 Strategies for metabolomic investigations. (Reprinted with permission of John Wiley & Sons, Inc. Dettmer, K., Aronov, P. A., and Hammock, B. D. Mass spectrometry-based metabolomics. *Mass Spectrom. Rev.* 2007, 26, 51–78. Copyright 2007.)

1.3.2 Metabolomics

The study of global metabolite profiles that are contained within a system at any one time representing a set of conditions is metabolomics.⁸ The system can be comprised of a cell, tissue, or an organism. Metabolomics has also been described as the measurement of all metabolite concentrations in cells, tissues, and organisms, while metabonomics involves the quantitative measurement that pathophysiological stimuli or genetic modification have upon the metabolic responses of multicellular systems.⁹ The system's genomic interaction with the environmental system condition results in the production of metabolites. Metabolites represent an extremely diverse and broad set of biomolecule classes that include lipids, amino acids, nucleotides, and organic acids and cover widely different concentration ranges.¹⁰ It has been estimated that there are from 2000 major metabolites¹¹ to 5000, with over 20,000 genes and 1 million proteins in humans.¹² Figure 1.4 illustrates the investigative strategies for two major approaches of mass-spectrometry-based metabolomics. The first strategy of the figure (left) is quantitative metabolic profiling, where generally predefined metabolites are targeted and measured to answer a biological question(s). The second strategy of the figure (right) is global metabolic fingerprinting, where the patterns or “fingerprints” of metabolites of a system are compared to measure changes due to a response of the system to disease, environmental changes, and the like. Table 1.1 lists some metabolomic-related definitions.

1.3.3 Lipidomics

Lipidomics has also experienced a dramatic increase in the amount of literature reported studies using mass spectrometry. Lipidomics involves the study of the lipid

TABLE 1.1 Metabolomic-Related Definitions

Metabolite	Small molecules that participate in general metabolic reactions and that are required for the maintenance, growth, and normal function of a cell. ^a
Metabolome	The complete set of metabolites in an organism.
Metabolomics	Identification and quantification of all metabolites in a biological system.
Metabolic Profiling	Quantitative analysis of set of metabolites in a selected biochemical pathway or a specific class of compounds. This includes target analysis, the analysis of a very limited number of metabolites, e.g., single analytes as precursors or products of biochemical reactions.
Metabolic Fingerprinting	Unbiased, global screening approach to classify samples based on metabolite patterns or “fingerprints” that change in response to disease, environmental, or genetic perturbations with the ultimate goal to identify discriminating metabolites.
Metabolic Footprinting	Fingerprinting analysis of extracellular metabolites in cell culture medium as a reflection of metabolite excretion or uptake by cells.

^a This is in contrast to xenobiotic or foreign compound metabolites, which may overshadow natural metabolites in an analytical procedure. However, they can be very valuable in evaluating the physiological status of the organism.

Source: K. Dettmer, P. A. Aronov, and B. D. Hammock, Mass spectrometry-based metabolomics. *Mass Spectrom. Rev.*, 26, 51–78, 2007. Copyright 2007. Reprinted with permission of John Wiley & Sons, Inc.

profile (or lipidome) of cellular systems and the processes involved in the organization of the protein and lipid species present,¹³ including the signaling processes and metabolism of the lipids.¹⁴ Lipids are a diverse class of physiologically important biomolecules that are often classified into three broad classes: (1) the simple lipids such as the fatty acids, the acylglycerols, and the sterols such as cholesterol, (2) the more complex polar phosphorylated lipids such as phosphatidylcholine and sphingomyelin, and (3) the isoprenoids, terpenoids, and vitamins. Recently, the Lipid Maps Organization has proposed an eight-category lipid classification (fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, prenol lipids, saccharolipids, and polyketides) that is based upon the hydrophobic and hydrophilic characteristics of the lipids.¹⁵ Past methodology for the analysis of lipids often relied heavily on thin-layer chromatography, which generally requires a substantial amount of sample and the detection is nonspecific, done either visually or by spray phosphorus. For specific identifications the spot is usually scrapped from the plate, the lipids extracted, and then either analyzed directly or reacted with other reagents to increase volatility or detection ability with chromophore or fluorophore labeling. Often mass spectrometry can be used to separate biomolecules as illustrated in a single-stage spectrum that shows the molecular species present. Mass spectrometric experiments can then be performed where a single analyte species of interest observed in the single-stage mass spectrum is isolated from all of the other species present and collision-induced decomposition is performed. This effectively fragments the molecular species, which is recorded and illustrated in a product ion spectrum. The structure of the fragmented species can then be ascertained from the product ions produced. Figure 1.5 illustrates an electrospray ionization (ESI) single-stage mass spectrum of a complex biological extract where a number of molecular ion species

10 INTRODUCTION AND BASIC DEFINITIONS

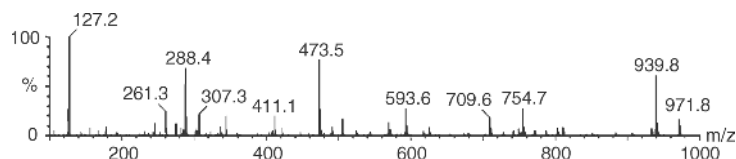


FIGURE 1.5 Electrospray ionization quadrupole–hexapole–quadrupole (ESI/QhQ) mass spectrum of the lithium adducts of a complex biological extract illustrating a number of biomolecules that are effectively separated in the gas phase.

are being observed as lithium adducts $[M + Li]^+$. The mass spectrometer has effectively separated these species according to their respective mass-to-charge ratios (m/z), producing a spectrum of peaks that represent individual biomolecular species. Using a type of mass spectrometer known as a quadrupole–hexapole–quadrupole mass spectrometer, the m/z 631 precursor ion species was isolated from all of the other species present and a tandem mass spectrometry experiment was performed as collision-induced dissociation (CID) of the precursor m/z 631 ion to produce the product ion spectrum illustrated in Figure 1.6. The quadrupole–hexapole–quadrupole mass spectrometer uses the inherent ability of a quadrupole mass analyzer to filter out all precursor ions except the one of interest, thus allowing it to pass through the system for the ensuing CID fragmentation study. Due to their filtering effect, quadrupoles are often referred to as mass filters. As illustrated in the product ion spectrum in Figure 1.6, there are two ranges of product ions, for the low range approximately m/z 43 to m/z 99 and for the high range approximately m/z 291 to m/z 365, that are used to deduce the structure and identity of the unknown biomolecule m/z 631. Using the mass spectrometric technique of CID and subsequent fragmentation, the unknown m/z 631 species was identified as 1,3-distearin. The process used in identifying unknowns such as the m/z 631 precursor biomolecule from its product ion spectrum will be extensively discussed in the ensuing chapters (see Chapters 5–10).

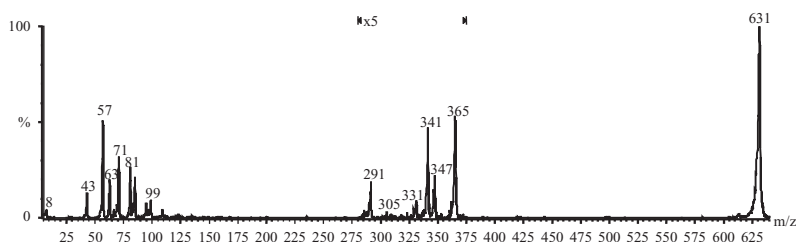


FIGURE 1.6 Electrospray ionization quadrupole–hexapole–quadrupole (ESI/QhQ) product ion mass spectrum of the lithium adduct precursor ion m/z 631 illustrated in Figure 1.5. Two product ion ranges, a high and a low, are observed that are used for structural identification of the unknown biomolecule. The m/z 631 species was identified as 1,3-distearin.

A recent review by Pulfer and Murphy¹⁶ offers an informative discussion of the analysis of the phosphorylated lipids by mass spectrometry with a focus upon electrospray as the ionization technique, while Griffiths¹⁷ covers the mass spectrometric analysis of the simple class of lipids (fatty acids, triacylglycerols, bile acids, and steroids). Recent examples of the application of mass spectrometry to biological sample lipid analysis includes a study by Lee et al.¹⁸ where the relatively new technique of electron capture atmospheric pressure chemical ionization mass spectrometry was used in quantitative work of rat epithelial cell lipidomes, the analysis of human cerebellum gangliosides by nanoelectrospray tandem mass spectrometry,¹⁹ the analysis of lyso-phosphorylated lipids in ascites from ovarian cancer patients,²⁰ the analysis of Amadori-glycated phosphatidylethanolamine in plasma samples from individuals with and without diabetes using a quadrupole ion trap mass spectrometer,²¹ and the identification of nonpolar lipids and phosphorylated lipids in tear.^{22,23}

A primary direction illustrated by these studies is for the understanding of biological systems in both normal and diseased states using the investigative technique of mass spectrometry. Proposed biological systems investigated can include studies of the expression of biomolecules from diseased states and stress models searching for relevant markers, the study of biological fluids for characterization, the study of biological processes intra- and extracellular, and the study of biomolecule interactions. Areas of mass spectrometric study of biomolecular systems include the characterization of the specific classes of proteins, lipids, carbohydrates, sugars, and so forth, the development of extraction, purification, and enrichment of biomolecules from complex biological samples, the development of quantitative analysis of biomolecules, the development of identification methods for biomolecules, and the identification and quantification of biomolecules in normal and diseased state expression studies and their function and importance. The discipline of lipidomics and the application of mass spectrometry to the analysis of lipids will be extensively covered in Chapter 8.

1.4 FUNDAMENTAL STUDIES OF BIOLOGICAL COMPOUND INTERACTIONS

The study of thermodynamic properties of complexes such as bond dissociation energies by CID tandem mass spectrometry for the measurement of the dissociation of gas-phase complexes has been a useful approach. For the study of the bond dissociation energy (BDE) of a cobalt carbene ion Armentrout and Beauchamp²⁴ introduced an ion beam apparatus and have since refined the measurement of noncovalent, collision-induced threshold bond energies of metal–ligand complexes by mass spectrometry. There are extensive examples of the use of CID tandem mass spectrometry²⁵ for the measurement of thermodynamic properties of gas-phase complexes including investigations of noncovalent interactions,^{26–29} bond dissociation energies,¹⁶ bond dissociation energies using guided ion beam tandem mass spectrometry,³⁰ gas-phase dissociative electron-transfer reactions,³¹ critical energies for ion dissociation

12 INTRODUCTION AND BASIC DEFINITIONS

using a quadrupole ion trap,²⁶ or an electrospray triple quadrupole,^{32,33} or a flowing afterglow triple quadrupole,^{34,35} and gas-phase equilibria.^{36–38}

During the ionization process, electrospray^{39–42} is an ionization method that is well known to produce intact gas-phase ions with minimal fragmentation. Species originating from solution are quite accessible by electrospray mass spectrometry for studies of gas-phase thermochemical properties. Another “soft” ionization approach to gas-phase ionic complex analysis is matrix-assisted laser desorption/ionization (MALDI); however, during crystallization with the matrix the complex’s character in the solution phase can be lost. However, using a MALDI ion source coupled to a hybrid mass spectrometer, Gidden et al.⁴³ derived experimental cross sections for a set of alkali metal ion cationized poly(ethylene terephthalate) (PET) oligomers. With the advent of soft ionization techniques such as electrospray, it is now possible to study intact, wild-type associations such as lipid–metal/nonmetal, lipid–lipid, lipid–peptide, lipid–protein, protein–protein, and metabolite–protein adducts and complexes of both noncovalent and covalent-type bonding. Electrospray effectively allows the transfer of intact associations within solution phase (physiological/aqueous, or organic solvent modified) to the gas phase without interruption of the weak associations, thus preserving the original interaction (however, specific and nonspecific interactions need to be deciphered). Methods for determining the dissociation energies of these types of associations that can be applied to these studies have been developed.⁴⁴

Figure 1.7 is the product ion spectrum of the diacylglycerol 1-stearin,2-palmitin illustrating the product ions produced upon collision-activated dissociation measured by an electrospray quadrupole–hexapole–quadrupole mass spectrometer (ESI/QhQ-MS). Using energy-resolved studies, it is possible to estimate the energies that were required to initiate bond breakage to form the product ions that are illustrated in Figure 1.7.

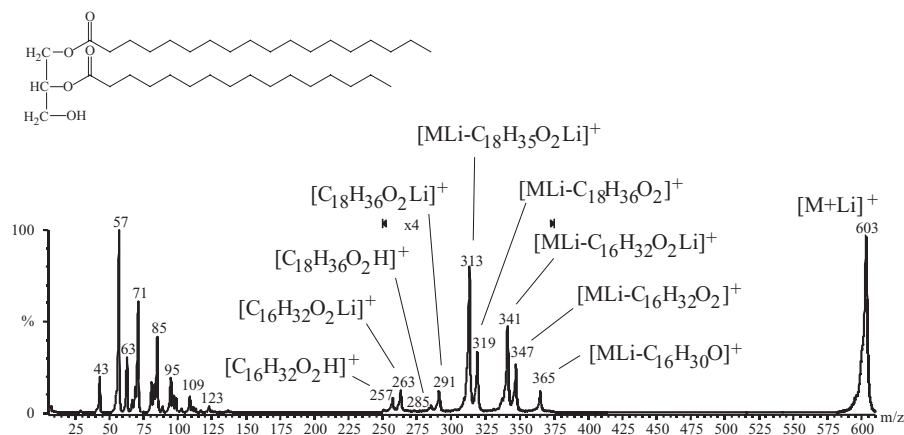


FIGURE 1.7 Product ion spectrum of the lithium adduct of the diacylglycerol 1-stearin,2-palmitin at m/z 603 illustrating the product ions generated by collision-induced dissociation.

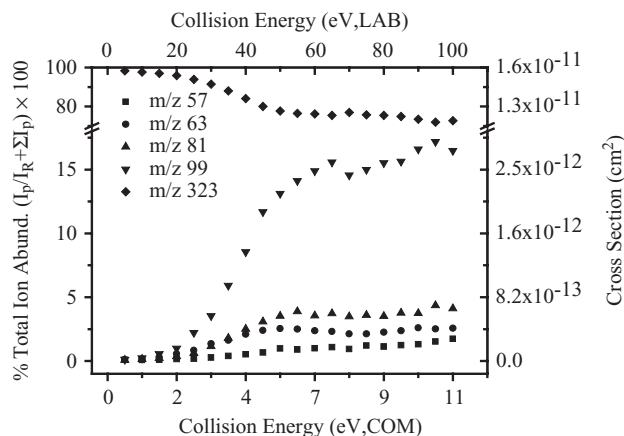
1.5 MASS-TO-CHARGE RATIO (m/z): HOW THE MASS SPECTROMETER SEPARATES IONS 13

FIGURE 1.8 Energy resolved breakdown graph illustrating product ion formation with increasing internal energy for the collision-induced dissociation of monpentadecanoin.

Figure 1.8 is a energy-resolved breakdown graph of the production of lower molecular weight ions in CID experiments of monpentadecanoin showing increased production with increasing internal energy. The experiments used to construct the plot in Figure 1.8 are performed by measuring the products produced by CID such as those illustrated in Figure 1.7 at increasing internal energies. These results can then be used to relate the measured products of the CID experiments to the dissociation energies involved in the bond cleavages. Figure 1.9 is an energy diagram describing the production of a selected number of low-molecular-weight product ions from the CID of monpentadecanoin. Energy-resolved experiments and the application of mass spectrometry to gas-phase thermochemistry will be covered further in Chapter 4.

1.5 MASS-TO-CHARGE RATIO (m/z): HOW THE MASS SPECTROMETER SEPARATES IONS

For an analyte to be separated by a mass analyzer and subsequently measured by a detector, it must first be in an ionized state. Often the analyte is in a neutral state whether in a solid or liquid matrix (e.g., in a soil sample, solid tissue sample, an aqueous solution, or as an organic solvent extract). Using various ionization techniques, the analyte is ionized and transferred into the gas phase in preparation for introduction into the mass analyzer (see Chapters 2 and 3). The ionization of the analytes and transfer into the gas phase are typically done in an apparatus called a source, which acts as a front-end preparation stage for the mass analysis of the analyte as a positively or negatively charged ion. An analyte M can be in the form of a protonated molecule giving the analyte species a single positive charge

14 INTRODUCTION AND BASIC DEFINITIONS

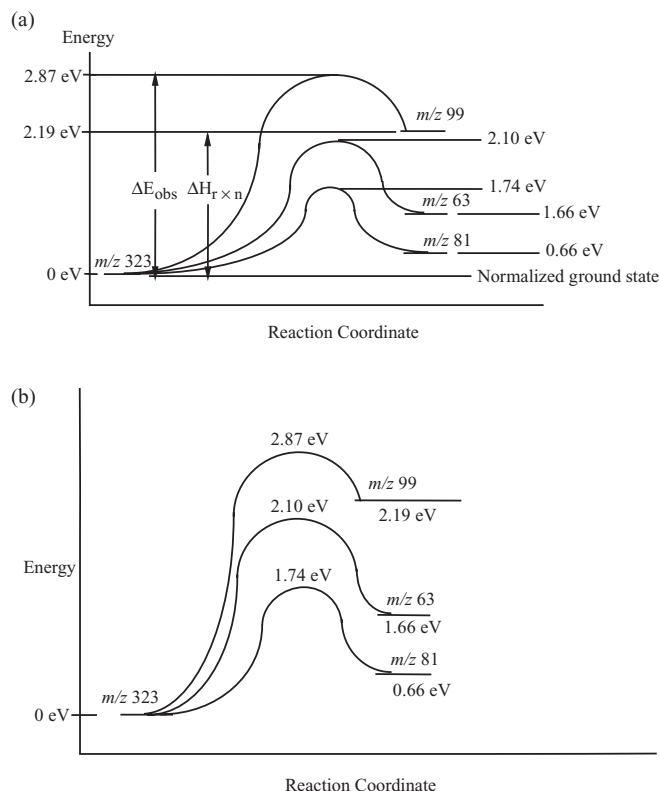


FIGURE 1.9 Energy diagram describing the formation of selected product ions from the collision-induced dissociation of monpentadecanoin.

$[M + H]^+$, a metal adduct such as sodium giving the molecular species a single positive charge $[M + Na]^+$, a chloride adduct giving the molecular species a single negative charge $[M + Cl]^-$, a deprotonated state giving the molecular species a single negative charge $[M - H]^-$, through electron loss giving the molecular species a single positive charge as a radical M^+ (also known as a molecular ion), and so on. All mass analyzers that are used in mass spectrometric analysis possess a characteristic means of relating an analyte species' mass to its respective charge (m/e), where m is the species mass and e is the fundamental charge constant of 1.602×10^{-19} C. The time-of-flight (TOF) mass analyzer affords a simple and direct example of the mass analyzer's physical relationship of analyte mass to analyte charge. The TOF mass analyzer separates analytes by time that have drifted through a preset described length drift tube. In the ionization source the analytes have all been given the same initial kinetic energy (KE); therefore, their physical description of $\frac{1}{2}mv^2$ are virtually constant for all of the ions for their drift through the drift tube. Using the relationship of $KE = zeV = \frac{1}{2}mv^2$, where V is the acceleration potential, and v is the velocity of

1.5 MASS-TO-CHARGE RATIO (m/z): HOW THE MASS SPECTROMETER SEPARATES IONS 15

the ion, an analyte with a larger mass m will possess a lower velocity v . The time (t) for the ions to arrive at the detector at the end of the drift tube is measured and is converted to a mass scale:

$$\text{KE} = zeV = \frac{1}{2}mv^2 \quad (1.1)$$

$$v = \left(\frac{2zeV}{m} \right)^{1/2} \quad (1.2)$$

$$t = \frac{L}{v} \quad L = \text{length of drift tube} \quad (1.3)$$

$$t = L \left(\frac{m}{2zeV} \right)^{1/2} \quad V \text{ and } L \text{ are fixed} \quad (1.4)$$

Solving for m/z :

$$t^2 = L^2 \left(\frac{m}{2zeV} \right) \quad (1.5)$$

$$\frac{m}{z} = \frac{2eVt^2}{L^2} \quad (1.6)$$

Thus, we have related the mass-to-charge ratio (m/z) of the analyte species as a positively or negatively charged ionic species to the drift time (t) of the ion through the drift tube while keeping the acceleration potential (V) and the length (L) of the drift tube fixed. This effectively allows the TOF mass analyzer to separate ionized species according to their mass-to-charge ratio. In general, an ion with a smaller mass and a larger charge will have the shorter drift time t . All mass analyzers must possess the physical characteristic of separating ionized species according to their mass-to-charge ratio. This characteristic aspect of general mass analyzers will be further discussed and illustrated in Chapter 3.

An example of a typical mass spectrum illustrating one usual design used is illustrated in Figure 1.10. The x axis of the mass spectrum represents the mass-to-charge (m/z) ratio measured by the mass analyzer, and the y axis is a percentage scale that has been normalized to the most abundant species in the spectrum. Often the

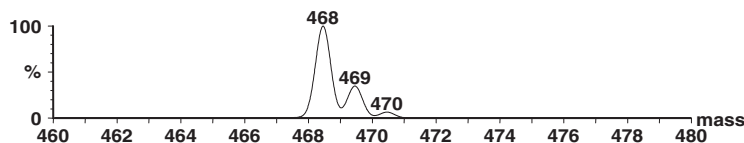


FIGURE 1.10 Typical mass spectrum illustrating three peaks at m/z 468, m/z 469, and m/z 470. The y axis is in percentage response and the x axis represents the mass-to-charge ratio (m/z).

16 INTRODUCTION AND BASIC DEFINITIONS

y axis may also be presented as an intensity scale of arbitrary units. In this particular mass spectrum there are three peaks illustrated at m/z 468, m/z 469, and m/z 470.

1.6 EXACT MASS VERSUS NOMINAL MASS

Mass analyzers with high enough resolution have the ability to separate the naturally occurring isotopes of the elements. Therefore, in mass spectra there is often observed a series of peaks for the same species. This is illustrated in Figure 1.11 for an m/z 703.469 species (the farthest most left peak with lowest mass). The isotopic peaks are at m/z 704.465 and m/z 705.480. The m/z 703.469 peak is known as the monoisotopic peak where the particular species is made up of ^{12}C , and, if containing hydrogen, oxygen, nitrogen, phosphorus, and the like, it will be comprised of the most abundant isotope for that element: ^1H , ^{16}O , ^{14}N , ^{31}P , and so forth. Organic compounds are primarily comprised of carbon and hydrogen. Therefore, it is these two elements that contribute the most to the isotopic pattern observed in mass spectra (mostly carbon contribution) and to the mass defect of the molecular weight of the species (mostly hydrogen contribution). The mass defect of a species is the difference between the molecular formula's nominal value and its exact mass. A molecular formula nominal mass is calculated using the integer value of the most abundant isotope and

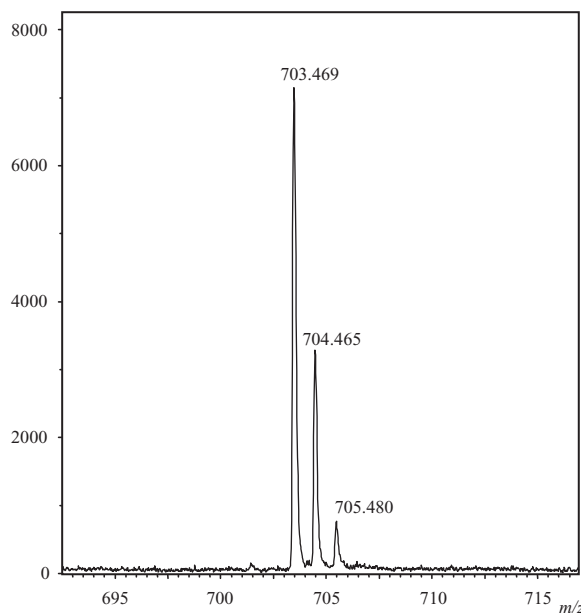


FIGURE 1.11 Mass spectrum of isotopically resolved peaks where m/z 703.469 species (the farthest most left peak of lowest mass) contains only ^{12}C and is known as the monoisotopic peak, the m/z 704.465 peak contains one ^{13}C , and the m/z 705.480 peak contains two ^{13}C .

a molecular formula exact mass (monoisotopic mass) is calculated using the most abundant isotopes' exact masses. For a formula of $C_{10}H_{20}O_3$, the nominal mass is $10 \times 12 \text{ amu} + 20 \times 1 \text{ amu} + 3 \times 16 \text{ amu} = 188 \text{ amu}$, and the exact monoisotopic mass is $10 \times 12 \text{ amu} + 20 \times 1.007825035(12) \text{ amu} + 3 \times 15.99491463(5) \text{ amu} = 188.141245 \text{ amu}$. Therefore, the mass defect is equal to $188.141245 \text{ amu} - 188 \text{ amu} = 0.141245 \text{ amu}$. For the mass spectrum shown in Figure 1.11 the second peak at m/z 704.465 is due to the inclusion of one ^{13}C into the structure of the measured species. The peak at m/z 705.480 is present due to the inclusion of two ^{13}C into the structure of the measured species.

The periodic table that the student is most familiar with lists the average weighted mass for the various elements listed in the table. For example, in the periodic table carbon is listed as 12.0107 amu, while in exact mass notation used in mass spectrometry carbon has an atomic weight of 12 amu. The abundances of the natural isotopes of carbon are ^{12}C (12.000000 amu by definition)⁴⁵ at 98.93(8)% and ^{13}C [13.003354826(17) amu] at 1.07(8)%. Therefore, the weighted average of the two carbon isotopes gives a mass of 12.0107 amu as is found in periodic tables. This is true of all the elements where the average atomic weight values in the periodic table represent the sum of the mass of the isotopes for that particular element according to their natural abundances (see Appendix 1 for a listing of the naturally occurring isotopes).

Some of the elements such as chlorine or bromine if present will also have a significant influence on the isotopic pattern observed in mass spectra. For example, chlorine has two isotopes: ^{35}Cl [34.968852721(69) amu at 75.78(4)% natural abundance] and ^{37}Cl [36.96590262(11) amu at 24.22(4)% natural abundance] that produces a more complex isotope pattern than that illustrated in Figure 1.10. The contribution of a chlorine atom to the isotopic pattern seen in mass spectra is illustrated in Figure 1.12 where the top spectrum is the isotope pattern for a molecular formula of $C_{30}H_{60}O_3$ (nominal mass of 468). In this isotope pattern there are three peaks observed for the ^{12}C monoisotopic peak at m/z 468, the inclusion of one ^{13}C at m/z 469, and the inclusion of two ^{13}C at m/z 470. If we take the same formula and add one chlorine

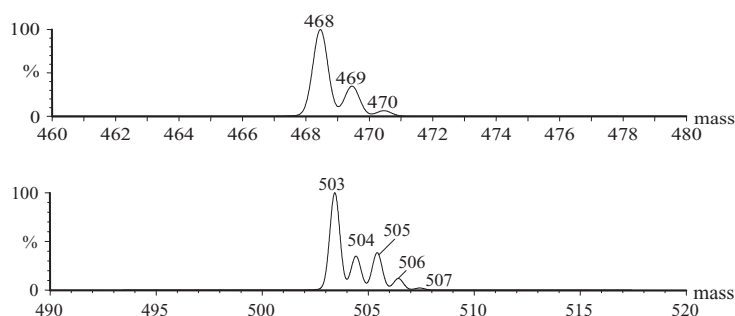


FIGURE 1.12 Isotope patterns for molecular formulas $C_{30}H_{60}O_3$ (top with m/z 468 nominal mass) and $C_{30}H_{60}O_3Cl$ (bottom with m/z 503 nominal mass).

18 INTRODUCTION AND BASIC DEFINITIONS

atom, giving a molecular formula of $C_{30}H_{60}O_3Cl$ (nominal mass of 503), we will see a more complex isotope pattern. This is illustrated in the bottom mass spectrum of Figure 1.12 where there are now five clear isotopic peaks present. The m/z 503 peak represents the ^{12}C and ^{35}Cl containing species, the m/z 504 peak represents the ^{12}C and ^{35}Cl containing species with one ^{13}C , the m/z 505 peak represents a mixture of a ^{12}C and ^{35}Cl containing species with two ^{13}C and a ^{12}C and ^{37}Cl containing species, the m/z 506 peak represents a ^{12}C and ^{37}Cl containing species with one ^{13}C , and the m/z 507 peak represents a ^{12}C and ^{37}Cl containing species with two ^{13}C . This can, in fact, be a useful tool when a more complex isotopic series is observed that contains more than three peaks in the identification of elements present. Most software packages used with mass spectrometers have the ability to calculate and display isotopic series for a given atomic or molecular formula and to perform isotopic pattern matching.

1.7 MASS ACCURACY AND RESOLUTION

Mass accuracy in mass spectrometry is related to the calibration of the mass analyzer to properly assign the true mass-to-charge ratio to a detected ion and to the resolution of the detector response, which is in the form of an intensity spike or peak within a mass spectrum. In calibrating a mass spectrometer a series of standard compounds, usually in the form of a multicomponent standard, are measured and related to the fundamental properties of the mass analyzer such as the drift time within a TOF mass spectrometers drift tube. An example of an electrospray TOF mass analyzer calibration is illustrated in Figure 1.13 for sodium iodide (NaI) clusters. The top

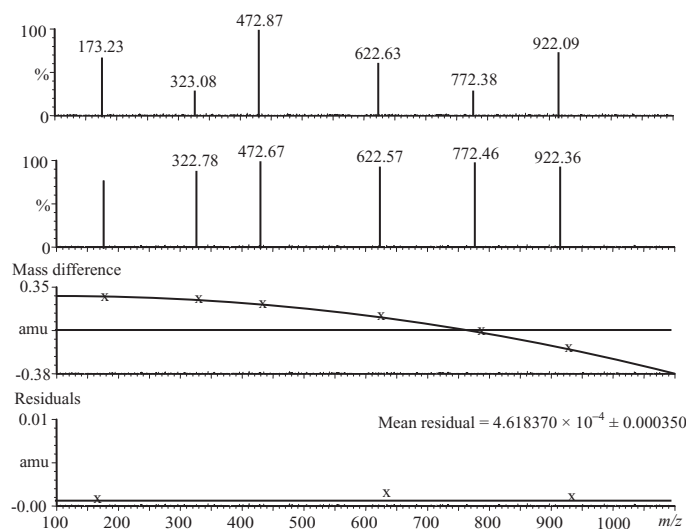


FIGURE 1.13 Calibration output for NaI clusters.

spectrum is the experimental spectrum obtained on the instrument in the laboratory. The second to top spectrum is a reference spectrum of a sodium iodide standard. A mass difference plot and residual plot are also displayed, showing the statistical results of the calibration fit. Calibrations of mass analyzers can be linear or polynomial; however, if more than two points are used for the calibration of the mass analyzer, a polynomial fit is generally used.

The mass accuracy in mass spectrometry is usually calculated as a parts-per-million (ppm) error where the theoretical mass (calculated as the monoisotopic mass) is subtracted from the observed mass, divided by the observed mass and multiplied by a 10^6 factor. Mass accuracy (ppm error) can thus be represented as

$$\text{Mass accuracy (ppm)} = \frac{m_{\text{observed}} - m_{\text{theoretical}}}{m_{\text{observed}}} \times 10^6 \quad (1.7)$$

In mass spectrometry mass resolution is generally calculated as $m/\Delta m$ where m is the m/z value obtained from the spectrum and Δm is the full peak width at half maximum (FWHM). This is illustrated in Figure 1.14 for the m/z 703.469 ion, which has an FWHM of 0.1797 m/z . The resolution of the m/z 703.469 would be calculated as $m/\Delta m = 703.469/0.1797 = 3914$. The narrower and sharper a peak is will result in a higher resolution value and a better estimate of the apex of the Gaussian-shaped peak, which thus results in a better estimate of the true value of the mass-to-charge ratio and therefore higher accuracy. In contrast to this, as a peak becomes

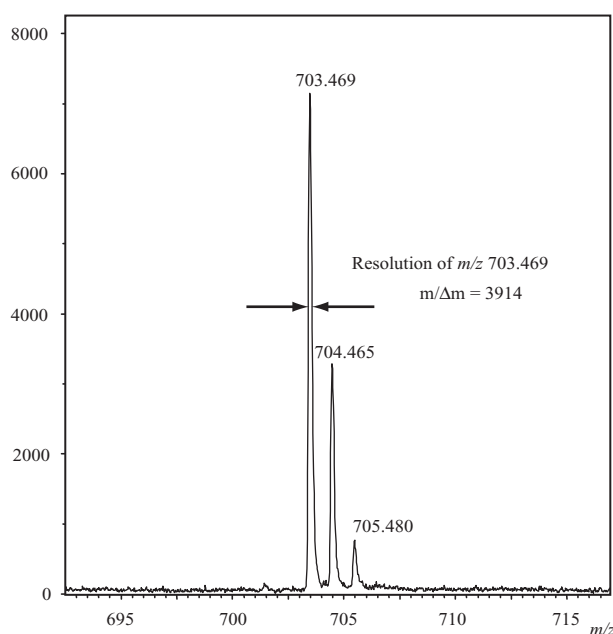


FIGURE 1.14 Full width at half maximum (FWHM) calculation of the resolution of m/z 703.469 as $m/\Delta m = 3914$.

20 INTRODUCTION AND BASIC DEFINITIONS

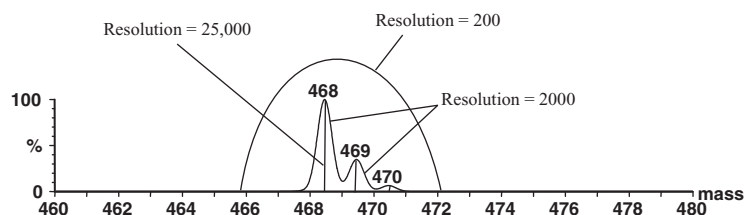


FIGURE 1.15 Effect of mass resolving power on the mass spectrum.

broader and wider, the apex of the peak becomes more distorted, resulting in a lower resolution value and thus reducing the accuracy of the estimate of the mass-to-charge ratio (m/z).

To get a better idea of the impact that the mass resolving power of a mass analyzer has upon mass spectra, the illustration in Figure 1.15 shows a comparison of a mass resolution of approximately 200 for the overall curve where none of the isotopic peaks have been resolved. A resolution of 200 will give an average mass value for the species being measured with a high degree of error for the exact mass (>500 ppm). The three isotope peaks are resolved at a resolution of 2000 and an intermediate accurate exact mass is obtainable. At a resolution of 25,000 the three isotopic peaks are completely baseline resolved and a mass accuracy typically less than 5 ppm can be obtained.

1.8 HIGH-RESOLUTION MASS MEASUREMENTS

There is a useful property that can be used when measuring the monoisotopic peak previously discussed in Section 1.6. For low-molecular-weight species (usually less than 2000 amu) measured as mass-to-charge ratio ions (m/z) in the gas phase using mass spectrometry, the monoisotopic peak will be the left most peak (lowest m/z value) of the isotopic peaks for that particular species, the most abundant peak in the isotopic series, and will contain only ^{12}C isotopes. Again, by definition and used as a standard the ^{12}C isotope has been assigned a mass of 12.00000000 amu. In bioorganic molecules the isotopic species most often encountered are carbon 12 as $^{12}\text{C} = 12.00000000$ amu, hydrogen 1 as $^1\text{H} = 1.007825035$ amu, nitrogen 14 as $^{14}\text{N} = 14.00307400$ amu, phosphorus 31 as $^{31}\text{P} = 30.973763$ amu, and oxygen 16 as $^{16}\text{O} = 15.99491463$ amu. When adding up the isotopes' mass contributions for a particular molecule (known as the theoretical molecular weight), such as a lithium adduct of cis-9-octadecenamide as $[\text{C}_{18}\text{H}_{35}\text{NOLi}]^+$, a cation in positive ion mode mass spectrometry, the molecular weight will include the mass error that is associated with the masses just listed for hydrogen (+0.007825035 amu), nitrogen (+0.00307400 amu), phosphorus (−0.026237 amu), and oxygen (−0.00508537 amu) where phosphorus and oxygen actually have a negative mass defect. The theoretical molecular weight for the lithium adduct of cis-9-octadecenamide as $[\text{C}_{18}\text{H}_{35}\text{NOLi}]^+$ is m/z 288.2879 (shown as a mass-to-charge m/z ion measured by mass spectrometry). To a certain

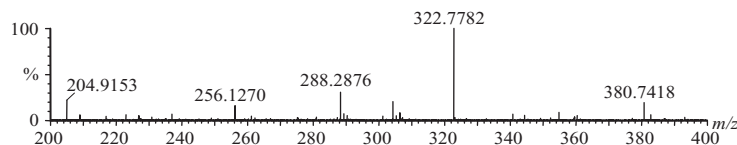
TABLE 1.2 Possible Formulas for m/z 288.2879 at a Mass Accuracy of 50 ppm

Theoretical Mass (Da)	Calculated Mass (Da)	Mass Difference (Da)	Mass Error (ppm)	Formula
288.2879	288.2879	0.0	0.0	$C_{18}H_{35}LiNO$
	288.2903	-2.4	-8.2	$C_{17}H_{38}NO_2$
	288.2855	2.4	8.4	$C_{19}H_{32}Li_2N$
	288.2815	6.4	22.3	$C_{14}H_{32}Li_2N_3O_2$
	288.2991	-11.2	-38.9	$C_{17}H_{35}LiN_3$
	288.3015	-13.6	-47.1	$C_{16}H_{38}N_3O$

accuracy we can derive the only molecular formula that will fit the theoretical mass of 288.2879 Da. At a mass accuracy of 50 ppm and using the most common atoms found in biomolecules (and also lithium 7 at $^7Li = 7.016005$ Da as we already have the luxury of knowing what type of cationized species it is), there exists six molecular formulas for a mass of 288.2879 Da. The six molecular formulas are listed in Table 1.2.

As can be seen in Table 1.2 a mass accuracy of 25 ppm will include the top four entries, at a mass accuracy of 10 ppm the top three will be included, and finally at a mass accuracy of 5 ppm only the top formula will be included, which is in fact the correct one. In this example we knew the true formula beforehand. However, oftentimes the mass spectrometrists will be working with unknown samples. In this case, the most accurate measurement of the monoisotopic peak is essential for reasonable molecular formula determination or confirmation. A benchmark or rule-of-thumb value of ≤ 5 ppm for the mass error is often used for consideration of an unknown's molecular formula derived from a high-resolution mass measurement. Figure 1.16 shows the results of a high-resolution mass measurement of the lithium adduct of cis-9-octadecenamide. The spectrum has been smoothed, centroided, and a lock mass technique has been used for fine adjustment of the calibration curve within the vicinity of the monoisotopic peak of interest. In this particular case the NaI cluster peak at m/z 322.7782 has been used as an internal standard for fine adjustment of the calibration used in collecting this spectrum.

The monoisotopic peak of cis-9-octadecenamide has a high-resolution mass measurement value of m/z 288.2876, as illustrated in Figure 1.16. Using the mass accuracy equation of Equation (1.7), we calculate an error of 1.0 ppm for the high-resolution mass measurement of cis-9-octadecenamide. Using the mass spectrometric approach

**FIGURE 1.16** High-resolution mass measurement of cis-9-octadecenamide at m/z 288.2876 as the lithium adduct $[C_{18}H_{35}NOLi]^+$. The NaI cluster peak at m/z 322.7782 was used for the internal calibration standard.

22 INTRODUCTION AND BASIC DEFINITIONS

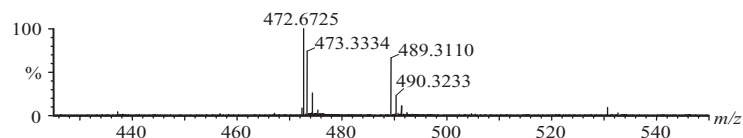


FIGURE 1.17 High-resolution mass measurement of unknown species at m/z 489.3110 as the sodium adduct $[M + Na]^+$. The NaI cluster peak at m/z 472.6725 was used for the internal calibration standard.

of high-resolution mass measurement and an error tolerance of ≤ 5 ppm, there is only one molecular formula that will match, demonstrating the utility of this methodology.

At higher molecular weights the possible molecular formulas that will match a high-resolution mass measurement will increase greatly for a mass error tolerance of ≤ 10 ppm. For example, for a high-resolution mass measurement of m/z 489.3110, illustrated in Figure 1.17 using the NaI cluster at m/z 472.6725 as the internal standard calibrant, gives 23 possible molecular formulas. The 23 molecular weight possibilities for the unknown species at m/z 489.3110 are listed in Table 1.3 for a mass error

TABLE 1.3 Possible Formulas for m/z 489.3110 at a Mass Accuracy of 10 ppm

Theoretical Mass (Da)	Calculated Mass (Da)	Mass Difference (Da)	Mass Error (ppm)	Formula
489.3110	489.3110	0.0	0.0	$C_{22}H_{51}O_7P_2$
	489.3110	0.0	0.1	$C_{27}H_{47}O_4PNa$
	489.3109	0.1	0.1	$C_{32}H_{43}ONa_2$
	489.3116	-0.6	-1.1	$C_{24}H_{49}N_2ONaP_2$
	489.3117	-0.7	-1.5	$C_{31}H_{41}N_2O_3$
	489.3099	1.1	2.2	$C_{21}H_{48}N_4O_3NaP_2$
	489.3099	1.1	2.2	$C_{26}H_{44}N_4Na_2P$
	489.3123	-1.3	-2.7	$C_{28}H_{43}N_4NaP$
	489.3123	-1.3	-2.7	$C_{23}H_{47}N_4O_3P_2$
	489.3094	1.6	3.4	$C_{24}H_{46}N_2O_6P_2$
	489.3093	1.7	3.4	$C_{29}H_{42}N_2O_3Na$
	489.3133	-2.3	-4.8	$C_{34}H_{42}ONa$
	489.3134	-2.4	-4.9	$C_{29}H_{46}O_4P$
	489.3086	2.4	5.0	$C_{25}H_{48}O_4Na_2P$
	489.3136	-2.6	-5.3	$C_{19}H_{45}N_4O_{10}$
	489.3140	-3.0	-6.1	$C_{26}H_{48}N_2ONaP_2$
	489.3077	3.3	6.8	$C_{26}H_{41}N_4O_5$
	489.3147	-3.7	-7.6	$C_{30}H_{42}N_4P$
	489.3069	4.1	8.3	$C_{22}H_{47}N_2O_6NaP$
	489.3069	4.1	8.4	$C_{27}H_{43}N_2O_3Na_2$
	489.3152	-4.2	-8.6	$C_{22}H_{46}N_2O_8Na$
	489.3064	4.6	9.5	$C_{25}H_{45}O_9$
	489.3157	-4.7	-9.7	$C_{36}H_{41}O$

tolerance of ≤ 10 ppm. The most abundant atoms that make up biomolecules were included in the molecular formula search and are C, H, N, O, P, and Na.

If the mass error tolerance is decreased to ≤ 5 ppm, it can be seen in Table 1.3 that there are still 14 possibilities for the identification of the unknown species molecular weight. If the mass error tolerance is decreased to ≤ 2 ppm, there are now still 5 possibilities, illustrating the utility of a high mass accurate measurement for decreasing the number of possible molecular formulas of an unknown species. Finally, the inclusion of the theoretical isotopic distribution can help to reduce the number of possible formulas associated with an unknown's molecular weight even further.

1.9 RINGS PLUS DOUBLE BONDS (r + db)

Due to the valences of the elements that make up most of the biomolecules that are analyzed by mass spectrometry, there is a very useful tool known as the “total rings plus double bonds” (r + db) that can be applied to molecular formulas such as $C_{12}H_{24}O_2$. The most general representation of the rings plus double bonds relationship is

$$r + db = \sum \text{Group IVA} - \frac{1}{2} \sum (H + \text{Group VIIA}) + \frac{1}{2} \sum \text{Group VA} + 1 \quad (1.8)$$

In mass spectrometry this expression is often simplified to only include the most common elements that typically make up the molecular ion species measured. This is generally presented as

$$r + db = x - \frac{1}{2}y + \frac{1}{2}z + 1 \quad (1.9)$$

for the molecular formula $C_xH_yN_zO_n$. For example, normal hexane has no rings plus double bonds, cyclohexane contains one ring plus double bonds and benzene contains four rings plus double bonds, as illustrated in Figure 1.18.

The rings plus double bonds relationship presented in Equations (1.8) and (1.9) are applicable to organic compounds and to odd electron (OE) molecular ions ($M^{+ \cdot}$) that are produced by electron ionization mass spectrometry (EI-MS). However, for even-electron (EE) ions that are produced by electrospray ionization mass spectrometry (ESI-MS) or matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) where the species has been ionized in the form of an acid adduct $[M + H]^+$, a metal adduct such as sodium $[M + Na]^+$, or a chloride adduct $[M + Cl]^-$, Equations (1.8) and (1.9) must be followed by the subtraction of 1/2. This is also a useful tool for interpreting whether a molecular ion is even electron or odd electron, as will be applied in the next section.

24 INTRODUCTION AND BASIC DEFINITIONS

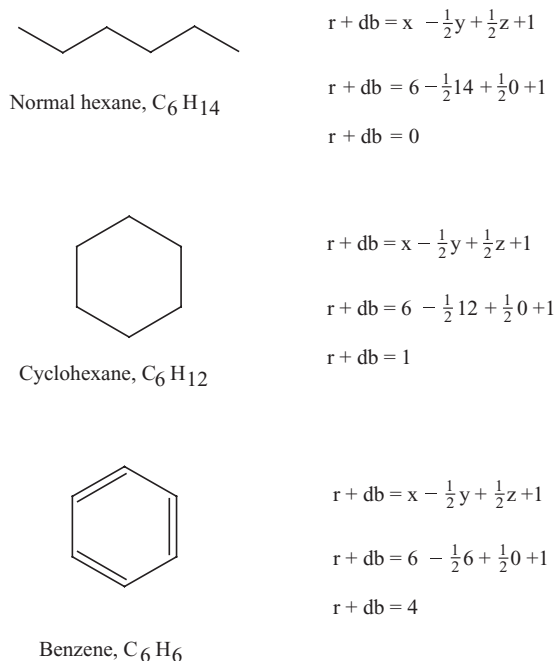


FIGURE 1.18 Application of the rings plus double bonds association for normal hexane, cyclohexane, and benzene using the simplified form of the equation for molecular formulas represented by C_xH_yN_zO_n.

1.10 NITROGEN RULE IN MASS SPECTROMETRY

Another useful tool used in mass spectrometry of biomolecule analysis is the so-called nitrogen rule. The nitrogen rule for organic compounds states that, if a molecular formula has a molecular weight that is an even number, then the compound contains an even number of nitrogen (e.g., 0N, 2N, 4N, . . . , etc.). The same applies to an odd molecular weight species, which will thus contain an odd number of nitrogen (e.g., 1N, 3N, 5N, . . . , etc.). The nitrogen rule is due to the number of valences that the different elements possess. The number of valences is the number of hydrogen atoms that can be bonded to the element. Most of the elements encountered in mass spectrometry either have an even mass and subsequent even valence or an odd mass and an odd valence. For example, the even mass/even valence elements are:

Carbon: ¹²C has a valence of 4, CH₄ = 16 Da (even mass and even number of N = 0).

Oxygen: ¹⁶O has a valence of 2, H₂O = 18 Da (even mass and even number of N = 0).

Silicon: ^{28}Si has a valence of 4, $\text{H}_4\text{Si} = 32$ Da (even mass and even number of N = 0).

Sulfur: ^{32}S has a valence of 2, $\text{H}_2\text{S} = 34$ Da (even mass and even number of N = 0).

The odd mass/odd valence elements are:

Hydrogen: ^1H has a valence of 1, $\text{H}_2 = 2$ Da (even mass and even number of N = 0).

Fluorine: ^{19}F has a valence of 1, $\text{HF} = 20$ Da (even mass and even number of N = 0).

Phosphorus: ^{31}P has a valence of 3 or 5, $\text{H}_3\text{P} = 34$ Da (even mass and even number of N = 0).

Chlorine: ^{35}Cl has a valence of 1, $\text{HCl} = 36$ Da (even mass and even number of N = 0).

However, nitrogen has an even mass but odd valence:

Nitrogen: ^{14}N has a valence of 3, $\text{NH}_3 = 17$ Da (odd mass and odd number of N = 1).

An odd number of nitrogen results in an odd mass such as ammonia NH_3 , which has a formula weight of 17 Da. An example of a N_2 -containing compound would be urea, $\text{CH}_4\text{N}_2\text{O}$ with a nominal mass of 60 Da. The nitrogen rule can be directly applied to odd-electron (OE) molecular ion species ($\text{M}^{+\bullet}$) that are generated with electron ionization (EI; see Chapters 2 and 3) because the ionization process has not changed the species molecular formula or molecular weight. However, this is not the case with processes such as electrospray ionization mass spectrometry (ESI-MS) or matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) where the species has been ionized in the form of an acid adduct $[\text{M} + \text{H}]^+$, a metal adduct such as sodium $[\text{M} + \text{Na}]^+$, or a chloride adduct $[\text{M} + \text{Cl}]^-$. In these cases the precursor ion is an even electron (EE) ion and the weight of the original molecule has changed while the valence has not. Here the exact opposite to the above-stated nitrogen rule that applied to organic compounds and odd electron molecular ions is the case. In other words the nitrogen rule for even electron precursor ions states that an ion species with an odd mass will have an even number of nitrogen (e.g., 0N, 2N, 4N, ..., etc.), and an ion species with an even mass will have an odd number of nitrogen (e.g., 1N, 3N, 5N, ..., etc.).

1.11 PROBLEMS

1.1. What are the six components that comprise a mass spectrometer?

1.2. To what does mass analyzer separate charged ionic species in the gas phase?

26 INTRODUCTION AND BASIC DEFINITIONS

- 1.3. Why is it important for the mass analyzer and detector to be under high vacuum?
- 1.4. What two types of pumps are used with mass spectrometric instrumentation, and what pressures do they obtain approximately?
- 1.5. To what three areas has mass spectrometry currently found an increased application?
- 1.6. Give a definition of proteomics, metabolomics, and lipidomics.
- 1.7. How is it that the soft ionization technique of electrospray allows gas-phase analysis of solution-phase associations? What are some examples of these associations?
- 1.8. What are a couple of examples of ionized analytes that are measured using mass spectrometry?
- 1.9. Given an acceleration voltage (KE) of 2000 eV, calculate the mass-to-charge ratio (m/z) of a biomolecule measured by a TOF mass spectrometer with a drift tube length of 2 m and a flight time of 68 μ s.
- 1.10. A biomolecule has a mass of 129 amu and a charge of 2+. If it is given an acceleration voltage of 1800 eV, what would be its drift time through a drift tube of length 2.3 m?
- 1.11. Calculate the exact mass (using the “relative atomic mass” of the highest abundance isotope in Appendix 1) and the weighted average mass (using the “standard atomic weight” in Appendix 1) for each of the following molecular formulas. What is the associated mass defect for each of the molecular formulas?
 - a. $C_{10}H_{32}O_2$
 - b. $C_{15}H_{28}N_2OPNa$
 - c. $C_{29}H_{42}N_2O_3K$
 - d. $C_{40}H_{75}N_2OP_3$
 - e. $C_5H_{10}O_3Cl_3$
- 1.12. Calculate the mass accuracy of the following observed masses for the following biomolecules according to their proposed formula:
 - a. $C_{24}H_{45}O_3N$ observed at m/z 395.4511
 - b. $C_{64}H_{120}O_{10}N_4P_2$ observed at m/z 1166.8398
 - c. $C_{48}H_{72}O_6$ observed at m/z 744.5310
 - d. $C_{78}H_{130}O_{12}N_8$ observed at m/z 1370.9932
- 1.13. Using the following data, calculate the resolution of the following observed mass spectral peaks:
 - a. m/z 342.4766 with peak width of m/z 0.6624
 - b. m/z 1245.6624 with peak width of m/z 0.0573
 - c. m/z 245.1191 with peak width of m/z 1.2444

- 1.14.** Using the rings plus double bonds formula decide whether the following ions are even-electron or odd-electron ions and draw their formulas as such (as an example the first one is done). Using the nitrogen rule list whether you would expect each ion to have an even or odd mass-to-charge (m/z) ratio value. What is that value?
- CH_4
 $r + db = 0$, odd electron (OE) as CH_4^+ , even mass ion with $m/z = 16$ Th.
 - NH_3
 - NH_4
 - H_3O
 - $\text{C}_2\text{H}_7\text{O}$
 - $\text{C}_{14}\text{H}_{28}\text{O}_2$
 - $\text{C}_6\text{H}_{14}\text{NO}$
 - $\text{C}_4\text{H}_{10}\text{SiO}$
 - $\text{C}_{29}\text{H}_{60}\text{N}_2\text{O}_6\text{P}$

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28 INTRODUCTION AND BASIC DEFINITIONS

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