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CHAPTER 9

Mass Spectrometry I

Principles and Instrumentation

definition:

Mass spectrometry (MS) is a technique for creating gas-phase ions from the molecules or atoms in a sample, separating the ions according to their mass-to-charge ratio, m/z , and measuring the abundance of the ions formed. MS is currently one of the most rapidly advancing fields of instrumental analysis. It has developed from an inorganic method used to prove that most elements exist as isotopes of differing masses to one of the cornerstone techniques used to elucidate the structure of biomolecules, especially high molecular weight (MW) proteins. MS provides the analyst with information as diverse as the structure of complex organic and biomolecules to the quantitative determination of ppb concentrations of elements and molecules in samples. *Applications:*

MS is an analytical technique that provides qualitative and quantitative information, including the mass of molecules and atoms in samples as well as the molecular structure of organic and inorganic compounds. MS can be used as a qualitative analytical tool to identify and characterize different materials of interest to the chemist or biochemist. MS is used routinely for the quantitative analysis of mixtures of gases, liquids, and solids. MS is used in many fields in addition to identifying and quantifying organic and biological molecules. These include atomic physics, physical chemistry, and geology.

This chapter will focus on MS principles and instrumentation for both organic and inorganic analysis. Chapter 10 will cover applications of MS for organic and inorganic analyses as well as interpretation of simple mass spectra for structural identification of organic compounds.

9.1 PRINCIPLES OF MS

The mass spectrometer is an instrument that separates gas-phase ionized atoms, molecules, and fragments of molecules by the difference in their mass-to-charge ratios. The mass-to-charge ratio is symbolized by m/z , where the mass m is expressed in **unified atomic mass units** and z is the number of charges on the ion. The statement found in many texts that MS separates ions based on mass is only true when the charge on the ion is a single + or -; in this case, m/z is numerically equal to the mass of the ion. While some MS methods do generate mostly +1 charged ions, many new techniques generate ions with multiple charges. *mass unit:*

The mass of an ion is given in **unified atomic mass units, u**. One unified atomic mass unit is equal to 1/12 of the mass of the most abundant, stable, naturally occurring isotope of carbon, ^{12}C . The mass of ^{12}C is defined as exactly 12 u. The abbreviation **amu**, for atomic mass unit, is now considered obsolete but may still be encountered in the literature. A synonym for the unified atomic mass unit is the **dalton (Da)**; $1 \text{ u} = 1 \text{ Da}$. In the Systeme International d'Unites (SI) unit of mass, $1 \text{ u} = 1.665402 \times 10^{-27} \text{ kg}$. Table 9.1 presents the exact masses for some common isotopes encountered in organic compounds.

Table 9.1 Comparison of Atomic Weights and Measured Accurate Isotope Masses for Some Common Elements in Organic Compounds

Element	Atomic Weight	Isotope	Mass ^a	% Abundance
Hydrogen ^b	1.00794(7)	¹ H	1.007825	99.99
		² H (or D)	2.01410	0.01
Carbon ^b	12.0107(8)	¹² C	12.000000 (defined)	98.91
		¹³ C	13.00336	1.1
Nitrogen ^b	14.0067(2)	¹⁴ N	14.0031	99.6
		¹⁵ N	15.0001	0.4
Oxygen ^b	15.9994(3)	¹⁶ O	15.9949	99.76
		¹⁷ O	16.9991	0.04
		¹⁸ O	17.9992	0.20
Fluorine	18.99840	F	18.99840	100
Phosphorus	30.97376	P	30.97376	100
Sulfur ^b	32.065(5)	³² S	31.9721	95.02
		³³ S	32.9715	0.76
		³⁴ S	33.9679	4.22
Chlorine ^b	35.453(2)	³⁵ Cl	34.9689	75.77
		³⁷ Cl	36.9659	24.23
Bromine	79.904(1)	⁷⁹ Br	78.9183	50.5
		⁸¹ Br	80.9163	49.5

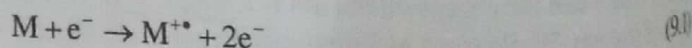
^a Many of the isotope masses have been determined by MS accurately to seven or more decimal places. Numbers in parentheses in the atomic weights represent the first uncertain figure.

^b The atomic weights for these elements and a few others are now given as ranges due to the variability of values in natural terrestrial materials. (Atomic weights are based on the 2009 values in *Pure Appl. Chem.*, 2011, 83, 359–396 as tabulated at <http://www.chem.qmul.ac.uk/iupac/AtWt/>.) With permission. Newer tables now provide a range of isotope abundances of some elements due to variations in Earthly sources. Other celestial bodies (e.g., Moon, Mars, asteroidal meteorites) display even more different isotope variations, which enables MS measurements to identify the particular source of some meteorites.

charge on ion:

The term z symbolizes the number of charges on the ion; this number may be positive or negative, such as +1, -1, +2, and +10. The number of charges is not the same as the total charge of the ion in coulombs (C). The total charge $q = ze$, where e is the magnitude of the charge on the electron, 1.6×10^{-19} C. Electron ionization:

For simplicity, the following discussion will focus on what happens to an organic molecule in one type of MS experiment. One method of forming ions from sample molecules or atoms is to bombard the sample with electrons. This method is called **electron ionization (EI)**:



where

M is the analyte molecule

e^- is the electron

M^{+z} is the ionized analyte molecule; this species is called the **molecular ion**

In many cases, only ions with a single positive charge are formed, that is, the number of charges on the ion, z , equals +1. The mass spectrometer separates ions based on the mass-to-charge ratio, m/z .

for ions with a single positive charge, m/z equals the mass of the ion in unified atomic mass units. For a molecular ion, m/z is related to the MW (also called the relative molecular mass) of the compound. The symbol M^+ indicates that a molecular ion from an organic compound is a radical cation formed by the loss of one electron. In most cases, molecular ions have sufficient energy as a result of the ionization process to undergo *fragmentation* to form other ions of lower m/z . All these ions are separated in the mass spectrometer according to their m/z values, and the abundance of each is measured.

A magnetic sector mass spectrometer is shown schematically in Figure 9.1. This instrument is a *single-focus* mass spectrometer. The gas-phase molecules from the sample inlet are ionized by a beam of high-energy (i.e., high-velocity) electrons passing closely among them. The rapidly changing electric field produced by the passage of an energetic electron can both eject electrons from the atom or molecule (ionization) and transfer sufficient energy to the molecule to cause its bonds to rupture (fragmentation). Only a very small percentage (0.1%–0.001%) of the molecules is ionized. A permanent magnet is often used to produce a magnetic field, which causes the beam electrons to follow a spiral trajectory of longer path length as they transit the ion source, thus increasing the ionization efficiency and improving sensitivity. However, too many ions in the ion source, especially when present from a high excess of unseparated matrix components, will add to and distort the instrument's operating fields from this *space charge*. The ions are then accelerated in an electric field at a voltage V . If each ion has a single positive charge, the magnitude of the

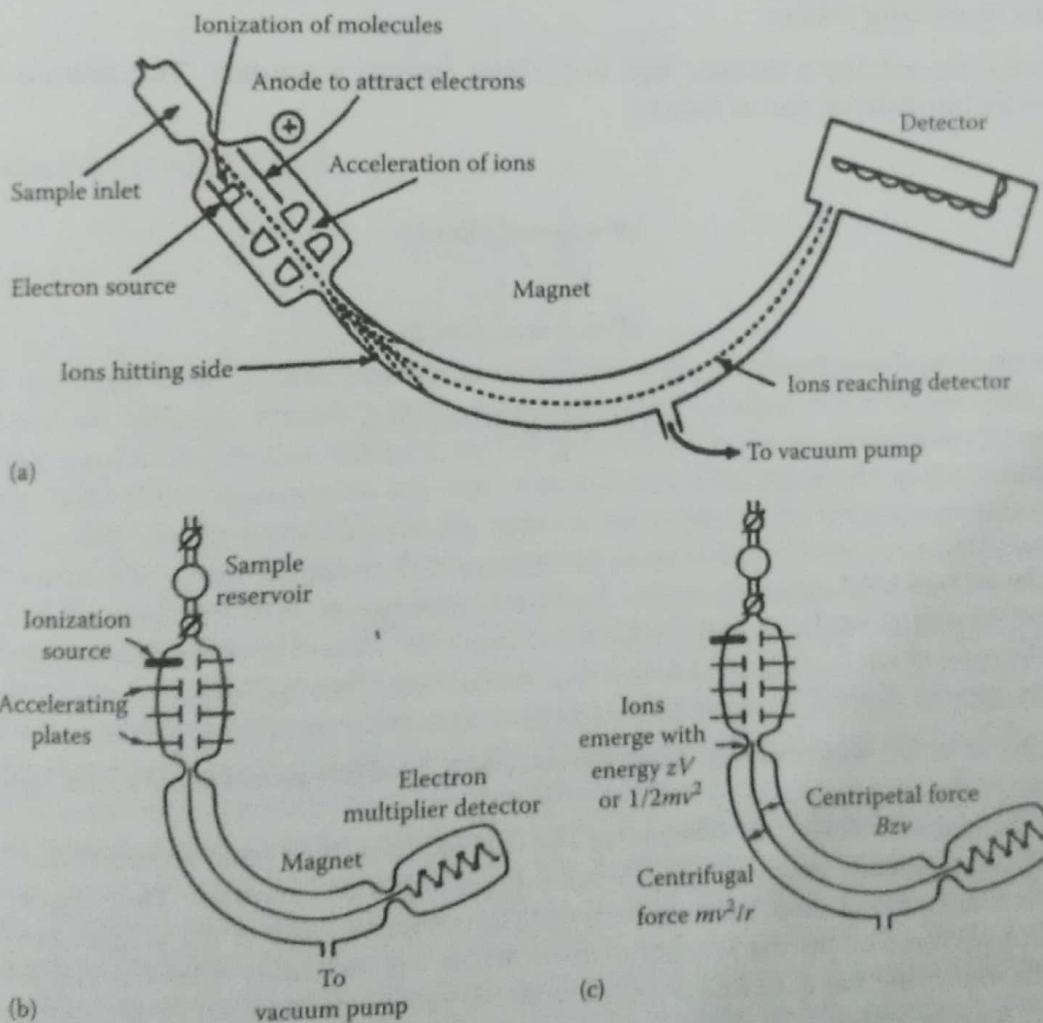


Figure 9.1 (a) Schematic magnetic sector mass spectrometer. (b) Schematic magnetic sector mass spectrometer showing a gas sample reservoir with fill and inlet valves and an electron multiplier (EM) detector. (c) Forces and energies associated with ions traveling through a magnetic sector mass spectrometer. For an ion to reach the detector, Bzv must equal mv^2/r .

Calculating m/z :
 charge is equal to $+1$. The energy of each ion is equal to the charge z times the accelerating voltage, zV . The energy acquired by an ion on acceleration is kinetic energy. It is important to note that the kinetic energy of an ion accelerated through a voltage V depends only on the charge of the ion and the voltage, not on the mass of the ion. The translational component of the kinetic energy is equal to $\frac{1}{2}mv^2$. The kinetic energy of all singly charged ions is the same for a given accelerating voltage; therefore, those ions with small masses must travel at higher velocity than ions with larger masses. That is, for ions with a single positive charge,

$$\frac{1}{2}mv^2 = zV \quad (9.2)$$

hence,

$$v = \left(\frac{2zV}{m} \right)^{1/2} \quad (9.3)$$

where

- m is the mass of the ion
- v is the velocity of the ion
- z is the charge of the ion
- V is the accelerating voltage

As m varies, the velocity v changes such that $\frac{1}{2}mv^2$ remains a constant. This relation can be expressed for two different ions as follows:

$$zV = \frac{1}{2}m_1v_1^2 \quad (\text{ion 1})$$

$$zV = \frac{1}{2}m_2v_2^2 \quad (\text{ion 2}) \quad (9.4)$$

$$\frac{1}{2}m_1v_1^2 = \frac{1}{2}m_2v_2^2$$

where

- m_1 is the mass of ion 1
- v_1 is the velocity of ion 1
- m_2 is the mass of ion 2
- v_2 is the velocity of ion 2

$v \propto \frac{1}{\sqrt{m}}$
 The velocity of an ion depends on its mass; the velocity is inversely proportional to the square root of the mass of the ion. *magnetic energy:*

After an applied voltage has accelerated the charged ions, they enter a curved section of a magnet of homogeneous magnetic field B and a fixed radius of curvature. This magnetic field acts on the ions, making them move in a circle. The attractive force of the magnet equals Bzv . The centrifugal force on the ion is equal to $\frac{mv^2}{r}$, where r is the radius of the circular path traveled by the ion. If the ion is to follow a path with the radius of curvature of the magnet, these two forces must be equal:

$$\frac{mv^2}{r} = Bzv \quad (9.5)$$

or

$$\frac{1}{r} = \frac{Bzv}{mv^2}$$

and

$$r = \frac{mv}{zB} \quad (9.6)$$

Substituting for v (Equation 9.3), we get

$$r = \frac{m}{zB} \left(\frac{2zV}{m} \right)^{1/2}$$

Squaring both sides, we have

$$r^2 = \frac{m^2}{z^2 B^2} \frac{2zV}{m} = \frac{m2V}{zB^2} \quad (9.7)$$

which can be rearranged to give

$$\frac{m}{z} = \frac{B^2 r^2}{2V} \quad (9.8)$$

Conclusion:

That is, the radius of the circular path of an ion depends on the accelerating voltage V , the magnetic field B , and the ratio m/z . When V and B are kept constant, the radius of the circular path depends on the m/z value of the ionized molecule. Ions of different m/z travel in circles with different radii; this is the basis of the separation by m/z . Ions with different paths are shown as the dotted lines in Figure 9.1a; only one particular m/z has the right r to pass through the mass spectrometer under a given V and B . The others, as shown, follow paths that cause them to hit the sides of the instrument and be lost. By varying V or B , we can select which m/z will pass through the system. Voltage scanning is cheaper and was used in early instruments with permanent magnets. Scanning the magnetic field strength is more sensitive and is easily done with modern electromagnets. (In reality, a curved magnet with a path of significant width could accommodate a range of different m/z values, and it is the width and position of the exit slit that determine precisely which ions finally get through to the detector in a real scanning magnetic sector mass spectrometer.) *magnetic scanning:*

In all modern magnetic sector mass spectrometers, the applied magnetic field B of the electro-magnet is varied while the accelerating voltage V is held constant. The radius of curvature of the magnetic sector of a given instrument is a constant, so only ions with a trajectory of radius r pass through. For a particular magnetic field strength, then, only ions with an m/z value that satisfies Equation 9.8 will exit the chamber. Consequently, for different values of B , ions with different mass-to-charge ratios will pass through the instrument to the detector, and by varying B , we can scan the range of mass-to-charge ratios of the sample ions and measure their abundance with a detector that either counts and sums individual ions or measures a current produced by their impact on the detector. A plot of abundance versus m/z is called a **mass spectrum**. The mass spectral data can be shown as a plot or as a table. A digitized mass spectrum of benzene, C_6H_6 , is shown in Figure 9.2a;

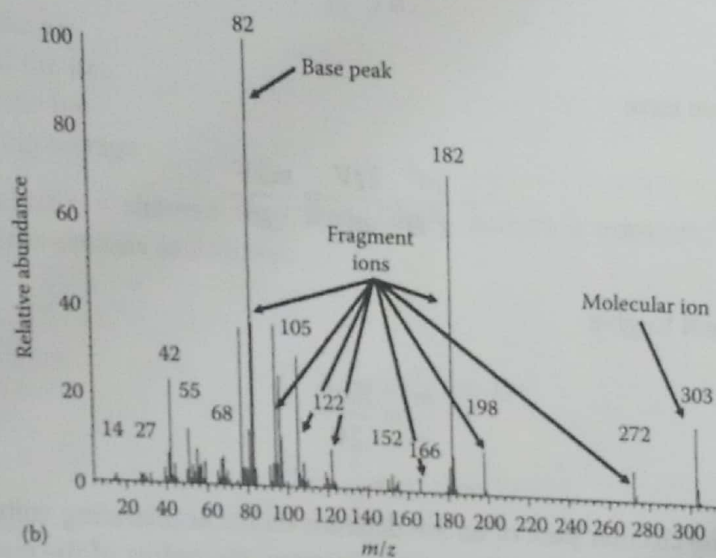
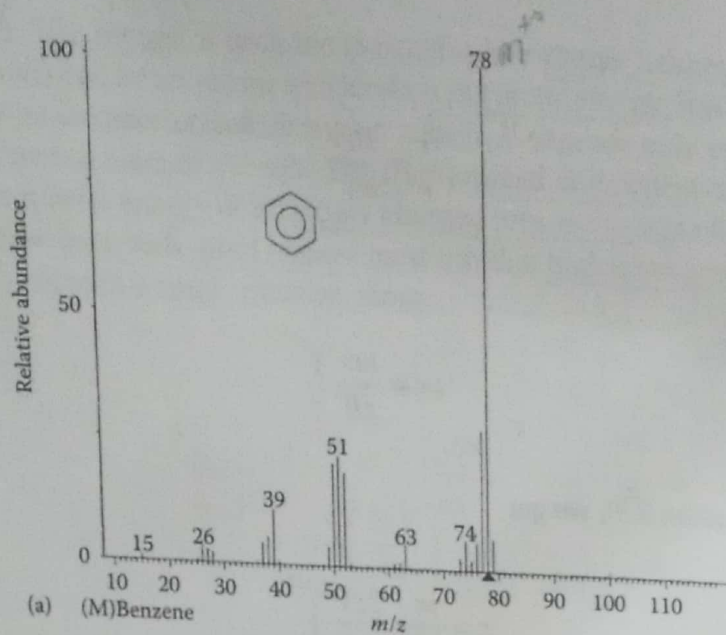


Figure 9.2 (a) A mass spectrum of benzene, C_6H_6 (MW = 78), plotted as relative abundance versus m/z , with the most abundant peak set to a relative abundance of 100. In this spectrum, the most abundant peak (or base peak) is the molecular ion peak at m/z 78, marked by the dark triangle on the x-axis. Some fragment ion m/z values are also marked on the spectrum. (b) A mass spectrum of cocaine. In this spectrum, the molecular ion peak is not the base peak; the fragment ion at m/z 82 is the most abundant ion. (The cocaine spectrum is courtesy of Dr. Robert Kobelski, CDC, Atlanta, GA.)

the plot is shown as a histogram. The most abundant peak in the spectrum, called the **base peak**, is scaled to 100, so the y-axis represents the **relative abundance** of the ions of each m/z value. The nominal mass of the benzene molecular ion is equal to the sum of the nominal masses of the C and H isotopes, so $6(12) + 6(1) = 78$; the most abundant peak in this benzene mass spectrum is the molecular ion peak at m/z 78. A number of fragment ions at lower values of m/z are also seen in this spectrum. Table 9.2 presents the same mass spectral data for benzene as shown in the plot. Figure 9.2b is a mass spectrum of cocaine, which exhibits many fragment ions. This example shows that the molecular ion is not always the most abundant ion. The base peak in the cocaine mass spectrum is the fragment ion at m/z 82. The m/z values and the fragmentation pattern enable the analyst to determine the MW and structure of organic compounds by MS. Detailed interpretation of simple organic mass spectra will be covered in Chapter 10.

There is a difference between the mass of an atom (or ion) and what chemists are used to thinking of as the atomic weight or MW of a species. Atomic weight is the average weight of all

Table 9.2 Mass Spectral Data for Benzene

m/z	Relative Abundance	m/z	Relative Abundance
37	4	53	0.80
37.5	1.2	63	2.9
38	5.4	64	0.17
38.5	0.35	73	1.5
39	13	74	4.3
39.5	0.19	75	1.7
40	0.37	76	6.0
48	0.29	77	14
49	2.7	78	100
50	16	79	6.4
51	18	80	0.18
52	19		

A.W = avg w isotopes

the isotopes of an element and MW is calculated from these average atomic weights. This is the value we would need to use if we wished to weigh out an exact number of moles of a substance whose component atoms have a distribution of different isotopes, which is usually the case. Each stable isotope (nuclide) of an element has an exact mass (more properly called a measured accurate mass), which has been determined accurately by MS. MS separates compounds containing ^{12}C atoms from compounds with ^{13}C atoms precisely because of the difference in mass between the isotopes. Table 9.1 gives a few examples of the atomic weights of some of the elements found in organic compounds as well as the measured accurate mass and abundance of the isotopes of the element. In a mass spectrum, a given ion is monoisotopic, not a weighted average. For example, the MW of acetone, $\text{C}_3\text{H}_6\text{O}$, is calculated from the atomic weights of the elements to be $(3 \times 12.011) + (6 \times 1.00797) + (1 \times 15.9994) = 58.0795$ g/mol. The molecular ion of acetone as measured by MS has a mass that consists of only contributions from the one most abundant isotope of each element, that is, ^1H , ^{12}C , and ^{16}O ; its mass can be calculated from the formula $^{12}\text{C}_3^1\text{H}_6^{16}\text{O}$ to be $(3 \times 12.000) + (6 \times 1.00783) + (1 \times 15.9949) = 58.0419$ u or 58.0419 g/mol if we had a mole of the monoisotopic compound. *m/e ratio:*

The term m/z is the correct term to use for the mass-to-charge ratio of an ion. Older literature used the term m/e ; however, the symbol e is used for the charge on the electron in coulombs and is *not* what goes into the divisor when the mass is given in unified atomic mass units, u. Two terms used in the older literature that are no longer acceptable for use in MS are "parent ion" for molecular ion and "daughter ion" for fragment ions. Ions do not have gender. The terms molecular ion and fragment ion should be used; "precursor ion" and "product ion" are used for tandem MS/MS experiments described later in this chapter.

9.1.1 Resolving Power and Resolution of a Mass Spectrometer

The **resolving power** of a mass spectrometer is defined as its ability to separate ions of two different m/z values. Numerically, the resolution is equal to the mass of one singly charged ion, M , divided by the difference in mass between M and the next m/z value that can be separated. For example, to separate two singly charged ions of 999 and 1001 Da requires a resolving power of $999/(1001 - 999) = 500$. That is,

$$\text{Resolving power} = \frac{M}{\Delta M} \quad (9.9)$$

Resolution range :

In practice, it is found that if we wish to distinguish between ions of 600 and 599 Da, the resolving power required is 600, or 1 Da in 600 Da. As a rule of thumb, if we wish to distinguish between ions differing by 1 Da in the 600 Da mass range, we need a resolving power of 600. If we need to distinguish between ions differing by 1 Da in the 1200 Da range, we need a resolving power of at least 1200. This is not a very high resolution. Some isotopes of different elements or molecular fragment ions composed of different combinations of atomic isotopes adding up to close to the same mass may produce ions differing by much less than 1 Da. To distinguish between these types of species, high-resolution MS, with resolution in the range of 20,000–100,000 or higher, is required.*

Both the magnetic sector and other designs of MS instruments control ion movement and trajectories by controlling the voltage fields from charge (electrons) on conductive metal components, often described as lenses, which focus and concentrate ions, or extractors, which move them between stages, or multipole rods with applied radio-frequency (RF) alternating voltages and polarities, which induce controlled resonant spiral orbits to select ions of a given m/z , etc. All these components must be interactively set to obtain the optimum MS ion peak shapes, ideally a Gaussian profile with the top of the symmetrical peak at a location on the mass axis corresponding to the actual m/z value. Early magnetic sector or quadrupole MS instruments used a great array of knobs and an oscilloscope readout to manually "tune" the instrument to this optimum (analogous to the much simpler process of tuning a radio to a particular station). As these components age or become contaminated, the voltages on the surfaces will shift, the MS peak shapes will deteriorate, and the instrument will require retuning. Depending on usage conditions, this might be required at the beginning of each day's runs. Most modern computerized instruments employ "autotune" algorithms, whereby the computer observes selected peaks from an autotune compound vapor (often perfluorotributylamine [PFTBA]) continuously infused into the ion source and iteratively adjusts all these interlocking voltages, checking selected MS peak shapes and positions, until an optimum set of tuning conditions is achieved.

The resolving power is determined by actual measurement of the mass spectral peaks obtained. The method for calculating ΔM must also be specified. Two methods are commonly used to indicate the separation between peaks, and these are shown in Figure 9.3. One definition is that the overlap between the peaks is 10% or less for two peaks of equal height; that is, the height of the overlap should not be more than 10% of the peak height. A second method is to use the full width at half maximum (FWHM) as a measure of ΔM . The FWHM method results in a resolving power twice that of the 10% overlap method, so it is important to state how the calculation was performed.

Resolving power for commercial mass spectrometers depends on the instrument design and can range from 500 to more than 1×10^6 . In general, the higher the resolving power, the greater the complexity and cost of the MS instrument.

Resolution is the value of ΔM at a given M and is often expressed in ppm. For the spectrometer with a resolving power of 600 earlier described, the resolution would be 1 part in 600 parts. To distinguish between $^{14}\text{N}_2^+$ with an exact mass of 28.0061 Da and $^{12}\text{C}^{16}\text{O}^+$ with an exact mass of 27.9949 Da, we would need a resolution of $(28.0061 - 27.9949) = 0.0112$ Da in 28 Da. To convert this resolution to ppm, divide 0.0112 by 28 and multiply by 1×10^6 ; a resolution of 400 ppm is required. The resolving power needed would be $27.9949/0.00112 = 2500$. **Mass accuracy** describes how well the top of the MS peak can be defined and kept stable and its position identified on the mass axis. Mass axis calibration is usually established by a separate portion of the tuning algorithm, which identifies the centroids of several mass peaks of precisely known mass in the spectrum of a suitable calibration compound and adjusts tuning parameters to place these at the appropriate positions on the mass axis. Like resolution, it can be expressed as a percentage or ppm value of the calibration mass values.