CHAPTER

HRMS: Fundamentals and Basic Concepts

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1.1 INTRODUCTION (TO HIGH-RESOLUTION MASS SPECTROMETRY)

1.1.1 BASIC CONCEPTS (UNITS AND DEFINITIONS)

Mass spectrometry (MS) is an analytical technique commonly used for qualitative and quantitative chemical analysis. MS measures the mass−charge ratio (m/z) of any analyte, of both organic and inorganic nature, which has previously been ionized. Only the ions are registered in MS, but the particles with zero net electric charge (molecules or radicals) are not detected. Therefore, MS does not directly measure mass, but it determines the m/z, being m the relative mass of an ion on the unified atomic scale divided by the charge number, z, of the ion (regardless of sign). The m/z value is a dimensionless number.

Because the mass of atoms and molecules is very small, the kilogram as standard international (SI) base unit cannot be used for its measurement. For that, a non-SI unit of mass, unified atomic mass unit (u) is used. At this point, in this introductory section, it is worth clarifying some basic terms (units and definitions) in MS according to the International Union of Pure and Applied Chemistry (IUPAC) recommendations (IUPAC, 1997; Murray et al., 2013).

The u also called Dalton (Da), is defined as 1/12th of the mass of one atom of ^{12}C at rest in its ground state, being 1 u = 1 Da = 1.660538921 (73) $\times 10^{-27}$ kg (number in parentheses indicates the estimated uncertainty). In this way, the mass of other atoms or molecules is expressed relative to the mass of the most abundant stable isotope of carbon, ^{12}C, and this value is dimensionless.

The z is defined as absolute value of charge of an ion divided by the value of the elementary charge of the electron (e) rounded to the nearest integer, being $e = 1.602177 \times 10^{-19}$ C. The m/z unit is the thomson (Th), although it is now a deprecated term, being 1 Th = 1 u/e = 1.036426×10^{-8} kg/C. For that, use of the dimensionless term m/z is accepted in the literature, and this criterion will be followed throughout this book.

Other basic concepts that are commonly used in MS will be shortly described to clarify the meaning of these throughout the following chapters.

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- *Atomic mass*: The number that represents the element's mass based on the weighted average of the masses of its naturally occurring stable isotopes. For example, the integer atomic mass of bromine is 80 Da. This is because there are only two naturally occurring stable isotopes of bromine, ^{79}Br and ^{81}Br, which exist in nature in about equal amounts. When the *relative mass* (Mr) of an ion, molecule, or radical is reported, it is based on the atomic masses of its elements.
- *Nominal mass*: Mass of a molecular ion or molecule calculated using the isotope mass of the most abundant constituent element isotope of each element (Table 1.1) rounded to the nearest integer value and multiplied by the number of atoms of each element. Example: nominal mass of $H_2O = (2 \times 1 + 1 \times 16)$ u = 18 u.
- *Monoisotopic mass*: Exact mass of an ion or molecule calculated using the mass of the most abundant isotope of each element. Example: monoisotopic mass of $H_2O = (2 \times 1.007825 + 1 \times 15.994915)$ u = 18.010565 u. The exact mass of the common elements and their isotopes are provided in Table 1.1.
- *Exact mass*: Calculated mass of an ion or molecule with specified isotopic composition.
- *Mass defect*: Difference between the nominal mass and the monoisotopic mass of an atom, molecule, or ion. It can be a positive or negative value.
- *Relative isotopic mass defect (RΔm)*: It is the mass defect between the monoisotopic mass of an element and the mass of its A+1 or its A+2 isotopic cluster (Thurman & Ferrer, 2010). For instance, RΔm for the pair ^{35}Cl:^{37}Cl is 0.0030 Da.
- *Average mass*: Mass of an ion or molecule weighted for its isotopic composition, i.e., the average of the isotopic masses of each element, weighted for isotopic abundance (Table 1.1). Example: average mass of $H_2O = (2 \times 1.00794 + 1 \times 15.9994)$ u = 18.01528 u.
- *Accurate mass*: Experimentally determined mass of an ion of known charge.
- *Mass accuracy*: Difference between the mass measured by the mass analyzer and theoretical value.
- *Resolution or mass resolving power*: Measure of the ability of a mass analyzer to distinguish two signals of slightly different *m/z* ratios.
- *Mass calibration*: Means of determining *m/z* values of ions from experimentally detected signals using a theoretical or empirical relational equation. In general, this is accomplished using a computer-based data system and a calibration file obtained from a mass spectrum of a compound that produces ions of known *m/z* values.
- *Mass limit*: Value of *m/z* above or below which *ions* cannot be detected in a *mass spectrometer.*
- *Mass number*: The sum of the protons and neutrons in an atom, molecule, or ion. If the mass is expressed in u, mass number is similar to nominal mass.
- *Most abundant ion mass:* The mass that corresponds to the most abundant peak in the isotopic cluster of the ion of a given empirical formula.

Table 1.1 Nominal, Isotopic, and Average Masses of Some Common Stable Isotopes

Element	Isotope	Abundance	Nominal Mass	Isotopic Mass	Average Mass
H	^{1}H	99.9885	1	1.007825	1.00794
	^{2}H	0.0115	2	2.014102	
C	^{12}C	98.93	12	12.000000	12.0110
	^{13}C	1.08	13	13.003355	
N	^{14}N	99.632	14	14.003074	14.00674
	^{15}N	0.368	15	15.000109	
O	^{16}O	99.757	16	15.994915	15.9994
	^{17}O	0.038	17	16.999131	
	^{18}O	0.205	18	17.999160	
F	^{19}F	100	19	18.998403	18.9984
Na	^{23}Na	100	23	22.989770	22.9898
Si	^{28}Si	92.2297	28	27.976927	28.0855
	^{29}Si	4.6832	29	28.976495	
	^{30}Si	3.0872	30	29.973770	
P	^{31}P	100	31	30.973762	30.9738
S	^{32}S	94.93	32	31.972072	32.0660
	^{33}S	0.76	33	32.971459	
	^{34}S	4.29	34	33.967868	
Cl	^{35}Cl	75.78	35	34.968853	35.4527
	^{37}Cl	24.22	37	36.965903	
Br	^{79}Br	50.69	79	78.918336	79.9094
	^{81}Br	49.32	81	80.916289	
I	^{127}I	100	127	126.904476	126.9045

1.1.2 LOW-RESOLUTION MASS SPECTROMETRY VERSUS HIGH-RESOLUTION MASS SPECTROMETRY

It should be noted that mass measurements in MS can be carried out at either low resolution (LRMS) or high resolution (HRMS). An LRMS measurement provides information about the nominal mass of the analyte (Dass, 2007), i.e., the *m/z* for each ion is measured to single-digit mass units (integer mass). However, exact mass is measured by HRMS, i.e., the *m/z* for each ion is measured to four to six decimal points (Ekman, Silberring, Westman-Brinkmalm, & Kraj, 2009). This is very useful to structure elucidation of unknown compounds for analytes having the same nominal mass, but with very small differences in their exact masses. As a result, by LRMS measurements it is not possible to differentiate between imazalil, $C_{14}H_{14}Cl_2N_2O$ ($14 \times 12 + 14 \times 1 + 2 \times 35 + 2 \times 14 + 1 \times 16 = 296$ u), and flunixin, $C_{14}H_{11}F_3N_2O_2$ ($14 \times 12 + 11 \times 1 + 3 \times 19 + 2 \times 14 + 2 \times 16 = 296$ u),

pesticides. However, this would be possible by using exact mass measurements, imazalil $C_{14}H_{14}Cl_2N_2O$ ($14 \times 12 + 14 \times 1.007825 + 2 \times 34.968852 + 2 \times 14.003074 + 1 \times 15.994915 = 296.048317$ u) and flunixin $C_{14}H_{11}F_3N_2O_2$ ($14 \times 12 + 11 \times 1.007825 + 3 \times 18.998403 + 2 \times 14.003074 + 2 \times 15.994915 = 296.077262$ u).

High-resolution mass spectrometers have evolved from the 1960s with the introduction of double-focusing magnetic-sector mass instruments (Picó, 2015). Next, Fourier transform ion cyclotron resonance (FT-ICR), time-of-flight (TOF), and Orbitrap mass analyzers were also introduced in the market. Also, hybrid HRMS instruments, such as quadrupole TOF (Q-TOF), ion trap (IT)-TOF, linear trap quadrupole (LTQ)-Orbitrap, or Q−Orbitrap, have been developed. These last analyzers provide tandem (MS/MS) or MS^n spectra of high resolution, in addition to accurate monoisotopic mass measurements, of great applicability both for the confirmation of target compounds and the identification of unknown compounds (Lin et al., 2015). The TOF and Orbitrap analyzers, single or hybrid instruments, are the most widely used in the analysis of organic contaminants, such as pesticide residues (Lin et al., 2015; Picó, 2015).

Among the main characteristics that define the performance of a mass analyzer are (Dass, 2007; de Hoffmann & Stroobant, 2007; Mcluckey & Wells, 2001) mass range, speed, efficiency, linear dynamic range, sensitivity, resolution (or its mass resolving power), and mass accuracy. The mass range is that over which a mass spectrometer can detect ions or is operated to record a mass spectrum. When a range of m/z is indicated instead of a mass range, this should be specified explicitly. The speed or scan speed is the rate at which the analyzer measures over a particular mass range. Efficiency is defined as the product of the transmission of the analyzer by its duty cycle, where the transmission is the ratio of the number of ions reaching the detector and the number of ions entering the mass analyzer, and the duty cycle can be described as the fraction of the ions of interest formed in the ionization step that are subjected to mass analysis.

Linear dynamic range is considered as the range over which ion signal is linear with analyte concentration. Sensitivity can be expressed as detection sensitivity or abundance sensitivity; the first is the smallest amount of an analyte that can be detected at a certain defined confidence level, while the second is the inverse of the ratio obtained by dividing the signal level corresponding to a large peak by the signal level of the background at one mass-to-charge unit lower or higher. A summary of these characteristics of high-resolution mass analyzers is shown in Table 1.2. As it can be observed, in terms of resolving power and accuracy, the FT-ICR analyzer presents the best values, followed by the recently introduced tribrid Orbitrap analyzer. TOF and Q-TOF analyzers have worse values, although the FT-ICR analyzer comprises the worst sensitivity.

Last but not least, two key characteristics of high-resolution mass analyzers are resolution (or its mass resolving power) and mass accuracy, which will be treated in more detail in the following two sections.

Table 1.2 Comparison of the Characteristics of Some High-Resolution Mass Spectrometry Analyzers

Analyzer	Mass Range	Speed	Linear Dynamic Range	Sensitivity	Resolving Power (FWHM)	Accuracy (ppm)
Magnetic sector	10,000	~1 s	10^9	10^6–10^9	100,000	<1
FT-ICR	10,000	~1 s	10^3–10^4	10^3–10^4	1,000,000	<1
TOF	>300,000	Milliseconds	10^6	10^6	30,000	3–5
Q-TOF	10,000	~Milliseconds	10^3–10^4	10^6	30,000	3–5
IT-TOF		~0.1 s	10^3–10^4	10^5	100,000	3–5
Exactive Orbitrap	4000	0.1 s	>5000	10^6	100,000	<3
LTQ-Orbitrap	4000	0.1 s	>5000	10^6	100,000/240,000	<3
Q-Orbitrap	8000	0.05 s	10^5	10^6	240,000	<2
Tribrid Orbitrap	6000	0.05 s	10^5	10^6–10^7	500,000	<1

FT-ICR, *Fourier transform ion cyclotron resonance;* FWHM, *full width at half maximum;* IT-TOF, *ion trap TOF;* LTQ, *linear trap quadrupole;* Q-TOF, *quadrupole TOF;* TOF, *time-of-flight. Adapted from Picó, Y. (2015). Advanced mass spectrometry. In Y. Picó (Ed.),* Comprehensive analytical chemistry, *Vol. 68. Amsterdam: Elsevier.*

1.2 RESOLUTION AND MASS RESOLVING POWER

Resolution or resolving power is the capacity of a mass analyzer to yield distinct signals for two ions with a small m/z difference (de Hoffmann & Stroobant, 2007). Unfortunately, there is confusion about these two concepts and also between mass resolving power and resolving power in MS, because the definitions provided by different documents are not exactly the same.

Dass (2007) defines the mass resolution of a mass spectrometer as its ability to distinguish between two neighboring ions that differ only slightly in their mass (Δm). According to this definition, it is the inverse value of the resolving power, $\text{RP} = m/\Delta m$, where m is the average of the accurate masses, $(m_1 + m_2)/2$, of the two neighboring ions. Xian, Hendrickson, and Marshall (2012) define the resolution as the smallest mass difference, $m_2 - m_1$ or Δm, between two mass spectral peaks such that the valley between their sum is a specified fraction (e.g., 50%) of the height of the smaller individual peak. A similar definition is given by Marshall, Hendrickson, and Shi (2002), as the minimum mass difference between two equal magnitude peaks such that the valley between them is a specified fraction of the peak height.

The IUPAC recommendations (Murray et al., 2013) define resolution *as* $m/\Delta m$, where m is the m/z of the ion of interest. Although depending on the method of measurement of $\Delta(m/z)$, it is possible to differentiate between the two concepts (Murray et al., 2013; Price, 1991). On one hand, resolution, as 10% valley, is the $(m/z)/\Delta(m/z)$ value measured for two peaks of equal height in a mass spectrum at m/z and $m/z + \Delta(m/z)$ that are separated by a valley for which the lowest point is 10% of the height of either peak, i.e., the peaks are resolved when the valley between the two m/z values is 10% of the height of either one (Fig. 1.1). For peaks of similar height separated by a valley, let the height of the valley at its lowest point be 10% of the lower peak, and the resolution should be given for a number of values of m/z. This 10% valley definition for the resolution is used with magnetic-sector analyzers (Ekman et al., 2009).

On the other hand, resolution, as peak width, expresses the $(m/z)/\Delta(m/z)$ value for a single peak, where $\Delta(m/z)$ is the width of the peak at a height, which is a specified fraction (50, 5, or 0.5%) of its maximum peak height (Fig. 1.1). The used fraction is often 50%, and $\Delta(m/z)$ is named as full width at half maximum (FWHM). FT-ICR, TOF, and Orbitrap analyzers use this 50% valley definition for set resolution (Ekman et al., 2009).

In addition, there is controversy in the definition of mass resolving power and resolving power in MS (IUPAC, 1997). The definition of the first term is similar to the definition of resolution indicated earlier (Murray et al., 2013), i.e., as a dimensionless ratio between $m/\Delta m$. Resolving power in MS is the ability of an instrument or measurement procedure to distinguish between two peaks differing in the quotient m/z by a small increment and expressed as the peak width in mass units. However, both terms have been unified in the current IUPAC definition as a measure of the ability of a mass spectrometer to provide a specified value of mass resolution.

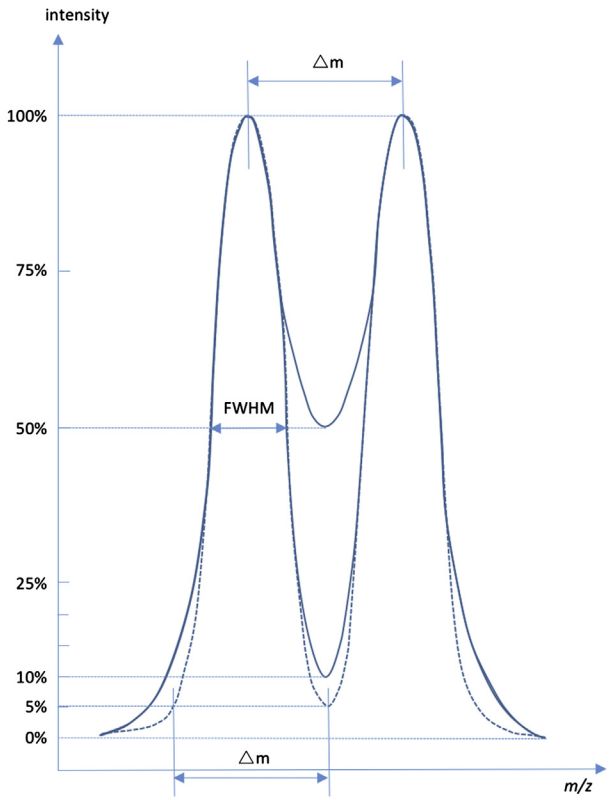

FIGURE 1.1

Methods of calculating mass resolving power.

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1.3 ACCURATE MASS MEASUREMENT: EXACT MASS AND MASS DEFECT

It is important to differentiate between accurate mass and exact mass. The first is the experimentally determined mass of an ion of known charge (Bristow and Webb, 2003; Sparkman, 2006) and it refers to a measured mass, while the second is the calculated mass of an ion or molecule with specified isotopic composition (Kim, Rodgers, & Marshall, 2006), and it refers to a calculated mass. Therefore, although an LR mass spectrometer can measure integer relative mass with high accuracy, the information obtained is not so complete as the measurement of accurate relative mass offered by HR mass spectrometers (Herbert & Johstone, 2003). The difference between the nominal mass and the monoisotopic mass of an atom, molecule, or ion, positive or negative value, is the mass defect.

The value of accurate mass measurement is illustrated in the following examples: (1) to distinguish compounds with the same integer nominal (molecular) mass in the same sample; (2) to determine the molecular formula or elemental composition for an unknown compound, which is helpful for its identification; and (3) to find out the fragmentation routes. As an example, Fig. 1.2 shows the fragmentation pattern for the flonicamid pesticide, which can be elucidated by HRMS.

In HRMS, the mass accuracy is the difference between the m/z value measured by the mass spectrometer and the theoretical m/z value. It can be reported as an absolute value; for instance, in millimass units (mmu) or millidalton (mDa):

$$\text{Mass accuracy(mmu)} = (m/z_{\text{measured}} - m/z_{\text{theoretical}}) \times 10^3$$

Also, it can be expressed as a relative value in parts per million (ppm):

$$\text{Mass accuracy(ppm)} = \left(\frac{m/z_{\text{measured}} - m/z_{\text{theoretical}}}{(m/z_{\text{theoretical}})}\right) 10^6$$

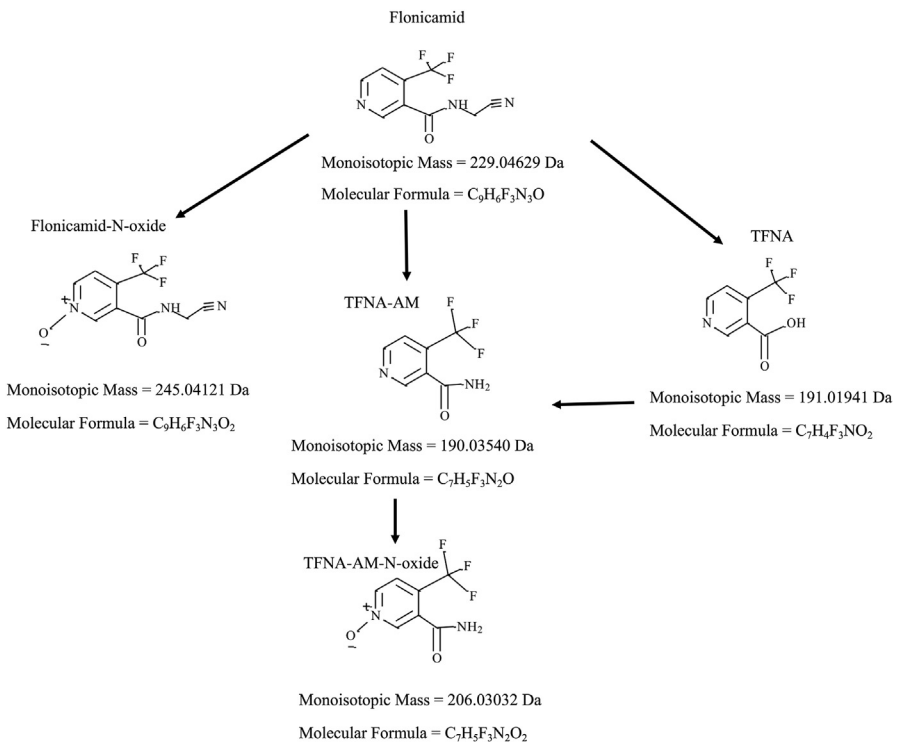

FIGURE 1.2

Fragmentation pattern for the flonicamid pesticide (TFNA: 4-trifluoromethylnicotinic acid; TFNA-AM: 4-trifluoromethilnicotinamide).

In general, an acceptable value of the measured mass should be within 5 ppm of the accurate mass (Gross, 1994). A key point to minimize error in accurate mass measurement is ensuring that the target ion is completely free of interfering ions, because these ions shift the mass of the target peak.

In general, high mass resolution and high mass accuracy depend on each other, because the latter tends to improve as the former is improved. HR allows to separate neighboring ions, and accurate mass can deliver molecular formulas (Gross, 2011). Therefore, it is important to note that HR alone does not equally imply measuring the accurate mass.

1.4 MASS CALIBRATION IN HIGH-RESOLUTION MASS SPECTROMETRY

Mass calibration is a relevant process in every mass spectrometer for a proper representation of ions in the *m*/*z* axis. It also results in a very important fact in HRMS where not only high mass resolution but also high mass accuracy is critical (Gross, 2011). For that, typically, mass reference compounds with a compilation of well-known *m*/*z* values are needed (Busch, 2004, 2005).

Calibration is frequently performed in an automatic or semiautomatic way by the mass spectrometer software when the list of ions of those mass calibration compounds are correlated with experimentally obtained *m*/*z* values. It is called external mass calibration if the mass calibration is stored in a calibration file for further measurements and the mass calibration standard is not used during acquisition of experimental mass spectra. Frequency of recalibration has influence on mass accuracy of the mass analyzer. The selection of the mass calibration compound depends on the ionization method and, of course, the mass analyzer used. However, (1) they should yield sufficient regularly spaced abundant ions across the entire scan range; (2) the reference ions should have negative mass defects to prevent overlap with typical compounds containing C, H, N, and O; and (3) they should be readily available, chemically inert, and sufficiently volatile. Some of the most common calibration standards and their masses and relative abundances can be found in literature (Dass, 2007). For example, perfluorokerosene (PFK) is often established as a mass calibration standard in electron ionization (EI). PFK provides numerous fragment ions that may be used up to *m*/*z* 700−1100 depending on the type of mixture used (commercially available from low to high boiling grades). Also, perflourotributylamine (FC-43) is also proposed as mass calibration standard thanks to its characteristics ions up to 614 in an EI spectrum (Sack, Lapp, Gross, & Kimble, 1984). When a high-mass calibration (i.e., up to 3000 u) is required, triazines and a mixture of fluorinated phosphazenes called Ultramark can be used as reference calibrants. For electrospray instruments, the most typical calibration standards are CsI, poly(-ethylene glycol) (PEG), poly(ethylene glycol) bis(carboxymethyl ether), poly(ethylene glycol monomethyl ether), and poly(propylene glycol). MALDI users also have several reference compounds available, such as α-CHCA matrix (dimer + H^+),

4-hydroxy-3-methoxycinnamic acid (trimer + Na^+), angiotensin I and II, bradykinin, substance P, desArg1-bradykinin, gramicidin, and autodigestion products of trypsin.

As an alternative, internal mass calibration can be performed. For that, the mass calibration standard is introduced using a second inlet system into the ion source, for instance, as a volatile standard. As an alternative, it can be mixed with the analyte before analysis. This last option presents more limitations than the use of alternative inlet systems.

Typical mass accuracy obtained by internal mass calibration used to be better than those obtained by external calibration. Some examples are 0.1−0.5 ppm with FT-ICR, 0.5−1 ppm with Orbitrap, 0.5−5 ppm with magnetic sector, or 1−10 ppm with TOF analyzers.

Fast atom bombardment instruments are sometimes internally calibrated with good mass accuracy by using the matrix peaks for a mass calibration but it is preferred a mixture of the standard with the analyte (matrix) if unwanted reactions are not observed and proper solubility of the analyte and standard. One typical mass calibration standard used is PEG with an average molecular weight of 600 u (PEG 600). In this sense, the reproducibility of mass calibration after several scan cycles is improved because of affection of magnets by hysteresis.

MALDI mass calibration can be compromised if thick sample layers are used with on-axis TOF instruments. However, orthogonal acceleration TOF analyzers present better results. In some cases, for example, in the analysis of synthetic polymers, the formation of evenly spaced oligomer ions can be used as internal mass calibration (Dienes et al., 1996). In the case of TOF analyzers, the conversion from a measured flight time to mass requires a mass calibration. The computer makes the calculations using proper algorithms once the values of flight time for a few calibrant masses are known. Sometimes, a second calibration step is requested to achieve enough accuracy (Ferrer & Thurman, 2009). It must be carefully controlled changes in flight distances or accelerating potentials to obtain mass accuracies of 1 ppm, but again, the internal mass calibration can correct such instrument factors with an automatic data processing carried out at the same time as that of the analysis of the sample. Generally, a mass calibration per day or week is enough to obtain a proper accuracy of m/z for many TOF instruments, but it is adequate to use internal mass calibration, especially when long analyses are performed (Chernushevich, Loboda, & Thomson, 2001).

FT-ICR mass analyzers with superconducting magnets are frequently very stable for many days of use in normal applications. In this case, a mass accuracy better than 1 ppm can be achieved in a wide mass range (Rodgers, Blumer, Hendrickson, & Marshall, 2000).

Some HR mass spectrometers such as double focusing systems (DFS) can be operated by multiple ion detection (MID) mode where the intensities of some ions typical of a target analyte can be continuously monitored to increase sensitivity, precision, and selectivity of the method. A monitoring window is selected if the

instrument is coupled to a chromatographic inlet device (gas or liquid chromatograph). For data acquisition, the magnet gets blocked in one mass and electric scans are carried out modifying the acceleration voltage. Each scan suffers a rectification of the mass calibration at the same time that experimental data are obtained. This technique is called lock-mass technique and improves mass accuracy increasing reliability of mass spectrometric data obtained. Resolution of the instrument can also be recalculated on each scan. It can also be improved doubling the calibration masses in a technique called Lock-plus-cali mass technique. This scan-to-scan mass calibration processed in the background improves confidence of the analytical data. For this internal mass calibration, the calibration standard is leaked continuously from the reference inlet system into the ion source. Two ion masses are selected from the reference substance: one mass that is below the analyte target masses and another one above the analyte target masses. The lowest mass is called "lock mass" and the highest is named "calibration mass." The magnet is locking the magnet at the start of each MID process and performing a mass calibration based on the lock mass. All analyzer jumps to the calibration and target masses by fast electrical jumps of the acceleration voltage. It provides a fine calibration in only a very few milliseconds (Thermo Fisher Scientific Inc., 2007). Fig. 1.3 shows the typical sequence of steps during a MID process in DFS instrument.

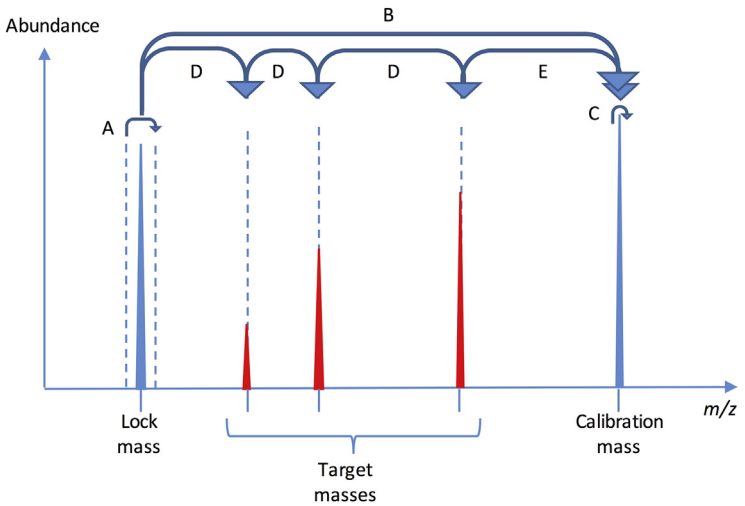

FIGURE 1.3

Internal mass calibration and target mass detection during a multiple ion detection (MID) process in a double focusing system (DFS) instrument: A, magnet locking and lock mass sweep. Mass calibration and resolution determination; B, electrical jump to calibration mass; C, calibration mass sweep and mass calibration; D, electrical jumps to target masses; E, electrical jump to calibration mass for mass calibration.

1.5 GENERAL CONSIDERATIONS

Empirical determination of a molecular formula for a substance can be very useful in organic MS. HRMS can be an alternative to traditional chemical methods based on the tedious, slow, and often inaccurate process of breaking down of a known weight of a molecule into its constituent elements and weight of them (Herbert & Johstone, 2003). It should be mentioned that the mass of an electron is very small compared with masses of any element, and therefore, frequently, the mass of M^+ is considered the same as that of M. Therefore, an HR mass spectrometer can be used to measure relative atomic, molecular, or fragment ion masses with high accuracy. An acceptable value of the measured mass should be within 5 ppm of the accurate mass (Gross, 1994).

It is essential that the ion of interest is properly resolved from all other neighboring ions because any interfering ion would introduce an error in mass measurement. Therefore, a high resolving power is very important for accurate mass measurements. For small molecules, a resolving power higher than, i.e., 10,000 is not critical. For example, a resolving power of 770 would be enough for mass-resolving $C_2H_4{}^{+\bullet}$ (28.031300) and $CO^{+\bullet}$ (27.994915). Nevertheless, a resolving power of at least 5500 is needed to separate $C_{13}H_{16}{-}C_2H_4{}^{+\bullet}$ and $C_{13}H_{16}{-}CO^{+\bullet}$ ions, even if the difference between both masses is 0.036385 u. Another difficulty is that the possible elemental composition becomes quite larger as the mass increases.

Full scan can be one of the simplest acquisition modes to obtain an accurate mass value when an internal mass calibration is carried out. Accurate masses of all ions can be determined in one single chromatographic run, and elemental composition can be achieved with an adequate accuracy of less than 5 ppm. Peak-matching mode is a more accurate mass measurement technique with a typical accuracy lower than 0.3 ppm. For that, only one ion is determined at a time, for example, using the MID technique described earlier. It is recommended that the mass of the mass calibration ion must be within 2% of the unknown mass to give the highest accuracy. A combination of slow scanning of the accelerating voltage and computer programs that improve signal averaging, smoothing, and peak centroiding improves mass accuracy (Hammar, Pettersson, & Carpenter, 1974).

A nominal mass may result from several combinations of elements, but only one composition can match an accurate mass. Nevertheless, more possible combinations can occur with an increased number of atoms in a molecule. A list of potential molecular formulas for various masses has been proposed (Beynon & Williams, 1963). Diverse algorithms and computer programs are available for elemental composition; many of them are available online.

Finally, it should be mentioned that RΔm is also a valuable tool for confirmation purposes. The sum of RΔm of all isotopes of the molecule is defined as the isotopic mass average (IMA), and Padilla-Sánchez et al. (2012) used this approach for the reliable identification of small pesticides such as ethephon and N-acetyl-glufosinate, obtaining an experimental $IMA_{(A+2)}$ value of −0.00286 Da for ethephon, which was within the theoretical interval of -0.0029 ± 0.0001 Da.

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