2.1 INTRODUCTION

Undoubtedly, the establishment of electrospray ionization (ESI) in the late 1980s (Fenn, Mann, Meng, Wong, & Whitehouse, 1989, p. 64; Yamashita & Fenn, 1984, p. 4451) and its enormous potential for biomedical applications prompted the development and growth of liquid chromatography—mass spectrometry (LC—MS) instrumentation. Several manufacturers launched LC—MS instrumentation in the mid-1990s with either ion trap or triple quadrupole (QqQ) analyzers, which established itself as the gold standard for quantitative trace analysis. Meanwhile, the use of high-resolution LC—MS instrumentation with time-of-flight (TOF) analyzers was commercially available from 1996 with the development of first hybrid quadrupole time-of-flight (Q-TOF) instruments. Micromass commercialized the first Q-TOF mass spectrometer in 1996 (Q-TOF-1), which was furnished with ESI source, quadrupole mass filter, hexapole collision cell, orthogonal acceleration, and microchannel plate detector. It featured a resolving power of 5000. Unfortunately, these instruments were originally applicable only to qualitative purposes such as the determination of molecular mass and elemental composition through accurate mass analysis. There were several issues (linear dynamic range due to detector saturation and scarce robustness) that limited the use of such instrumentation for quantitative purposes.

The last two decades have witnessed an unprecedented growth of mass spectrometric instruments of increasing high-mass resolving power. Such ability enabling accurate mass measurements permits the determination of elemental compositions for small molecules up to 500–1000 Da in mixtures containing thousands of chemical components (Marshall & Hendrickson, 2008, p. 579). There has also been an outstanding growth in the use of high-resolution instrumentation for quantitative application. This fact has been supported by relevant improvements of instrument performance in terms of resolution, sensitivity, and speed. Among them, in 2004, several vendors (Agilent, Bruker, Waters) offered updated and upgraded versions of TOF instruments, with improvements on electronics to prevent detector saturation, temperature control of flight tube, and continuous accurate mass calibration systems (dual sprayer, MassLock, etc.), which broadened the range of applications and enable more versatile and user-friendly operation. In 2005, the
orbital ion trapping analyzer (Orbitrap), an alternative concept of high-resolution instrumentation—probably one of the major breakthroughs in mass spectrometry in the last decades—was launched by Thermo Fisher Scientific. Its advantageous features, particularly related to resolving power, have fostered lately the development of high-performance TOF analyzers with enhanced performance and solutions to challenge the current Orbitrap lead.

Besides TOF and Orbitrap, high-resolution mass spectrometry (HRMS) can be also carried out using Fourier transform ion cyclotron resonance (FT-ICR) first commercialized in 1983 (Nier, Yergey, & Gale, 2015) and magnetic sector instruments. Provided the current literature and the number of instruments sold and manufacturers offering these technologies, it seems that the interest for these technologies is diminishing. In gas chromatography—high-resolution mass spectrometry (GC—HRMS), for years GC has been coupled to HR magnetic sector instruments, mostly for dioxin analysis. Yet, GC—HRMS is the reference instrumentation for analysis of many persistent organic pollutants (Eljarrat & Barceló, 2002, p. 1105; Hernández et al., 2012, p. 1251; Van Babel & Abad, 2008, p. 3956) particularly dioxins, including polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans. However, recently there has been a growing interest in GC—HRMS with TOF and Orbitrap mass analyzers (Mol, Tienstra, & Zomer, 2016, p. 161; Peterson, Balloon, Westphall, & Coon, 2014, p. 10,044; Peterson, Mcalister, Quarmby, Griep-Raming, & Coon, 2010, p. 8618; Van Mourik, Leonards, Gaus, & De Boer, 2015, p. 259) partly associated with the introduction of atmospheric pressure chemical ionization (APCI) sources (Van Babel et al., 2015, p. 9047), which ultimately may replace magnetic sector instruments within the next decade.

In this chapter, an overview of the more commonly used HRMS instrumentation viz. those capable of featuring resolving power above 10,000 (full width at half maximum, FWHM) is provided, including orthogonal acceleration/reflectron TOF mass spectrometers and Fourier transform high-resolution mass spectrometers (Orbitrap and FT-ICR instruments) coupled with either LC or GC. Along with the ability to provide accurate mass measurements, these instruments offer several hardware combinations yielding hybrid instruments [coupled with ion traps and/or quadrupoles and even ion mobility (IM) spectrometry modules] providing interesting possibilities in terms of acquisition mode to face different qualitative and quantitative applications. On the other hand, the combination of accurate mass measurements with available databases (often free on the Web) permits unprecedented capability to scrutinize samples and their content in an automated fashion.

### 2.2 Principles of High-Resolution Mass Spectrometry Analyzers

In this section, the principles of the main HRMS analyzers used nowadays are briefly outlined. A thorough explanation of the physical principles and the involved

2.2.1 TIME-OF-FLIGHT

TOF is a relatively mature technology (Fjeldsted, 2016, p. 19). Actually, the concept of TOF mass analysis was first proposed by Stephens in 1946 (Stephens, 1946, p. 691), and subsequently developed by Cameron and Eggers and referred to as a “velocitron” in 1948 (Cameron & Eggers, 1948, p. 605). The basic principle of TOF instrument relies on the velocity-dependent separation and subsequent detection of ions as they travel through a flight tube. Charged ions produced are accelerated into TOF tube and are allowed to drift along the path. Provided that velocity is dependent on mass-to-charge ($m/z$) ratio, the lighter ions will fly faster and reach the detector earlier. There are two main types of TOF analyzers: linear and of orthogonal acceleration. Whereas the use of linear time-TOFs was associated with pulsed ionization methods such as laser beams with matrix-assisted laser desorption/ionization (MALDI) experiments, the latter is the most commonly used configuration nowadays. **Fig. 2.1** shows the scheme of an orthogonal acceleration TOF. It consists of...
an ionization interface, which is followed by an ion transfer section (ion optics) and focusing region. The continuous ion beam emerging from the ion source is sharpened within the ion optic path and sent toward the pulser. A high-voltage pulse is applied to the pusher plate to orthogonally accelerate pockets of ions into the flight tube.

Wiley and McLaren (1955, p. 1150) realized the importance of space focusing. They found that small differences on the position of the ions created in the ion source and the distances to the detector affected resolving power dramatically. Ions created at the back of the ion source volume had a longer distance to travel to the detector than those that were created closer to the front of the ion source volume. They demonstrated that with the appropriate increase in voltage at the back pusher plate within the ion source it was possible to significantly reduce the loss of resolution because of the origin of the ions within the source. This advance was connected to the early development of the first TOF commercial instrument offered by Bendix in the mid-1950s (Fjeldsted, 2016, p. 19; Wiley, 1956, p. 817).

Therefore, during TOF analysis, it is important that all ions receive an equal acceleration to, as much as possible, reduce spreading in time. The focusing of the ion beam before reaching the pusher and the following orthogonal acceleration become critical to ensure equal starting conditions for all ions regardless of their individual masses and thermal energy. Thus, the initial spatial and ion energy spread among the ions is minimized. Mamyrin (1966) proposed the use of an ion mirror (referred to as a reflectron) in the flight path to compensate for the spread in the initial energy of the ions. Mamyrin showed that when a proper reflecting field was established, ions with greater energy proportionally travel further into the reflectron before being reflected with the effect that the arrival time of the ions at the detector shows a greater tolerance to the effects of their initial energy spread. Therefore, a careful design of the reflectron permits a compensation of speed differences, as caused by the remaining energy difference among the accelerated ions. In addition, the implementation of the reflectron doubled the flight path length to achieve longer flight times and therefore increased resolution.

TOF instruments are of pulsed nature. The use of ion packets is central to TOF analyzers. Initially, the creation of ion packets was generated by employing a pulsed ionization source such as an electron ionization filament or a beam deflector. In the latter approach, a pulsed ion packet was created through deflection by gating, or momentarily steering, the ion beam into the TOF analyzer. In this sense, the concept of orthogonal acceleration was proposed by Dawson and Guilhaus (1989, p. 155). A continuous beam of ions was momentarily pushed in a direction perpendicular to the direction of the continuous ion beam. The packet of orthogonally directed ions enters the TOF tube that is therefore oriented perpendicular to the continuous ion beam rather than in-line. With this configuration, the energy spread is substantially minimized. The introduction of the orthogonal TOF geometry was of paramount importance for adapting TOF to atmospheric pressure ionization sources, such as ESI, and the subsequent growth in TOF applications in LC/MS.

The detector—located at the end of the flight path—records the arrival time and the number of incoming ions. The square of the flight time is proportional to the $m/z$
of the detected ion. Hence, masses (m/z) can simply be calculated after an instrument calibration process. Because of the fast ion flight times, a single push cannot be used to record a spectrum. Hundreds of consecutive pushes are summed to produce a single, averaged spectrum instead (corresponding to one chromatographic data point). A single pulser push accelerates only the ions passing above the surface of the pusher plate. The next push should not be started before the heaviest ion accelerated by the preceding push has reached the detector. This results in duty cycles clearly below 100%. The very short ion flight times within the flight tube put extreme importance on the frequency of the detection device and the following signal processing. In the past, fast time-to-digital conversion (TDC) detectors were only able to measure the flight time, but could not count the number of the simultaneously incoming ions at a given time. Such devices have been replaced by analog-to-digital conversion (ADC) detectors, which unlike TDC devices can account for the number of ions simultaneously hitting the detector plate, thus enabling the expansion of the dynamic range to up to four decades. Yet this point remains as one of the main weaknesses of TOF detectors; they undergo saturation from a threshold intensity, which not only affects the measured signal amplitude but also can result in a relevant mass shift of the ions being affected by saturation.

2.2.2 FOURIER TRANSFORM ION CYCLOTRON RESONANCE

FT-ICR was first described by Marshall and Comisarow in 1974 (Comisarow & Marshall, 1974, p. 282; Comisarow & Marshall, 1996, p. 581). Yet, it is the instrument providing the highest performance in terms of resolving power. In fact, the resolving power of either a magnetic sector or a TOF depends on the path length during the experiment, 7 m being the path length of the highest resolution magnetic sector and between 1 and 2.5 m in current TOF analyzers (leaving aside multireflextron TOF instruments). In contrast, an ion with m/z 1000 at a standard 9.4 T magnet exhibits cyclotron frequencies of 144,346 Hz and thus, has an effective path length higher than 9 km in 1 s (2π × 0.01 m × 144,346 Hz = 9070 m).

FT-ICR enables the accurate determination of m/z of ions based on the cyclotron frequency of the ions in a fixed magnetic field (Comisarow & Marshall, 1996, p. 581; Marshall & Hendrickson, 2008, p. 579). The ions are trapped in a Penning trap (a magnetic field with electric trapping plates) where they are excited (at their resonant cyclotron frequencies) to a larger cyclotron radius by an oscillating electric field orthogonal to the magnetic field. After the excitation field is removed, the ions are rotating at their cyclotron frequency in phase (as a “packet” of ions). These ions induce a charge (detected as an image current) on a pair of electrodes as the packets of ions pass close to them. The resulting signal is called a free induction decay, transient, or interferogram that consists of a superposition of sine waves. The signal is digitalized and subjected to discrete fast Fourier transformation to yield a spectrum of ion cyclotron frequencies, which may then be converted to a spectrum of m/z (Marshall, Hendrickson, & Jackson, 1998, p. 1). The expression that describes
the rotation of the ions at a cyclotron frequency \( \nu_c \) (Hz) in the spatially uniform magnetic field \( B \) is:

\[
\nu_c = \frac{eB}{2\pi m}
\]

in which \( B \) is the magnetic field, \( m \) is the mass, and \( e \) is the elementary charge. At room temperature, typical ion cyclotron orbital radii are at the submillimeter level. Considering that ions with a certain \( m/z \) rotate with random phase, it is necessary to resonantly excite the ions with an oscillating or rotating electric field to yield a spatially coherent packet of ions of each given \( m/z \). The motion of the different ion packets gives rise to a time-domain signal consisting of the difference in current induced on a pair of opposed electrodes (Fig. 2.2).

Nowadays, only one vendor commercializes a portfolio of FT-ICR instruments (Bruker Daltonics, Bremen, Germany). Up to three different models are offered including a hybrid quadrupole FT-ICR 7-Tesla (T) (solarisX XR) and 12-T or 15-T high-field solarisX XR with resolving power up to 600,000 at \( m/z \) 400 with 1 s transient (and up to 10,000,000 resolving power depending on magnet and acquisition time). To enable a flexible range of experiments, these instruments permit a wide range of fragmentation techniques including in-source collision-induced dissociation (CID), CID in collision cell, electron-capture dissociation (ECD), electron-transfer dissociation (ETD), and sustained off-resonance irradiation (SORI) CID in cell. In the past, Thermo also offered a hybrid linear ion trap (LTQ)-FT-ICRMS, but it was withdrawn in 2014, with the emergence of high-field Orbitrap, which nearly matched the performance of FT-ICR systems. The applications foreseen for this type of instrumentation are petroleomics (Cho, Ahmed, Islam, & Kim, 2015, p. 248; Nikolaev, 2015, p. 421; Schwemer, Rüger, Sklorz, & Zimmermann, 2015, p. 11,957) and state-of-the art proteomics research (Marshall & Chen, 2015, p. 410; Nicolardi, Bogdnov, Deelder, Palmblad, & Van

![FIGURE 2.2](image.png)

**FIGURE 2.2**

Summary of Fourier transform mass spectrometry based detection of ions in Fourier transform ion cyclotron resonance mass spectrometry: (A) excited ion cyclotron rotation, (B) differential image-current signal from opposed detection electrodes, (C) frequency-domain signal, and (D) mass spectrum obtained by calibrated frequency-to-\( m/z \) conversion. A typical acquisition of 1 s compatible with liquid separation permits resolving power above 400,000 (depending on the magnet used). Higher acquisition time permits increased values of resolving power above 1,000,000. For details, see Comisarow and Marshall (1996, p. 581) and Marshall and Hendrickson (2008, p. 579).
Der Burgt, 2015, p. 27,133), being the application of FT-ICR in pesticide testing scarcely addressed as the requirements and maintenance cost of such sophisticated instrumentation are not met by standard routine laboratories (Gilbert-López et al., 2013, p. 419).

2.2.3 ORBITRAP

The Orbitrap mass analyzer is the first high-performance mass analyzer that employs trapping of ions in electrostatic fields, together with a sophisticated ion injection process, which enables high resolution, mass accuracy, and excellent sensitivity for addressing numerous analytical applications in both research and routine analysis. The principle of Orbitrap is based on the Kingdon ion trap described in 1923 (Kingdon, 1923, p. 408). It was a trapping device consisting of a charged wire stretched along the axis of an enclosed metal can. The wire establishes an electrostatic field within the can, and ions that possess sufficiently high tangential velocity orbit the wire, rather than directly colliding with it (Eliuk & Makarov, 2015, p. 61). It was first commercially introduced in 2005 (Eliuk & Makarov, 2015, p. 61). It provides a very convenient balance between the advantages of a benchtop instrument featuring high resolution approaching FT-ICR performance, whereas the instrument size, laboratory space requirements, and regular operational maintenance are more comparable to Q-TOF instruments.

A thorough explanation of the mass analysis process is detailed elsewhere (Eliuk & Makarov, 2015, p. 61). This mass analyzer is based on the confinement of ions in an electrostatic potential well (Makarov, 1999, US 5886346; Makarov, 2000, p. 1156; Makarov & Hardman, 2006, US 6998609 B2) created between two carefully shaped electrodes: an inner coaxial spindled-shaped electrode and an outer (barrel-like) electrode, which is actually composed of two symmetrical halves electrically isolated from each other, set out for two purposes: establishment of the ion trapping fields and as receiver plates for image-current detection. The electrodes are precisely machined so that the electrostatic attractions of the ions to inner electrode are finely balanced by centrifugal forces, which cause the ions to orbit around the spindle. Previously, ions are first accumulated on an external injecting device (C-trap) that traps the ions in gas-filled quadrupole being then injected tangentially into the mass analyzer in short pulses (Fig. 2.3). Besides, an axial field causes the ions to oscillate harmonically along the spindled-shaped electrode. The outer electrodes allow differential image-current detection. These image currents produced by the oscillating ions are detected, followed by a fast Fourier transform (FT) to convert the time-domain signal to frequency domain and then to $m/z$ spectrum. Here the resolution is directly proportional to number of harmonic oscillations detected. The resolving power can be enhanced by increasing the gap between inner and outer electrodes providing higher field strength for a given voltage. As the maximum acquisition time is limited in Orbitrap, the resolution power is not as high as FT-ICR. However, commercial Orbitrap analyzer provides a nominal resolution power as high as 500,000 at FWHM (Table 2.1).
2.3 TIME-OF-FLIGHT MASS SPECTROMETRY: INSTRUMENT CONFIGURATION AND MAIN FEATURES

2.3.1 STAND-ALONE ELECTROSPRAY IONIZATION TIME-OF-FLIGHT AND HYBRID QUADRUPOLE TIME-OF-FLIGHT INSTRUMENTATION

The Micromass Q-TOF-1 (1996) was the first commercially available Q-TOF instrument launched in 1996, featuring a resolving power of 5000 (Morris et al., 1996, p. 889). Later, other small manufacturers including Perceptive, Analytica or Bradford, Sensar, and JEOL introduced ESI-TOFs. In 1999, Micromass—part of Waters—updated the Q-TOF system (Q-TOF-2) with an increase in the resolving power up to 10,000 and presented a stand-alone TOF instrument (Waters LCT). A similar instrument was also commercialized by Applied Biosystems (now Sciex) around 2000 (Applied Biosystems Mariner ESI-TOF). In 2001, Waters upgraded the performance of Q-TOF (Q-TOF Ultima) using W-type flight path providing a resolving power of 17,500. After that, in 2002 Applied Biosystems (now Sciex) launched the Q-STAR XL, its first Q-TOF, which was updated in 2008 (Q-STAR Elite) with improvements in detector design and increased resolving power.

FIGURE 2.3
Cross section of the C-trap ion accumulation device and the Orbitrap mass analyzer with an example of an ion trajectory. During the voltage ramp, the ion packets enter the Orbitrap mass analyzer forming rings that induce current which is detected by the amplifier.

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Table 2.1 Overview of the Technical Specifications and Characteristics of Time-of-Flight (TOF) and Orbitrap

<table>
<thead>
<tr>
<th>Mass Analyzer Type</th>
<th>Manufacturer</th>
<th>Instrument Name</th>
<th>Resolving Power (FWHM Defined at m/z)</th>
<th>Mass Accuracy</th>
<th>m/z Range</th>
<th>Acquisition Speed (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q-TOF</td>
<td>Bruker</td>
<td>MicroOTOF-Q II</td>
<td>20,000 (m/z 922)</td>
<td>&lt;2</td>
<td>50</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Daltonics</td>
<td>MaXis impact</td>
<td>40,000 (m/z 386)</td>
<td>&lt;1</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MaXis 4G</td>
<td>60,000 (m/z 1222)</td>
<td>&lt;0.6</td>
<td>50</td>
<td>30 (MS), 10 (MS/MS)</td>
</tr>
<tr>
<td>Waters</td>
<td></td>
<td>XEVO G2 Q-TOF</td>
<td>22,500 (m/z 956)</td>
<td>&lt;1</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Synapt G2-S HDMS</td>
<td>50,000 (m/z 956)</td>
<td>&lt;1</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>Waters</td>
<td></td>
<td>6500 Q-TOF series</td>
<td>42,000 (m/z 922)</td>
<td>&lt;1</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Agilent</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sciex</td>
<td>TripleTOF 4600</td>
<td>30,000 (full-range)</td>
<td>&lt;0.5</td>
<td>5–40,000</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TripleTOF 5600</td>
<td>35,000 (full-range)</td>
<td>&lt;0.5</td>
<td>5–40,000</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TripleTOF 6600</td>
<td>40,000 (full-range)</td>
<td>&lt;0.5</td>
<td>5–40,000</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LC–MS-IT-TOF</td>
<td>10,000 (m/z 1000)</td>
<td>3</td>
<td>50–5000</td>
<td>10</td>
</tr>
<tr>
<td>IT-TOF</td>
<td>Shimadzu</td>
<td>Q-Exactive</td>
<td>140,000 (m/z 200)</td>
<td>&lt;1</td>
<td>50–4000</td>
<td>12 at RP</td>
</tr>
<tr>
<td></td>
<td>Thermo</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>17,500</td>
</tr>
<tr>
<td></td>
<td>scientific</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q-Orbitrap</td>
<td>Thermo</td>
<td>Orbitrap Elite</td>
<td>240,000 (m/z 400)</td>
<td>&lt;1</td>
<td>50–4000</td>
<td>8 at RP</td>
</tr>
<tr>
<td></td>
<td>scientific</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>17,500</td>
</tr>
<tr>
<td>LTQ-Orbitrap</td>
<td>Thermo</td>
<td>Orbitrap Fusion</td>
<td>500,000 (m/z 200)</td>
<td>&lt;1</td>
<td>50–6000</td>
<td>18 at RP</td>
</tr>
<tr>
<td></td>
<td>scientific</td>
<td>Lumos tribrid</td>
<td></td>
<td></td>
<td></td>
<td>17,500</td>
</tr>
</tbody>
</table>

FWHM, full width at half maximum; HDMS, high-definition mass spectrometry; IT-TOF, ion trap time-of-flight; LTQ-Orbitrap, linear ion trap Orbitrap.
Chapter 2 HRMS: Hardware and Software

The second generation of TOF instruments emerged by 2004, enabling a significant enhancement of both linearity and robustness. Three major vendors (Waters, Agilent Technologies, and Bruker) offer either completely new or completely transformed instruments. Waters first came up with both LCT TOF Premier and Q-TOF Premier using 4 GHz TDC detection and programmable enhanced dynamic range, which decreases the ion beam intensity to reduce detector saturation, extending the quantitation capabilities of the instrument. These TOF instruments featured continuous accurate mass calibration (MassLock). Both Bruker and Agilent introduced ESI-TOF instruments in 2004 with 10,000 resolving power, being the equivalent Q-TOF instruments launched in 2005 (micrOTOF-Q) and 2006 (Agilent 6510 Q-TOF), respectively, with resolving power of c. 15,000 in both cases. Follow-up products (micrOTOF-Q II operating at 2 GHz and 6520 Q-TOF operating at 4 GHz) enabled resolutions of about 20,000. These two instruments used ADC instead of TDC, featuring—a dynamic range of up to five decades. A comprehensive explanation on the details of each technology is given elsewhere (Fjeldsted, 2003, 2016, p. 19). An example of the typical hardware configuration of a current Q-TOF instrument is illustrated in Fig. 2.4, where the schematics of Q-TOF Agilent 6550 is shown including a dual ion funnel ion sampling and a two-stage ion mirror (Fjeldsted, 2016, p. 19).

2.3.2 IMPROVEMENTS OF CURRENT (QUADRUPOLE) TIME-OF-FLIGHT INSTRUMENTATION

The improvement of TOF instrumentation can be attributed to different aspects, which are interrelated so that it is difficult to isolate the contribution of each individual factor to the overall performance.

**Ionization step.** First of all, ion production and atmospheric sampling is a key aspect on the overall sensitivity. Significant progress has been made from early electrospray experiments in the 1980s. The use of a nebulizing gas was proposed to increase the efficiency of ion production. To accelerate droplet size reduction, heated gases were then used (Turbo V source, Sciex). Then, different manufacturers came up with similar heated ESI based sources, such as the Jet Stream from Agilent, first implemented in 2008 Q-TOF 6530. Jet Stream (Agilent) consists of an enhanced ESI technology employing concentrically applied heated gas providing an average fivefold sensitivity increase compared with nonthermally enhanced ESI design (although this may vary depending on each individual compound).

**Ion sampling.** Major efforts have also been carried out for the improvement of ion sampling in the mass spectrometer. For this purpose, a balance between sampling orifice/inlet capillary diameter and vacuum demands must be achieved. Additionally, the ion transfer step has also been improved with more efficient ion guide technologies such as the ion funnels (Agilent) and other ion guide devices [QJet ion guide (Sciex) or Stepwave (Waters)], permitting an increase in the ions entering the mass spectrometer with the subsequent improvement of sensitivity.
The use of radio frequency (RF) fields exerts a force on ions over a wide range of pressure and mass. Based on this principle, the QJet ion guide designed by Sciex improved efficiency in separating ions from the noncharged species (neutrals), improving the focusing of captured ions into the mass spectrometer. Another approach (StepWave) was presented by Waters and first implemented in 2008 in the Synapt G2 hybrid instrument based on the use of a set of ion guides to shift the ion beam axis offset to the atmospheric pressure sampling inlet. RF and direct current (DC) potential are applied onto a set of ion concentric rings with overlapping apertures, so that the ions are shifted off the main axis into smaller off-axis concentric apertures, which focus the ion beam and direct it to the subsequent mass analysis stages while neutral gas passes along the principle axis being diverted to the exhaust.

Based also on the use of stacked ring RF ion guides, R.D. Smith et al. showed that ion confinement could be achieved at pressures in the range from 0.1 to 30 torr (Kelly, Tolmachev, Page, Tang, & Smith, 2010, p. 294), when constructing
a set of ring electrodes having a linear decrease in the inner diameters in the last section of the stack (the so-called ion electrodynamic funnel), which could be used individually or as a pair in succession (dual ion funnel) with the second having a lower operating pressure than the preceding funnel. These RF ion funnel technologies enable highly efficient ion capture and may provide up to a 10-fold increase in the atmospheric sampling efficiencies and overall sensitivity (Fjeldsted, 2016, p. 19). The use of multiplexed (hexabore) capillaries has also been proposed to increase ion sampling efficiency (Kelly et al., 2010, p. 294).

**Resolving power and flight path length.** Unlike Orbitrap, the resolving power in TOF analyzer increases with \( m/z \) values. Several solutions have improved the overall performance of commercial TOF instrumentation particularly in terms of resolving power, attaining values up to 50,000 or even higher. Most of the improvements should be attributed to both ion optics (focusing of ions before TOF analysis) and detection electronics including the increase in the detector operation frequency of ADC TOF detectors (from 1 to 5 GHz) (Fjeldsted, 2016, p. 19). Besides, there is another obvious strategy that increases resolution (increasing the flight path length), although at the expense of sensitivity. This can be accomplished by using either longer flight tubes (up to 1.5 m) or multiple reflection by placing a second mirror between the ion pusher and detector, reflecting the beam twice, thus doubling the effective flight path length [such as \( W \)-optics mode in Waters, \( N \)-optic design (Sciex) or folded path technology enabling up to 64 reflections (LECO)]. Ion mirror (reflectron) has also been improved through the use of two-stage mirrors, which compensate ion energy spread from the ion pusher. Nevertheless, reflectrons not only double the flight path length, but also are responsible for a relevant loss of recorded ion abundances. Hence, users of such multireflectron instruments have to come across a compromise between sensitivity and selectivity. Last, but not least, flight tube temperature control in the system also reduces the internal energy spread of the ions with the same mass, so that an increase in resolution is also achieved.

**Ion detection.** Ion detection and digitization have also undergone significant improvements in the last decade. It seems that most of the manufacturers have found that multichannel plate—based detectors with ADC are the best choice to avoid detector saturation and lack of linearity (Fjeldsted, 2016, p. 19). However, the use of very fast TDC conversion and a multianode detector is also used in instruments offering excellent performance such as Sciex TripleTOF 5600 Q-TOF (Fig. 2.5) (Andrews, Simons, Young, Hawkridge, & Muddiman, 2011, p. 5442). A detailed discussion on this matter is described by Fjeldsted (2003, 2016, p. 19).

**Accurate mass measurements and mass calibration.** Mass accuracy is central to HRMS. This fact is particularly important in TOF instruments, as they are generally less robust than FT-MS instruments in terms of the stability of accurate mass measurements and the subsequent accuracy (ppm mass errors). This is a key parameter, as small changes in the experimental conditions (such as laboratory temperature) usually affect the actual time measurements of ions TOF, provided the change in drift tube path length due to temperature changes (small thermal expansion or contraction of the flight tube length). A high-performance temperature
control assembly of the drift tube is mandatory to assess mass accuracy performance of TOF instruments.

For this purpose, mass axis calibration is an important process for obtaining reliable results. Mass calibration consists of comparing the observed experimental mass measurement of one or two known ions with their (theoretical) exact mass. Calibration may be internal (i.e., the reference masses are for ions of known elemental composition in the same mass spectrum of the analyte) or external (i.e., reference masses from a mass spectrum of another analyte acquired under similar conditions). Internal calibration is typically at least twofold more accurate than external (Marshall et al., 1998, p. 1). Accurate mass correction is usually performed in full-scan (FS) data, as the ions selected are available in all acquired spectra through the run. However, when recording accurate mass spectra in MS/MS mode with precursor ion selection, the lack of such calibrating ions hampers the accuracy of product ion spectra.

In the first generation of TOF instruments, external mass calibration was applied before the chromatographic run. This approach did not deliver results better than $5-10$ ppm relative mass error because of the significant drifting of instrumental status (e.g., flight tube temperature). To overcome this situation, various strategies have been proposed to provide continuous accurate mass calibration in commercial
TOF instruments, allowing them to feature routine relative mass errors in the range of 1–3 ppm, when using mass calibration devices such as MassLock (Waters), the dual sprayer solution (Agilent Technologies), or similar technologies presented by other manufacturers such as the TwinSprayer (Sciex), which is an independent calibrant delivery path proposed in the recently commercialized compact Sciex Q-TOF (Sciex X500R Q-TOF) dedicated for small molecule applications (Fig. 2.6). These assemblies provide a compensating mechanism to keep flight times constant for a given fixed mass (reference solution). First, Waters proposed the discontinuous periodical infusion of a lock mass solution by a switching device that alternatively permitted the flow from two independent ESI sprayers (the reference solution and the analytical sample) (Eckers et al., 2000, p. 3683; Wolff et al., 2001, p. 2605). This strategy is not flawless because it introduces mass signals—unrelated to the sample—into both the spectrum and the data file, which are used to calibrate the entire data set at the end of the run. Likewise, Agilent proposed the use of a dual sprayer assembly, where a calibrant solution was continuously sprayed—orthogonal to the analytical sprayer—and introduced in the mass spectrometer (Fig. 2.6). This solution provided excellent results even enabling the measurement of the mass of an ion using twin fragment ions (Ferrer & Thurman, 2005, p. 3394). However, although they are orthogonal, there might be some interaction between the analytical and reference sprayers, which may yield signal suppression if the concentration/abundance of the reference solution analytes is not controlled. As mentioned previously, the newest Sciex Q-TOF (Sciex X500R Q-TOF) makes use of an

![FIGURE 2.6](image)

Examples of continuous calibration systems for mass calibration in time-of-flight instrumentation: (A) Agilent MSD TOF (2004); (B) Sciex X500R (2016).

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independent calibrant delivery path embedded in the analytical sprayer (Fig. 2.6), which also provides excellent accuracy figures, thanks also to the sophisticated temperature control of the flight tube.

### 2.3.3 ION MOBILITY QUADRUPOLE TIME-OF-FLIGHT

A relatively recent development with great potential in the detailed analysis of complex samples is the introduction of gas-phase IM as an additional dimension of separation within LC–MS. The combination of IMS with MS (IM-MS) incorporates shape and size as additional orthogonal dimensions to chromatography and mass spectrometry. It takes advantage of the differences in drift times of charged species in an inert gas with size and shape [in quantitative terms expressed as the collisional cross section (CCS)] being the key determinants of their mobilities (Beucher, Dervilly-Pinel, Prevost, Monteau, & Le Bizec, 2015, p. 9234; Fjeldsted, 2016, p. 19). As this separation takes place in the millisecond timescale, it is compatible (at the expense of some sensitivity) with both chromatographic separation (peak widths of a few seconds) and TOF ion detection system (ion flight times in the microsecond range and ion recording on the detector surface in the nanosecond scale). This approach proves very valuable in the separation of isomers that are undistinguishable solely based on accurate mass measurements. With slight differences in their CCS and thus drift times, such isomers can be resolved in this additional third dimension (fourth with MS/MS experiments) of LC–HRMS instrumentation (Beucher et al., 2015, p. 9234).

The use of IM is not new, although in the past IM-MS experiments were restricted to homemade instruments (Lapthorn, Pullen, & Chowdhry, 2013, p. 43). The progress and developments in hybrid IM mass spectrometry instrumentation in the last decade have prompted the commercial availability of various instruments from different vendors opening the door for a wider acceptance of this technique. The first commercial IM-mass spectrometry instrument became available around 2006 (Giles et al., 2004, p. 2401; Thalassinos et al., 2004, p. 55), the so-called Synapt high-definition mass spectrometry system (Waters) (Fig. 2.7), which was based on the use of high-transmission traveling-wave ion mobility (TWIM) cell. A follow-up version enabled a resolution of 40 (Pringle et al., 2007, p. 1). This quadrupole ion mobility separation time-of-flight mass spectrometry (Q-IMS-TOF MS) consists of a quadrupole unit followed by high-performance IM separation. It consists of three traveling wave—enabled stacked ring ion guides (TWIGs), namely the trap TWIGs, ion mobility separator (IMS), and the transfer TWIG. The trap TWIG has the function of trapping and releasing ions into the IMS, while the transfer TWIG functions to deliver the ions into the TOF analyzer (Fjeldsted, 2016, p. 19). This module also permits fragmentation of ions within the trap and in transfer TWIG. Ions are separated as they pass through IMS based on their size, charge, and cross section. Q-IMS-TOF provides excellent data-dependent (DDA) and data-independent acquisition (DIA), which are useful in metabolite profiling to detect and identify even low-level metabolites from endogenous matrices associated with high background noise.
Recently, a new commercial IMS-Q-TOF instrument has been launched (Kurulugama, Imatani, & Taylor, 2013) based on the use of a uniform low-field drift tube and ion funnel sampling and focusing that allows high-performance IM (RP of c. 80) keeping also high sensitivity (Fig. 2.8) (Kurulugama et al., 2013). The separation is carried out based on the different drag force that ions undergo in the drift tube while they move through it because of the electric field applied. The countercurrent drug force is due to the collision of the ions with the buffer gas molecules. The drag force experienced by the ions depends on their collision cross sections (a function of size and shape, electric charge, and mass), so that ions with larger cross sections are slowed more easily by collisions with the buffer gas in the drift tube.

Differential ion mobility (DMS) has also been coupled in Sciex QTOF instruments (Sciex SelexION available in TripleTOF 5600+ and 6600) as a means to increase selectivity, enable isobaric separations, and reduce chemical background noise. DMS is—according to some authors—also known as High-field asymmetric waveform ion mobility (FAIMS) (Guevremont, 2004, p. 3; Kanu, Dwivedi, Tam, Matz, & Hill, 2008, p. 1), which has also been implemented with LC–TOFMS instruments using a chip operated at ambient pressure (Brown et al., 2010, p. 9827; Brown et al., 2012, p. 4095). A thorough explanation on the fundamentals and theory of separation for both FAIMS and DMS is available elsewhere (Kolakowski & Mester, 2007, p. 842).
The applications of LC–IMS-Q-TOF in small molecule applications are relatively scarce despite this technique being available for nearly one decade. Limited literature is currently available in routine small molecule studies such as food safety, environmental, or forensics applications (Beucher et al., 2015, p. 9234; Far, Delvaux, Kune, Eppe, & De Pauw, 2014, p. 11,246; Fjeldsted, 2016, p. 19; Goscinny, Joly, De Pauw, Hanot, & Eppe, 2015, p. 85; Smith et al., 2013, p. 76; Stephan et al., 2016, p. 6545). The results obtained are generally superior in terms of selectivity, as no interfering compounds occur at the same retention time, exact mass, and drift time, yielding cleaned mass spectra or extracted ion chromatograms, although at the expense of some loss of sensitivity and overall system ruggedness. Besides LC sample introduction, supercritical fluid extraction coupled to ion mobility high-resolution mass spectrometry (SFC-Q-TWIM-TOFMS) (Beucher et al., 2016) using a Synapt G2 with TWIM coupled with Q-TOFMS mass analysis has also been proposed.
2.3.4 HYBRID ION TRAP TIME-OF-FLIGHT

The ion trap time-of-flight (IT-TOF) (Shimadzu) is a relatively new type of hybrid tandem mass spectrometer that combines the unique MS^n capability of quadrupole ion trap (QIT) with high resolution/accurate mass for both MS and MS^n modes provided by the TOF analyzer. Therefore, accurate mass measurements of the different MS/MS stages may provide the actual composition of each fragment to foster structure elucidation, as it may also be carried out in a hybrid LTQ Orbitrap. The main technological challenges are the efficient introduction of ions into the QIT and the simultaneous ejection of trapped ions to the TOF.

In the LC–MS-IT-TOF, ions are injected into the ion trap in pulses that are produced through the action of a combination of the skimmer, octapole, and lens optics (compressed ion injection). In creating these pulses, the ions are first accumulated in the octapole for a fixed period of time, with more ions accumulated at longer accumulation times for higher signal intensity where needed. The value for the ion accumulation time can be freely set in the method file. However, when the automatic sensitivity control feature is activated, the ion accumulation time in the octapole is adjusted automatically and instantaneously during the analysis to effectively avoid saturation of the detector (Shimadzu, 2009). Thus, the octapole acts as an ion gate, holding and controlling ions before release into the ion trap itself. The ion trap performs two functions: pulses ions into the TOF mass analyzer at a precise start time and achieves high CID efficiency experiments as pulsed argon gas eliminates CID energy mass dependence. Studies using LC–IT-TOF for pesticide screening or the determination of other contaminants in food are relatively scarce (Li, Zhang, Ma, Li, & Guo, 2015, p. 316; Zhan, Xing, Sun, Ting, & Chew, 2015).

2.3.5 GAS CHROMATOGRAPHY—TIME-OF-FLIGHT AND GAS CHROMATOGRAPHY—QUADRUPOLE TIME-OF-FLIGHT

The development of GC-compatible APCI and atmospheric pressure photoionization interfaces and their combination with HRMS has revitalized and raises back the interest in GC–MS, given its attractive features for nontarget analysis. The use of soft ionization techniques promotes the formation of molecular ions as compared to the commonly observed extensive fragmentation under EI conditions and thus results in an increase in sensitivity at detecting the molecular ion. In contrast, the use of a hybrid instrument with precursor ion isolation and dedicated fragmentation in a collision cell is required for unambiguous confirmation with MS/MS experiments.

In 2011 LECO began commercialization of Pegasus GC–HRT instrument with folded flight path technology enabling high resolution with high spectral production frequency (200 Hz) and three resolution operational modes: unit mass, high resolution (>25,000 FWHM), and ultrahigh-resolution (featuring R = 50,000 although with limited mass range) based on up to 64 reflections—up to 20 m effective path length.
These features—including scan speed—map well against the requirements of comprehensive gas chromatography systems (GC × GC) and may find several applications (Zimmermann et al., 2014). Agilent also commercializes a dedicated GC-Q-TOF instrument with electron impact ionization, which has been applied to pesticide testing in fruits and vegetables (Belmonte et al., 2015, p. 2162; Zhang et al., 2012, p. 39) (Fig. 2.9).

**2.4 ORBITRAP ANALYZERS: INSTRUMENT CONFIGURATIONS AND MAIN FEATURES**

Different Orbitrap instruments (Thermo Scientific) have come out in the last decade. A detailed summary of each of the variants commercialized is provided in Table 2.2 (Martins, Bromirski, Prieto Conaway, & Makarov, 2016, p. 3). The main features are described, organized from the simplest configuration (stand-alone Orbitrap) to more complex instrument architectures (tribrid Orbitrap) rather than by the date of commercialization.

**Stand-alone Orbitrap (Exactive).** Until the introduction of the Exactive (stand-alone Orbitrap) in 2009 (Bateman, Kellmann, Muenster, Papp, & Taylor, 2009, p. 1441), the relatively high cost and complexity of Orbitrap hybrid platforms (LTQ-Orbitrap) limited their implementation in routine laboratories such as food safety, environmental monitoring, and clinical and toxicological testing. The Exactive is
<table>
<thead>
<tr>
<th>Family</th>
<th>Name</th>
<th>Launch Year</th>
<th>Front End</th>
<th>Orbitrap Analyzer</th>
<th>Other Analyzers</th>
<th>Fragmentation Methods</th>
<th>Other Features</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Research-Type Instruments</strong></td>
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<tr>
<td></td>
<td>LTQ-Orbitrap</td>
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</tr>
<tr>
<td></td>
<td>Classic (Makarov et al., 2006, p. 2113)</td>
<td>2005</td>
<td>Capillary</td>
<td><strong>Standard 3.5 kV</strong></td>
<td>LT, 2</td>
<td>CID</td>
<td>Discovery version (2007)</td>
</tr>
<tr>
<td></td>
<td>XL (Olsen et al., 2007, p. 709)</td>
<td>2007</td>
<td>As above</td>
<td>As above</td>
<td>As above</td>
<td>CID, HCD, (ETD)</td>
<td>MALDI version 2008 (Stupat et al., 2009, p. 1451)</td>
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<td></td>
<td>Velos (Olsen et al., 2009, p. 2759)</td>
<td>2009</td>
<td><strong>S-lens</strong></td>
<td>As above</td>
<td>Dual-pressure LT</td>
<td>As above</td>
<td></td>
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<td></td>
<td>Elite (Michalski et al., 2012)</td>
<td>2011</td>
<td>As above</td>
<td><strong>High-field 3.5 kV</strong></td>
<td>As above</td>
<td>As above</td>
<td>eFT signal processing</td>
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<td></td>
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<td></td>
<td>(Lange, Damoc, Wieghaus, &amp; Makarov, 2014, p. 16)</td>
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<tr>
<td></td>
<td>Orbitrap Fusion</td>
<td></td>
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<tr>
<td></td>
<td>Classic (Senko et al., 2013, p. 11,710)</td>
<td>2013</td>
<td>As above</td>
<td><strong>High-field 5 kV</strong></td>
<td>QMF, LT</td>
<td>CID, HCD, (ETD)</td>
<td>(Internal calibration)</td>
</tr>
<tr>
<td></td>
<td>Lumos</td>
<td>2015</td>
<td><strong>HCCT and ion funnel</strong></td>
<td>As above</td>
<td>Segmented QMF</td>
<td>As above</td>
<td>As above</td>
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<tr>
<td><strong>Routine-Type Instruments</strong></td>
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<tr>
<td></td>
<td>Exactive</td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Classic (Bateman et al., 2009, p. 1441)</td>
<td>2008</td>
<td>Capillary</td>
<td><strong>Standard 5 kV</strong></td>
<td>None</td>
<td>(HCD)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Plus and plus EMR</td>
<td>2012</td>
<td>S-lens</td>
<td>As above</td>
<td>None</td>
<td>As above</td>
<td>eFT, EMR version (2013)</td>
</tr>
<tr>
<td></td>
<td>Classic (Rose et al., 2012, p. 1084)</td>
<td>2011</td>
<td>As above</td>
<td>As above</td>
<td>None</td>
<td>HCD</td>
<td>eFT</td>
</tr>
<tr>
<td></td>
<td>Plus</td>
<td>2013</td>
<td>As above</td>
<td>As above</td>
<td>Segmented QMF</td>
<td>As above</td>
<td>eFT, inject prefiltering</td>
</tr>
<tr>
<td></td>
<td>Focus</td>
<td>2014</td>
<td>As above</td>
<td><strong>High-field 5 kV</strong></td>
<td>QMF</td>
<td>HCD</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HF (Scheltema et al., 2014, p. 3698)</td>
<td>2014</td>
<td>As above</td>
<td>As above</td>
<td>QMF</td>
<td>HCD</td>
<td>eFT</td>
</tr>
<tr>
<td></td>
<td>Q-Exactive GC</td>
<td>2015</td>
<td><strong>In vacuum EI/CI</strong></td>
<td>As above</td>
<td>As above</td>
<td>As above</td>
<td>As above</td>
</tr>
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</table>

CID, collision-induced dissociation; eFT, enhanced Fourier transform; EMR, enhanced mass range; ETD, electron-transfer dissociation; fETD, front-end electron-transfer dissociation; HCCT, high-capacity transfer tube; HCD, higher-energy collision-induced dissociation; LT, linear trap; LTQ, linear ion trap Orbitrap; QMF, quadrupole mass filter. **Bold** denotes first appearance of a feature; references belong to the first publications devoted to the corresponding instrument. Reproduced from Elsevier, with permission. Copyright 2016 [Martins, C.P.B., Bromirski, M., Prieto Conaway, M.C., & Makarov, A.A. (2016). Orbitrap mass spectrometry: Evolution and applicability. In S. Pérez, P. Eichhorn, & D. Barceló (Eds.), Applications of time-of-flight and Orbitrap mass spectrometry in environmental, food, doping and forensic analysis (p. 3). Amsterdam: Elsevier].
a relatively compact benchtop instrument, which only consists of a stand-alone Orbitrap analyzer (omitting the ion trap using in hybrid LTQ-Orbitrap platforms). Thus, it does not have the ability to perform ion/mass selection. Because the total ion population is collected in the C-trap and injected into the Orbitrap analyzer, an automated gain control mechanism was implemented for reproducible ion injection.

The Exactive permits high-resolution/accurate mass (HR/AM) screening of known and unknown compounds with high sensitivity—approaching QqQ instruments in the MRM mode—and selectivity (high resolution up to 100,000 and mass accuracy lower than 5 ppm without internal calibration). Thanks to the high intrascan dynamic range (over four decades of magnitude) and fast polarity switching (both positive and negative HR spectra at 70,000 within a second), the development of (quantitative) screening methods based on exact mass and RT of compounds with sensitivity approaching MRM methods is relatively straightforward. FS screening also allows retrospective examination of data based on a posteriori hypothesis of additional compounds of interest, because of the FS data acquisition rather than strictly targeting specific ions of interest as it is done with standard QqQ analyses. Besides FS, the Exactive can also be operated in pseudo-MS/MS mode without precursor ion isolation, using a high-energy collision cell (HCD), which was optional—not available in all Exactive units. This mode of analysis, referred to as all-ion fragmentation (AIF) is similar to analogous techniques available on instruments sold by Agilent, Sciex, or Waters (all-ion mode, MS\(^{\text{ALL}}\), and MS\(^{\text{E}}\) respectively). This mode is really convenient as it permits parallel acquisition of FS of intact molecules plus the characteristic fragmentation of all species, although at the expense of a lack of the selectivity provided by the absence of precursor ion isolation step.

Recently, two additional versions of Exactive were launched: Exactive Plus and Exactive Plus EMR. The first one included the same Orbitrap analyzer used in Q-Exactive, thus providing a resolving power of up to 140,000. The EMR stands (enhanced mass range) version permits acquisition over the range \(m/z\) 300 to \(m/z\) 20,000 and is intended for applications addressing structural studies of high—molecular weight biomolecules.

**Hybrid quadrupole Orbitrap (Q-Exactive).** To perform precursor ion isolation and fragmentation experiments (similarly to the equivalent Q-TOF or QqQ instruments), the Exactive mass spectrometer was combined with a front quadrupole with the mission to act as a mass filter and to isolate targeted precursor ions that were subsequently fragmented in the HCD cell (similar to that used in the stand-alone Orbitrap) and mass analyzed in the Orbitrap (Fig. 2.10). The so-called “Q-Exactive” instrument came out in 2011 (Rose, Damoc, Denisov, Makarov, & Heck, 2012, p. 1084). To achieve faster acquisition rates, an advanced processing technique [enhanced Fourier Transform (eFT)] for transforming the transient detection signal produced by the Orbitrap mass analyzer was first implemented with the Q-Exactive. Its use combined with fast quadrupole isolation and HCD fragmentation provided improved data quality and acquisition rates. The HCD cell or the C-trap is filled with ions while the previous MS/MS detection cycle is ongoing. High resolution could be acquired at a rate up to 12 Hz, thus avoiding the problem of low speed for accurate mass MS/MS common to hybrid LTQ-Orbitraps.
Recently, three new versions of Q-Exactive have been launched: an upgraded version of the classic Q-Exactive with enhanced ion optics and transmission (advanced quadrupole technology and advanced active beam guide) (Q-Exactive Plus); an entry-level and more affordable version of Q-Exactive for routine laboratory applications (Q-Exactive Focus), with a resolving power up to 70,000 \( m/z \) 200, a scan rate of 12 Hz at 17,500 \( m/z \) 200, and full MS/MS capability; and finally an advanced version for high-end high-throughput proteomics applications using the high-field Orbitrap analyzer (Q-Exactive HF) featuring a resolving power of 240,000 (at \( m/z \) 200 and 1.5 spectra per second) (Table 2.3).

**Table 2.3** Acquisition Speed and Resolution of Q-Exactive, Orbitrap Elite, and Q-Exactive HF

<table>
<thead>
<tr>
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<tr>
<td>Resolution Setting ( m/z ) 400 (FWHM)</td>
<td>Resolution Setting ( m/z ) 400</td>
<td>Resolution Setting ( m/z ) 200 (FWHM)</td>
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<tr>
<td>Spectra per Second (Hz)</td>
<td>Spectra per Second Hz</td>
<td>Spectra per Second (Hz)</td>
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<tr>
<td>12,500</td>
<td>12</td>
<td>15,000</td>
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<tr>
<td>25,000</td>
<td>7</td>
<td>30,000</td>
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<tr>
<td>50,000</td>
<td>3</td>
<td>60,000</td>
</tr>
<tr>
<td>100,000</td>
<td>1.5</td>
<td>120,000</td>
</tr>
<tr>
<td>240,000</td>
<td>1.2</td>
<td>240,000</td>
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FWHM, full width at half maximum.
Hybrid linear ion trap Orbitrap (LTQ-Orbitrap). The hybrid linear ion trap (LTQ-Orbitrap) was the first commercial instrument to incorporate an Orbitrap mass analyzer (Fig. 2.11). This configuration provides flexibility to undertake different experiments, including MS and MS\textsuperscript{n} spectra using either the Orbitrap for high resolution (up to 120,000 FWHM at \(m/z\) 400) and mass accuracy or the ion trap. One of the most commonly used operation mode is high FS HR/AM acquisition using data-dependent MS/MS scan in the ion trap. The LTQ-Orbitrap instrument features scan rates in the 4—5 Hz range in MS/MS with nominal mass detection or 3 Hz MS/MS spectra with accurate mass (Makarov et al., 2006, p. 2113). In addition, to obtain structural information not possible with CID fragmentation, additional fragmentation techniques such as ETD reagent ion source and HCD were implemented.

High-Field (LTQ) Orbitrap (Orbitrap Elite). The high-field version of the Orbitrap analyzer was first introduced in 2011 as a part of the Orbitrap Elite instrument (Michalski et al., 2012) (Fig. 2.11). Later, it was also utilized in a high-end version of Q-Exactive (Q-Exactive HF) intended for advanced proteomics applications and also in the tribrid Orbitrap Fusion. The high-field Orbitrap effectively doubles the operating frequency of the previous version, and combined with the eFT technique it permitted high resolving power of 24,000 at \(m/z\) 400 for a 768-ms transient (acquisition time) yielding about fourfold increase in

![FIGURE 2.11](image-url)

(A) Schematic of the linear ion trap (LTQ)-Orbitrap mass spectrometer with traditional ion trap followed by Orbitrap mass spectrometer. (B) Schematic of the Orbitrap Elite mass spectrometer with S-lens, dual-pressure linear ion trap, high-energy collision-induced dissociation (HCD) cell, electron-transfer dissociation (ETD) option, and high-field Orbitrap mass analyzer.

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resolving power at the same transient length compared to the earlier standard Orbitrap. Thus, the high-field Orbitrap can be operated at higher acquisition rates for the same resolution, ultimately enabling a faster acquisition cycle and/or increasing the number of MS/MS analyses in a given run. The high-field Orbitrap Elite instruments with appropriate modifications and acquisition times of c. 3 s were reported to provide resolving power above 1,000,000 for \textit{m/z} 300–400 (Denisov, Damoc, Lange, & Makarov, 2012, p. 80). In addition, the increase in resolving power/acquisition rate provides significant advantages in quantitative experiments such as SILAC.

**Tribrid (LTQ/Q-Orbitrap).** The tribrid Orbitrap instrument (Orbitrap Fusion and Orbitrap Fusion Lumos) consists of a quadrupole mass filter, an Orbitrap, and a linear ion trap mass analyzers (Fig. 2.12). The high-field Orbitrap used features 500,000 resolving power at \textit{m/z} 200. The system is conceived so that operation can be fully parallelized, maximizing the use of the ion current for the execution of complex acquisition modes, because of its ability to simultaneously isolate ions with one analyzer and separately detect ions in the two remaining analyzers (Senko et al., 2013, p. 11,710). For instance, precursor ion mass isolation can be performed with either the quadrupole or the ion trap while fragmentation can be generated by HCD, CID, ETD, or the novel electron-transfer and higher-energy collision dissociation fragmentation type at any level of MS\textsuperscript{n}. In addition, these precursor and fragment ions can be detected in either the Orbitrap or ion trap analyzers, providing a plethora of possibilities to conduct complex experiments to elucidate chemical
structures. This instrument is designed particularly for complex bioanalysis applications rather than small molecule analysis. Unfortunately, its relatively high cost—considering the standard prizes for benchtop HRMS instruments for small molecule application—dramatically reduces the implementation of such systems for food safety or environmental testing applications.

**GC-Q-Orbitrap.** The use of Orbitrap analyzer for GC analysis was first reported in 2010 based on a hybrid linear ion trap-Orbitrap LC instrument (Peterson et al., 2010, p. 8618; Peterson et al., 2014, p. 10,044). Later, a dedicated GC—quadrupole Orbitrap (GC—Q-Orbitrap) with electron impact ionization was conceived instrument (Peterson et al., 2010, p. 8618; Peterson et al., 2014, p. 10,044) using a high-field Orbitrap analyzer, offering the possibility of four modes of operation including FS high-resolution MS, selected ion monitoring (SIM) with quadrupole isolation, “AIF” MS/MS with beam-type CID in an HCD cell, and dedicated MS/MS high-resolution spectra with precursor ion isolation in the quadrupole and fragmentation in the HCD cell. At a resolution of 60,000, the Exactive GC system has a scan speed of c. 7 Hz (Thermo Scientific, 2016). The scan rate increases up to 18 Hz at a resolution of 12,500 \((m/z\ 272)\), and up to 23 Hz (32 ms transient) at a resolution of 10,000 \((m/z\ 200)\), providing low relative mass errors typically within 2 ppm (Thermo Scientific, 2016), which anticipates a broad range of application in the small molecule research field including metabolomics (Peterson et al., 2014, p. 10,044) or food safety testing (Mol et al., 2016, p. 161). An example is shown in Fig. 2.13 for the peak of hexachlorobenzene and the effect of resolution/scan rate on the number of points defining a chromatographic peak with c. 6-second width.

### 2.5 Acquisition Modes in High-Resolution Mass Spectrometry

In recent years, the traditional acquisition in HRMS workflow has evolved toward allowing a wide range of tools to identify or quantify compounds. This evolution has been made possible by improvements in HRMS instrumentation. As stated before, the introduction of new hybrid instruments including Q-TOF, Q-IMS-TOF, IT-TOF, Q-Orbitrap, etc. has allowed change in how mass spectrometry users work. A brief summary of the different tools is shown in Fig. 2.14. As could be observed, two types of data acquisition could be employed, namely data dependent and data independent.

#### 2.5.1 Data-Dependent Acquisition

According to Mann et al. DDA is mode of data collection in MS/MS in which a fixed number of precursor ions whose \(m/z\) values were recorded in a survey scan (FS single-mass) are selected in real time using predetermined rules and are subjected to a second stage of mass selection in an MS/MS analysis (Fig. 2.14) (Mann,
After acquiring the product ion mass spectra, the system returns back to the survey scan. Precursor ion scan (PIS) is one of the most used in the single-step MS/MS experiment, in which a second mass analyzer is set at the mass of the selected product ion; the first mass analyzer is scanned from that mass upward, resulting in a mass spectrum that contains signals for all the precursor ions.

**FIGURE 2.13**
Gas chromatography (GC)—Q-Orbitrap analysis of hexachlorobenzene (63 pg on column) automated gain control (AGC) target set at 1e6. Scan rate versus mass resolving power. Extracted ion chromatograms for m/z 283.80,945 (hexachlorobenzene). Sticks represent intensities for individual scans (no smoothing applied), MA: peak area. For details, see (Mol et al., 2016, p. 161).

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Hendrickson, & Pandey, 2001, p. 437; Murray et al., 2013, p. 1515). After acquiring the product ion mass spectra, the system returns back to the survey scan.

**Precursor ion scan.** Precursor ion scan (PIS) is one of the most used in the single-step MS/MS experiment, in which a second mass analyzer is set at the mass of the selected product ion; the first mass analyzer is scanned from that mass upward, resulting in a mass spectrum that contains signals for all the precursor ions.
ions that dissociate to the selected product ion. This mode could be carried out by the different hybrid mass spectrometers available including Q-TOF and Q-Orbitrap. However, this is limited because these MS/MS experiments have to be fixed previously. So, it was necessary to have a suspect compound. Thus, the analyst must have a more comprehensive understanding of system studied.

**Ion intensity-dependent.** In this DDA acquisition method, the user has to fix an ion intensity threshold. Then, the compounds, which have reached this ion intensity threshold, will be subjected to MS/MS experiments. It can be concluded that there is no need for any previous knowledge of the m/z values of the precursors (Ma & Chowdhury, 2013, p. 1285). However, the main drawback of this tool is the sensitivity of the analytes. It would be very complicated to obtain MS/MS spectra of low-level metabolites using intensity-dependent acquisition because it will mainly select high-intensity endogenous ions in biological matrices for MS/MS and MS^n acquisition (Ladumor et al., 2016, p. 199).

**Pseudo neutral loss-dependent acquisition.** Certain compounds under the action of CID could present a specific neutral loss. Thus, the MS/MS acquisition is triggered by this fragmentation in pseudo neutral loss—dependent acquisition. DDA is based on the acquisition of two consecutive FS. The first is performed at low collision energy (CE) (i.e., 5 eV), followed by another scan with a higher CE ramping (i.e., 20–40 eV). Then, the specific m/z differences of ion pairs (neutral loss) between consecutive low and high collision energy full-scan MS are obtained. The MS/MS acquisition will be triggered when the exact neutral falls within the specified mass tolerance window (Ladumor et al., 2016, p. 199; Ma & Chowdhury, 2013, p. 1285).
**List dependent.** In this approach the user has to build an inclusion list of accurate masses of target or suspect compounds. The different compounds of this list present a very specific mass tolerance range, which has been fixed by the user. Then, the precursor ions are predicted with the help of in silico tools such as MetaSite (Molecular Discovery), MetabolitePilot (AB Sciex), and MetWorks (Thermo Electron Corporation) in FS mode at real time. Finally, the instrument will obtain the product ion (MS/MS) spectrum of the ion of interest. This approach has proven to be a powerful tool for acquiring product ion spectra for low-level metabolites in complex biological matrices, because, the number of false positives due to the matrix is reduced (Ma & Chowdhury, 2013, p. 1285). However, the development of an accurate mass inclusion for every compound could considerably reduce the laboratory-throughput.

**Isotope pattern-dependent.** Isotope pattern data—dependent MS/MS has been developed for selective data acquisition of analytes that display a distinct isotope pattern. This acquisition uses some elements such as Cl and Br. It is well known that these elements have unique isotopic patterns that can be easily recognized in their mass spectra. Thus, the software is programmed to detect any ion with unique isotopic pattern in the full MS survey scan (Ma, Wen, Ruan, & Zhu, 2008, p. 1477). Once a listed metabolite ion is found in the survey scan, MS/MS acquisition of the metabolite is automatically triggered. DDA based on isotope pattern—dependent could be extended to compounds that contain synthetically incorporated isotopes (e.g., $^{13}$C$^-$, $^2$H$^-$, $^{18}$O$^-$, $^{15}$N$^-$, etc.) or the radiolabeled compound ($^{14}$C$^-$) with a distinct $^{12}$C/$^{14}$C isotopic pattern (Ma & Chowdhury, 2013, p. 1285).

**Parallel reaction monitoring.** Parallel reaction monitoring (PRM) is the closest scan mode to working with a QqQ mass analyzer using SRM. A list of targeted precursor ions, retention times, and CEs are used, providing the most sensitive and selective quantitation results in very complex matrices. PRM is carried out by Q-Exactive instruments (Law & Lim, 2013, p. 551). In PRM mode a precursor ion list has to be developed by the user. Then a full fragment-ion spectrum of each precursor ion is recorded continuously throughout the entire LC separation. Although precursor ion spectrum is not registered in standard PRM mode, one can still use an alternative setting to acquire a full MS scan or SIM spectrum at an expense of duty cycle (termed targeted DDA or directed DDA) (Schmidt, Gehlenborg, & Bodenmiller, 2008, p. 2138) to conduct MS$^1$- and MS$^2$-based analyses. The main advantage of PRM is the use of ultrahigh-resolution Orbitrap mass analyzer that is able to separate interferences from the true signals, thus significantly enhancing the selectivity of the method compared to the conventional SRM approach (Gallien, Duriez, Demeure, & Domon, 2012, p. 148). The high resolving power of the Orbitrap mass analyzer provides a significant improvement on the selectivity of measurements. Thus, low limits of quantification and detection are generally reached in spite of the high complexity of the samples, especially for the highest values of transient time. This is explained by the theoretical increase in S/N ratio in proportion to the square root of transient time in the FT spectra (Rauniyar, 2015, p. 28,566) and by the discrimination of nearly isobaric product ions generated from coisolated precursors.
in complex samples. However, the main handicap of this acquisition mode is that the number of precursor ions is limited because this number depends on the duty cycle or transient length of the (Orbitrap) mass analyzer and the chromatographic conditions. The use of different tools including parallelization (up to 10 precursor windows at one time), time-scheduling, and relaxation on the Orbitrap resolving power could increase the number of monitored precursor ions.

### 2.5.2 DATA-INDEPENDENT ACQUISITION

It should be clear that DDA modes are highly dependent from prior information collected by the analyst (Arnhard, Gottschall, & Pitterl, 2015, p. 405), because, the precursor ions should be previously fixed and analyzed by MS/MS. DIA has now become a powerful alternative for quantification and identification proposes (Doerr, 2014, p. 35). This acquisition mode could be considered as a simple and generic mode, which is based on nonspecific CID. Therefore, the MS/MS spectra are obtained in a nonselective manner.

**Full scan.** Until relatively recently, the analysis of unknown compounds was carried out by TOF or Orbitrap mass spectrometer. FS or full-spectrum mode is the most widely used acquisition data mode in HRMS. In FS, the mass spectra are continuously acquired within a fixed period of time (mostly ≤1 s) (Niessen & Falck, 2015, p. 1). Additionally, the range of m/z studied could be selected. However, FS is not adequate for confirmation propose of unknown compounds. To overcome this problem, a very interesting tool is in-source CID fragmentation (Abranko, García-Reyes, & Molina-Díaz, 2011, p. 478), where a pseudo-MS/MS experiment takes place in an intermediate pressure section of the mass spectrometer, between the atmospheric pressure source and the high vacuum of the mass spectrometer. Ions are generated in ionization chamber, being introduced in the vacuum region. Then, ions could be accelerated under various voltage conditions, prompting collisions with surrounding species, which can produce sufficient energy to yield (diagnostic) fragment ions. Thus, it would be possible to obtain MS/MS spectra using a TOF instruments.

**PIS without precursor ion isolation.** This acquisition mode was devolved for metabolite using with Q-TOF or Orbitrap technology [MS\(^E\) (Waters), all-ion mode (Agilent), MS\(^{ALL}\) (Sciex), and all-ion fragmentation (Thermo Scientific)]. In this approach, no precursor ion isolation is undertaken. Thus, two FSs have to be applied, one at low CE and the other one at a higher CE range. The mass spectra obtained from low CE provide intact molecular ion information. On the other hand, the mass spectra from the high CE contain MS/MS information (Ma & Chowdhury, 2013, p. 1285). However, the interpretation of the obtained product ion spectra is a challenging task, because of the presence of fragment interferences from coeluting components and background matrices. So, it was necessary to obtain good chromatographic performance to obtain peaks without a fragment ions derived from the interference. In Orbitrap, AIF works in the same way of MS\(^E\). In this case, low-energy spectrum is obtained from the C-Trap and the high-energy mass spectrum is obtained from the HDC cell in a Q-Orbitrap instrument (Geiger, Cox, & Mann, 2010, p. 2252).
Nonselective MS/MS spectra. This strategy has been developed by SCIEX. It is a very useful way to obtain MS/MS spectra TripleTOF 5600 system. This DIA called sequential window acquisition of all theoretical fragment-ion spectra (SWATH) is an alternative approach that combines a high-specificity DIA method with a novel targeted data extraction strategy to mine the resulting fragment ion data sets. The SWATH strategy is based on a recurring cycle of a survey scan and a Q1 isolation strategy. At the beginning, a survey scan with low CE covers the user-defined mass range (Q1 set to full transmission). The mass range then is consecutively scanned using predefined Q1 narrow ion windows (20 Da), applying a range of CEs to produce product ion spectra, which are analyzed by the TOF analyzer (Roemmelt, Steuer, Poetzsch, & Kraemer, 2014, p. 11,742). In a wide mass range the Q1 window could be stepped, obtaining FS composite MS/MS spectra at each step, with an LC compatible cycle time. To collect this large amount of data, it was necessary that instrument has a high scan speed (100 Hz). The main advantage of SWATH is that the user need not select a list of specific target compounds, because this step will be carried in a postacquisition step (Ladumor et al., 2016, p. 199).

2.5.3 POSTACQUISITION APPROACHES
There are several postacquisition algorithms to ease the interpretation of mass spectra data. Among them, mass defect filter (MDF) has been developed to improve the detection of common and uncommon compounds. MDF mode could filter out most of interferences in a complex matrix. To erase this undesirable information, the software establishes a mass defect range of the common compounds studied. The interference ions, for which m/z is outside of this mass range, will not be considered. It should be noted that the use of HRMS is mandatory because the m/z difference between interference and analytes could be quite small. This approach has been successfully applied to resolve metabolite ions and isobaric interferences when they were separated by greater than ~50 mDa in a typical mass range of 200–1000 Da (Ladumor et al., 2016, p. 199; Zhang, Zhang, Rayb, & Zhu, 2009, p. 999). MDF has been used as a postacquisition data processing method. In fact, most of commercial brands have available software with this tools. On the other hand, this approach has been applied for real-time acquisition using a Q-TOF (TripleTOF) instrument (Bloomfield & Le Blanc, 2009).

2.6 DATABASES AND THE INTERNET RESOURCES FOR HIGH-RESOLUTION MASS SPECTROMETRY
Up to date, the analysis of untargeted compounds has been carried out in multiple steps (Tautenhahn et al., 2012, p. 826). First, mass spectrometry data for each of the samples are acquired. Then, the different unknown peaks have to be selected by the differences found between a group of samples. These compounds are identified manually in a database by m/z ratios of the peaks of interest. Finally, to obtain an unequivocal identification, tandem mass spectrometry (MS/MS) data
from the sample could be compared with MS/MS data obtained from bibliography or a commercially available standard. Thus, untargeted workflow could be quite daunting. The use of free-access mass spectrometry database is an alternative to facilitate identification of compounds in the untargeted workflow. There are more and more complementary mass spectrometry data compilations (Oberacher, 2013, p. 312). In this section an overview of the most relevant database is shown.

**MassBank.** MassBank was the first mass spectra database of small chemical compounds for life sciences (<3000 Da) (MassBank, 2016). This repository was designed to share the obtained mass spectral data among the scientific community (Horai et al., 2010, p. 703). Thus, MassBank data could be useful for the chemical identification and structure elucidation of chemical compounds detected by mass spectrometry. Among other features, MassBank supplies (1) tandem mass spectra acquired on broad range of mass spectrometers as well as different ion sources; (2) a friendly interface, which could be used to find mass spectrum by chemical name or molecular formula; and (3) an easy way to search similar spectra on a neutral loss-to-neutral loss basis.

**ChemSpider.** ChemSpider is a free online service that delivers information of more than 57 million structures from hundreds of data sources (Chemspider, 2016). These sources include data from government databases, commercial chemical supplier catalogs, academic and commercial website. Thus, it could be considered as the richest single source of structure-based chemistry information. For identification purposes, ChemSpider is useful to obtain MS and MS/MS spectra from known compounds. The spectra can only be found by systematic name, trade name, SMILES, InChI, or CSID. However, it is not possible to obtain information of compounds, which have not been partially identified before.

**METLIN Metabolomics Database.** This web-based database has been developed by the Scripps Research Institute (California, USA) (METLIN, 2016). This database is designed to provide, among others, structural and physical data on known endogenous metabolites, drugs, and pesticides and their metabolites and high-accuracy mass spectrum data from known compounds and their derivatives (Smith et al., 2005, p. 747). In addition, a wide MS/MS spectra collection has been developed. This information has been obtained on an Agilent 6510 Q-TOF instrument by collecting compound-specific reference spectra at three different CEs. The mass spectrometer is operated in both ESI positive and negative modes. Currently, there are more than 700,000 high-resolution MS/MS spectra available. It should be noted that this number is continuously expanding.

As an example, an unknown compound search is shown in Fig. 2.15. In METLIN screen data search the experimental m/z has to be selected. Additionally, the user could indicate the mass error or ionization mode, or delimit the search between either the molecular ions or several adduct ions (Fig. 2.15A). Then, several compounds were displayed according to the mass error (Fig. 2.15B). Finally, the user can select the MS spectra or MS/MS spectra at four different CEs. So, the tandem MS data that METLIN provides are therefore particularly valuable to researchers who are in the early stages of metabolite identification.
FIGURE 2.15
Basic search for an unknown compound using METLIN database: (A) m/z settings; (B) list of candidates; (C) MS and MS/MS spectrum with different collision energies.
A new database namely isoMETLIN has been developed through METLIN (Cho et al., 2014, p. 9358; IsoMETLIN, 2016). This database could facilitate the identification of metabolites that have been isotopically labeled. Thus, in the field of pesticide analysis it could be a powerful tool, as the number of isotope labeled species is gradually increasing. This database allows to search all computed isotopologues derived from METLIN on the basis of \( m/z \) values and specified isotopes of interest, such as \(^{13}\text{C}\) or \(^{15}\text{N}\). In addition, isoMETLIN provides experimental MS/MS data on hundreds of isotopomers.

**The Spectral Data Base for Organic Compounds.** The Spectral Data Base for Organic Compounds (SDBS) (2016) is a free online site developed by National Institute of Advanced Industrial Science and Technology in Japan. SBDS contains six different types of organic compounds including an electron impact mass spectrum (EI-MS), a Fourier transform infrared spectrum (FT-IR), a \(^1\text{H}\) nuclear magnetic resonance (NMR) spectrum, a \(^{12}\text{C}\) NMR spectrum, a laser Raman spectrum, and an electron spin resonance (ESR) spectrum. This database has more than 25,000 MS spectra, which were obtained by the electron impact method.

**Human Metabolome Database.** The Human Metabolome Database (HMDB) is an online database of small molecule metabolites found in the human body. This freely available electronic database contains detailed information about 42,000 small molecule metabolites. The project has been developed by the Canadian Institutes of Health Research, Alberta Innovates—Health Solutions, and by The Metabolomics Innovation Centre (Human Metabolome Database, 2016). This database was designed to give helpful information about (1) chemical data, (2) clinical data, and (3) molecular biology/biochemistry data. In the field of mass spectrometry, to identify or characterize human metabolites, HMDB allows to query NMR spectroscopy, GC MS spectrometry and LC/MS spectrometry data. In fact, HMDB includes nearly 10,000 NMR, GC MS, and LC/MS spectra. Additionally, there are other databases related to HMDB including Urine Metabolome, DrugBank, Toxin and Toxin Target Database (T3DB), Small Molecule Pathway Database (SMPDB), and Food Database (FooDB). The HMDB provides information about 3100 small molecule metabolites found in human urine (Urine Metabolome Database, 2016). Up to date, the DrugBank database (Drugbank, 2016) helps to get information about more than 8200 drug and drug metabolites. T3DB (Toxin and Toxin Target Database, 2016) contains information on 3673 toxins such as pollutants, pesticides, drugs, and food toxins. SMPDB (Small Molecule Pathway Database, 2016) has more than 618 pathway diagrams. FooDB is the most recent database in HMDB site (Food Database, 2016). This database contains information on 28,000 food components and food additives. Among others, FooDB allows to obtain data on (1) MS and MS/MS spectra, (2) nomenclature, and (3) concentrations in various foods.

**Fiehn Library.** This database has been developed by West Coast Metabolomics Center (California, USA) (Kind et al., 2009, p. 10,038). The library has been developed using several mass spectrometers including TOF or single quadrupole using electron impact ionization. Currently, this database contains over 1000
identified metabolites. MS spectra along with retention time allow to obtain a comprehensive metabolic profiling.

**mz Cloud.** mz cloud is a free database, which contains information on 6000 compounds and more than 1,518,041 high-resolution MS/MS spectra (m/z Cloud Database, 2016). This spectra information is shown to the user with spectral trees. Thus, the users could find the information in a more intuitive way. Most data displayed have been collected with Thermo mass spectrometers. MS/MS and multistage MSn spectra were acquired at different CEs, precursor m/z, and isolation widths using CID and HCD. mz Cloud provides information about (1) the chemical structure, (2) computationally and manually annotated fragments (peaks), (3) identified adducts and multiply charged ions, and (4) molecular formulas, predicted precursor structure. Additionally, mz cloud will allow registered users to create individual, public, or private spectral libraries increasing the laboratory workflow.

A summary of mz cloud interface work is illustrated in Fig. 2.16, on the search of MS-spectra information of dopamine. First of all, the user should indicate the compound name (Fig. 2.16A). In this section, additional complementary information for instance related to the mass spectrometer and ionization mode used is available. Then, the user can select the different MSn spectra available in the spectral tree (Fig. 2.16B). Finally, parameter such as CID or HCD applied in the selected MSn spectrum could be checked.

The following databases are not readily available. However, because of their wide use and acceptance by scientific community it is worthwhile mentioning them. These databases are the Wiley Registry MS/MS and the NIST MS/MS database.

**The Wiley Registry MS/MS.** This MS/MS spectral library includes nearly 775,500 MS/MS spectra and 741,000 chemical structures. Thus, sensitive, specific, and robust identification of small molecules such as illicit drugs, pharmaceutical compounds, pesticides, and other small bioorganic molecules could be carried out (Oberacher, 2013, p. 312). These MS/MS spectra were obtained with different high-resolution mass spectrometers (Q-TOF) employing positive or negative ESI at least at 10 different CEs (Wiley, 2016). Its full potential has been checked in several cross-validation studies using sample spectra extracted from tandem mass spectral libraries from literature and acquired on various types of tandem mass spectrometers (Oberacher, Whitley, & Berger, 2013, p. 487).

**The NIST MS/MS database.** This database was developed by National Institute of Standards and Technology (NIST, Maryland, USA). NIST MS/MS database, which is widely used for scientific community, is supplied by John Wiley and Sons (NIST Standard Reference Database, 2016). The aim of this library is to provide reference mass spectral data for the identification of compounds through the fragmentation of their ions generated by ESI. MS spectra were obtained using several types of mass spectrometers, including low- and high-resolution mass spectrometers, including ion trap, QqQ, and Q-TOF. ESI electronic impact ionization has been employed. NIST MS/MS database currently provides more than 193,119 and 41,165 MS/MS spectra of small molecules and biologically active peptides, respectively (NIST Standard Reference Database, 2016).
FIGURE 2.16
Basic search for a suspect compound using m/z cloud database: (A) selection of the suspect compound; (B) MS^n spectra available in the spectral tree; (C) MS or MS/MS spectrum selected.
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