Mass Analyzers and Mass Spectrometers 7

Anthony M. Haag

Abstract

Mass spectrometers are comprised of three main components: an ion source, a mass analyzer, and a detector. Ionization of the analyte occurs in the ion source and the resulting ions are counted at the detector. However, it is the mass analyzer that is responsible for determing the mass-to-charge ratio (*m*/*z*) of the ions (Jennings KR, Dolnikowski GG, Method Enzymol 193:37–61, 1990). Therefore, it is primarily the analyzer that allows the mass spectrometer to serve its primary goal – determining the mass of the analytes being measured. This becomes important in the field of molecular biology, where biomolecules may be of low molecular weight or often take on multiple charges (z) after ionization (Fenn JB, Mann M, Meng CK, Wong SF, Whitehouse CM, Science 246:64–71, 1989). For this reason, the choice of analyzer is dependant on the properties of the analyte after ionization and the requirements of the experiment being performed.

Keywords

Mass spectrometer • Mass analyzer • Quadrupole mass analyzer • Ion trap mass analyzer • Time-of-flight (TOF) mass analyzer • FT-ICR mass analyzer • Orbitrap mass analyzer • Tandem mass analyzer • Triple quadrupoles tandem mass analyzer • Q-TOF tandem mass analyzer • TOF/TOF tandem mass analyzer • Product ion scan • Precursor ion scan • Neutral loss scan • Selected reaction monitoring

7.1 Introduction

Mass spectrometers are comprised of three main components: an ion source, a mass analyzer, and a detector. Ionization of the analyte occurs in the ion source. The mass analyzer then resolves ions

A.M. Haag (✉)
Department of Pathology and Immunology, Baylor College of Medicine, Houston, TX 77030, USA

Texas Children's Microbiome Center, Texas Children's Hospital, Houston, TX 77030, USA
e-mail: Anthony.Haag@bcm.edu

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based on their mass-to-charge ratio (m/z) [1]. Ions most often impact a detector to produce a signal that is recorded. A mass spectrum is a plot of the relative abundance of ions against their m/z. It is primarily the analyzer that allows the mass spectrometer to serve its primary goal – determining the mass of the analyte being measured. Because analyzers only measure the m/z of ions, some amount of mass spectral interpretation is often required by the mass spectrometrist. This becomes important in the field of molecular biology, where biomolecules often take on multiple charges (z) after ionization [2]. For this reason, the m/z of a compound will often be a fraction of the actual mass (m) of the ion.

There are many different analyzer designs available. Along with their ability to resolve ions of different m/z, several analyzers are also capable of trapping and storing ions. Thus, such analyzers can function in a multitude of roles. The most common types of analyzers in commercial production include quadrupole, ion trap, time-of-flight (TOF), and Fourier transform analyzers (ion cyclotron [ICR] and Orbitrap), along with numerous combinations or hybrids of these analyzers. The choice of mass analyzer depends on a number of factors and experimental considerations. Such factors may include but are not limited to

1. The desired m/z range to be analyzed
2. The mass of the analyte
3. The required resolving power of the analyzer
4. The ability of the analyzer to interface with the ion source of the mass spectrometer
5. The limit of detection required

Because there is no single mass analyzer that is suitable for all applications, most laboratories will employ different mass spectrometers that utilize different analyzers. The most commonly utilized mass analyzers are discussed below.

7.2 Quadrupole

The quadrupole mass analyzer continues to be one of the most popular types of mass analyzer in use. Quadrupole mass analyzers are often employed in benchtop mass spectrometers due to their low cost, compact design, durability and reliability. For these reasons they have become the workhorse analyzer in the pharmaceutical industry. They are often used in tandem with each other such as in triple quadrupole mass spectrometers or with other mass analyzers such as time-of flight (TOF) [3].

A quadrupole analyzer is essentially a mass filter, due to its ability to discriminate and filter ions of different m/z [4]. Quadrupoles consist of four cylindrical or hyperbolic rods in parallel with each other (Fig. 7.1). Rods opposite each other are electrically connected together and a radio frequency (RF) potential is applied. A direct current (DC) potential is then superimposed over of the RF potential. The combination of RF and DC potential causes ions to oscillate as they pass through the quadrupole in the z-direction. Depending on the DC potential and frequency of the RF field, only ions of a particular m/z will have stable trajectories. Those ions that have unstable trajectories will collide into the rods and be filtered out. By varying the DC and RF potentials, ions of different m/z can be scanned or "filtered" through the quadrupoles [5].

A quadrupole or other multipole (hexapole or octupole) can also operate in an "RF-only" mode, in which the DC potential is reduced and only an RF potential is applied to the rods. This allows all ions to pass through the multipole, thereby transforming the quadrupole analyzer into a device for transmitting ions from one

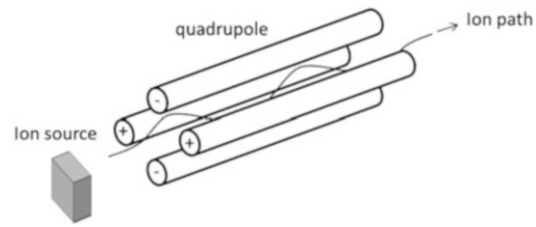

Fig. 7.1 A quadrupole mass analyzer consists of four metallic rods connected to both an RF and DC field. Ions entering the quadrupole will oscillate as they pass through the field between the quadrupole rods. Ions with stable oscillation trajectories will pass through while those that are unstable will collide with the rods

area of the mass spectrometer to another, such as moving ions from the ionization source into another analyzer. Thus, RF-only multipoles can act as ion transmission guides within a mass spectrometer where needed. RF-only multipoles can also act as collision cells for performing collision-induced dissociation (CID) [6]. When an inert gas is introduced into the collision cell and the RF-energy on the multipoles is increased, ions that are transmitted through the collision cell will undergo fragmentation via CID. By varying the RF-energy, the amount of ion fragmentation can be controlled.

As mentioned earlier, a major advantage of quadrupole mass spectrometers is their low cost and compact shape and size which makes them ideal for most laboratories. They are made by a variety of different manufacturers and have proven to be rugged and reliable for long periods of time, thus require little maintenance. They have excellent stability over long periods of time, thereby reducing the need for repeated calibrations. Because quadrupole analyzers have fast duty cycles and the need for a continuous flux of ions, they easily interface to both gas chromatography (GC) and liquid chromatography (LC) equipment [7, 8]. However, this makes quadrupole analyzers less suitable for pulsed ion sources such as matrix assisted laser desorption/ionization (MALDI). Also, quadrupole analyzers suffer from both limited mass ranges and poor resolution. This puts them at a disadvantage when analyzing large molecular weight compounds that may not form multiply charged ions or complex mixtures of compounds with similar masses.

7.3 Ion Trap

The ion trap mass analyzer is a modification of the quadrupole mass analyzer [9]. The 3D ion trap, also known as a Paul Trap, was the most common ion trap until the twenty-first century [10, 11]. Recently, the 2D linear ion trap has become more popular because of its numerous advantages over 3D traps in most commercially available equipment. The 3D traps consist of two hyperbolic electrode plates facing each other and a hyperbolic ring electrode placed in between them (Fig. 7.2). Using an oscillating RF field and a superimposed DC electric field, similar to that in quadrupoles, ions are trapped between the electrodes. In order to act as an analyzer, ions of different m/z are selectively ejected from the trap by varying the RF potential. The ejected ions are then registered at the detector. 2D traps, often referred to as linear traps, are equivalent to quadrupoles but a potential field is applied to each end of the quadrupole in order to trap the ions within the quadrupole itself. Ions can be selectively ejected either axially or radially depending on the design of the 2D trap.

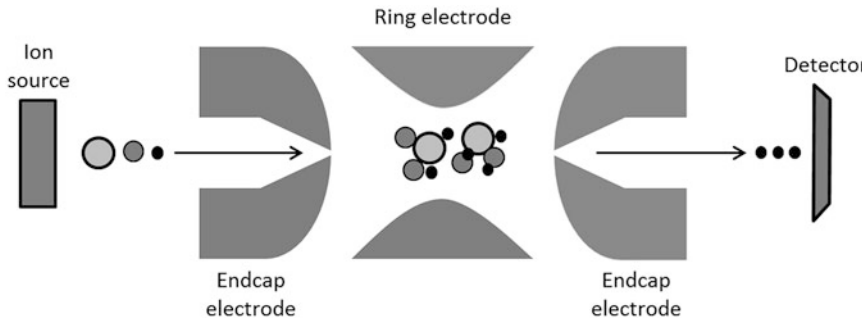

Fig. 7.2 In a 3D trap, ions enter a small opening in the endcap of one of the electrodes. An RF field is placed on the ring electrode, trapping the ions toward the center of the ion trap. The stability of ions within the trap is based on the RF frequency, the m/z of the ions, and the amplitude of the RF field. Ions may be selectively ejected at the opposite endcap electrode from which they entered by increasing the voltage of the RF field on the ring electrode

Because ion traps have the ability to accumulate ions over time, mass spectrometers that utilize them are known for their improved sensitivity. Much like their quadrupole counterpart, ion trap analyzers have the advantage of having a small and compact size, making them very affordable in most mass spectrometers. For this reason, they have played a major role in expanding the field of proteomics. Much of the early developments in identification of proteins in a complex mixture were performed on mass spectrometers utilizing ion trap analyzers [12, 13].

One of the biggest disadvantages to ion trap analyzers is their low resolving power. Even at slow scan speeds, ion trap analyzers (particularly 3D models) have only single unit mass resolution. With the advancement of other types of analyzers that have faster speed, better mass accuracy and superior resolution, there has been a shift away from performing proteomic analysis on mass spectrometers using only ion trap analyzers. However, due to their geometry, 2D analyzers still find widespread use in hybrid instruments, particularly those that utilize them as a precursor mass filter when performing tandem MS.

7.4 Time-of-Flight

Although Time-of-flight (TOF) analyzers have been around for some time, it has been the advent of MALDI ionization (which allowed for the easy analysis of large biomolecules) that propelled TOF analyzers to the forefront [14, 15]. TOF analyzers are the easiest to conceptualize as illustrated in (Fig. 7.3). In its simplest form, a TOF analyzer consists primarily of a flight tube and an acceleration grid that acts to accelerate a "packet" of ions from the ionization source to the MS detector [16]. Essentially, if two ions of different m/z are accelerated from the ion source with the same kinetic energy and allowed to drift through a field free region of the flight tube, then their arrival times at the detector will be different.

The equation for kinetic energy for any mass is

$$E_k = \frac{1}{2}mv^2$$

wherein E_k is the kinetic energy of the ion after acceleration, m is the mass of the ion and v is its velocity. Ion velocity remains constant after acceleration as it moves through the field free

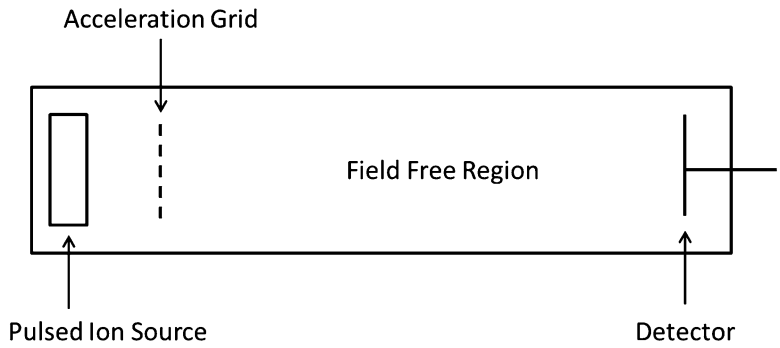

Fig. 7.3 Ions generated by the ion source are accelerated by placing a pulsed electric potential on the acceleration grid. The accelerated ions then drift through a field free region of a flight tube where they are separated based on their m/z. The greater the ion mass, the slower the drift through the flight tube. When an ion hits the detector, the mass spectrometer determines the time it took for the ion to drift through the flight tube. The drift time through the flight tube is proportional to the m/z of the ion

region of the flight tube. Because this velocity remains static, then velocity is given by

$$v = \frac{d}{t}$$

wherein d is the distance the ion travels and t is the time it takes for the ion to travel from its acceleration point to the detector. Substituting v into the kinetic energy equation results in

$$E_k = \frac{1}{2}m(d/t)^2$$

Solving for the mass of the ion yields

$$m = \frac{2E_k t^2}{d^2}$$

Because the initial kinetic energy (E_k) of the ions and the length of the flight tube (d) remain constant, mass is strictly a function of the time it takes for the ions to be detected after initial acceleration (time-of-flight).

TOF analyzers today also employ the use of a reflectron which reflects the ion path back in the direction of the ion source before being detected [17]. This allows for corrections in the small differences in initial kinetic energies of the ions that may occur during acceleration [18]. Other methods such as delayed extraction are also employed in order to increase resolution [19, 20]. After ions are formed, delayed extraction introduces a small delay (usually on the order of a few hundred nanoseconds) in the electric pulse of the acceleration grid before the ions are accelerated. This small delay allows the ions formed after ionization to equilibrate and have a more uniform average momentum before acceleration.

Due to the high ion transmission efficiencies of TOF analyzers, they can achieve the widest mass range of all mass analyzers. TOF analyzers allow for the separation of ions with masses of only a few Daltons to well over 100 kDa [21]. This makes them the analyzer of choice for observing singly charged high mass biomolecules such as proteins [22]. Because of their ability to simultaneously measure the masses of many peptides, TOF analyzers have been the most popular analyzer for performing peptide mass fingerprinting [23, 24]. Although new tandem analyzer configurations have allowed TOF analyzers to be interfaced ion sources that provide a continuous flux of ions, they have initially been employed with only pulsed ion sources such as MALDI.

7.5 FT-ICR

FT-ICR analyzers determine m/z by measuring the cyclotron frequency of ions in a fixed magnetic field [25]. Ions are first introduced into a Penning trap, a device similar to a 3D ion trap but using a magnetic field to trap ions rather than an electric field. The ions are injected into the magnetic field from the source as a "packet". The ions then experience a Lorentz force, which causes them to assume a circular motion in a plane perpendicular to the magnetic field (Fig. 7.4). The angular frequency, also known as the cyclotron frequency, is described by the equation

$$\omega_c = \frac{qB}{m}$$

where ω_c is the angular frequency of the ions in radians, m is the mass of the ion, q its charge and B is the strength of the magnetic field. However, because the ions are not in phase when initially introduced into the trap and typically have very small orbits, it is impossible to detect them. In order to detect these ions, they must be coherently excited to a larger radius within their plane of motion. This is accomplished by exciting the ions with a limited frequency sweep of a broadband RF field [26]. This excitation coherently places the ions in a higher cyclotron orbit, which allows them to be detected. As the ions are detected over time by the receiver plates, their signal intensity is digitized with respect to time and converted to a frequency spectrum via a Fourier transform. The cyclotron frequencies of the ions are proportional to their m/z.

One of the biggest advantages of FT-ICR analyzers are their very high mass accuracy and

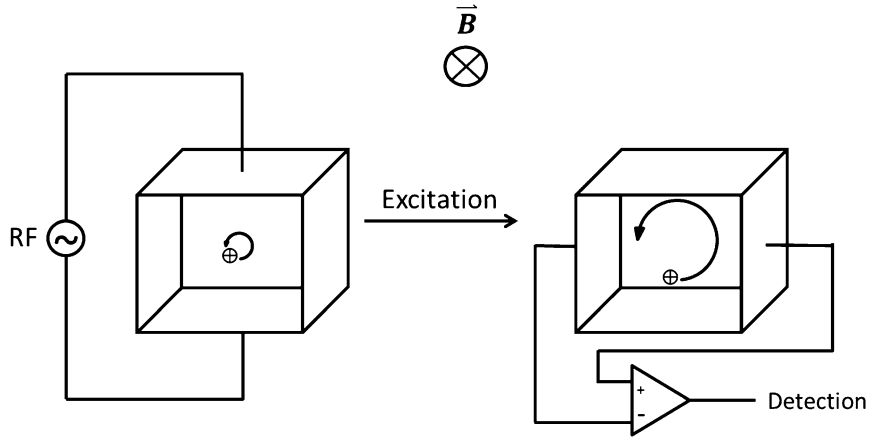

Fig. 7.4 Ions are injected into a magnetic field for which they then undergo a small cyclotron frequency perpendicular to the magnetic field. A brief broadband RF pulse excites the ions into a larger and coherent cyclotron orbit. The circular motion of the ions in the magnetic field is detected by the receiver plates and a Fourier transform converts the signal to a frequency spectrum. The angular frequency of the ions is determined by their m/z

resolving power. One million resolution has been reported on instruments with magnetic field strengths as low as 1 T [27]. All aspects of FT-ICR improve with higher magnetic field: increased resolution, increased mass accuracy, increased number of ions that can be put in the cell, decreased ion coalescence, etc. Most commercially available FT-ICR analyzers operate in magnetic field ranges between 7 and 12 T. This high resolution and mass accuracy is very useful when determining the elemental composition of small molecules based on their "mass defect" [28]. For example, two compounds, one with the empirical formula C_6H_{12} and the other with the empirical formula C_5NH_{10}, both appear to have the same mass of 84 Da. However, when calculating their mass with very high precision, C_6H_{12} has an exact mass of 84.09389 Da and $C_5H_{10}N$ has an exact mass of 84.08131 Da, a difference of 0.01258 Da. This is due to slight differences in the binding energies in the nuclei of the carbon and nitrogen atoms, thus causing a slight shift in their atomic mass. Unlike many other analyzers with lower resolving power, FT-ICR analyzers have the ability to obtain empirical formulas directly from mass data.

Another major application for FT-ICR is in the field of proteomics where high mass accuracy is often required. In order to maintain isotopic resolution of large molecular weight ions with multiple charge states, very high resolution must be employed. For example, a 50 KDa protein, regardless of charge state, would require a resolution of 50,000 of the analyzer in order to observe isotopic peaks. In order to perform top-down sequencing of proteins, it is preferable to have isotopic resolution of the protein and its MS/MS products. Because of the resolving power of FT-ICR, entire large proteins can be sequenced and identified when performing tandem MS. Post-translational modifications within an isolated protein can also be identified without having to first perform chemical or enzymatic cleavage of the isolated protein as required in bottom-up approaches [29].

Due to the need for very strong magnetic fields, FT-ICR analyzers require the use of large superconducting magnets. This introduces two major problems. First, large magnet sizes require large amounts of lab space to be available. This may also include the need for high laboratory ceilings in order to perform maintenance. Second, superconducting magnets require liquid helium as a coolant in order for them to operate. The cost of liquid helium is high and often beyond the budget of many small laboratories. The initial cost of most FT-ICR instruments is also very high.

Mass spectrometers that utilize FT-ICR also suffer from slow scan speeds compared to other analyzers such as time-of-flight. This makes it impractical for many LC-tandem MS experiments, such as Multi-dimensional Protein Identification Technology (MudPIT), where many different co-eluting peptides need to be analyzed at very high scan rates in order to collect as much tandem MS data as possible.

7.6 Orbitrap

Similar to the FT-ICR analyzer, the orbitrap is also a type of analyzer that makes use of a Fourier transform to convert a signal, produced by ions oscillating in a trap, from the time domain to a frequency domain [30, 31]. Unlike FT-ICR analyzers, which use a magnetic field to induce oscillation in the ions, orbitrap analyzers use an electric field to induce these oscillations [32].

The orbitrap mass analyzer is composed of three main parts, an inner spindle electrode covered by two hollow outer concave electrodes facing each other. The two outer electrodes are separated by a thin ring of dielectric material (Fig. 7.5). A voltage potential is applied between the inner and outer electrodes, creating a linear electric field between them. Ions are introduced tangentially into the orbitrap as a "packet" between the inner and outer electrodes through a hole machined into one of the outer electrodes. Due to the electric field between the inner and outer electrodes, the ion packet is bent towards the inner electrode while the tangential velocity of the ions creates an opposing centrifugal force. At a specific potential between the inner and outer electrodes, the ions remain in a spiral path around the inner electrode. However, due to the conical shape of the electrodes, a harmonic axial oscillation in the ions is induced. The outer electrodes also act as receiver plates that detect the back and forth axial harmonic motion of the ions. This signal image is digitized and transformed from the time domain to the frequency domain. Similar to FT-ICR, the axial harmonic frequencies are proportional to the m/z of the ions.

One of the major advantages of the orbitrap analyzer is its high resolving power, resulting in its use as a replacement for FT-ICR analyzers for many applications, particularly those involving proteomics. In general, FT-ICR analyzers are superior to orbitraps in the low molecular weight range, thus making them ideal for low mass

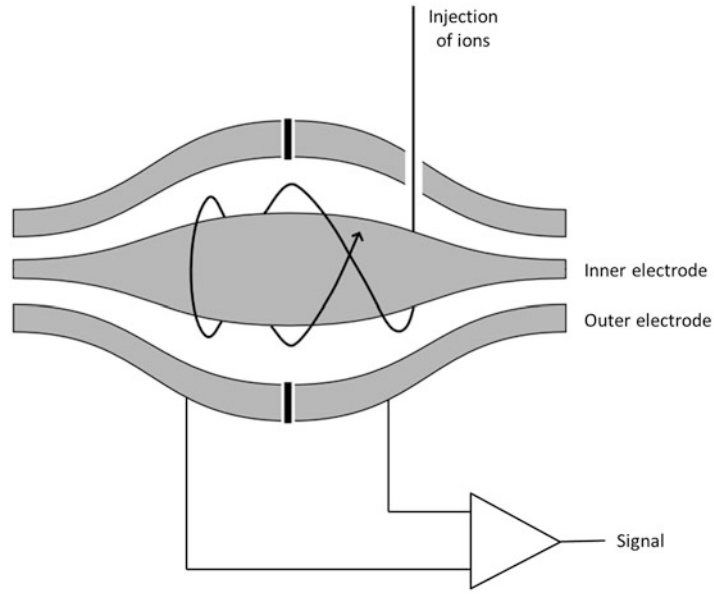

Fig. 7.5 In an orbitrap analyzer, ions enter through an opening in one of the outer electrodes. The entry of the ions is tangential to the inner electrode. At a particular potential between the inner and outer electrodes, the ions will continuously spin around the inner electrode. Ions will oscillate back and forth along the axis of the inner electrode. This oscillation is detected and transformed via a Fourier transform to obtain a mass spectrum

compounds. However, there is a fast decrease in the resolving power of FT-ICR analyzers at higher *m*/*z*. This decrease in resolving power of FT-ICR analyzers is inversely proportional with an increase in the *m*/*z* being measured. With orbitrap analyzers, this decrease in resolving power is inversely proportional to the square-root of the *m*/*z* being measured. Therefore, orbitrap analyzers often have better resolving power than FT-ICR analyzers at higher *m*/*z* [33]. This property can give the orbitrap an advantage when analyzing high molecular weight compounds such as proteins. Recently, there has been a move from bottom-up proteomic analysis to top-down analysis. Because top-down analysis requires very high resolving power, it was limited to FT-ICR analyzers and beyond the affordability of most MS labs. Orbitrap analyzers have been instrumental in overcoming this difficulty and have therefore pushed the advancement of top-down proteomics.

There are also a number of other advantages to orbitrap analyzers. Unlike the large size and operating costs of instruments utilizing FT-ICR, orbitrap instruments are much smaller and require very little maintenance. Orbitrap analyzers also do not use magnetic fields to operate, and therefore cryogenic refrigerants such as liquid helium are not necessary and operating costs are kept low. Although counterintuitive, the resolving power of the orbitrap analyzer is increased by the decrease in size of the analyzer. The main limitation to improved orbitrap design has been the tolerances needed in the machining process during manufacture. As machining processes improve, smaller orbitrap analyzers will no doubt continue to decrease the overall size of mass spectrometers that utilize them. Their smaller design will also allow for faster acquisition rates and higher resolution.

Improvements in orbirap analyzer design will continue to provide faster scan speeds and duty cycles. However, even with major improvements expected in the future, they will continue to be slower than that of TOF analyzers. This makes orbitrap analyzers potentially less ideal for performing MudPIT experiments where fast acquisition rates may outweigh the need for very high mass resolution or accuracy. Orbitrap analyzers are also very prone to space-charge effects and therefore the amount of ions entering the analyzer must be monitored by the MS software in order to trap a limited amount of ions.

7.7 Tandem Mass Analyzers

Mass spectrometers that utilize two or more mass analyzers consecutively are known as tandem mass spectrometers [34, 35]. Tandem MS analysis is the process by which the first analyzer is used to select ions of a particular *m*/*z* value, subject those ions to CID (as described in RF-only multipoles), and then analyze the resulting product ions using a second mass analyzer. CID is also sometimes referred to as collision-activated dissociation (CAD) and is a process by which ions are fragmented by colliding them with chemically inert gas (typically argon or nitrogen) at low pressure (~10^{-5} torr). The fragmentation occurs due to converting some of the kinetic energy from the collision of the analyte ion with inert gas atoms to internal energy of the ions, thus resulting in bond breakage of the analyte ion molecules [36]. These product ions formed from CID often provide information about the structure of the analyte molecules.

7.7.1 Triple Quadrupoles

Triple quadrupole mass spectrometers are one of the most commonly sold types of mass spectrometer and are one of the best examples of using analyzers in tandem [37]. In a triple quadrupole mass spectrometer, three sets of quadrupole analyzers are used in sequence (Fig. 7.6). The first analyzer is often referred to as Q1 and can scan across a range of *m*/*z* values or selectively filter ions of a selected *m*/*z*. Those ions that pass through Q1 then enter a second set of quadrupoles that are referred to as Q2. Unlike a quadrupole that operates as an analyzer, Q2 is used exclusively as a collision cell to fragment the selected ions from Q1. The product ions

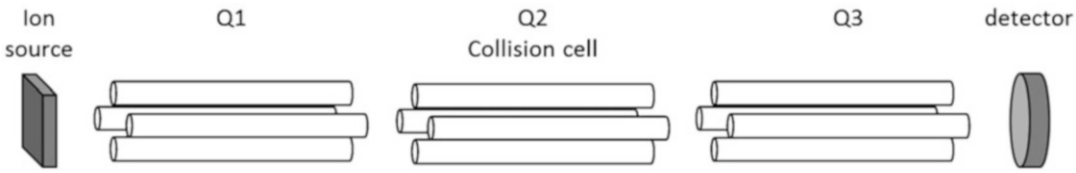

Fig. 7.6 A triple quadrupole mass spectrometer consists of three quadrupole analyzers used in tandem. Ions enter the first quadrupole, Q1. Ions that pass through Q1 enter into quadrupole Q2, where ions undergo CID. The resulting fragment ions are then filtered through the final quadrupole, Q3

Table 7.1 Table of different scan modes of triple quadrupole mass analyzers

	Q1	Q2	Q3
Product ion scan	Fixed *m/z*	CID	Scan full range
Precursor ion scan	Scan full range	CID	Fixed *m/z*
Neutral loss scan	Scan full range	CID	Scan full range Q3=Q1-Δm/z
Selected reaction monitoring	Fixed *m/z*	CID	Fixed *m/z*

formed in this process can then be either scanned through the final set of quadrupoles, Q3, to obtain a mass spectrum or Q3 can be fixed in order to monitor a particular ion. The combination of fixed or scanning modes of Q1 and Q3 determine the type of scan performed [38, 39]. The most common scan modes are described below and in Table 7.1.

Product ion scan – In a product ion scan, Q1 remains fixed such that only ions of a selected *m*/*z* are filtered through the quadrupole. These ions are then fragmented via CID through Q2. The resulting product ions are then scanned and analyzed in Q3. Once the product ions are recorded, Q1 can then fix on a new *m*/*z* and the process repeated. This technique is often used in order to determine structural information of specific analytes. For example, in a bottom-up proteomics approach, the sequence of many peptides eluting off a chromatographic column can be sequenced.

Precursor ion scan – In a precursor ion scan, Q1 is scanned across the entire *m*/*z* range of the analyzer. The precursor ions subsequently pass through Q2 for CID. However, Q3 is kept fixed such that only product ions of a specific *m*/*z* are filtered through the quadrupole. The mass chromatogram is plotted as the intensity of the ions exiting Q3 with respect to the *m*/*z* value that they originated from in Q1. In other words, precursor ion scanning allows one to determine the *m*/*z* of all precursor ions that have the same product ion. This is valuable in proteomics when one wants to identify all peptides that may have the same functional

group. For example, performing a precursor ion scan at $m/z = 216$, a signature immonium ion for phosphotyrosine, allows one to selective identify peptides that may contain phosphotyrosine.

Neutral loss scan – A neutral loss scan is a technique to track ions before and after the loss of a neutral group. Both Q1 and Q3 are scanned simultaneously over the entire m/z range but with Q3 offset from the Q1 by an amount that corresponds to the loss of a neutral fragment from the ion. Using this method, all precursors that undergo the loss of the same neutral fragment can be monitored. Similar to precursor ion scanning, this technique can be a powerful tool for quickly and selectively identify peptides that are post-translationally modified such as those that have been phosphorylated. An example is in the identification of peptides with phosphorylated serine or threonine. Performing low energy CID on peptides that are phosphorylated will often result in the loss of phosphoric acid (H_3PO_4, m/z = 98) from the parent ion.

Selected reaction monitoring – Selected Reaction monitoring (SRM), sometimes referred to as multiple reaction monitoring (MRM), is a popular scanning technique for the quantification of compounds in a mixture. Q1 is fixed to allow only precursors of a particular m/z to filter through the quadrupole. CID then occurs in Q2 and all fragments sent to Q3. Q3 is fixed to only allow product ions of specific m/z to filter through. Thus, specific signature fragment ions originating from a compound of known mass can be monitored. This technique essentially allows for a single known compound to be monitored in real time. One caveat is that although the mass and potential m/z values for Q1 can be easily determined for a compound of interest, the m/z values of the product ions of that compound must be known prior to designing the SRM experiment. This can be solved but first performing a product ion scan of the compound of interest and recording the m/z of all product ions.

7.7.2 Q-TOF

Quadrupole analyzers prefer to operate efficiently when there is a continuous stream of ions from the ion source. However, TOF analyzers prefer a pulse or packet of ions. In order for the two analyzers to work in tandem, the TOF analyzer is placed in an orthogonal configuration after the quadrupole analyzer [40]. This configuration allows ions that are filtered through the quadrupole to be injected orthogonally into the TOF analyzer as a packet using a set of pusher and puller plates between the two analyzers (Fig. 7.7) [41].

Some of the biggest advantages to Q-TOF tandem analyzers are their higher mass accuracy, higher resolution and increased scan speed as compared to triple quadrupole mass analyzers and thus their ability to easily interface to liquid chromatography and perform very fast tandem MS. This allows many spectra to be acquired when there are many co-eluting compounds in a chromatographic run. Although the resolution of the data is not of the same quality as that when analyzed by an orbitrap or FT-ICR analyzer, it is far superior to that obtained by standard quadrupole or ion traps.

7.7.3 TOF/TOF

Time-of-flight/time-of-flight (TOF/TOF) is a method by which two TOF analyzers are used in tandem and CID is performed between the two TOF analyzers (Fig. 7.8) [42]. This allows one to perform tandem MS on biological compounds such as peptide and oligonucleotides that often are ionized by ionization methods such as MALDI [43]. Because of the speed at which TOF analyzers operate, sample analysis in both the MS and MS/MS level can be performed very rapidly.

In order to perform tandem MS in a TOF/TOF analyzer, very fast electronic switching must occur in a series of steps. First, ions of different m/z are separated through the flight tube based on their velocity. Second, ions of a particular m/z are

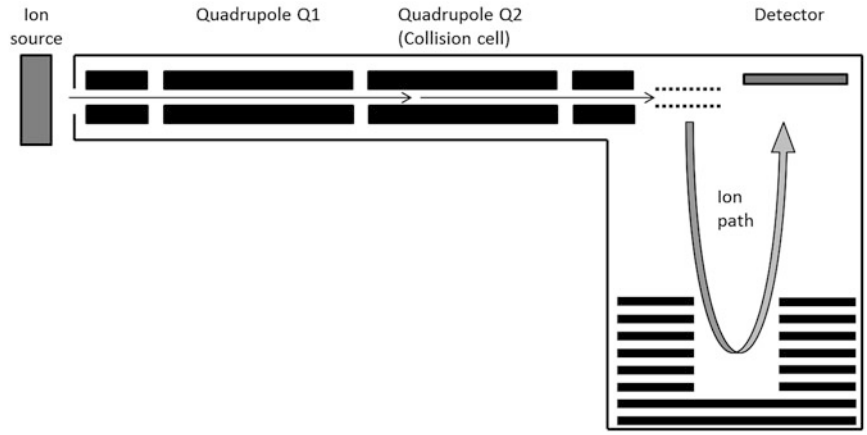

Fig. 7.7 The QTOF analyzer is a hybrid of triple quadrupole analyzer and a time-of-flight analyzer. It is analogous to a triple quadrupole system but with the exception that the last quadrupole is replaced by a time-of-flight analyzer

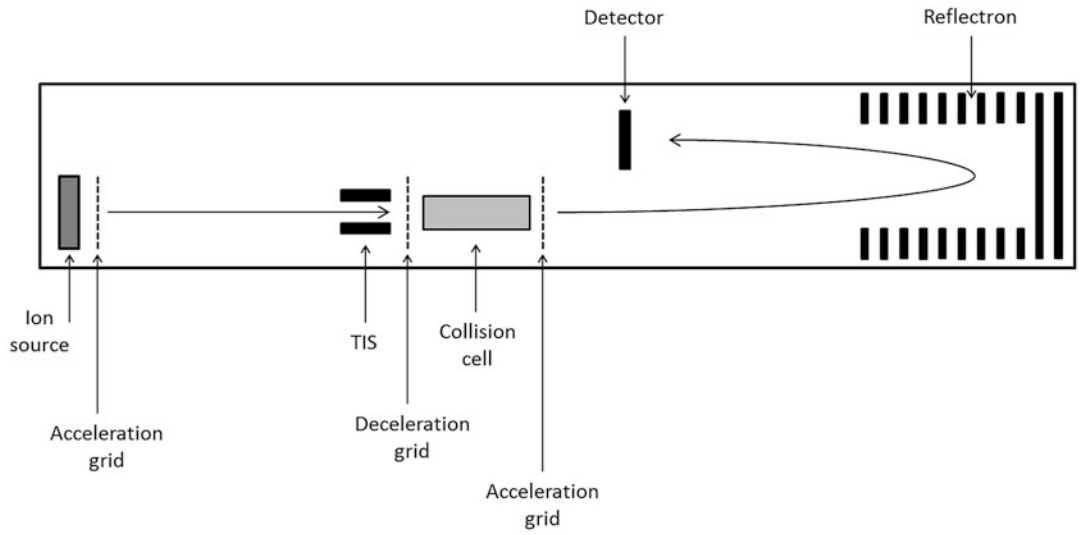

Fig. 7.8 TOF/TOF analyzers combine two TOF analyzers in tandem. Ions are accelerated in the first TOF. A timed ion selector allows ions of a particular m/z to pass. The selected ions are then decelerated before passing into a collision cell where they undergo CID. The resulting fragment ions are re-accelerated into the second TOF and their time-of-flight is measured to obtain a mass spectrum

selected while filtering out all others. This precursor ion selection is often performed using a Bradbury-Nielsen gate which is essentially a timed-ion-selector (TIS) that filters ions based on their arrival time to the gate [44]. Third, the selected precursor ions are then passed to a set of ion optics that de-accelerates them to a much slower velocity. Fourth, the ions then pass through a collision cell for CID. Finally, the product ions formed are re-accelerated into a second flight tube and analyzed. The fast analysis of this analyzer combination, combined with MALDI ion sources, make it ideal for the analysis of peptides from tryptic digests.

7.7.4 Other Tandem Analyzer Combinations

There are other combinations of mass analyzers which are far too numerous to list. In principle, the combination of mass analyzers, regardless of

their type, allow the mass spectrometrist to perform tandem MS. The choice of combination is dependent on many necessary factors such as resolution, acquisition speed (duty cycle), mass accuracy, etc. For example, if high resolution of a product ion is required but not that of its precursor, the first analyzer may be a quadrupole or ion trap and the second analyzer an orbitrap or an FT-ICR. Newer instruments have a multitude of different analyzers that may be utilized in a number of different configurations. As newer combinations of analyzers continue, the variety of tandem MS methods will also continue to grow.

7.8 Other Analyzers

Although there are a number of other types of analyzers, those that have been described herein comprise the majority of analyzers currently used in mass spectrometers. There have been many other analyzers that were once popular but have been overtaken by the current selection of analyzers for a multitude of reasons. For example, magnetic sector analyzers were one of the first analyzers used in mass spectrometry. They can have high resolution (~200,000), good stability, and significant mass accuracy, but unfortunately suffer from their large size, low resolution for precursor ion selectivity, and slow scan speeds. For these reasons magnetic sector instruments have been less ideal for interfacing to LC. Other analyzers have found a niche market for a number of reasons. The QTRAP analyzer allows a triple-quad mass spectrometer to act as a quadrupole and linear ion trap tandem mass spectrometer. Although there are a number of advantages to this type of analyzer, the demand has not propelled it to the point that it has become one of the primary analyzers used in proteomics.

There is no doubt that newer analyzers will be developed along with improvements in current ones. These advancements will continue to push the limits of current mass spectrometry. Because of the complex nature of the proteomics field, the necessity for many different avenues of approach to problem solving by mass spectrometry will undoubtedly continue to grow.

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