10 Mass Spectrometry and Hyphenated Instruments in Food Analysis

Tomas Cajka, Jana Hajslova, and Katerina Mastovska

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10.1 INTRODUCTION

Mass spectrometry (MS) has come a long way since the record of the first mass spectra of a simple low-molecular weight substance by Thomson in 1912 [1]. Especially over the past decades, MS has been the subject of many developments. Particularly, the hyphenation of MS to capillary gas chromatography (GC) and liquid chromatography (LC) and also the development of novel ionization techniques caused extensive spreading of MS to food analysis. Also, the introduction of relatively inexpensive quadrupole and ion trap mass analyzers and, at the end of the last century, the rediscovery of time-of-flight mass analyzers allowed the use of this sophisticated instrumental technique in both research as well as routine applications [2–4].

In this chapter, we would like to give the readers basic information of crucial components of various MS instrumentation, introducing also the up-to-date trends and advancements in this rapidly developing area.

10.1.1 Mass Spectrum

MS is an instrumental technique based on the separation of ions in vacuum, in the gas phase, according to their mass-to-charge ratios (m/z). The analyzed molecules have to be first ionized, forming a molecular or pseudomolecular ions, depending on the ionization technique (see below for details). If the (pseudo)molecular ion contains sufficient internal energy, it undergoes further fragmentation, which leads to formation of fragment ion(s). Generally, each fragment ion derived from the molecular ion can again undergo fragmentation. All these formed ions are separated in the mass spectrometer according to their m/z and are detected. A mass spectrum of the molecule is then produced, which represents a distribution chart of the abundances (y-axis) of ions versus their m/z values (x-axis). The peak with the highest intensity in the spectrum is called the *base peak* and the spectrum is generally normalized to the abundance (intensity) of this peak (i.e., its intensity is assigned 100%).

A mass spectrum obtained under the conditions of electron ionization (EI) can be interpreted to provide structural information as demonstrated in Figure 10.1 on an example of a pesticide dichlorvos. *Molecular ion* is undoubtedly the single most important piece of information because it determines the molecular weight (MW). Mass spectrometrists use isotopic MW, which is based on the isotopic weights of the most common (abundant) isotope of each element in the molecule. Whereas, average MW, based on the elements' average atomic weights in nature, is typically used in other fields. For instance, the isotopic MW of dichlorvos is 220 Da, whereas the average MW is 221 Da (when expressed in integer numbers). It should be noted that, for high-resolution mass spectrometric instruments, the isotopic MW is typically expressed in a decimal form (e.g., 219.9459 Da for the example of dichlorvos given previously). The molecular ion is usually the most abundant peak that appears in the heaviest mass isotope pattern in the spectrum (ion m/z 220 in Figure 10.1). In some cases, however, fragmentation can be too extensive, leaving little or no trace of a molecular ion, which makes the determination of MW difficult or even impossible. In such cases, ''soft'' ionization techniques are typically applied leading to enhanced formation of the (pseudo)molecular ion (Section 10.2.1.2).

Isotopic peaks, resulting from the natural isotope abundances of the individual elements, also provide very useful information. As an example can be used natural chlorine that consists of 75% ^{35}Cl-isotopes and 25% ^{37}Cl-isotopes and, consequently, each fragment ions containing chlorine can

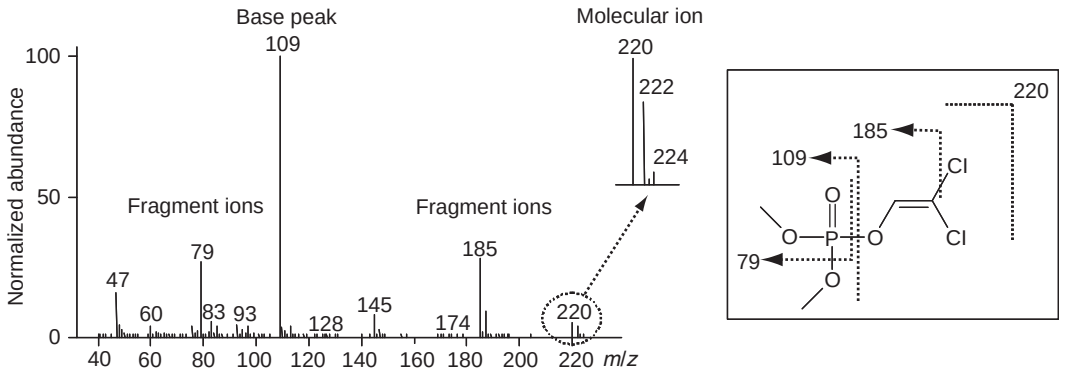

FIGURE 10.1 Mass spectrum of dichlorvos (MW = 220), obtained under the conditions of EI, and its main characteristics.

be identified by its typical chlorine isotopic pattern. In Figure 10.1, these ion patterns are represented by the ions m/z 185 and 187 of the fragment containing one chlorine atom, i.e., $[C_4H_7{}^{35}ClO_4P]^+$ and $[C_4H_7{}^{37}ClO_4P]^+$, and the ions m/z 220, 222, and 224 in the molecular ion pattern containing two chlorine atoms, i.e., $[C_4H_7{}^{35}Cl_2O_4P]^+$, $[C_4H_7{}^{35}Cl^{37}ClO_4P]^+$, and $[C_4H_7{}^{37}Cl_2O_4P]^+$. On the other hand, the base peak m/z 109, i.e., $[C_2H_6O_3P]^+$, does not contain any chlorine atom and consists only of elements that do not offer characteristic ion patterns, thus only one dominating peak is present (the other isotopic peaks have intensity <2%). The isotopic peaks can be obtained also for other elements such as bromine, sulphur, carbon, etc., therefore introduce very characteristic patterns, as shown in Figure 10.2. On the contrary, mono-isotopic elements such as fluorine, iodine, phosphorus, and also hydrogen provide only a single peak in the mass spectrum.

10.2 INSTRUMENTATION

A mass spectrometer consists of three fundamental parts: an ion source, a mass analyzer, and a detector (Figure 10.3). Initially, the analyzed sample has to be introduced into the ion source of the instrument, where the sample molecules undergo the ionization process. These ions are then extracted into the analyzer where they are separated according to their m/z. The separated ions

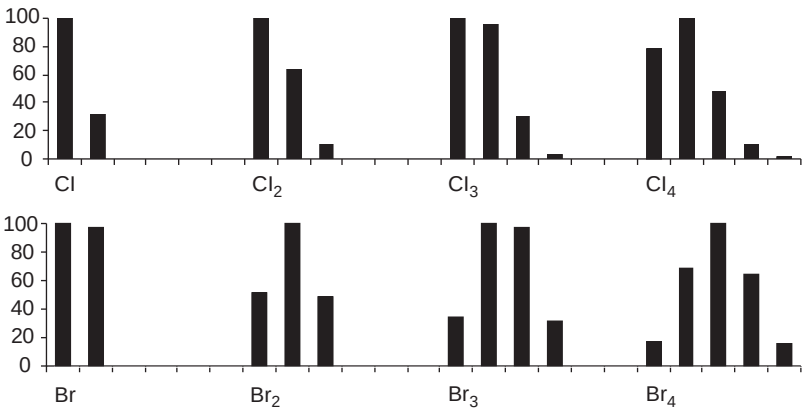

FIGURE 10.2 Isotopic patterns for 1–4 chlorine and bromine atoms in an ionized molecule/fragment.

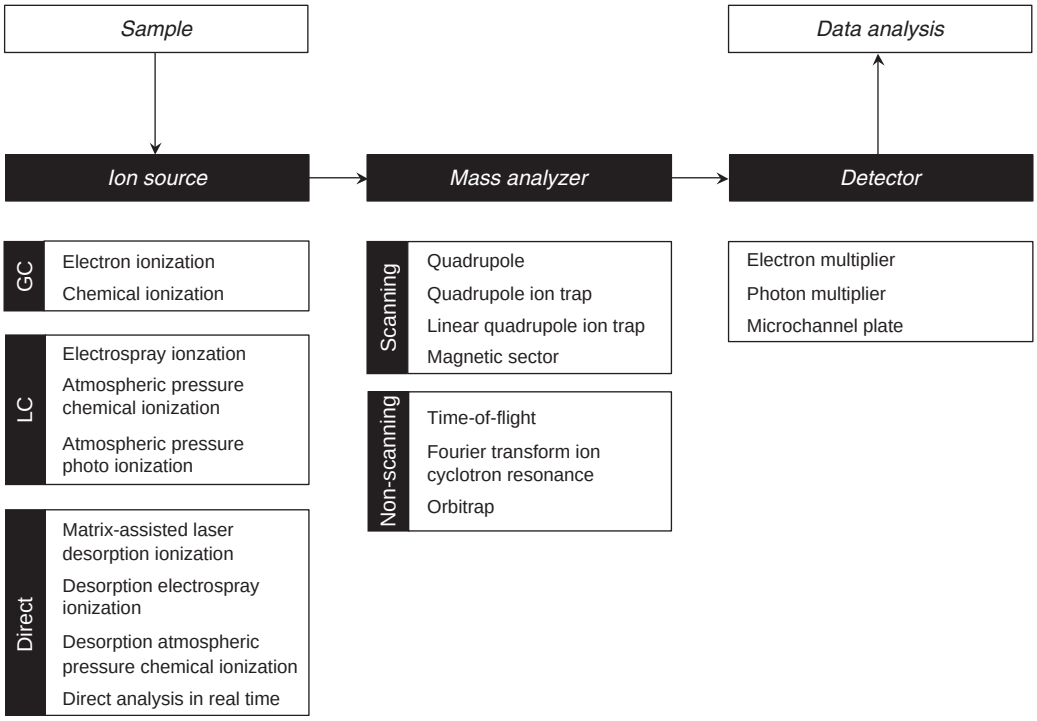

FIGURE 10.3 Overview of mass spectrometric technique setups.

are detected and the signal containing information about the m/z values is stored together with their relative abundance (intensity) as a mass spectrum.

To allow the ions traveling from one end of the instrument to the other part without any hindrance originated from air molecules, both the analyzer and detector (and often the ion source as well) are kept under high vacuum.

The sample can be introduced to the ion source directly, or after its previous separation involving either GC or high-performance LC (HPLC); thus, the sample is separated into a series of components, which then enter the mass spectrometer for their subsequent analysis. There are several ionization methods available, each having its own advantages and limitations. The fitness of the ion source depends on factors such as the polarity, molecular weight, thermal lability of the analytes, and complexity of the examined sample (Figure 10.4).

In GC–MS, electron ionization and chemical ionization (CI) represent the fundamental ionization techniques. On the basis of the scientific literature abstracted in SciFinder Scholar the EI was used in approx. 95% of all food GC–MS applications, while the rest of applications (5%) employed CI. In LC–MS, electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), and atmospheric pressure photoionization (APPI) represent ionization techniques widely employed. On the basis of SciFinder Scholar, ESI was the most often reported ionization technique in food LC–MS applications (approx. 80%), while APCI and APPI were more rarely employed (18% and 2%, respectively). In addition, the direct ionization techniques such as matrix-assisted laser desorption ionization (MALDI), desorption electrospray ionization (DESI), desorption atmospheric pressure chemical ionization (DAPCI), and direct analysis in real time (DART) can be employed for sample characterization.

A wide range of mass analyzers are nowadays available. While quadrupole, quadrupole ion trap, linear quadrupole ion trap, and magnetic sector represent scanning instruments, in which the mass spectrum is recorded by gradual changing of electrical and magnetic field; the other group of mass

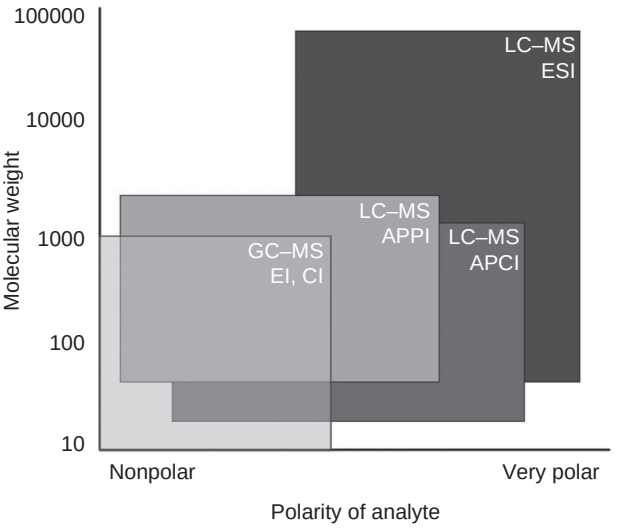

FIGURE 10.4 Application scope of various ionization techniques coupled to GC and LC.

analyzers such as time-of-flight, Fourier transform ion cyclotron resonance (FT-ICR) analyzer, and orbitrap are nonscanning instruments, in which entire mass spectra are obtained simultaneously.

The detector allows to monitor the ion current and, after its amplification, to record the data in the form of mass spectra. The most common types of MS detectors include the electron multiplier, the photon multiplier, and the microchannel plate (MCP) detector.

10.2.1 Ion Sources for Gas Chromatography

10.2.1.1 Electron Ionization

The EI (EI, formerly called electron impact) process starts by the acceleration of electrons through an electric field. These energetic electrons interact with neutral molecules (M). Upon collision, the molecule loses an electron and becomes a particle with an odd number of electrons and a positive charge. This radical cation ($M^{\bullet+}$) is typically called molecular ion.

$$M + e^- \rightarrow M^{\bullet+} + 2\,e^-$$

If the molecular ion contains sufficient internal energy, it undergoes fragmentation, which leads to the formation of fragment ion(s); either a radical ($R^{\bullet}$) and an ion with an even number of electrons (EE^+), or a neutral molecule (N) and a new (odd ion) radical cation ($OE^{\bullet+}$) can be considered. Each fragment ion derived from the molecular ion can undergo further fragmentation.

$$\begin{array}{rcl} M^{\bullet+} & \longrightarrow & EE^+ + R^{\bullet} \\ & \longrightarrow & OE^{\bullet+} + N \end{array}$$

The fragmentation reactions are initiated at the site of the unpaired electron and the positive charge in the precursor ion. The most preferred radical and charge site in the molecular ion is assumed to be a loss of the molecule's electron of lowest energy in the order of $\sigma < \pi < n$ electrons originating from sigma bonds, double bonds, or nonbonding electron pairs.

The energy required for the ionization process is called the ionization energy and can vary between 0 and over 100 eV. A positively charged species (i.e., molecular ions) begin to appear at

low intensities at around 10 eV. Increasing of this ionization potential leads to the formation of fragment ions. Standard mass spectra are obtained typically at 70 eV because maximum ion intensity is observed at this value, and mass spectra are reproducible and characteristic independently of the type of instrument.

Under standardized conditions, the electrons are produced in the EI source from a heated filament and are energized by accelerating them through a potential of 70 eV. The moving electrons directed across the source are forced into a beam by a magnetic field. This electron beam then interacts with sample molecules that have been vaporized. The ions are extracted from the source by an electric field and passed into the analyzer as an ion beam or as an ion packet.

Under these circumstances, virtually identical spectra can be obtained regardless of mass spectrometers equipped with the EI source as long as the electron energy is the same. This fact has led to the compilation of extensive mass spectra libraries (e.g., the NIST—National Institute of Standard and Technology library and the Wiley library). EI is therefore preferred for the identification of unknowns, determination of molecular structure, and confirmation of target analyte identity through consistent ion abundance ratios and library spectra matching.

10.2.1.2 Chemical Ionization

CI represents a low energy or ''soft'' ionization technique and is therefore very suitable for those less volatile or thermally labile molecules that do not yield molecular ions by EI. For CI, a suitable reagent gas is introduced into the ion source at a concentration that largely excesses the amount of the analytes (e.g., 10^4:1). The reagent gas is usually ionized as in EI. The formation of primary ions is followed by reactions between those primary ions and the noncharged molecules of gas producing the CI reagent ions as well as the thermal electrons.

In *positive chemical ionization* (PCI), the ion source is filled with a reagent gas (e.g., methane), at a relatively high pressure (0.1–100 Pa), which undergoes EI, producing an excess of reagent ions.

$$CH_4 + e^- \rightarrow CH_4^{\bullet +} + 2e^-$$

Sample molecules are subsequently ionized by the reagent gas ions *via* proton transfer, producing pseudomolecular ions $[M + H]^+$ and, depending on the choice of a reagent gas, adduct ions may be formed.

$$CH_4^{\bullet +} + CH_4 \rightarrow CH_5^+ + CH_3^{\bullet}$$

$$M + CH_5^+ \rightarrow [M + H]^+ + CH_4$$

The pseudomolecular ions generally have low internal energy and, consequently, they are less prone to the fragmentation than the molecular ions generated under EI conditions; in this way unambiguous molecular weight information can be obtained. Due to little or no fragmentation, PCI is less suitable for confirmation. This can be, however, highly appreciated in some analyses, since the pseudomolecular ion is more intensive and specific than any lower-mass fragment ions. In other words, PCI can offer both increased sensitivity and improved detectability due to reduced chemical noise from background or co-eluting analytes, resulting in increased signal to noise ratio (S/N).

The production of a large population of low-energy electrons during the CI operation utilizes another ionization technique: negative chemical ionization (NCI), alternatively called electron capture negative ionization (ECNI) or negative ion chemical ionization (NICI). The basic mechanism of this technique is similar to that of an electron capture detector: a low-energy electron is captured by an electronegative sample molecule (*ABC*), forming the molecular anion (by the resonance capture, dissociative capture, or ion-pair formation mechanisms), which may undergo fragmentation, depending on its structure.

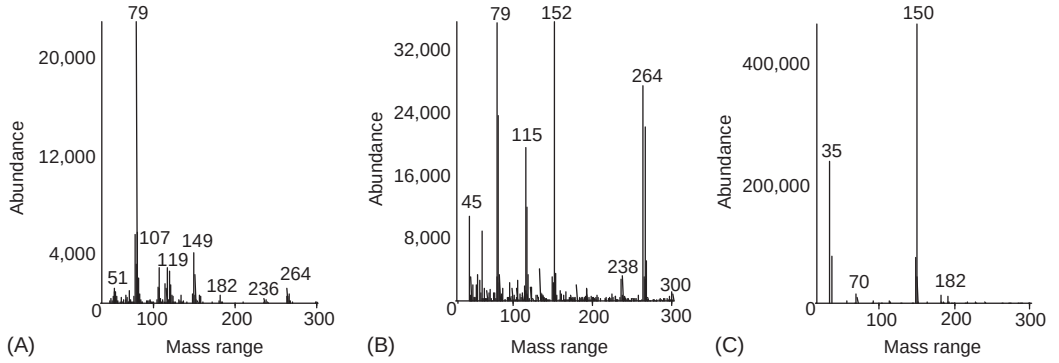

FIGURE 10.5 The (A) EI, (B) PCI, and (C) NCI mass spectra of a pesticide captan (MW = 299). The ionization techniques influence the extent of fragmentation and ion intensities. Methane was used as a CI reagent gas.

$$ABC + e^- \rightarrow ABC^-$$
$$ABC + e^- \rightarrow AC + B^-$$
$$ABC + e^- \rightarrow AB^+ + C^- + e^-$$

The main advantages of NCI compared to EI and PCI include a possibility of up to 100-fold improvement in sensitivity, and higher degree of selectivity, since only a limited number of analytes, such as those containing a halogen atom, a nitro group, or an extended aromatic ring system, are prone to efficient electron capture. Figure 10.5 compares the effect of EI, PCI, and NCI ionization techniques on the fragmentation and ion intensities in the resulting GC–MS spectra of a selected analyte.

10.2.2 Ion Sources for Liquid Chromatography

Compared to GC–MS, the LC–MS coupling was slower in its development mainly due to the technical problems that derived from the introduction of liquids into a mass spectrometer with a high vacuum required for separation of ions. The online LC–MS coupling requires the availability of an interface or restrictor that allow to keep the total mass flow entering the mass analyzer at values compatible with the pumping capacity of the vacuum system. The main interfaces adopted for this purpose include the use of the moving belt interface, particle beam interface, direct liquid introduction, continuous flow fast atom bombardment, thermospray, and atmospheric pressure ionization (API). In the following paragraphs, the attention will be paid to the most frequently used LC–MS ion sources coupled in current practice: ESI, APCI, and APPI.

It should be noted that LC–API-MS interface is considerably influenced by the composition of liquid entering the detector, i.e., the type and amount of organic mobile phase modifiers and volatile buffers, and also the type and amount of sample matrix components. These substances present in the injected sample can cause serious quantification problems when co-eluted with the analyte of interest; either by suppression or enhancement of the analyte signal. It is assumed that matrix components influence the efficiency of the ionization processes in API interface (causing a mutual positive or negative effect in the amount of ions formed from the target analyte). Those components may also influence the ion formation in the ionization process by altering the surface tension of electrospray droplets and by building adduct ions or ion pairs with the analytes. As a result of matrix suppression/enhancement phenomena, the response of an analyte in pure solvent standard may differ significantly from that in matrix sample. Therefore for quantification purposes, calibration using solvent-based external standards can provide biased results, especially in the analysis of complex samples, such as food [5].

10.2.2.1 Electrospray Ionization

ESI represents a very soft ionization technique, resulting typically in a single peak of (de) protonated molecular ion ($[M+H]^+$ in positive-ion mode and $[M-H]^-$ in negative-ion mode) or molecular ion adducts with, e.g., sodium, potassium, or ammonia. ESI is suitable for ionic analytes and other relatively polar compounds with MW ranging from less than 100 Da to more than 1,000,000 Da. In the analysis of large molecules, such as proteins, ESI generates multiply charged ions, which reduces the m/z ratio, effectively extending the mass range of mass spectrometers.

During ESI, the sample is dissolved in a polar solvent and introduced though a narrow stainless steel capillary (nebulizing needle, ESI needle) either from a syringe pump or as the effluent flow from a LC. Flow rates are typically in a range of 1 μL/min to 1 mL/min. Using a high voltage (typically 2.5–4 kV), applied to the tip of the capillary, it results in the formation of a strong electric field and the sample emerging from the tip is dispersed into an aerosol, which consists of highly charged droplets. A nebulizing gas (usually nitrogen) flowing around the outside of the capillary helps to direct the spray towards the mass analyzer. By solvent evaporation, the charged droplets diminish their size by the assistance of a drying gas (warm flow of nitrogen), which passes across the front part of the ion source (Figure 10.6). After the droplets reach the point that the surface tension cannot sustain the charge (so-called the Rayleigh limit), ''Coulombic explosion'' occurs and the droplets are disintegrated. This process repeats until analyte ions evaporate from the droplet. The (multiply) charged analyte ions then pass through a sampling cone or orifice into a vacuum region, and from there through a small aperture into the mass analyzer.

10.2.2.2 Atmospheric Pressure Chemical Ionization

APCI is a somewhat similar ionization technique to CI in GC–MS. However, while the latter ionization requires a vacuum, the APCI occurs at atmospheric pressure. In APCI, the analyte solution is introduced from a direct inlet probe or an LC eluate at a flow rate between 2 μL/min and 2 mL/min into a pneumatic nebulizer, where it is converted into a vapor by a nitrogen beam. Created droplets are then displaced by the gas flow through a heated quartz tube (desolvation/vaporization chamber). After desolvation, the gas-phase solvent molecules are ionized by primary ions (such as $N_2^{\bullet+}$) produced by the corona discharge, which has the same function as the electron filament in CI. The primary ions collide with the vaporized solvent molecules to form secondary reactant gas ions, which then undergo repeated collisions with the analyte resulting in the proton transfer and formation of analyte ions. Similarly to ESI, the APCI spectra are characterized by predominant molecular species and adduct ions with very little fragmentation. The ions then enter the mass spectrometer through a tiny inlet and are focused towards the analyzer.

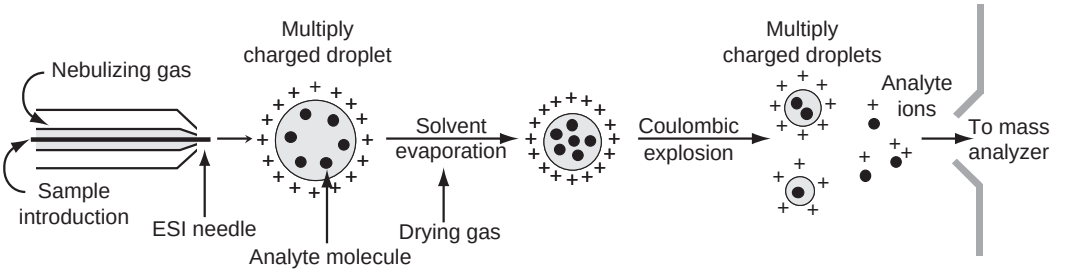

FIGURE 10.6 Illustration of droplet and ion formation in electrospray ionization (ESI).

10.2.2.3 Atmospheric Pressure Photoionization

APPI represents a novel, alternative ionization method for LC–MS. The ionization process in APPI is initiated by 10 eV photons, which are emitted by a krypton discharge lamp. The photons can ionize compounds that possess ionization energies below their energy (10 eV), which includes most of the larger molecules (analytes), but leaves out most of the typically used gases and solvents. Under these conditions, the analytes can be ionized selectively, with minimum background interference. In addition, as the ionization of the analytes is dependent on the ionization energy of the analyte rather than its proton affinity like in ESI and APCI, the ionization of molecules of relatively low polarity is also possible.

10.2.3 Ion Source for ICP-MS

The sample for inductively coupled plasma (ICP)-MS has to be introduced into the plasma as an aerosol, usually as a liquid sprayed through a nebulizer. Under the conditions of high temperature (6,000–10,000 K) in the plasma, the sample is atomized and ionized, creating positively charged atomic ions. While the larger aerosol droplets are removed from the gas stream by a spray chamber, the remaining smaller droplets are swept into the central channel of an argon plasma followed by their drying, decomposition, and dissociation into individual atoms in the plasma. These atoms are converted to positively charged ions before their extraction into the vacuum system for the detection creating the ICP-MS spectrum, which represents the elemental composition of the sample. The most important features of ICP-MS, compared to other elemental analysis techniques, are extreme sensitivity, selectivity, and simultaneous multi-element capability. The only elements that cannot be directly measured by this technique are hydrogen, helium, neon, argon, and fluorine [6].

10.2.4 Ion Sources for Direct MS Analysis

10.2.4.1 Matrix-Assisted Laser Desorption Ionization

MALDI is mainly suitable for thermolabile and/or nonvolatile organic compounds with high molecular mass (e.g., peptides, proteins, glycoproteins, oligoproteins, and oligonucleotides). Before the analysis, the sample is pre-mixed with a solvent containing highly UV-absorbing matrix compound. This mixture is dried before the analysis removing the liquid solvent used in preparation. The obtained deposit of matrix–analyte solid solution then undergoes the ablation of bulk portions of this solid solution by intense pulses of laser for a short duration (Figure 10.7). The irradiation by the laser induces rapid heating of the crystals as a result of the accumulation of a large amount of energy in the condensed phase through excitation of the matrix molecules. The rapid heating leads

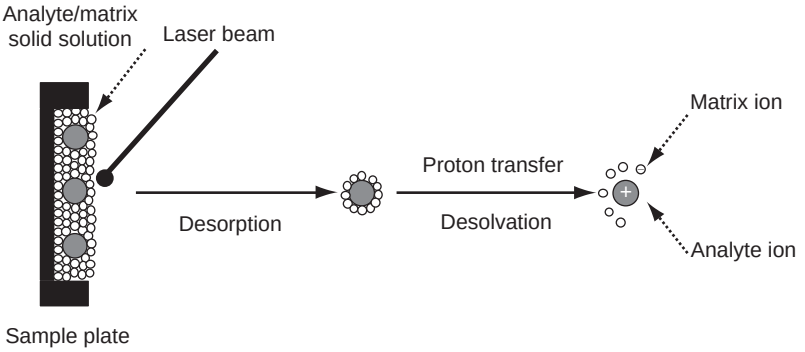

FIGURE 10.7 Principle of matrix-assisted laser desorption ionization (MALDI).

to sublimation of the deposits and their expansion into the gas phase followed by evaporation of the matrix molecules away from the clusters to leave the free analyte ions in the gas phase. The analyte ions, created by proton transfer from ionized matrix molecules, are then extracted into the mass spectrometer for the analysis [7].

In most MALDI instruments, the desorption/ionization process takes place in vacuum, thus the sample is not accessible during the analysis (similarly to EI or CI in GC–MS). Although atmospheric pressure MALDI (APMALDI) has been recently introduced, it still requires sample dilution/coating with a UV-absorbing matrix compound and operation under enclosed conditions to protect the operator from potential exposure to laser radiation [8].

Also, APMALDI and atmospheric pressure ionization techniques, such as ESI, APCI, or APPI, require that samples have to be exposed to elevated temperatures and electrical potentials, ultraviolet irradiation, laser radiation, or a high-velocity gas stream. Recently introduced ionization methods described below in Sections 10.2.4.2 and 10.2.4.3 do not pose these limitations, requiring essentially no sample preparation and allowing full access to the sample while mass spectra are being recorded.

10.2.4.2 Desorption Electrospray Ionization and Desorption Atmospheric Pressure Chemical Ionization

DESI is carried out by directing electrosprayed charged droplets and ions of solvent onto the analyzed sample surface (Figure 10.8). The spray is directed at an insulating sample or an analyte deposited on an insulating surface such as polytetrafluoroethylene. The desorbed ions are sampled with a commercial mass spectrometer equipped with an atmospheric interface connected via a transfer line made either of metal or an insulator. Similarly to ESI, the resulting mass spectra show mainly singly or multiply charged molecular ion of the analyte [9].

DAPCI offers an alternative option for those compounds that do not provide sufficient ion intensity by DESI. DAPCI has been shown to provide increased sensitivity for compounds of moderate polarity. The DAPCI technique uses nitrogen sheath gas and a solvent from which ions are produced by a corona discharge. Reagent ions formed in the corona discharge region react with desorbed analyte molecules, creating protonated or deprotonated molecular ions depending on the polarity of the ionization mode [10].

10.2.4.3 Direct Analysis in Real Time

DART is based on the atmospheric pressure interactions of long-lived electronic excited-state atoms or vibrionic excited-state molecules with the sample and atmospheric gases. A gas (typically helium or nitrogen) flows through a chamber where an electrical discharge produces ions, electrons, and excited-state (metastable) atoms and molecules. Most of the charged particles are removed as

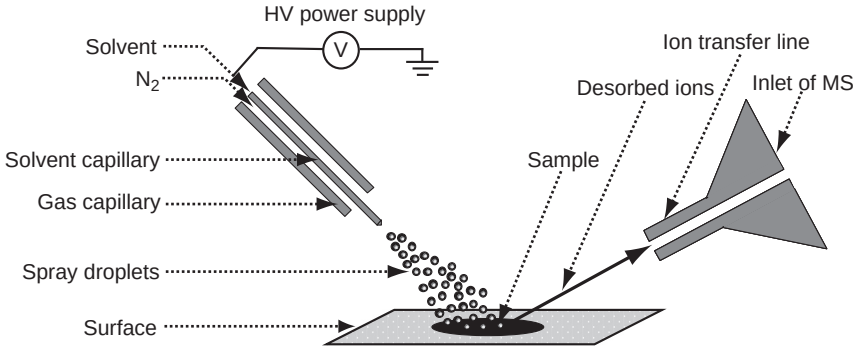

FIGURE 10.8 Desorption electrospray ionization (DESI) schematic.

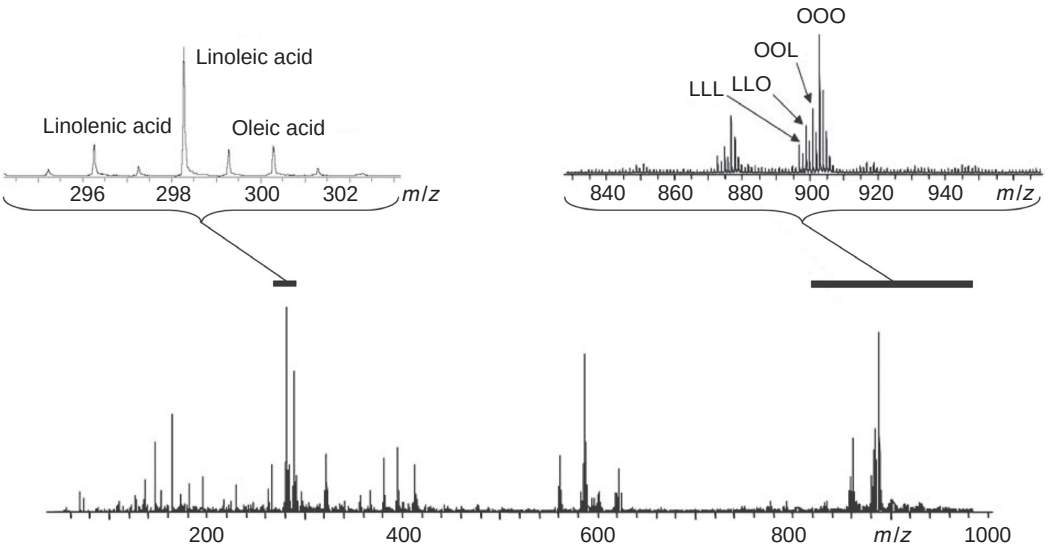

FIGURE 10.9 DART mass spectrum of olive oil. (Reproduced with permission from JEOL(Europe), Zaventem, Belgium.)

the gas passes through perforated lenses or grids and only the neutral gas molecules (including metastable species) remain. A perforated lens or grid at the exit of DART acts as an electrode to promote ion drift toward the orifice of the atmospheric pressure interface of the mass spectrometer. DART produces relatively simple mass spectra characterized by $[M]^+$ or $[M+\mathrm{H}]^+$ in positive-ion mode, and $[M]^-$ or $[M-\mathrm{H}]^-$ in negative-ion mode. As opposed to DESI, DART does not use any solvent, but its applicability is probably limited to smaller organic molecules [11]. Figure 10.9 shows the DART mass spectrum of olive oil characterizing its lipid composition.

10.2.5 Mass Analyzers

Once the molecules are converted into charged particles, they undergo mass analysis in a mass analyzer. The choice of an optimal mass analyzer is determined by several key operating parameters that are briefly summarized below [12–14]. The general specifications and features of selected mass analyzers hyphenated to both GC and LC are shown in Tables 10.1 and 10.2.

- *Mass range* represents the range of m/z over which a mass analyzer can separate and then detect/record ions. In GC, analyte volatility/thermolability effectively dictates the upper mass limit, thus the majority of mass spectrometers combined with GC operate typically up to m/z 1000. In LC, however, the mass range is often increased up to several orders higher m/z.
- *Mass resolution/mass resolving power* is the ability of a mass analyzer to separate two ions of similar mass. On the basis of the recent IUPAC (the International Union of Pure and Applied Chemistry) provisional recommendation on standard definitions of MS terms (drafted in 2006 [15]), mass resolution is defined as the smallest mass difference Δm between two equal magnitude peaks, so that the valley between them is a specified fraction of the peak height. Mass resolving power is then defined as the observed mass divided by the difference between two masses that can be separated, $m/\Delta m$. The procedure by which Δm was obtained and the mass at which the measurement was

TABLE 10.1
General Specifications and Features of Selected Mass Analyzers Coupled to GC

Criteria	Quadrupole	Triple Quadrupole	Ion Trap	DF Magnetic Sector	High-Speed TOF	High-Resolution TOF
Mass range	Up to 1,200 Da	Up to 1,500 Da	Up to 1,000 Da	Up to 4,000 Da	Up to 1,000 Da	Up to 1,500 Da
Mass accuracy	0.1–0.2 Da	0.1–0.2 Da	0.1–0.2 Da	<5 ppm		<5 ppm
Maximal spectral acquisition speed	12,500 amu/s (i.e., theoretically 25 Hz for m/z 50–550 Da)		5 scans/s (m/z 50–550 Da)	7 scans/s (m/z 50–550 Da)	500 spectra/s (m/z 50–550 Da)	20 spectra/s (m/z 50–550 Da)
Acquisition mode	Full scan, SIM	Full scan, SIM, SRM, MRM	Full scan, SRM, MRM	Full scan, SIM	Full spectra	Full spectra
Sensitivity	>pg in full scan fg in SIM	>pg in full scan <pg in MS/MS	>pg in full scan	fg in SIM	pg	fg–pg
Linear dynamic range (orders of magnitude)	>5	>5	4–5	>5	4	4
Versatility	EI, PCI, NCI	EI, PCI, NCI	EI, PCI, NCI	EI, PCI, NCI	EI	EI, PCI, NCI
Mass resolution/mass resolving power	Unit mass	Unit mass	Unit mass	>10,000 (10% valley definition)	Unit mass	>7,000 (FWHM)
Cost	+	+++	+	++++	+++	+++
MS/MS	None	MS^2	MS^n, $n = 2$–10	Only with special configuration	None	None

Note: EI, electron ionization; MS, mass spectrometry; NCI, negative chemical ionization; PCI, positive chemical ionization; SIM, selected ion monitoring; SRM, selected reaction monitoring; MRM, multiple reaction monitoring; pg, picogram; fg, femtogram.

TABLE 10.2
General Specifications and Features of Selected Mass Analyzers Coupled to LC

Criteria	Quadrupole	Triple Quadrupole	Ion Trap	High-Speed TOF	High-Resolution TOF
Mass range	Up to 1,000–3,000 Da	Up to 1,000–3,000 Da	Up to 2,000–6,000 Da	Up to 6,000 Da	Up to 30,000 Da
Mass accuracy	0.1–0.2 Da	0.1–0.2 Da	0.1–0.2 Da	<15 ppm	<3 ppm
Acquisition mode	Full scan, SIM	Full scan, SIM, SRM, MRM	Full scan, SRM, MRM	Full spectra	Full spectra
Maximal spectral acquisition speed	≈6 scans/s (m/z 50–1,000 Da)		≈2 scans/s (m/z 50–1,000 Da)	100 spectra/s (m/z 50–1,000 Da)	20 spectra/s (m/z 50–1,000 Da)
Sensitivity	>pg in full scan <pg in SIM	>pg in full scan <pg in MS/MS	>pg in full scan	>pg	pg
Linear dynamic range	>5	>5	4–5	>3	4
Versatility	ESI, APCI	ESI, APCI, APPI	ESI, APCI	ESI	ESI, APCI, APPI
Mass resolution/mass resolving power	Unit mass	Unit mass	Unit mass	>2,000 FWHM	>12,000 FWHM
Cost	+	+++	+	+++	+++
MS/MS	None[a]	MS^2	MS^n, $n = 2$–10	None[a]	None[a]

Note: APCI, atmospheric pressure chemical ionization; APPI, atmospheric pressure photoionization; ESI, electrospray ionization; FWHM, full width at half maximum; MS, mass spectrometry; SIM, selected ion monitoring; TOF, time-of-flight; SRM, selected reaction monitoring; MRM, multiple reaction monitoring; pg, picogram; fg, femtogram.

[a] MS/MS option only when hyphenated, such as Q-TOF.

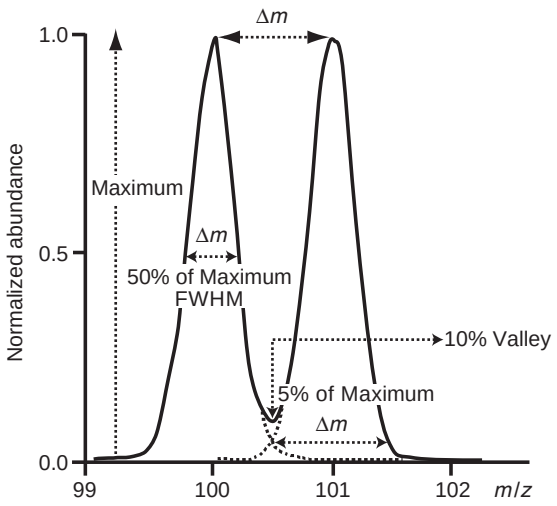

FIGURE 10.10 Relationship between the two definitions of mass resolution.

made should be reported. It should be noted that mass resolution is also often expressed as $m/\Delta m$ ratio in current MS practice, i.e., not as Δm as recommended by the IUPAC. Figure 10.10 demonstrates two different ways that are used in practice to obtain Δm and, consequently, to evaluate mass resolution and mass resolving power. The 10% valley definition is based on the mass difference between two mass peaks separated by a 10% valley, while full width at half maximum (FWHM) definition expresses Δm as the peak width of a given mass peak measured (in mass units) at 50% of its height. The FWHM definition usually gives a number twice the magnitude of the 10% valley definition. The most mass analyzers operate at so-called unit mass resolution (or actually at mass resolving power <1000). High-resolution MS instruments provide improved selectivity, thus reduced chemical noise and, consequently, increased S/N in complex samples.

- *Mass accuracy* is the deviation between measured mass (accurate mass) and calculated mass (exact mass) of an ion expressed as an error value (mDa, ppm). This parameter is important for structural interpretation allowing confirmation of the target analyte identity and the calculation of elemental composition of ''unknowns.'' The unit-resolution mass spectrometers provide mass accuracy of approx. 0.1–0.2 Da (e.g., 200.0 and 200.1 Da), while the high-resolution instruments operate at mass accuracy <5 ppm (e.g., 200.000 and 200.001 Da).
- *Spectral acquisition speed* represents time required for recording of a mass spectrum or selected ion(s). For scanning instruments terms scans/s, s/scan, or s/decade are commonly used; for nonscanning instruments a term spectra/s is preferred. *Maximal* spectral acquisition speed is a critical parameter in detection of very narrow peaks generated during fast chromatographic separation.
- Selection of *acquisition mode* allows the use of different modes of mass spectra collection in scanning instruments. Both magnetic sector and quadrupole instruments can be operated in full scan and selected ion monitoring (SIM); in the latter case structural information is sacrificed in favor of detection sensitivity.
- *Detectability* (or often expressed as *sensitivity*) of the instrument, which is characterized by the instrument manufacturers as minimal S/N ratio at a given concentration of a reference

compound. For this purpose, compounds such as octafluoronaphthalene or hexachlorobenzene are typically used in GC–MS, and reserpine in LC–MS.

- *Linear dynamic range* is the range of analyte concentrations, over which the detector proportionally responds to concentration changes. For common scanning instruments, this range varies between 5–6 orders of magnitude; the nonscanning instruments, such as TOFMS, offer linear dynamic range of typically 4 orders of magnitude in maximum. Narrow dynamic range makes the quantification complicated, especially if samples with largely varying concentrations of target analytes have to be analyzed.
- *Availability of tandem MS function*, which is a method involving at least two stages of mass analysis, either in conjunction with a dissociation process or a chemical reaction that causes a change in the mass or charge of particular ion. MS/MS methods involve activation of selected ions (called precursor or parent ions), typically by collision with an inert gas, sufficient to induce fragmentation (resulting in ions called product or daughter ions). Basically, two different approaches in MS/MS exist: *in space* by coupling of two physically distinct parts of instrument (e.g., in triple quadrupole, see Section 10.2.5.8); or *in time* by performing a sequence of events in an ion storage device (e.g., in ion trap, see Section 10.2.5.2). The main tandem MS/MS scan modes are (Figure 10.11) (1) *product ion scan*, which involves selection of an ion of interest, its activation, and mass analysis of the product ions in full scan mode, (2) *precursor ion scan* represents opposite process compared to the product ion scan, providing information about all precursor ions that react to produce a selected product ion, (3) *neutral loss scan* is a scan that determines all precursor ions that react to the loss of a selected neutral mass, (4) *selected reaction monitoring* (SRM) monitors a single transition from a precursor to a product ion, (5) *multiple reaction monitoring* (MRM) is used if several different reactions are monitored in one time window, and (6) MS^n *scans*, which is commonly applied on ion trap analyzers (Section 10.2.5.2).
- *Versatility*, in terms of the use of alternative ionization techniques, extends the scope of applications.
- *Cost* is undoubtedly an important factor when considering an MS system purchase. In routine practice, less expensive scanning instruments (quadrupole and ion trap analyzers) are widely used. Although there has been a decrease in cost of other mass analyzers, such as TOF or sector, during the recent years, unfortunately, their cost is still substantially higher (approx. 2–3 times for TOF and approx. 6 times for sector) compared to low-resolution quadrupole or ion trap instruments.

10.2.5.1 Quadrupole

The quadrupole represents the most popular mass analyzer mainly due to its relatively low cost, ruggedness, reliability, and the simplicity of operation. The quadrupole mass analyzer (often considered as a ''mass filter'') consists of four (hyperbolic or cylindrical) rods placed in a radial array (Figure 10.12). Opposite rods are charged by positive direct-current (DC) voltage, while adjacent rods have the opposite (negative) charge applied. Ions are introduced into the quadrupole field by means of a low accelerating potential. An appropriate combination of DC and radiofrequency (RF) fields on the quadrupole rods allows to pass only the ions of one particular m/z at a time. Ions with a nonstable trajectory through the quadrupole collide with the quadrupole rods, thus are not detected using the given DC and RF potential settings.

The quadrupole can be operated in two modes: (1) full scan of a selected mass range (e.g., m/z 50–550) and (2) SIM. In the latter mode, sensitivity of an analyte is enhanced by monitoring only a few selected m/z ions (typically 1–20), but the spectral information is sacrificed, which must be taken into account.

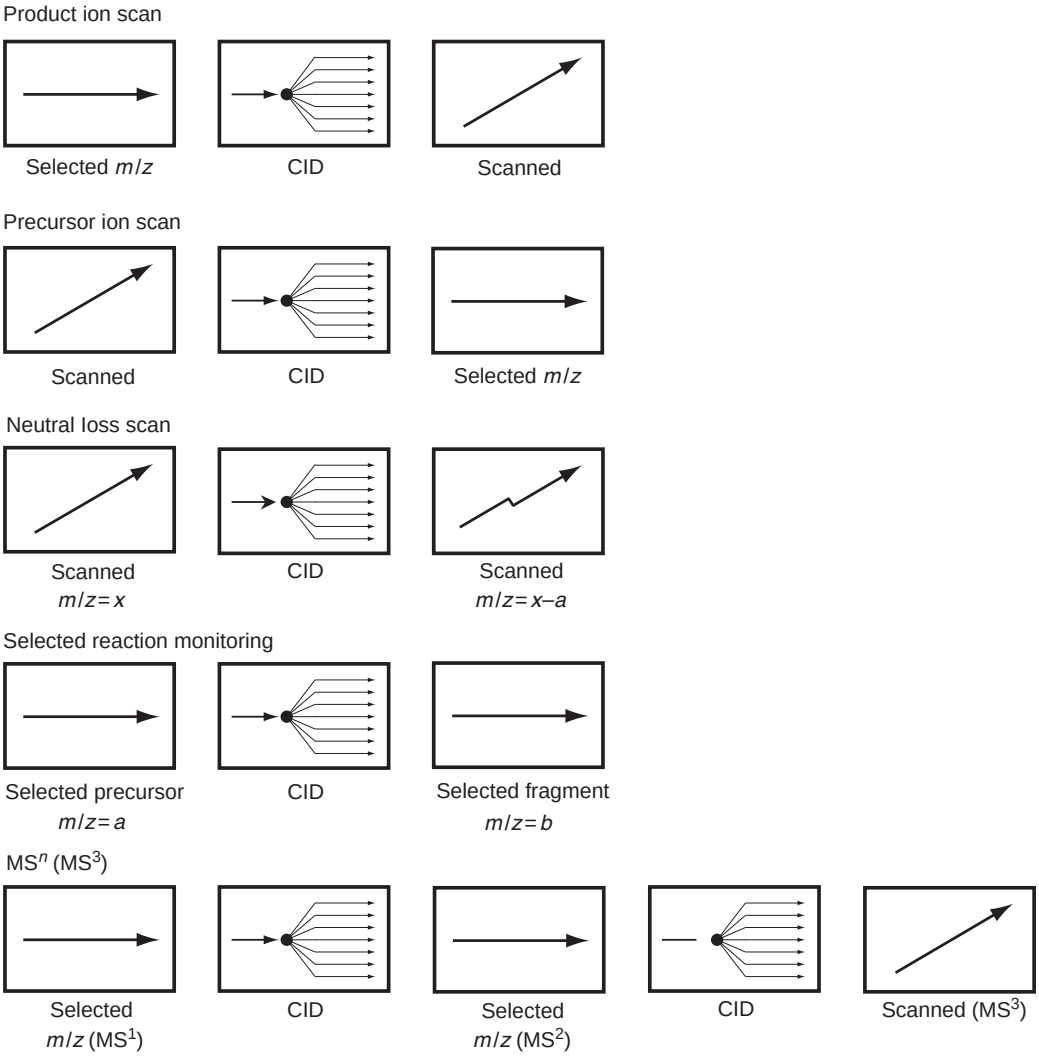

FIGURE 10.11 Various setups in tandem MS (CID stands for collision-induced dissociation).

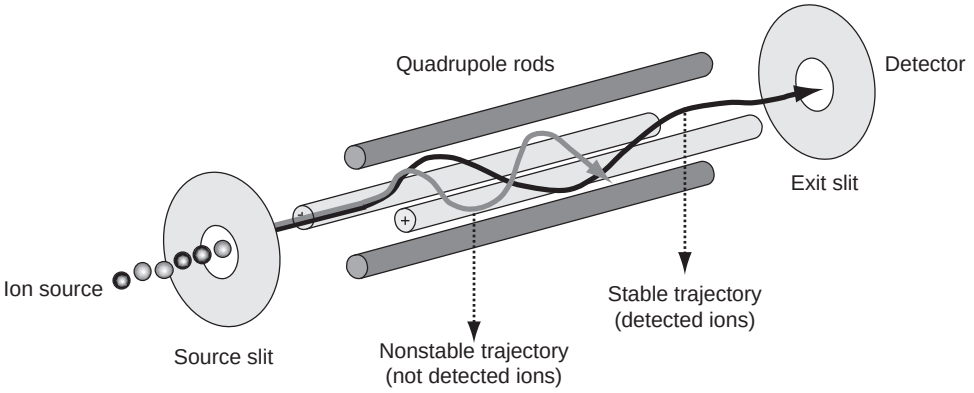

FIGURE 10.12 Quadrupole mass analyzer.

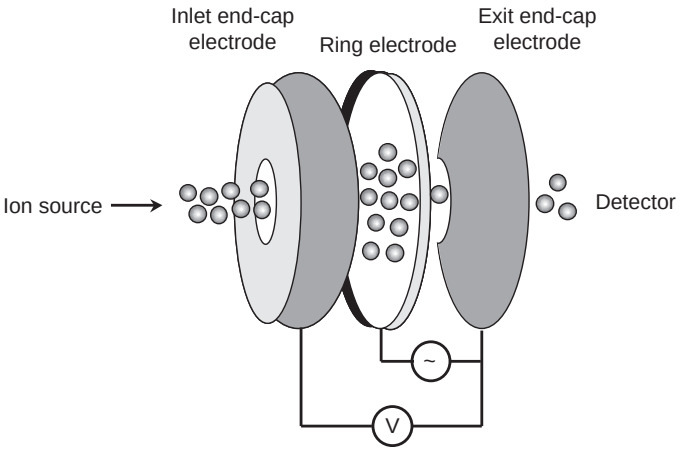

FIGURE 10.13 Quadrupole ion trap mass analyzer.

10.2.5.2 Quadrupole Ion Trap (3D Trap)

The quadrupole ion trap consists of three cylindrically symmetric electrodes (two end-caps and a ring), see Figure 10.13. The ions of selected m/z range are trapped by an RF potential, which is formed between end-cap electrodes and a ring electrode. Increasing of the RF potential leads to the formation of unstable trajectories of ions that rapidly deflect in the direction of the exit end-cap electrode followed by their detection in increasing m/z order.

Although the quadrupole ion traps allow both full scan and SIM acquisition, there is no increase in detection sensitivity in SIM mode that is typically observed with quadrupole or sector instruments. Enhanced selectivity (increased of S/N) can be obtained in MS^n mode (mostly MS/MS because orders $n > 3$ are not practical for relatively small molecules). The MS/MS is often performed by means of collision-induced dissociation (CID), where an RF voltage is applied to the end-cap electrodes to isolate precursor ions of a selected m/z value. After ionization, the application of an excitation voltage together with collision with helium buffer gas lead to the formation of monitored product ions.

The main advantage of using MS/MS is the significant reduction of the chemical noise, which can originate from different sources (e.g., matrix compounds, co-eluting analytes, column bleed, contamination from an ion source). The effect of multiple MS/MS steps on S/N ratio illustrates Figure 10.14. Although each MS step leads to a loss in the analyte signal intensity, the noise decreases even more rapidly, resulting in significant improvements in S/N ratio as the number of MS steps increases in MS^n [16].

10.2.5.3 Linear Quadrupole Ion Trap (2D Trap)

The linear quadrupole ion trap differs from the 3D ion trap as it traps ions in the radial dimension by an RF quadrupole field and by static DC potentials applied to the ends of a four-rod structure. Upon ejection, ions emerge radially over the length of the quadrupole-rod structure and can be detected using conventional detection system. The 2D trap has several advantages over the 3D trap. Since there is no quadrupole electric field in the z direction the ion injection and extraction efficiencies can be nearly 100%. In addition, as compared to the 3D trap, which has a limited capacity for ion storage due to its small strapping volume, the 2D trap has almost one order of magnitude increase in ion capacity, thus also in the linear dynamic range [17].

10.2.5.4 Time-of-Flight

The (orthogonal acceleration) time-of-flight (TOF) mass analyzer consists of a pulsing electrode, a flight tube, and a reflectron. The generated ions are accelerated to get constant kinetic energy

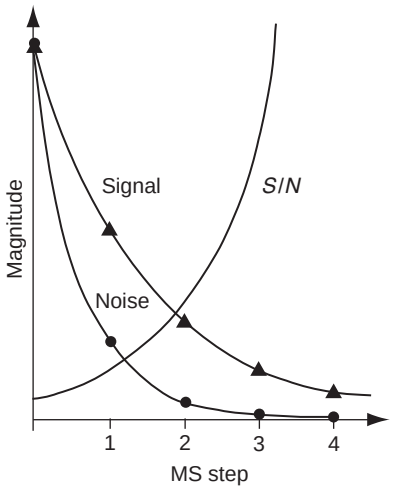

FIGURE 10.14 Effect of the number of MS steps in MS^n on signal (S), noise (N), and S/N. (Reproduced from Cooks, R.G. and Busch, K.L., *J. Chem. Edu.*, 59, 926, 1982. With permission.)

followed by their ejection into a mass analyzer's flight (drift) tube using pulsed electric-field gradient, which is oriented orthogonally to the ion beam. This orthogonal acceleration has a positive influence on mass resolving power, which is further enhanced by using a reflectron (ion mirror). This device consists of a series of ring electrodes with increasing voltage creating retarding fields. The ions with higher energy penetrate more deeply inside it, extending the time of their reflection. Consequently, the ions of the same m/z value with different initial energies hit the detector at almost the same time (Figure 10.15). In addition, the mass resolving power is substantially improved by making the ions pass twice along the TOF flight tube before reaching the detector. The times of arrival at the detector are proportional to the square root of respective m/z values [14,18].

It should be noted that the mass analyzer efficiency of TOFMS instruments is as high as 25% in full spectra acquisition. This value is significantly higher as compared to that obtained by scanning instruments such as quadrupole (~0.1%) [19]. This fact implies availability of full spectra information even at ultra-trace levels of a particular compound and, consequently, the possibility to carry out its identification on the basis of library search. Furthermore, since the TOF is nonscanning mass analyzer, all ions are recorded simultaneously, thus there are no changes in the ratios of analyte ions across the peak during the acquisition of the mass spectrum and, consequently, no spectral skewing

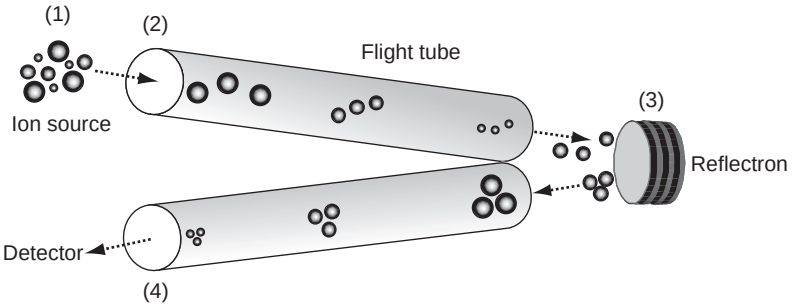

FIGURE 10.15 Time-of-flight mass analyzer. (1) Pulse of ions from the orthogonal accelerator (spatial focusing); (2) separation of ions according to their flight times; (3) focusing of kinetic energy of ions; and (4) separation of focused ions according to their flight times, which correspond to their weight.

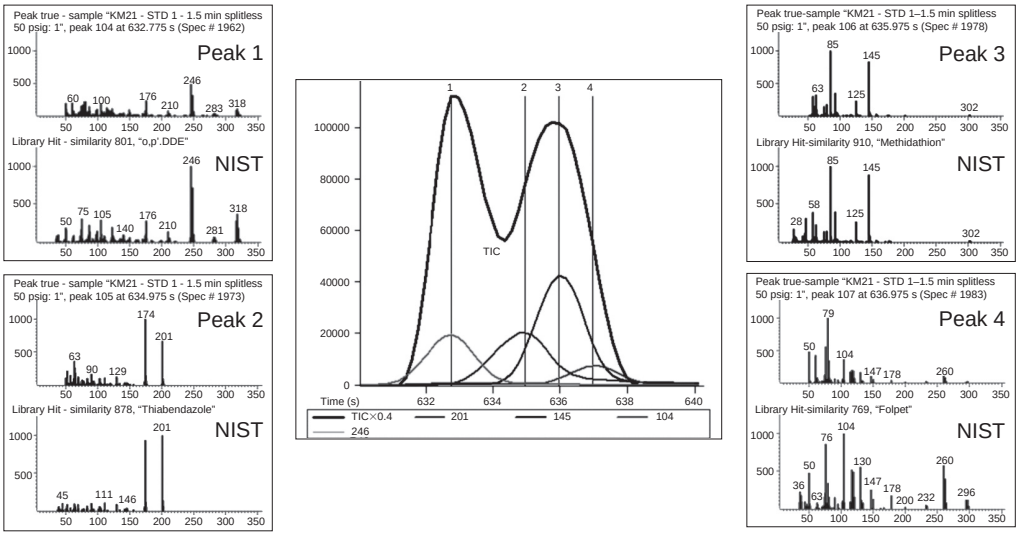

FIGURE 10.16 Demonstration of spectral deconvolution of four closely co-eluted compounds eluting within 8 s. Peak 1 = *o*,*p*′-DDT (m/z 246), peak 2 = thiabendazole (m/z 201), peak 3 = methidathion (m/z 145), and peak 4 = folpet (m/z 104).

(observed commonly by scanning instruments) is encountered. This allows TOF feature-automated deconvolution of partially overlapped peaks on the basis of increasing/decreasing ion intensities in collected spectra and background subtraction followed by identification using library search (Figure 10.16).

Although the deconvolution function (employing software correction for spectral skewing) is currently available also for scanning instruments, e.g., in the AMDIS software (Automated Mass Spectral Deconvolution and Identification System) provided by the NIST free of charge [20]), the low signal intensity during full spectra acquisition as well as relatively low acquisition rate of common scanning instruments can be a drawback that limits potential of this function when coupled to fast GC separations. Very narrow chromatographic peaks (<2 s at the baseline) generated under these conditions require a detector with high acquisition rates and, at the same time, with high sensitivity during the acquisition of full mass spectra, such as TOF, to fully utilize the advantage of spectral deconvolution [12,13].

Nowadays, two types of TOFMS instruments differing in their basic characteristics are available. One type uses high-resolution analyzers (7000 FWHM) providing only moderate acquisition speed (up to 20 spectra/s), and the second type are unit-resolution instruments that feature high acquisition speeds (up to 500 spectra/s) [14].

Figure 10.17 illustrates the main advantage of using a high-resolution TOFMS instrument. As mentioned earlier, the use of high mass resolving power significantly reduces chemical noise that can originate from various sources (mostly from matrix co-extractives), resulting in improved limit of detection (LOD). It should be also noted that the mass measurement accuracy allows determination of the mass of molecular and fragment ions. Thus, using this approach, the identification of the analyte can be based not only on retention time and mass spectrum, but also on elemental composition determination, which brings a new dimension to the identification/confirmation process.

10.2.5.5 Magnetic Sector

The *single focusing magnetic sector mass analyzer* consists of a magnetic sector, in which the magnetic field is used for separation of the ions with different m/z ratios. The ions entering the mass

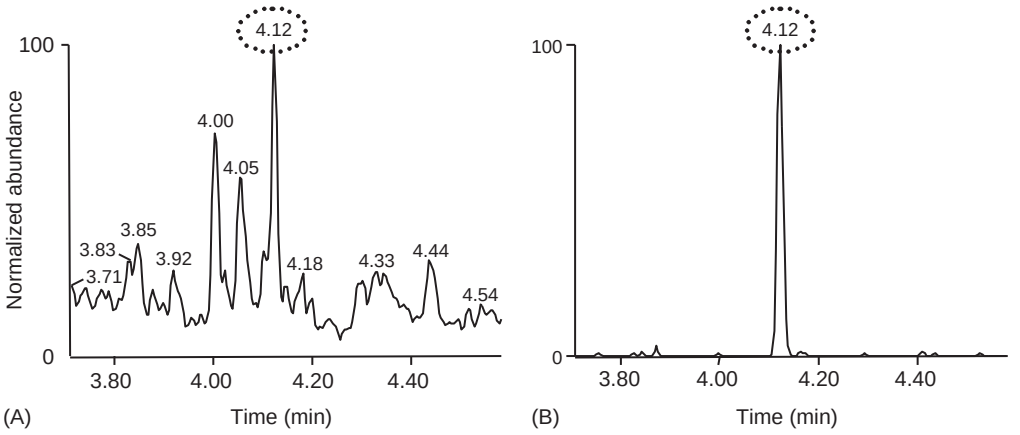

FIGURE 10.17 GC–HRTOFMS chromatograms of phosalone ($t_R = 4.12$ min) at concentration 0.01 mg/kg in baby food. Target ion m/z 182.0009 extracted using different mass windows. While by using (A) 1 Da mass window (setting corresponds to a unit mass resolution instrument) the peak-to-peak (PtP) S/N ratio was only 7:1, setting the mass window as narrow as (B) 0.02 Da led to a distinctly improved PtP S/N value of 63:1. Mass measurement accuracy allowed determination of the mass of phosalone's quantification ion $[C_8H_5{}^{35}ClNO_2]^+$ with the error as low as −0.9 mDa.

analyzer are initially accelerated to a high velocity using an electric field. The ions then pass through a magnetic sector where the magnetic field is applied in a direction perpendicular to the direction of ion motion. Although a magnetic sector separates the ions according to their m/z, the mass resolution is affected by the fact that ions leaving the ion source do not have exactly the same energy thus do not have exactly the same velocity. Coupling a magnetic sector with an electrostatic sector (double focussing magnetic sector mass analyzer) (Figure 10.18) allows to focus the kinetic energy of the ions. As a result, higher mass resolving power is achieved. The simplest way to operate a magnetic sector mass analyzer is to keep the accelerating potential and the electric sector at a constant potential and vary the magnetic field. Ions with a constant kinetic energy but differing in their m/z are focused at the detector slit at different magnetic field strengths. Usually, the electric

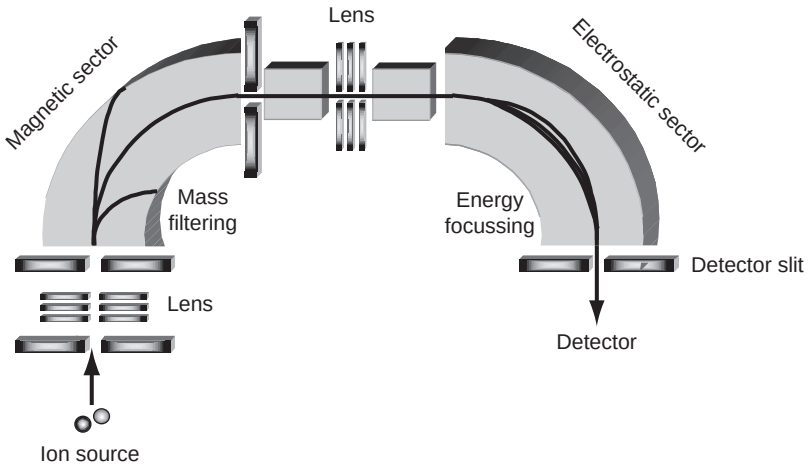

FIGURE 10.18 Double-focusing magnetic sector mass analyzer.

sector is held constant at a value, which passes only ions with the specific kinetic energy. The mass resolving power of a magnetic sector is influenced by the setting of slit widths. Higher mass resolving power is obtained by decreasing the slit widths, thus the number of ions that reach the detector is reduced. Double focussing magnetic sector mass analyzers provide very high reproducibility, high mass resolving power, high sensitivity, and a high dynamic range. However, their use is limited due to their size and higher cost compared to other mass analyzers, such as quadrupole and ion trap.

A double focussing magnetic sector mass analyzer has been employed in a number of applications for many years and is still being used to solve specific problems in different application areas. A very high capacity to remove the signals originating from matrix interfering compounds in the determination of the analytes typically present at ultra-trace concentrations (e.g., in the analysis of polychlorinated dibenzo-*p*-dioxins and -furans) is achieved when employing SIM mode at a mass resolving power of 10,000 (10% valley definition). Under these conditions, the presence of abundant matrix components in the analyzed extracts does not interfere with the detection process and high level of mass accuracy can still be obtained.

Another use represents the determination of complex mixtures of contaminants, such as polychlorinated terphenyls. In this particular case, not only the matrix interferences, but also problems due to interferences between fragment ions of congeners with different degree of chlorination may occur. In order to eliminate these specific interferences, a mass resolving power higher than 10,000 (10% valley definition) is required [3].

10.2.5.6 Fourier Transform Ion Cyclotron Resonance

The FT-ICR mass analyzer performs the mass analysis in a cubic cell that consists of pair of trapping plates, excitation plates, and detector plates (Figure 10.19). After trapping the ions in the cell, they are excited to a larger cyclotron radius by an oscillating electric field perpendicular to the fixed magnetic field. A packet of the ions is also formed during the excitation. The signal is then detected as an image current on a pair of receivers. The resulting signal consists of a superposition of sine waves, from which a regular mass spectrum is obtained by applying Fourier transformation.

The most important features of this type of mass analyzer are its extremely high mass resolving power (even 10^7 is possible) and sensitivity.

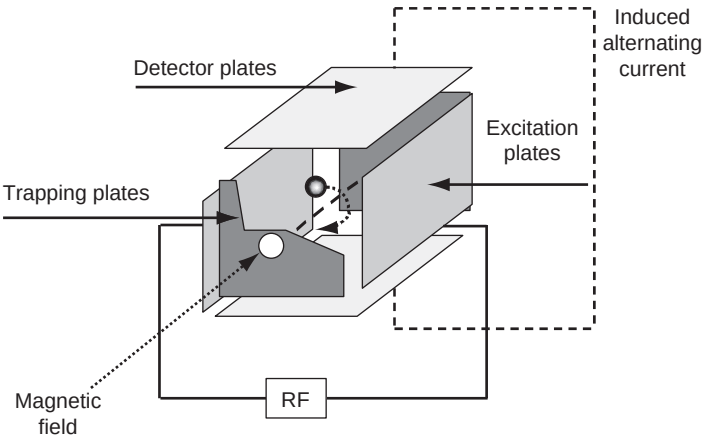

FIGURE 10.19 FT-ICR mass analyzer.

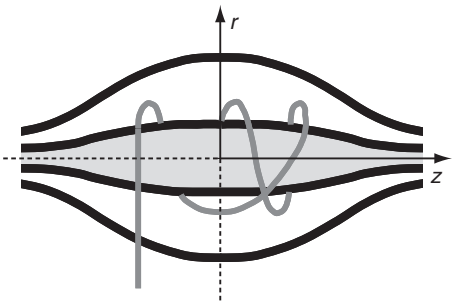

FIGURE 10.20 Orbitrap mass analyzer.

10.2.5.7 Orbitrap

The orbitrap represents a special case of an ion trap. Contrary to a conventional ion trap, which uses RF to hold the ions inside the trap, the moving ions in orbitrap are trapped in an orbit around a spindle-shaped electrode by an electrostatic field (Figure 10.20). The electrode confines the ions, which orbit around the central electrode and oscillate back and forth along long axis of the central electrode. This oscillation can be detected as an image current on the two halves of an electrode encapsulating the orbitrap. Similarly to the FT-ICR MS, Fourier transformation is employed to obtain oscillation frequencies for ions differing in their m/z. The features of the orbitrap include high mass resolving power (up to 150,000), large space charge capacity, high mass accuracy (2–5 ppm), and high mass range (m/z 6000) [21,22].

10.2.5.8 Hybrid Instruments

Different types of mass analyzers can be coupled together forming hybrid instruments. Triple quadrupole (QqQ) represents the most common known example. Other commercially available hybrid instruments include quadrupole/linear ion trap (Q-LIT), quadrupole/time-of-flight (Q-TOF), magnetic sector/time-of-flight, and time-of-flight/time-of-flight (TOF-TOF).

The widely used triple quadrupole consists of two single quadrupoles with a collision cell in between. The ions are directed from the ion source into the first quadrupole, where the precursor ion is selected for MS/MS reaction in the collision cell (usually a hexapole). The product ions are then separated in the second quadrupole and recorded by the detector. Figure 10.21 illustrates the

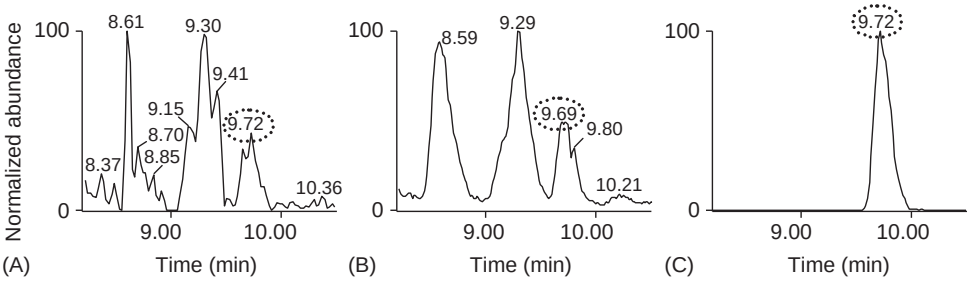

FIGURE 10.21 Liquid chromatography (LC)–mass spectrometry (MS[/MS]) analysis of thiodicarb ($t_R = 9.72$ min) in baby food at a concentration of 0.1 mg/kg. (A) MS^1, data acquired in full scan of m/z 50–1000 (m/z 355 displayed); (B) MS^1, data acquired in SIM mode (m/z 355 displayed); and (C) MS/MS, data acquired in MRM (transition m/z $355 > 87.9$ displayed). The PtP S/N values 6.4:1, 9.4:1, and 125:1, respectively.

TABLE 10.3
Comparison of Selected Single and Tandem Mass Spectrometry Instruments

Mass Analyser	Sensitivity in Full Spectra Acquisition	Selectivity	Mass Accuracy	Linear Dynamic Range
Q	+	+	+	+++
IT (MS^1)	++	+	+	++
Sector	+	+++	+++	+++
HRTOF	+++	++	+++	++
HSTOF	+++	+	+	++
QqQ	+	+++	+	+++
IT (MS^2)	+	+++	+	++
QTOF	++	+++	+++	++

Note: HRTOF, high-resolution time-of-flight; HSTOF, high-speed time-of-flight; MS, mass spectrometry; Q, quadrupole; QqQ, triple quadrupole; Q-TOF, quadrupole/time-of-flight; IT, ion trap.

improvement in selectivity, when employing tandem MS versus single MS (both in full scan and SIM) in the LC–MS (/MS) analysis.

If the last quadrupole in the series is replaced by TOF mass analyzer, the *quadrupole/time-of-flight* mass spectrometer is obtained, which combines the simplicity of quadrupole with high efficiency of a TOF mass analyzer. As in QqQ, the sample is introduced to the ion source, followed by focusing of the ions using the hexapole ion bridge into the first quadrupole, where the precursor ion is selected. The ions are ejected into the collision cell (a hexapole), where argon is typically used for their fragmentation. The product ions are collected into the TOF region, where separation of ions similar to that of a single TOF analyzer occurs. Due to the high mass analyzer efficiency, the detection of ions across the full mass range of the Q-TOF instrument is significantly higher (approx. 10–100 times) compared to that provided by QqQ under the same conditions. In addition, the Q-TOF systems offer high-resolution capability of up to 10,000 FWHM mass resolving power, allowing high-resolution and accurate mass measurement of the product ions generated in the collision cell [23].

Table 10.3 summarizes the advantages and limitations of single and tandem MS instruments in terms of sensitivity, selectivity, mass accuracy, and linear dynamic range.

10.2.6 Detectors

All mass analyzers separate the ions for their individual m/z values, what is followed by the recording of the number (abundance) of ions at each m/z value to give a mass spectrum. The ion detectors can be divided into three classes: (1) *point ion collectors* that detect the arrival of all ions sequentially at one point (e.g., electron multiplier, photon multiplier), (2) *array collectors* that detect the arrival of all ions simultaneously along a plane (e.g., MCP detector), and (3) detectors that consists of a pair of *metal surfaces* within the mass analyzer region, which the ions only pass near as they oscillate and only a weak image current is produced in a circuit between the electrodes (this principle is employed only for FT-ICR MS and orbitrap).

In an *electron multiplier*, the ions reach the first plate (dynode) of an electron multiplier and then the ejected electrons are accelerated through an electric potential to a second dynode. This process is typically repeated 10–12 times (according the number of used dynodes), which gives the amplifications of 10^6. The final flow of electrons provides an electric current that can be further increased by electronic amplification.

The *photon multiplier* is made up of two conversion dynodes, a phosphorescent screen, and a photomultiplier. Considering the positive mode, secondary ions are accelerated towards the dynode that holds the negative potential. Secondary electrons that are generated are accelerated towards the phosphorescent screen, where conversion into photons occurs, followed by their detection by the photomultiplier. The amplification ranges between 10^4–10^5. Both electron multiplier and photon multiplier are detectors typically used for quadrupole, ion trap, and sector instruments.

The MCP detector consists of a large number of miniature electron multiplier elements placed side by side over a plane. The surface of the MCP is metal coated, serving as electrodes. A voltage applied between the electrodes produces an electric-field gradient. When an ion hits the front part of the inner walls of the channel at this point, multiple secondary electrons are emitted. These electrons are then accelerated by the electric-field gradient inside the channel, and repeatedly hit the walls on the opposite side, emitting secondary electrons. At the end of this process, the electrons are captured by the anode, which produces an electrical signal. The amplification factor of the MCP is a few thousand at maximum. To achieve an amplification factor of 10^6, two MCPs are usually used (Dual MCP). The MCP is commonly used for detection of ions in TOFMS.

10.2.7 Miscellaneous

10.2.7.1 High-Field Asymmetric Waveform Ion Mobility Spectrometry

High-field asymmetric waveform ion mobility spectrometry (FAIMS) represents a new technique that separates gas-phase ions at atmospheric pressure based on the difference between ion mobility at high and low electric fields. In FAIMS, a mixture of ions is introduced between two electrodes, to which a high-voltage asymmetric waveform is applied to oscillate the ions in the alternating strong and weak electric fields. The ions drift toward an electrode depending on their differences in mobility in those electric fields. A compensation voltage is then applied to the electrodes to stop the drift of selected ions, which are transmitted to mass analyzer, while the others collide with the electrodes.

FAIMS coupled to MS provides a rapid, sensitive, and selective detection of ions. When coupled with MS, the FAIMS is located between an atmospheric pressure ion source (e.g., ESI) and the inlet of the mass spectrometer. This approach allows to separate isobaric ions and isotopes, reduce the chemical background, and simplify spectra of complex mixtures [24,25].

10.2.7.2 Supersonic Molecular Beam MS Interface

Supersonic molecular beam (SMB) interface is a unique instrumentation that is currently in the process of commercialization for the GC–EI-MS. In GC–SMB-MS, a nozzle of 0.1 mm is placed between the GC outlet and the MS. As eluted compounds from the separation column pass through the small opening, they form the SMBs. SMBs are characterized by intramolecular vibration supercooling, unidirectional molecular motion with controlled hyperthermal kinetic energy (1–20 eV), mass focusing, and capability to handle very broad range of column flow rates (up to 240 mL/min is possible with a prototype instrument) compared to a ''conventional'' flow of 1 mL/min typical for GC. The low thermal energy creates unique mass spectral properties that have several advantages compared to conventional GC–EI-MS: (1) increased selectivity as a result of enhanced intensity of molecular ion that occurs for most molecules at the low temperatures of SMB, (2) increased speed of analysis due to the use of very high gas flow rates, (3) extended scope of analyzed compounds including the analysis of both thermally labile and low-volatility compounds, (4) versatility in selection of injection techniques and column dimensions for fast GC separation, and (5) better peak shapes since the tailing effects that typically occur in the MS ion source are suppressed [26,27].

10.2.7.3 Imaging Mass Spectrometry

Imaging mass spectrometry (IMS) is a procedure used to form chemically selective images of objects based on the MS detection of ions desorbed from its surface. This is achieved through ionization from a clearly identified point on a flat sample and performing a raster of the analyzed

sample by moving the point of ionization over the sample surface. The collected data (positional data and m/z intensities) are converted into images that map distribution of target compounds in tissues or in various other materials [28,29]. For MS imaging, either secondary ion mass spectrometry (SIMS) or MALDI (Section 10.2.4.1) can be used as desorption/ionization methods.

In SIMS, the surface of the sample is bombarded by high energy ions (typically Cs^+, Ar^+, O_2^+, O^-, and Ga^+ at 1–30 keV), which leads to emission of both neutral and charged particles from the test piece. The emitted secondary ions are then extracted by an electrical potential and analyzed using a mass spectrometer. Basically, there are three different variants of this technique: (1) *static SIMS* used for submonolayer elemental analysis, (2) *dynamic SIMS* used for obtaining compositional information as a function of depth below the surface, and (3) *imaging SIMS* used for obtaining compositional images of the surface.

While imaging SIMS provides information on the spatial distribution of the elements as well as molecular structures of low-molecular mass compounds (<1000 Da), MALDI allows to obtain spatial distribution and molecular structure information about higher molecular mass compounds (<100,000 Da) [29].

10.3 FOOD ANALYSIS APPLICATIONS

A food matrix is very complex; in addition to major components such as lipids, proteins, and saccharides, a wide range of other natural minor compounds are contained (e.g., vitamins, aroma and flavor compounds, pigments). Under certain conditions, contaminants and other hazardous compounds may be present in food matrices as a consequence of human activities or due processing practices.

During the recent years, MS techniques have proved to be an excellent tool for qualitative characterization and quantitative determination of various food components because of their high sensitivity and specificity. In this part, we briefly discuss the application of MS, mainly coupled to chromatographic techniques (GC–MS, LC–MS), in the analysis of the most important natural and contaminant substances in food. It should be noted, however, that the sample preparation practice plays a crucial role in obtaining required parameters of particular analytical method. Not only the method performance characteristics such as detection limits, accuracy and ruggedness, but also its speed, labor demands, and (consequently) the cost of an analytical procedure depend on selected extraction and cleanup strategy and their efficiency.

10.3.1 Natural Substances in Food

Lipids. The applicability of both LC–APCI-MS and LC–APPI-MS has been successfully evaluated in the analysis of mixtures of neutral lipids, such as triacylglycerols and sterols. Those large neutral molecules are typically troublesome in obtaining the structural characterization by using other methods, such as GC–MS and LC–ESI-MS [2,30]. For the analysis of polar lipids (e.g., phospholipids), both LC–ESI-MS and LC–APCI-MS are the methods of choice. Applications of GC–EI-MS to lipid analysis include mainly the characterization of acylglycerol and sterol fractions [2,30,31].

Peptides and proteins. The predominant analytical approaches applicable to protein food research (proteomics) involve MALDI-TOFMS and LC–ESI-MS. The MALDI-MS is useful for obtaining fingerprint of the protein composition of various food samples, thus, rapid and accurate evaluation of the authenticity is possible. The LC–ESI-MS employing TOF, represents a tool for accurate mass determination of proteins. In addition, LC–ESI-MS/MS (or MALDI-TOF/TOF) allows analysis of proteins for elucidation purposes as well as the characterization of low-molecular mass peptides [32].

Saccharides. MS techniques employing different ionization modes and the hyphenated techniques play an important role in the structural elucidation of sugars. MALDI-TOFMS represents a powerful tool for the characterization of polysaccharides in various food samples. LC–MS with fast atom bombardment (FAB) interface, although not so frequently used anymore, has been proved valuable for precise isotopic measurements for saccharides and for their structural characterization. For the

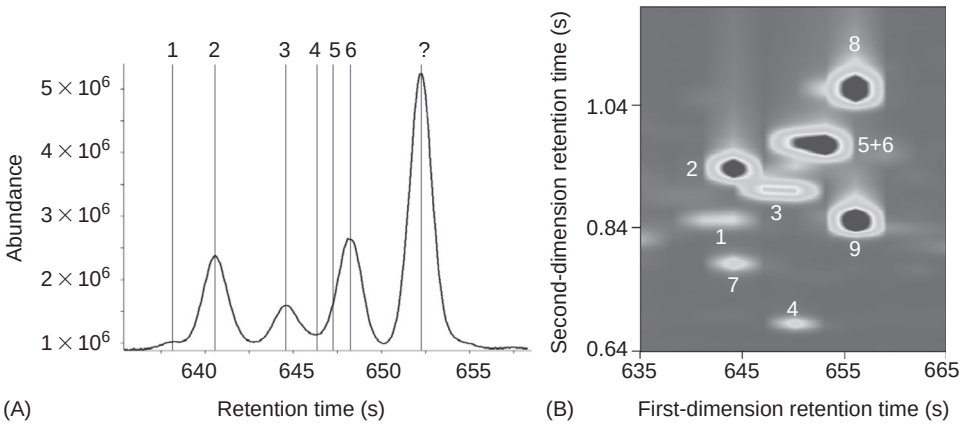

FIGURE 10.22 Comparison of separation of selected honey volatiles in two GC systems: (A) 1D-GC–TOFMS, and (B) GC×GC–TOFMS (TIC records shown). Marked compounds: (1) nonan-2-one, (2) linalool oxide, (3) dehydro-*p*-cymene, (4) undecane, (5) nonan-2-ol, (6) linalool, (7) terpinolen, (8) hotrienol, (9) nonanal; (?) complete co-elution of hotrienol and nonanal in 1D-GC system. Compound (7) not identified in 1D-GC. Compounds (5) and (6) partially co-eluted in GC×GC. (Reproduced from Cajka, T. et al., *J. Sep. Sci*., 30, 534, 2007. With permission.)

characterization of mono- and oligosaccharides by GC–MS, a derivatization procedure is required to remove hydrogen bonding [2].

Vitamins. Many HPLC-based methods that employ conventional detectors for detection of different groups of vitamins lack sensitivity and/or selectivity. The LC–MS technique with ESI and APCI (depending of the examined compounds) followed by their MS detection enables reliable identification/quantification of these nonvolatile, thermally labile biologically active compounds [2].

Aroma and flavor compounds. GC–MS has a considerable potential in the separation and characterization of food aroma and flavor compounds. The potential of this technique can be further increased by coupling with solid phase microextraction (SPME). This solvent-free, inexpensive sampling technique enables isolation of a wide range of analytes present in food crops and products by their extraction from its headspace and concentration in the fibre coating. During the last few years, GC–MS equipped with various analyzers, such as quadrupole and ion trap, allowed obtaining information on aroma and flavor compounds [33]. Recently, the application of high-speed TOF mass analyzer combined with comprehensive two-dimensional (GC × GC) has been used as a tool for rapid and comprehensive analysis of these compounds (Figure 10.22) [34,35].

Typically, EI is preferred for the characterization of the volatile fraction, since it allows library searching based on the EI mass spectra. CI is used mostly to confirm MW of the compounds of interest. It should be noted that under certain conditions, the use of retention indexes is important for identification/confirmation of aroma and flavor compounds.

10.3.2 Food Contaminants

Modern pesticides represent a group of compounds possessing a wide range of physico-chemical properties. With regards to this fact and considering a number of registered active ingredients (almost 1000), the analysis of multiple residues in various food matrices is obviously a difficult task [36]. Nowadays, both GC–MS and LC–MS/MS are of a growing popularity in this field. The types of MS instruments used for pesticide analysis include single mass analyzers (quadrupole, ion trap, and TOF) as well as hybrid instruments (triple quadrupole, quadrupole/time-of-flight). When employing SIM and MS/MS modes, settings of time segments are typically needed, which limits

the number of targeted analytes that can be detected in a particular time. The trade-off with SIM relates to the difficulty of identifying analytes due to fewer ions monitored and higher chance of matrix interferences as compared to MS/MS [37]. A recent progress in instrumentation design as well as the use of fast recording electronics together with improvements of signal processing techniques has led to rediscovery of TOF mass analyzers (both types, high-resolution and high-speed) for the determination of a wide range of pesticide residues [14,18,38]. Various ionization techniques are also possible in GC–MS; nevertheless, in the case of multiresidue analysis, EI is commonly preferred. CI (both PCI and NCI) as a softer ionization technique tends to give lower LODs depending on the pesticide, but it is not as widely applicable in multiclass pesticide methods and does not provide as much structural information about the analyte as EI. Regarding the LC–MS/MS, among the various interfacing systems developed during the past years, ESI and APCI have improved the feasibility of identification of pesticides of different chemical structures in foods at concentrations lower or comparable to those obtainable by GC–MS [39,40]. LC–MS/MS provides a complimentary tool to GC–MS for the analysis of more polar or thermolabile pesticides, which were previously analyzed by GC–MS with a various degree of difficulty or only after derivatization.

Polychlorinated biphenyls (*PCBs*). GC–MS represents a reliable technique for PCBs quantification, particularly given by the availability of ^{13}C-labeled PCB standards. Although the EI mode is often used, the NCI allows obtaining lower LODs of these analytes. Both single quadrupole (SIM) and ion trap (MS/MS) are frequently employed in the routine analysis of PCBs [41]. The use of high-resolution MS permits quantification of lower PCBs differing by two chlorines because of the high mass resolving power that allows unbiased measurement of ions. In addition, the application of both high-resolution and high-speed TOFMS (the latter in combination with GC×GC) represents a tool successfully applied in the analysis of PCBs [42,43].

Polychlorinated dibenzo-p-dioxins and dibenzofurans (*PCDDs/PCDFs*). Quantitative determination of PCDDs/PCDFs occurring in biotical matrices at ultra-trace levels is typically performed using GC coupled to high-resolution MS (sector analyzer) [43]. Although PCI/NCI techniques can be used for their determination, a majority of laboratories employ EI. High-resolution systems (mass resolving power of 10,000) provide higher selectivity compared to unit mass resolution instruments especially when the levels of potentially interfering compounds are too great. However, this instrumentation is very expensive, bulky, and requires operation by a highly trained specialist. Therefore, alternate analytical instruments (less expensive) have been investigated for dioxin analysis in several laboratories. GC–MS/MS employing ion trap analyser [44,45] and GC×GC–TOFMS [43,46] have been reported as a valuable technique for improved selectivity in dioxin analysis. In the case of GC–MS/MS, the high selectivity is obtained due to formation of characteristic dioxin fragment ions produced by the secondary ionization, while in GC×GC–TOFMS the improvement of selectivity is achieved employing the secondary column with different polarity that can better separate the target compounds from co-eluting matrix components.

Brominated flame retardants (*BFRs*) are represented by various groups of compounds, the most known being polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCD), and tetrabromobisphenol A (TBBPA). The determination of PBDEs is performed by GC–MS operated either in EI or NCI mode. The low-resolution MS is more routinely applied compared to the high-resolution MS that requires more experienced users and is much more costly and labor intensive [47]. The high-resolution MS (sector) has several advantages over low-resolution MS (e.g., increased sensitivity and selectivity), but is almost exclusively operated in EI mode. For low-resolution MS, NCI, in addition of EI, can be applied to obtain an increased sensitivity for higher-brominated BDE congeners. Recently, application potential of high-resolution TOFMS under NCI conditions in the analysis of PBDEs has been demonstrated [48]. The EI is preferred in the analysis of PBDEs, whenever the identification of mixed organohalogenated compounds

occurring in sample extracts has to be carried out. Another advantage of EI mode is the possibility to use ^{13}C-labelled internal standards. This is not applicable in NCI, since generally only the $[Br]^-$ ions (m/z 79 and 81) are monitored. The main benefits of NCI include efficient ionization, lower LODs, and less fragmentation compared with EI. Special attention has to be paid to analysis of BDE-209 (decabromodiphenyl ether). This high-molecular weight congener is sensitive for higher temperatures and the higher susceptibility for degradation in the GC system. For that reason, analysis of BDE-209 is typically carried out on a relatively short GC column (10–15 m) with a thin film of stationary phase (0.1–0.2 μm), which reduces the residence time of this congener [49].

Traditionally, analysis of HBCD has been carried out using GC–MS, typically in NCI mode by monitoring the $[Br]^-$ ions for a higher sensitivity. However, this technique does not allow quantification of individual diastereomers of HBCD (α-, β-, and γ-HBCD, each having two enantiomers) since they are not separated using common GC stationary phases; moreover, they undergo the interconversion above 160°C. Contrary to GC, reversed-phase LC–ESI-MS/MS or LC–APCI-MS/MS employing nonpolar (C_{18}, C_{30}) or chiral HPLC columns for their separation represent a versatile tool for the isomer-specific determination of HBCD isomers [49]. As regards tetrabromobisphenol A (TBBPA), acidification and derivatization are required before the GC–MS analysis, while LC–ESI-MS allows its direct determination [49].

Polycyclic aromatic hydrocarbons (*PAHs*). GC–EI-MS operated in SIM mode represents probably the most common GC technique for determination of PAHs in food matrices [50], although HPLC with a fluorescence detector (FLD) is also often routinely used. The problem encountered in the analysis of PAHs is separation of isomers and limited EI fragmentation, which does not allow reliable confirmation at ultra-trace levels. Alternatively, the LC–APCI-MS allows determination of PAHs without derivatization (post column), which is typically required in LC–ESI-MS [51]. The recently developed APPI enhances the ionization of the PAH analytes, thus, decreasing LODs [52].

Veterinary drug residues. In recent years, LC–MS-based strategies have been widely applied for determination and confirmation of veterinary drug residues in food samples. LC–MS revolutionizes this field, enabling quantitative and confirmatory multiresidue, multiclass analysis of veterinary drug residues, independent of their chemical structure or biological activity, thus replacing traditional single residue/class immunochemical or microbial methods [53]. The use of GC–MS-based methods is also possible, although their main limitation is the need of extensive cleanup and time-consuming derivatization procedures prior to their GC analysis since most of these compounds are relatively polar [2].

Mycotoxins. The GC–MS determination of mycotoxins is performed either by direct analysis or (mostly) employing various chemical derivatizations. The derivates can be detected selectively to very low levels using NCI, but also EI or PCI. Although quadrupole MS (SIM) is mostly used, the high-molecular weight of derivatized analytes predetermines their molecules for MS^n-measurements, especially in complex matrices like foodstuff and beverages. Recently, LC–ESI-MS/MS and LC–APCI-MS/MS methods, allowing the screening of mycotoxins of various classes within a single run, have been developed. These LC–MS-based methods represent a popular tool in mycotoxin analysis due to the improvement of sensitivity and avoiding extensive cleanup procedures and derivatization steps compared to GC–MS methods [54–56].

10.3.3 Processing Contaminants

Acrylamide. GC–MS methods employing either quadrupole or ion trap/triple quadrupole (tandem MS) can be used in EI or CI mode for the analysis of acrylamide in foods. Acrylamide is often derivatized (brominated) prior to the GC–MS analysis. A direct GC–MS determination without a derivatization step is also possible when a suitable sample preparation method is employed removing acrylamide precursors (asparagine and reducing sugars) from the analyzed sample, thus

preventing potential acrylamide formation from these precursors during the GC determinative step [57,58]. Alternatively, HRMS (sector or TOF) instruments are applied in the acrylamide analysis, since due to the high mass resolution power interfering compounds (chemical noise) with ions close to those of acrylamide can be significantly reduced. In this way improvement of S/N ratio can be effectively achieved. Besides GC–MS, recent studies pay more attention to methods employing LC–ESI-MS/MS techniques with triple quadrupole for the routine analysis because this instrumental technique applied for the quantitative analysis of acrylamide has high sensitivity and avoids potential problems with acrylamide formation that may occur in the direct GC–MS analysis [59].

Chloropropanols. The analysis of 1,3-dichloropropan-2-ol (1,3-DCP), 3-monochloropropane-1,2-diol (3-MCPD), and 2-monochloropropane-1,2-diol (2-MCPD) is based on GC–MS after their previous derivatization. Quadrupole mass analyser operated in SIM mode or ion trap analyser in MS/MS are typically used for their detection [60,61].

Heterocyclic amines. Determination of these mutagenic and carcinogenic substances classified as the IQ type (aminoimidazoazaarenes) and non-IQ type (pyrolytic heterocyclic amines) is based on the GC–MS methods. However, since most of these compounds are polar and nonvolatile, a derivatization step is usually performed before their analysis. This step can be effectively avoided using LC–MS with a single quadrupole or HRTOFMS, which allows selective and sensitive detection. The selectivity can be further enhanced by using LC–MS/MS with either ion trap or triple quadrupole MS instruments. Both ESI and APCI can be used for ionization [62,63].

10.3.4 Migrants from Packaging

Phthalate and adipate esters. GC–MS represents the key method for the determination of these packaging contaminants [64].

Epoxy compounds. The most widely used technique for the analysis of bisphenol A diglycidyl ether (BADGE), bisphenol F diglycidyl ether (BFDGE), and their hydrolyzed and chlorinated derivates is reversed-phase HPLC coupled to FLD. Since this is not a confirmatory technique, GC–MS methods were developed to confirm the presence of these compounds. However, this technique requires a derivation step. The use of HPLC methods with either APCI or ESI for the identification of these substances has been recently reported [65].

10.4 CONCLUSION AND FUTURE TRENDS

Over the past few years, there has been a substantial progress in technologies employing the MS technique in food analysis. In this context, a wide range of analytical methods involving both GC–MS and LC–MS(/MS) have been reported to detect, identify, quantify, and confirm various naturally occurring as well as xenobiotic substances in food chain. The most important developments in MS include the implementation of new ion sources and also mass analyzers. The multimode (online) LC-sources with simultaneous ESI and APCI ion generation provide new possibilities in terms of obtaining more structured information about the analyzed samples within a single run. The multimode GC sources applicable for EI and CI (not operated simultaneously) without a need for instrument's hardware modification may allow confirmation of analytes that can be troublesome under EI conditions. A distinctive trend in GC–MS analysis involves the rapidly growing interest in the applications that employ GC–TOFMS for both target and nontarget analysis of many classes of (semi)volatile organic compounds occurring in food. The high-speed TOFMS instruments, that are ideal for combining with fast GC, GC×GC, and ultra-performance LC (UPLC) provide a powerful technique for the identification and quantification of a wide range of compounds present in complex food matrices. On the other hand, the high-resolution TOFMS instruments allow also accurate mass measurements, which can be appreciated for calculation of analyte elemental

composition for the identification of ''unknown'' compounds and/or confirmation target analyte identity. It can be expected that in the near future some GC–TOFMS instruments will replace mass analyzers such as sector since the former ones are easier to operate and less costly. In LC–MS, both single (high-resolution TOFMS) and hybrid instruments (QqQ and Q-TOF) are becoming increasingly popular, mainly due to their enhanced selectivity and improved quantification and confirmation capabilities as compared to single quadrupole analyzers and ion traps, respectively.

Finally, the availability of sophisticated data systems and data-processing algorithms enabling automated and faster data handling represents another challenge, important for implementation of MS technique into the routine use.

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REFERENCES

1. Thomson, J.J., Further experiments on positive rays, *Phil. Mag*., 24, 209, 1912.
2. Careri, M., Bianchi, F., and Corradini, C., Recent advances in the application of mass spectrometry in food-related analysis, *J. Chromatogr. A*, 970, 3, 2002.
3. Santos, F.J. and Galceran, M.T., Modern developments in gas chromatography–mass spectrometry-based environmental analysis, *J. Chromatogr. A*, 1000, 125, 2003.
4. Nunez, O., Moyano, E., and Galceran, M.T., LC–MS/MS analysis of organic toxics in food, *TrAC-Trend Anal. Chem*., 24, 683, 2005.
5. Hajslova, J. and Zrostlikova J., Matrix effects in (ultra)trace analysis of pesticide residues in food and biotic matrices, *J. Chromatogr. A*, 1000, 181, 2003.
6. Todoli, J.L. and Mermet, J.M., Sample introduction systems for the analysis of liquid microsamples by ICP-AES and ICP-MS, *Spectrochim. Acta B*, 61, 239, 2006.
7. Karas, M. and Hillenkamp, F., Laser desorption ionization of proteins with molecular masses exceeding 10,000 daltons, *Anal. Chem*., 60, 2299, 1988.
8. Laiko, V.V., Baldwin, M.A., and Burlingame, A.L., Atmospheric pressure matrix-assisted laser desorption/ionization mass spectrometry, *Anal. Chem*., 72, 652, 2000.
9. Takats, Z. et al., Mass spectrometry sampling under ambient conditions with desorption electrospray ionisation, *Science*, 306, 471, 2004.
10. Cotte-Rodriguez, I. et al., Desorption electrospray ionization of explosives on surfaces: Sensitivity and selectivity enhancement by reactive desorption electrospray ionization, *Anal. Chem*., 77, 6755, 2005.
11. Cody, R.B., Laramee, J.A., and Durst, H.D., Versatile new ion source for the analysis of materials in open air under ambient conditions, *Anal. Chem*., 77, 2297, 2005.
12. Mastovska, K. and Lehotay, S.J., Practical approaches to fast gas chromatography–mass spectrometry, *J. Chromatogr. A*, 1000, 153, 2003.
13. Mastovska, K., Instrumental aspects and application of (ultra)fast gas chromatography–mass spectrometry, in Niessen, W. (Ed.), *Encyclopedia of Mass Spectrometry*, Elsevier, Oxford, 2006, p. 73.
14. Cajka, T. and Hajslova, J., Gas chromatography–time-of-flight mass spectrometry in food analysis, *LC GC Eur*., 20, 25, 2007.
15. Murray, K.K. et al., Standard Definition of Terms relating to Mass Spectrometry (UIPAC Recommendations 2006). Available: http://www.iupac.org/reports/provisional/abstract06/murray_prs.pdf via the Internet. Accessed on Feb 1 2007.
16. Cooks, R.G. and Busch, K.L., Counting molecules by desorption ionization and mass spectrometry/mass spectrometry, *J. Chem. Edu*., 59, 926, 1982.
17. Hopfgartner, G. et al., Triple quadrupole linear ion trap mass spectrometer for the analysis of small molecules and macromolecules, *J. Mass Spec*., 39, 845, 2004.
18. Cajka, T. and Hajslova, J., Gas chromatography–high-resolution time-of-flight mass spectrometry in pesticide residue analysis: Advantages and limitations, *J. Chromatogr. A*, 1058, 251, 2004.

19. Guilhaus, M., Selby, D., and Mlynski, V., Orthogonal acceleration time-of-flight mass spectrometry, *Mass Spec. Rev*., 19, 65, 2000.
20. AMDIS Download Page. Available: http://chemdata.nist.gov/mass-spc/amdis/ via the Internet. Accessed on Feb 1 2007.
21. Scigelova, M. and Makarov, A., Orbitrap mass analyzer—overview and applications in proteomics, *Proteomics*, 6, 16, 2006.
22. Hu, Q. et al., The Orbitrap: A new mass spectrometer, *J. Mass Spec*., 40, 430, 2005.
23. Ferrer, I. and Thurman, E.M., Liquid chromatography/time-of-flight/mass spectrometry (LC/TOF/MS) for the analysis of emerging contaminants, *TrAC-Trend Anal. Chem*., 22, 750, 2003.
24. Guevremont, R., High-field asymmetric waveform ion mobility spectrometry: A new tool for mass spectrometry, *J. Chromatogr. A*, 1058, 3, 2004.
25. Guevremont, R. et al., Ion trapping at atmospheric pressure (760 Torr) and room temperature with a high-field asymmetric waveform ion mobility spectrometer, *Int. J. Mass Spectrom*., 193, 45, 1999.
26. Amirav, A., Gordin, A., and Tzanani, N., Supersonic gas chromatography/mass spectrometry, *Rapid Comm. Mass Spec*., 15, 811, 2001.
27. Fialkov, A.B. et al., Sensitivity and noise in GC–MS: Achieving low limits of detection for difficult analytes, *Int. J. Mass Spec*., 260, 31, 2007.
28. Stoeckli, M. et al., Imaging mass spectrometry: A new technology for the analysis of protein expression in mammalian tissues, *Nat. Med*., 7, 493, 2001.
29. Todd, P.J. et al., Organic ion imaging of biological tissue with secondary ion mass spectrometry and matrix-assisted laser desorption/ionisation, *J. Mass. Spec*., 36, 355, 2001.
30. Cai, S. and Syage, J.A., Atmospheric pressure photoionization mass spectrometry for analysis of fatty acid and acylglycerol lipids, *J. Chromatogr. A*, 1110, 15, 2006.
31. Canabate-Diaz, B. et al., Separation and determination of sterols in olive oil by HPLC-MS, *Food Chem*., 102, 593, 2007.
32. Careri, M. and Mangia, A., Analysis of food proteins and peptides by chromatography and mass spectrometry, *J. Chromatogr. A*, 1000, 609, 2003.
33. Kataoka, H., Lord, H.L., and Pawliszyn, J., Applications of solid-phase microextraction in food analysis, *J. Chromatogr. A*, 880, 35, 2000.
34. Adahchour, M. et al., Comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometric detection for the trace analysis of flavour compounds in food, *J. Chromatogr. A*, 1019, 157, 2003.
35. Cajka, T. et al., Solid phase microextraction–comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry for the analysis of honey volatiles, *J. Sep. Sci*., 30, 534, 2007.
36. Mastovska, K., Food & Nutritional Analysis: (q) Pesticide residues, in Worsfold, P., Townshend, A., and Poole, C., (Eds.), *Encyclopedia of Analytical Science*, Elsevier, Oxford, 2005, 251.
37. Lehotay, S.J. and Mastovska, K., Determination of pesticide residues, in Otles, S. (Ed.), *Methods of Analysis of Food Components and Additives*, Taylor & Francis, Boca Raton, 2005, p. 329.
38. Zrostlikova, J., Hajslova, J., and Cajka, T., Evaluation of two-dimensional gas chromatography–time-of-flight mass spectrometry for the determination of multiple pesticide residues in fruit, *J. Chromatogr. A*, 1019, 173, 2003.
39. Pico, Y. et al., Control of pesticide residues by liquid chromatography-mass spectrometry to ensure food safety, *Mass Spec. Rev*., 25, 917, 2006.
40. Alder, L. et al., Residue analysis of 500 high priority pesticides: Better by GC–MS or LC–MS/MS? *Mass Spec. Rev*., 25, 838, 2006.
41. Ahmed, F.E., Analysis of polychlorinated biphenyls in food products, *TrAC-Trend Anal. Chem*., 22, 170, 2003.
42. Dallüge, J., Roose, P., and Brinkman, U.A.Th., Evaluation of a high-resolution time-of-flight mass spectrometer for the gas chromatographic determination of selected environmental contaminants, *J. Chromatogr. A*, 970, 213, 2002.
43. Focant, J. et al., Comprehensive two-dimensional gas chromatography with isotope dilution time-of-flight mass spectrometry for the measurement of dioxins and polychlorinated biphenyls in foodstuffs: Comparison with other methods, *J. Chromatogr. A*, 1086, 45, 2005.
44. Eppe, G. et al., PTV-LV-GC/MS/MS as screening and complementary method to HRMS for the monitoring of dioxin levels in food and feed, *Talanta*, 63, 1135, 2004.

45. Loran, S. et al., Evaluation of GC-ion trap-MS/MS methodology for monitoring PCDD/Fs in infant formulas, *Chemosphere*, 67, 513, 2007.
46. Hoh, E., Mastovska, K., and Lehotay, S.J., Optimization of separation and detection conditions for comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry analysis of polychlorinated dibenzo-*p*-dioxins and dibenzofurans, *J. Chromatogr. A*, 1145, 210, 2007.
47. Covaci, A., Voorspoels, S., and de Boer, J., Determination of brominated flame retardants, with emphasis on polybrominated diphenyl ethers (PBDEs) in environmental and human samples—a review, *Environ. Int*., 29, 735, 2003.
48. Cajka, T. et al., Challenges of gas chromatography–high-resolution time-of-flight mass spectrometry for simultaneous analysis of polybrominated diphenyl ethers and other halogenated persistent organic pollutants in environmental samples, *J. Sep. Sci*., 28, 601, 2005.
49. Covaci, A. et al., Recent developments in the analysis of brominated flame retardants and brominated natural compounds, *J. Chromatogr. A*, 1153, 145, 2007.
50. Stolyhwo, A. and Sikorski, Z.E., Polycyclic aromatic hydrocarbons in smoked fish—a critical review, *Food Chem*., 91, 303, 2005.
51. Takino, M. et al., Determination of polycyclic aromatic hydrocarbons by liquid chromatography–electrospray ionization mass spectrometry using silver nitrate as a post-column reagent, *J. Chromatogr. A*, 928, 53, 2001.
52. Itoh, N., Aoyagi, Y., and Yarita, T., Optimization of the dopant for the trace determination of polycyclic aromatic hydrocarbons by liquid chromatography/dopant-assisted atmospheric-pressure photoionization/mass spectrometry, *J. Chromatogr. A,* 1131, 285, 2006.
53. Gentili, A., Perret, D., and Marchese, S., Liquid chromatography-tandem mass spectrometry for performing confirmatory analysis of veterinary drugs in animal-food products, *TrAC-Trend Anal. Chem*., 24, 705, 2005.
54. Nielsen, K.F. and Thrane, U., Fast methods for screening of trichothecenes in fungal cultures using gas chromatography–tandem mass spectrometry, *J. Chromatogr. A*, 929, 75, 2001.
55. Sforza, S., Dall'Asta, C., and Marchelli, R., Recent advances in mycotoxin determination in food and feed by hyphenated chromatographic techniques/mass spectrometry, *Mass Spec. Rev*., 25, 54, 2006.
56. Zöllner, P. and Mayer-Helm, B., Trace mycotoxin analysis in complex biological and food matrices by liquid chromatography–atmospheric pressure ionisation mass spectrometry, *J. Chromatogr. A*, 1136, 123, 2006.
57. Mastovska, K. and Lehotay, S.J., Rapid sample preparation method for liquid and/or gas chromatographic-mass spectrometric analysis of acrylamide in various food matrices, *J. Agric. Food Chem*., 54, 7001, 2006.
58. Dunovska, L. et al., Direct determination of acrylamide in food by gas chromatography–high-resolution time-of-flight mass spectrometry, *Anal. Chim. Acta*, 578, 234, 2006.
59. Zhang, Y., Zhang, G., and Zhang, Y., Occurrence and analytical methods of acrylamide in heat-treated foods: Review and recent developments, *J. Chromatogr. A*, 1075, 1, 2005.
60. Chung, W., Hui, K., and Cheng, S., Sensitive method for the determination of 1,3-dichloropropan-2-ol and 3-chloropropane-1,2-diol in soy sauce by capillary gas chromatography with mass spectrometric detection, *J. Chromatogr. A*, 952 185, 2002.
61. Hamlet, C.G. and Sutton, P.G., Determination of the chloropropanols, 3-chloro-1,2-propandiol and 2-chloro-1,3-propandiol, in hydrolysed vegetable proteins and seasonings by gas chromatography/ion trap tandem mass spectrometry, *Rapid Com. Mass Spec*., 11, 1417, 1997.
62. Barcelo-Barrachina, E. et al., Evaluation of reversed-phase columns for the analysis of heterocyclic aromatic amines by liquid chromatography—electrospray mass spectrometry, *J. Chromatogr. B*, 802, 45, 2004.
63. Toribio, F. et al., Ion-trap tandem mass spectrometry for the determination of heterocyclic amines in food, *J. Chromatogr. A*, 948, 267, 2002.
64. Balafas, D., Shaw, K.J., and Whitfield, F.B., Phthalate and adipate esters in Australian packaging materials, *Food Chem*., 65, 279, 1999.
65. Pardo, O. et al., Determination of bisphenol diglycidyl ether residues in canned foods by pressurized liquid extraction and liquid chromatography–tandem mass spectrometry, *J. Chromatogr. A*, 1107, 70, 2006.