Recent developments in gas chromatography–mass spectrometry for the detection of food chemical hazards

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1.1 The combination of gas chromatography and mass spectrometry

1.1.1 Introduction

Gas chromatography–mass spectrometry (GC–MS) is a synergistic combination of the high-resolution separation of the components of a mixture with selective and sensitive mass detection. The term “hyphenation” or “tandem” is widely used to describe the possible combination of two or more instrumental analytical methods in a single run. GC separates chemical mixtures into individual components while MS identifies/quantifies the components at a molecular level.

In 1957, Holmes and Morrell demonstrated the first coupling of GC with MS (Holmes and Morrell, 1957), shortly after the development of gas–liquid chromatography (James and Martin, 1952), and it became commercially available in 1967.

The coupling of a gas chromatograph and a mass spectrometer in GC–MS allows a much more accurate chemical identification than applying either technique alone (Figure 1.1). The role of the GC technique is to separate the components from a complex mixture and identify or quantify those components. In order to identify
the components, the retention characteristics of an unknown component/analyte are compared with those of reference materials determined under identical experimental conditions. Even if the retention characteristics of an unknown and a reference material are identical, the two compounds may not be the same, because there are so many compounds involved and this affects the specific retention characteristics.

MS can identify compounds with a high degree of confidence, but it may require a very pure sample or standard. If a compound is part of a complex mixture, the mass spectrum obtained will contain ions from all of the compounds in the mixture, resulting in the identification being challenging. The combination of the two processes allows specific compounds separated by GC to be introduced into the mass spectrometer which can then be identified. In particular, those compounds with similar or identical retention characteristics in GC having different molecular or fragment masses in MS can be differentiated.

### 1.1.2 Basic gas chromatography

GC is more than 60 years old (James and Martin, 1952) and is a unique and versatile technique. In its initial stages of development, it provided separation and quantitative analysis for sample components both volatile at the temperatures used to achieve separation and thermally stable (Martin and Synge, 1941). However, it has also been applied to a wide range of nonvolatile compounds that can be conveniently converted into volatile derivatives. As an analytical tool, GC can be used to separate and analyze gaseous, liquid, or solid samples in some instances. The techniques of derivatization or pyrolysis GC can be utilized if the sample to be analyzed is nonvolatile.
The key parts of a typical gas chromatograph include (Figure 1.2): a gas supply system to supply carrier gas as the mobile phase, an injection system to deliver sample or sample solution into the column, the chromatographic column where separation occurs, an oven as a thermostat for the column, a detector to detect the presence of the compounds in the gas stream as it leaves the column, and a data system to record and display the chromatogram (Eiceman, 2000).

The retention characteristics of specific compounds depend upon their boiling point and the specific interactions with the stationary phase. The carrier gas, for the most part, is the means of delivering compounds of a sample through the column, but the choice of possible gases is restricted. The primary property of a suitable gas that affects the chromatographic result is its flow rate. The stationary phase in the chromatographic column, considered to be the most important item in a gas chromatograph, is the primary determinant in retention and separation. It may be a porous polymeric adsorbent or a liquid deposited on an inert solid support material. An ideal stationary phase is selective and has different adsorptivities between different components, to ensure separation, and a wide range of operating temperatures. It has to be chemically stable and have a low vapor pressure at high operating temperatures. It is common to classify stationary phases according to the polarity.

1.1.3 Typical mass analyzers and MS detectors in GC–MS

MS employs ionization and mass analysis of compounds to determine the elemental composition of a sample and the masses of particles and of molecules, and for elucidating the chemical structures of molecules. A mass spectrometer includes an ionizer, an extraction system, a mass analyzer and a detector.

The ionizer converts some portion of the sample into ions under vacuum conditions. There are two well-accepted standard types of ionization techniques in GC–MS, namely electron impact ionization (EI) and chemical ionization (CI). EI is considered to be a hard ionization technique, the energy of the electrons being high enough to produce highly reproducible fragmentation patterns of small molecules. In contrast, CI, in which ions are formed due to reaction with a reagent gas, is a softer ionization technique and fewer fragments are formed. Moreover, since
the fragmentation pattern depends on the properties of the reagent gas, different structural information can be obtained from different reagent gases (Canellas et al., 2012).

The extraction system removes ions from the sample for transmission to the mass analyzer.

The mass analyzer is the component of the mass spectrometer that separates the fragment ions, which have different masses, according to mass-to-charge ratios (m/z) and outputs them to the detector, where they are detected and subsequently converted into a digital output. Different general types of mass analyzers, such as quadrupole, ion trap (IT), and time-of-flight (TOF), have been implemented for the coupling of GC and MS.

The quadrupole consists of four parallel poles, where oscillating electric fields are created to select a certain range of mass/charge ratios passed through the system at any time. A single-stage linear quadrupole mass analyzer can be considered as a mass filter and is referred to as a transmission quadrupole. A common variation of the transmission quadrupole is the triple-quadrupole mass spectrometer.

An IT analyzer uses a combination of electric or magnetic fields to trap ions in a field. According to its principles of operation, it can be specified as three-dimensional quadrupole ion traps (Paul and Steinwedel, 1953), quadrupole linear ion traps (Schwartz et al., 2002), Fourier transform ion cyclotron resonance (FT-ICR) (Marshall et al., 1998), and Orbitraps (Kingdon, 1923).

The TOF analyzer is based on the kinetic energy and velocity of the ions, and uses an electric field to accelerate the ions through a constant homogeneous electrostatic field and then measures the time over a known distance (Cotter, 1994). If the particles all have the same charge, the kinetic energies will be identical and their velocities will depend only on their masses. Lighter ions will reach the detector first.

A detector determines the value of an indicator quantity and thus provides data for calculating the abundances of each ion present.

1.1.4 New development in GC–MS and sample preparation

Although the GC technique today is mature, reproducible, and reliable, there are still some areas that need to achieve better accuracy, lower detection limits, and higher selectivity with faster and easier methods using more robust and highly versatile instruments.

In recent years, GC–MS coupled to other systems has been developed and is widely used efficiently in current GC–MS studies, such as GC coupled with tandem mass spectrometry, two-dimensional GC coupled with mass spectrometry, and fast-GC–MS.

Tandem mass spectrometry (MS/MS) refers to multiple steps of MS selection, with some form of ions subjected to two or more sequential stages of analysis
1.1 THE COMBINATION OF GAS CHROMATOGRAPHY AND MASS SPECTROMETRY

(Todd, 1991). GC coupled with tandem mass spectrometry (GC–MS/MS) can achieve higher selectivity, higher sensitivity, and lower detection limits, and is usually necessary for ultra-trace analysis.

Two-dimensional GC (GC×GC) refers to a type of chromatographic technique in which the compounds are sequentially separated by passing them through two different columns. The second column has a different stationary phase from the first column, so that compounds that are poorly separated in the first column may be completely separated in the second column. Alternatively, the two columns might run at different temperatures. GC×GC increases the peak capacity to accommodate all compounds in a sample that may contain several hundred or even thousands of constituents (Davis and Giddings, 1983). Hence it can significantly enhance the resolving power of a separation (Liu and Phillips, 1991).

Fast-GC–MS is a technique that increases the speed of analysis. Basically, fast-GC is accomplished by using a short column, smaller column film thickness, low carrier gas viscosity, and/or large column diameter while manipulating specific operating parameters, such as linear velocity and oven temperature ramp rates. However, the trade-off for increased speed is a poorer separation efficiency. With the high selectivity of MS detection, the full-scan mode reduces the reliance on the chromatographic separations and can lead to faster analysis times.

Low-pressure gas chromatography (LP-GC) is a fast chromatographic technique that involves the use of a relatively short (10 m) large-diameter column connected with a restriction capillary (0.1–0.25 mm diameter and of appropriate length) at the inlet end. In this manner, the larger diameter analytical column is operated under low pressure generated by the MS vacuum pump while the restriction precolumn provides a positive inlet pressure to allow standard GC injection techniques (de Zeeuw et al., 2002).

In order to deal with the analysis of a large number of compounds in complicated food samples, effective sample preparation is essential to achieve good analytical results (Núñez et al., 2012). In most cases, compounds in the matrix could affect the analysis of specific analytes. Hence a selective clean-up step may be required in order to minimize the effect of all these possible interferences. Solid-phase extraction (SPE) and liquid–liquid extraction (LLE) are highly attractive and promising approaches for the purification, selective extraction, and enrichment of analytes in complex samples.

SPE is a separation process in which compounds are dissolved or suspended in a liquid phase (mobile phase) that is then passed through a solid phase (stationary phase) and separated into desired analytes and undesired interferences. LLE, also known as solvent extraction, is a method of separating compounds according to their relative solubilities in two different immiscible liquids phases, usually water and an organic solvent. It involves the extraction of a substance from one liquid phase into another liquid phase.
1.2 Analysis of pesticide residues in foods

The analysis of pesticides in food samples continually attracts considerable attention because of concerns about the possible long-term effects of human and animal exposure to even low levels of pesticides. Monitoring multiple pesticide residues is one of the most important aspects in minimizing potential hazards to human health from food contamination and from the standpoint of consumer safety.

As a result, analysts are beginning to focus on health-based residue limits for contaminants, and detection and quantitation at extremely low levels are essential. Following solvent extraction of a food sample, background matrix components are present at levels much higher than those of the targeted pesticide compounds, affecting the detection of trace levels of the pesticide residues. This necessitates improvements in the extraction, clean up and detection of pesticides in food samples. GC–MS can be used to achieve the selective detection of targeted pesticide components in the presence of the complex matrix (Schachterle et al., 1994).

1.2.1 Sample preparation

In recent years, analysts have emphasized the need for the development and use of analytical methods that are able to determine many pesticide residues in food products, such as insecticides, fungicides, and other biochemical compounds applied in agricultural practice. Because of the complex matrix of food samples, a wide variety of interfering compounds have to be eliminated by means of time-consuming clean-up procedures. A variety of capillary GC–MS-based methods have been developed for the analysis of multiple pesticide residues.

Nguyen et al. (2010) selected the LLE technique for the routine analysis of pesticide residues in soybean oil. After LLE, the co-extracted fat in the organic solvent was reduced by centrifugation based on the difference in the masses of the soybean oil and of the extraction solvent. Next, owing to the significant difference between the melting points of fat (below 40 °C) and studied pesticides (normally above 250 °C), the co-extracted fat can be separated from the pesticides by freezing. The co-extracted fat in the organic extract was precipitated in the frozen form at −20 °C in a freezer, whereas the pesticides remained dissolved in the cold organic solvent. Thus, the frozen co-extracted fat could be easily removed by centrifugation. The centrifugation and freezing pre-clean-up step dramatically helped reduce the mass of co-extracted fat in the extract after LLE by more than 94%. Although the mass of the remaining fat in the extract was still far from the criterion of a fat residue less than 0.25 mg g⁻¹ required for GC analysis, this pre-clean-up step helped reduce the use of an extra amount of sorbent in further dispersive SPE clean-up. Dispersive SPE clean-up on Florisil gave the cleanest chromatogram from the extract according to the GC–MS trace and the highest mean recoveries from 82 to 107%, with good relative standard deviation (RSD) from 2 to 15% for all the target pesticides in comparison with dispersive SPE clean-up on PSA (primary secondary amine), C₁₈, and
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bulk PSA and Florisil. Hence Florisil was selected as the sorbent for the dispersive SPE clean-up procedure. The recoveries obtained ranged from 80 to 114% and the RSDs were from 2 to 14% for spiking levels of 0.040, 0.080, and 0.160 mg kg\(^{-1}\), respectively. The limits of quantification (LOQs) of almost all the compounds were below the maximum residue limits (MRLs) established by the Korean legislation for soybean oil.

de Pinho et al. (2010a) optimized a simple and practical method for the identification and quantification of pesticides (chlorpyrifos, \(\lambda\)-cyhalothrin, cypermethrin, and deltamethrin) in honey samples. The method was based on liquid–liquid extraction and low-temperature purification using acetonitrile–ethyl acetate (6.5 mL + 1.5 mL) as the solvent for extraction. A final clean-up step with 2 g of Florisil was performed before analysis by GC with electron-capture detection (ECD). The technique was validated satisfactorily with an efficiency exceeding 85% and a linear chromatographic response for the tested pesticides, ranging from 0.033 to 1.7 \(\mu\)g g\(^{-1}\) with correlation coefficients higher than 0.99. Detection and quantification limits were lower than 0.016 and 0.032 \(\mu\)g g\(^{-1}\), respectively. The proposed method was applied to 11 honey samples. Chlorpyrifos and \(\lambda\)-cyhalothrin residues were found in two samples at concentrations below the established MRLs. The presence of these compounds was confirmed by MS in the selected ion monitoring (SIM) mode (GC–MS–SIM).

de Pinho et al. (2010b) also developed a simple and inexpensive method based on solid–liquid extraction with low-temperature purification (SLE-LTP) to determine chlorpyrifos, \(\lambda\)-cyhalothrin, cypermethrin and deltamethrin in tomato samples. The extraction technique was based on the partitioning of analytes between a solid matrix and a water-miscible organic phase at room temperature (25 °C). By decreasing the temperature to 20 °C, the liquid phase solidified, entrapping the solid matrix, and the liquid supernatant consisted of organic solvent containing the analytes. The advantage of this technique is that it is possible to obtain relatively pure extracts that can be directly analyzed by GC in a single step with no need for clean-up. SLE-LTP was applied to the analysis of pesticides in tomatoes, showing good efficiency (recovery rate above 79%) and other characteristics of interest. Compared with matrix solid-phase dispersion (MSPD), the extracts were always purer and of less intense color because of the carotenoids. Small changes in the polarity of the extraction mixture could be successfully adapted to the extraction of other pesticides. These characteristics make the extraction technique promising for other matrices and pesticides.

Qu et al. (2010) developed a rapid, economical, and efficient method for the determination of the residues of 20 organophosphorus pesticides in leeks. The amount of the analyzed sample was decreased to 3.0 g to reduce the level of chlorophyll in the extraction. The leek samples were heated in a microwave oven for 10 s to inactivate enzymes. Acetic acid (HAc) (0.1%) was added with acetonitrile as the extraction solvent to prevent the target compounds from degrading. HAc was also used to prepare a buffer solution with sodium acetate to regulate the pH at 6–7. The extracts were cleaned up by dispersive solid-phase extraction using PSA and graphitic carbon black to remove polar matrix components. The authors considered that the method could effectively decrease the chlorophyll in the final extract and protect
the chromatographic system. Moreover, compared with other analytical methods described for the detection of pesticide residues in leeks, this proposed method is easy, convenient, time saving, and economical. This approach can be applied to other leafy green vegetables containing large amounts of chlorophyll. Moreover, the selectivity of the triplequadrupole analyzer was clearly demonstrated in this investigation and was validated to be efficient in the determination of organophosphorus pesticides in vegetables with sulfur-containing compounds.

Sandra et al. (2003) developed the method of stir-bar sorptive extraction (SBSE) for the enrichment of pesticides from vegetables, fruits, and baby foods. A sample of ~15 g of a vegetable, fruit, or baby food was accurately weighed into a 100 mL flask and 30 mL of methanol were added. The mixture was homogenized using an Ultra-Turrax mixer for 5 min and the flask was then placed in an ultrasonic bath for 15 min. A fraction (~10 mL) of the blend was placed in a closed 20 mL vial and centrifuged for 5 min at 5000 rpm. A 1 mL volume of the supernatant methanol phase was placed in a 20 mL headspace vial and 10 mL of HPLC (high-performance liquid chromatography)-grade water were added. An SBSE stir bar, 10 mm long and coated with a 0.5 mm PDMS layer (24 mL), was added and the mixture stirred for 60 min at 1000 rpm. After sampling, the stir bar was removed with tweezers, dipped briefly in doubly distilled water, placed on lint-free tissue to remove residual droplets, and finally placed in the liner of a thermal desorption system. For quantification, 5 μL of the appropriate pesticide standard solutions in methanol were added to the sample before Ultra-Turrax mixing and ultrasonic treatment.

García-Rodríguez et al. (2012) developed a matrix solid-phase dispersion (MSPD)-GC–MS method for the determination of multiple residues of pesticides in seaweed samples. This method was fast because the on-column clean-up step was performed immediately after extraction. The method used anhydrous sodium sulfate as dispersant with Florisil and graphitized carbon black as clean-up adsorbents and of hexane–ethyl acetate (3:2) mixture as eluent. It was validated for different seaweed matrices and provided good selectivity, accuracy, precision, and sensitivity. The identification and quantification 17 pesticides at low concentration levels were feasible and the LOQs obtained were lower than the MRLs established by European legislation. The method was successfully applied to the analysis of pesticides in edible and wild seaweed samples from the Galician coast (NW Spain). The method was then applied to the analysis of 11 seaweed samples, of which 10 showed the presence of at least one pesticide at concentrations from below the LOQ to 3.6 ng g⁻¹. 2-Phenylphenol and three pyrethroid pesticides (tetramethrin, cypermethrin, and empenthrin) were detected and the results showed that there were very low concentration levels. Comparison of the performance of this method with that of a previously developed method using microwave-assisted extraction (MAE) large-volume injection (LVI) GC–MS/MS showed similar recovery ranges for the analytes and good correlation with the results from 11 seaweed samples treated and analyzed by the two methods.

Mezcua et al. (2009) described for the first time the application of various analytical methodologies to facilitate appropriate selection in the combined determination
of the two important substances isocarbophos and isofenphos-methyl in peppers at concentrations in the low-g kg\(^{-1}\) range. The proposed methods consisted of a sample treatment step based upon QuEChERS (quick, easy, cheap, effective, rugged, and safe) extraction method followed by quantitative analyses by GC–MS and GC–MS/MS. The proposed methodologies involved a liquid–liquid extraction with acetonitrile followed by a clean-up step with dispersive solid-phase extraction using PSA as the sorbent material. Recovery studies performed on peppers spiked at different levels (10 and 50 g kg\(^{-1}\)) yielded average recoveries in the range 85–98% with RSD values below 8%.

Georgakopoulos et al. (2011) used a QuEChERS method to determine 0.01–0.2 mg kg\(^{-1}\) of phorate, diazinon, chlorpyrifos, and methidathion in three low-fat baby food matrices. The quantities of octadecyl (C\(_{18}\)) sorbent used differed with fortification levels and matrix fat, based upon a central composite experimental design (CCD). A CCD for two independent variables (fortification level and amount of C\(_{18}\) sorbent) each at two levels with five star points, three replicates, and five center points was employed to fit a polynomial model (response surface). Quantification was performed by nitrogen–phosphorus detection (NPD) coupled with GC, using matrix-matched standards. The highest (\(p < 0.05\)) recoveries were observed for methidathion, the lowest fortification levels for a specific amount of C\(_{18}\) and the lowest amounts of C\(_{18}\). In meals containing vegetables (1.9% fat) and lamb (3.0% fat), 180–210 mg of C\(_{18}\) provided recoveries from 67 to 105% and absence of co-extracts. Yogurt dessert (4.5% fat) required 200–230 mg of C\(_{18}\) for similar results. Recoveries could also be predicted with <20% error by a polynomial model. The results suggested that modified QuEChERS could be effectively used in the analysis of pesticide residues in low-fat baby meals.

Yu et al. (2010) developed a new method to determine and confirm amitraz and its main metabolite, 2,4-dimethylaniline, in food animal tissues using GC–ECD and GC–MS. This method was based on a new extraction procedure using accelerated solvent extraction (ASE). It consisted of an n-hexane–methanol extraction step, a clean-up step using a BakerBond octadecyl C\(_{18}\) silica-bonded cartridge, hydrolysis, and derivatization to 2,4-dimethyl-7-fluorobutyramide for GC–ECD analysis. For confirmation using GC–MS, hydrolysis and derivatization were not needed. The parameters for the extraction pressure, temperature and cycle of ASE, clean-up, derivatization, and the analysis procedure were optimized. Spike recoveries from 50–300 \(\mu\)g kg\(^{-1}\) levels were found to be between 72.4 and 101.3% with RSDs less than 11.5% in GC–ECD, from 5 to 20 \(\mu\)g kg\(^{-1}\) levels were found to be between 77.4 and 107.1% with relative standard deviation less than 11.6% in GC–MS. The limit of detection (LOD) and LOQ were 2 and 5 \(\mu\)g kg\(^{-1}\), respectively. This rapid and reliable method can be used for the characterization and quantification of residues of amitraz and its main metabolite, 2,4-dimethylaniline, in swine, sheep and bovine liver and kidney samples.

Viñas et al. (2009) described a new solvent-free method for the sensitive determination of seven strobilurin fungicides (azoxystrobin, metominostrobin, kresoxim-methyl, trifloxystrobin, picoxystrobin, dimoxystrobin, and
pyraclostrobin) in baby food samples. Direct immersion solid-phase microextraction (DI-SPME) coupled to GC–MS-SIM, was employed. All analyses were performed with 2 g of sample, 14 mL of sample extract, and sample extract buffered at pH 5. Optimal extraction conditions were 60 °C for 40 min under continuous stirring using a polydimethylsiloxane–divinylbenzene fiber. Desorption was carried out at 240 °C for 4 min. The standard additions method was recommended and quantitation limits ranged from 0.01 to 0.4 ng g⁻¹ at a signal-to-noise ratio (S/N) of 10, depending on the compound. Recoveries obtained for spiked samples were satisfactory for all of the compounds. The method was validated according to the European Commission Decision 2002/657/EC. Different baby foods were analyzed by the proposed method and none of the samples contained residues above the detection limits.

Examples of the application of sample preparation methods with GC–MS analyses are presented in Table 1.1.

### 1.2.2 Development of GC–MS methods for the determination of pesticides in foods

**GC–MS**

Laboratory manuals published by different agencies (FDA, 1994; General Inspectorate for Health Protection, 1996) and reviews on this general area of analysis (e.g., Holland and Malcolm, 1992) showed that most pesticide residue analyses are performed using multi-residue methods involving solvent extraction, clean-up, determination by GC, and confirmation by MS.

The major analytical method used in the Swedish monitoring of pesticide residues in fruits and vegetables is a capillary GC multi-residue method, developed by the National Food Administration (NFA), in which samples were prepared for analysis by ethyl acetate extraction and gel permeation chromatography (GPC) clean-up. With this method, it was possible to detect, quantify, and confirm residues of more

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<td>Example</td>
<td>Reference</td>
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<td>LLE</td>
<td>de Pinho et al., 2010a; Nguyen et al., 2010</td>
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<td>SPE</td>
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<td>SLE-LTP</td>
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<td>SBSE</td>
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<td>MSPD</td>
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<td>QuEChERS</td>
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<td>ASE</td>
<td>Yu et al., 2010</td>
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<td>DI-SPME</td>
<td>Viñas et al., 2009</td>
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than 160 pesticides (Andersson and Bergh, 1991) with the routine confirmation process carried out by using GC–MS.

Valverde et al. (1997) assessed the NFA GC multi-residue method to determine residues of pyraclofos in fruits and vegetables. The relative retention times of pyraclofos in the OV-1701–FPD (flame photometric detector) and SE-30–TSD (thermionic specific detector) column-detector systems used for GC analysis (using parathion as reference) were 2.11 and 2.04, respectively. The corresponding relative response factors were 0.34 and 0.40. The mass spectrum obtained for pyraclofos using a PAS-1701 column GC–MS system suggested the use of the peaks at \( m/z \) 360 (molecular and base peak), 194, and 139 to confirm trace levels of pyraclofos by GC–MS-SIM. Recovery values obtained with the complete NFA GC multi-residue method for pyraclofos in tomato and strawberry samples spiked at levels of 0.05 and 0.40 mg kg\(^{-1}\) ranged from 72 to 111%. The LOQ of pyraclofos was determined as 0.05 mg kg\(^{-1}\), but levels of 0.02 mg kg\(^{-1}\) could be determined from the SE-30–TSD chromatographic response.

Vukovic et al. (2012) developed an LC–GC technique combined with electro-spray ionization (ESI), (LC–MS/MS) and GC–MS technique for the detection of 50 pesticides in baby foods. The QuEChERS method was used as a sample preparation procedure. The recoveries were investigated at three spiking levels (5, 10, and 50 \( \mu \)g kg\(^{-1}\)) and the results obtained showed compliance with current European Union (EU) requirements with a few exceptions. The LOQs for most of the pesticides tested were below the EU MRL (10 \( \mu \)g kg\(^{-1}\)), except for deltamethrin, cypermethrin, fenvalerate, phosalone, and beta-cyfluthrin (LOQs were 10 \( \mu \)g kg\(^{-1}\)). Both techniques were applied in the analysis of 50 samples of baby foods manufactured in Serbia.

**GC–TOF-MS**

de Koning et al. (2003) discussed a procedure for the fully automated analysis of food samples by means of difficult matrix introduction gas chromatography–time-of-flight mass spectrometry (DMI-GC–TOF-MS). After extraction, the samples required very little clean-up and were injected in a micro-vial that was held in a liner. The liner was then placed in the injector and the contents of the vial were thermally desorbed and led directly to the capillary GC column. Vials containing fruit extracts were placed in the sample tray of a sample preparation robot and a direct thermal desorption (DTD) liner containing a micro-vial was placed in the liner tray. The robot subsequently took an aliquot of an extract from the sample vial and introduced it into the micro-vial. Then, the injector was opened automatically and the DTD liner was put into place. After closure of the injector head, the sample solvent was vented. Then, the compounds were thermally desorbed from the micro-vial and transferred to the capillary GC column. After GC–TOF-MS analysis, the data were processed automatically using a peak deconvolution algorithm. The practicability of the procedure was demonstrated by analyzing spiked grape and pineapple samples down to the 1–10 ng g\(^{-1}\) concentration level.
Walorczyk and Gnusowski (2006) demonstrated that low-pressure (LP) GC coupled with triple-quadrupole Ms can be an efficient analytical tool for the rapid identification and quantification of pesticide residues in vegetables. Under the optimized conditions, the analysis time was reduced to 13.3 min with the LP-GC approach, which corresponded to an almost threefold gain in speed compared with conventional GC (37 min).

The authors also concluded that the LP-GC technique is generally superior to conventional GC with respect to linearity, accuracy, and precision. Additionally, lower LODs were achievable using LP-GC combined with MS in all three data acquisition modes (scan, SIM, and MS/MS). This allowed the presumption that LP-GC-based methods (especially those utilizing the highly sensitive and specific MS/MS detection mode) might be of practical value in application areas requiring reliable determinations at very low concentration levels, such as the determination of pesticides in baby foods.

Maštovská et al. (2001) developed a fast method for analyzing 20 representative pesticides by using LP-GC–MS. No special techniques for injection or detection with a common quadrupole GC–MS instrument were required for this approach. The LP-GC–MS technique used a 10 m × 0.53 mm i.d. analytical column with 1 μm film thickness coupled with a 3 m × 0.15 mm i.d. restriction capillary at the inlet end. Thus, the conditions at the injector were similar to those in conventional GC methods, but sub-atmospheric pressure conditions prevailed throughout the analytical column (MS provided the vacuum source). Optimal LP-GC–MS conditions were determined that achieved the fastest separation with the highest signal-to-noise ratio in MS detection (SIM mode). Owing to the faster flow rate, thicker film, and lower pressure in the analytical column, this distinctive approach provided several benefits to the analysis of the representative pesticides versus a conventional GC–MS method, which included: (i) a threefold gain in the speed of chromatographic analysis; (ii) substantially increased injection volume capacity in toluene; (iii) heightened peaks with 2 s peak widths for normal MS operation; (iv) reduced thermal degradation of thermally labile analytes, such as carbamates; and (v) owing to the larger sample loadability, lower detection limits for compounds not limited by matrix interferences. The optimized LP-GC–MS conditions were evaluated in ruggedness testing experiments involving repetitive analyses of the 20 diverse pesticides spiked in a representative food extract (carrot), and the results were compared with those of the conventional GC–MS approach. The matrix interferences for the analyte ions were worse for a few pesticides (acephate, methiocarb, dimethoate, and thiabendazole) in LP-GC–MS, but similar or better results were achieved for the other 16 analytes, and the sample throughput was more than doubled by using this approach.

Maštovská et al. (2004) optimized the operating parameters and evaluated the performance characteristics of LP-GC–MS for the analysis of multiple pesticide residues in food crops. Two LP-GC–MS column configurations, A and B, were
tested in various experiments. These two configurations employed the same restriction capillary at the inlet end, but different analytical columns attached to the vacuum provided by MS. In addition to the pesticide solutions prepared in the solvent used for initial optimization and evaluation of speed, separation efficiency, and peak characteristics, the LP-GC–MS systems were subjected to tests involving repeated injections of pesticides in matrix extracts.

Arrebola et al. (2003) developed an LP-GC–MS/MS method after a fast and simple extraction of vegetable samples with dichloromethane and without clean-up. The use of this GC technique reduced the total time required to determine 72 pesticides to less than half of the usual time (31 min), increasing the capability of a routine laboratory. The use of a guard column and a plug of CarboFrit in the glass liner in combination with LP-GC was evaluated. The method was validated with LOQs low enough to determine the pesticide residues at concentrations below the MRLs stated by legislation. In order to assess its applicability to the analysis of real samples, 25 vegetable samples previously analyzed using conventional capillary GC–MS/MS were analyzed using the LP-GC–MS/MS method. The results obtained by the two techniques showed differences of less than 0.01 mg kg\(^{-1}\).

Hercegová et al. (2005) studied a sample preparation method based on single solvent phase extraction and solid-phase extraction (SPE-NH\(_2\)) clean-up in combination with fast capillary GC to determine 18 selected pesticides belonging to various chemical classes in apples, the common raw material for baby food production, and baby food, at concentration levels ≤10 μg kg\(^{-1}\) (MRL). The possibilities of using MS detection and ECD in the fast-GC of samples with complex matrices at ultra-trace levels of pesticide residues were studied and compared. MS detection in the SIM mode provided higher selectivity than ECD. Optimization of extraction and the simplification of the whole process of sample preparation was carried out. The recoveries obtained at a concentration level of 5 μg kg\(^{-1}\) (the required value for the LOQ in baby food) with MS detection were >90%, except for dimethoate (77.7%) and captan (46.4%). For the majority of compounds, the LOQs obtained were at least one order of magnitude lower than 5 μg kg\(^{-1}\). The reproducibility of GC–MS measurements of matrix-matched standards expressed as RSD was <11%, except for captan and cypermethrin.

**GC–MS/MS**

GC coupled to MS can be used to achieve the selective detection of targeted pesticide components in the presence of a complex matrix. However, the detection limits attainable are set by the levels of interfering ions from the matrix, which can obscure the signal from the target compounds.

Tandem mass spectrometry (MS/MS) has been shown to be a valuable technique in the analysis of pesticide residues in complex matrices, with increased selectivity, excellent LODs, and improved signal-to-noise ratios (Haib et al., 2003; Vidal et al., 2004; Patel et al., 2005).
Schachterle et al. (1994) demonstrated excellent sensitivity, a wide dynamic range, and effective elimination of the chemical background. GC–MS/MS was compared with standard GC–EI-MS and GC–CI-MS techniques for the analysis of malathion in orange extract. GC–MS/MS is the most selective and sensitive technique, virtually eliminating the background interference. Product ion mass spectra were invariant across the chromatographic peak and excellent linearity was observed over a range from 2.2 pg μl\(^{-1}\) to 2.2 ng μl\(^{-1}\) for malathion. The detection limit achieved using GC–MS/MS was estimated as 0.5 μg kg\(^{-1}\) in the orange oil matrix (S/N = 5).

Walorczyk et al. (2006) combined fast-GC and MS/MS to determine pesticide residues in six tomato samples and demonstrated the applicability of the developed method for the analysis of real samples. LP-GC–MS experiments were performed on an HP-5 10 m × 0.32 mm i.d. × 0.25 μm film thickness analytical column connected to a 2.5 m × 0.15 mm i.d. uncoated restriction precolumn at the inlet end. For comparison purposes, conventional GC–MS analysis was performed on an RTX-5 30 m × 0.25 mm i.d. × 0.5 μm film thickness column. Under the optimized conditions, the analysis time was reduced to 13.3 min with the LP-GC approach, which corresponds to an almost threefold gain in speed versus conventional GC (37 min). Despite the poorer separation power of the LP-GC column, the experiments conducted with tomato and onion extracts spiked with 78 pesticides validated that LP-GC–MS was of practical value for performing full-scan screening analysis. Moreover, the rate of false-negative results was higher in the case of conventional GC–MS and LP-GC–MS permitted the correct identification of pesticides at lower levels since the peaks were improved in both size and shape. Validation experiments were performed on a sample of 12 categories of pesticides for comparison of the performance characteristics of the LP-GC and GC approaches with the mass spectrometer operated in scan, SIM, and MS/MS modes. The LP-GC column set-up interfaced to the MS detector was found to be superior to conventional GC with respect to the linearity, accuracy, and precision obtained. In addition, lower LODs in real extracts were achieved using the LP-GC approach.

Leandro et al. (2005) developed a GC–MS/MS method for the determination of 12 priority pesticides and transformation products (e.g., metabolites) specified in the EU Baby Food Directive 2003/13/EC. Prior to GC–MS/MS analysis, co-extractives were removed from the acetonitrile extracts using dispersive solid-phase extraction with octadecyl (200 mg) and PSA (50 mg) sorbents. The clean-up proved essential for the satisfactory long-term chromatographic performance during the analysis of a range of representative commercially preprepared baby food samples. Extracts spiked with pesticides at 1–8 μg kg\(^{-1}\) yielded average recoveries in the range 60–113% with RSDs of less than 28%.

Qu et al. (2010) developed a rapid multi-residue method for the determination of 20 organophosphorus pesticide residues in leeks by GC coupled to triple-quadrupole MS (GC–QqQMS/MS). Recoveries of the 20 organophosphorus pesticides ranged from 81.0 to 109.4% with RSDs below 10.4%. The LODs were 0.07–1.5 μg kg\(^{-1}\) and the LOQs ranged from 0.25 to 5 μg kg\(^{-1}\). Ten leek samples were analyzed for method validation.
Mezcua et al. (2009) described two analytical methods based on GC coupled with various MS analyzers (GC–MS and GC–MS/MS) for the identification, confirmation, and quantitation of two non-EU-authorized insecticides (isocarbophos and isofenphos-methyl) in pepper samples. No significant differences in the performances of the two methods were observed in terms of sensitivity and LOD, although the unambiguous confirmation capabilities provided by MS/MS could not be achieved with a single-quadrupole analyzer. It is clear that the information provided by the ion trap MS/MS method, a second-generation full-scan mass spectrum, exceeds that provided by a single-quadrupole SIM method. The potential of the proposed methods was tested by analyzing real samples, with excellent selectivity and sensitivity, thus allowing the unambiguous identification of trace levels of these insecticides in pepper samples.

MS/MS can be conceived in two ways: tandem in space (e.g., triple-quadrupole, QqQ) or in time (e.g., ion trap, IT). The QqQ and IT modes are commonly interfaced to GC; however, there has not been any direct comparison between them in the analysis of pesticide residues so far. Garrido Frenich et al. (2008) studied and compared the performance of GC coupled to these two analyzers (GC–QqQ-MS/MS and GC–IT-MS/MS) for the analysis of pesticide residues and its application in food analysis. The large-volume injection (LVI) technique together with programmed-temperature vaporization (PTV) was applied. For this purpose, 19 pesticides, including organochlorine and organophosphorus pesticides and pyrethroids, were analyzed in both systems. MS data, performance characteristics (linearity, intra-day and inter-day precision) and the influence of the nature of the matrix on the analysis of low concentrations of the analytes were compared. The target compounds were analyzed in a solvent and in two representative food matrices including cucumber (high water content) and egg (high fat content). The MS data and intra-day precision were similar in the QqQ and IT modes, but the inter-day precision was significantly worse in the QqQ mode. Linearity (expressed as the determination coefficient, $R^2$) in the range 10–150 μg L$^{-1}$ was adequate in both systems; however, better $R^2$ values were obtained with the QqQ analyzer in high and low concentration ranges (1–50 and 1–750 μg L$^{-1}$, respectively). The influence of the nature of the matrix on the analysis of low analyte concentrations for each analyzer was also evaluated. The performances of the QqQ and IT modes were similar in cucumber and solvent. However, the QqQ mode provided better sensitivity for egg samples performed with selected reaction monitoring (SRM).

**GC × GC–MS**

GC × GC–TOF-MS is a powerful separation and identification technique and is very suitable for the analysis of complex food samples containing hundreds or even thousands of GC-amenable compounds. When using GC × GC, the analytes of interest can be better separated from each other than using conventional one-dimensional (1D) GC. What is more important, a better separation could also be achieved for the analytes from interfering matrix compounds, and this tended to be a serious
problem in the 1D-GC–MS procedures. Consequently, the quality of the TOF-MS mass spectra obtained by GC×GC–MS is much better than that of spectra obtained with 1D-GC–MS, as illustrated in this study for a series of pesticides. The recording of two, rather than one, retention times and the excellent analyte detectability affected by the peak sharpening during modulation added to the attractiveness of GC×GC-based analyses. Therefore, GC×GC–TOF-MS can be used to simplify the complicated sample preparation procedures via analyte–matrix preseparation (Dallüge et al., 2002).

1.3 Analysis of contaminants formed during food processing

Contaminants can be formed in certain foods during their processing. They are generated by chemical reactions between compounds that are natural components of the food and/or a food additive that is intentionally added to the food. These reactions could generate potentially harmful compounds. The presence of processing-induced chemicals cannot be entirely avoided. Examples of food processing-induced chemicals include acrylamide, heterocyclic amines, furan, and polycyclic aromatic hydrocarbons.

1.3.1 Acrylamide

In April 2002, research groups from the University of Stockholm and the Swedish NFA reported the presence of acrylamide (2-propenamide) in a wide range of carbohydrate-rich fried or baked food samples (Swedish National Food Administration, 2002).

Acrylamide is formed during the Maillard reaction in starchy foodstuffs (Mottram et al., 2002; Stadler et al., 2002). It is a well-known compound that has the properties of neurotoxicity, genotoxicity, and potential human carcinogenicity (Tareke et al., 2002; Zhang, 2007). Therefore, the development of sensitive and reliable analytical methods for trace amounts of acrylamide in a variety of foods was indispensable. GC and LC are the main analytical techniques used to determine acrylamide. However, they are not appropriate for the determination of acrylamide at low μg kg⁻¹ levels. The development and validation of sensitive and reliable analytical methods for the quantification of low levels of acrylamide in different food matrices was considered to be essential (Joint Institute for Food Safety and Applied Nutrition (JIFSAN), 2002).

Pittet et al. (2004) developed a quantitative method for the determination of trace levels (<50 μg kg⁻¹) of acrylamide in cereal-based foods. The method is based on extraction of acrylamide with water, acidification and purification with Carrez I and II solutions, followed by bromination of the acrylamide double bond. The reaction
product (2,3-dibromopropionamide) was extracted with ethyl acetate–hexane (4:1 v/v), dried over sodium sulfate, and cleaned up through a Florisil column. The derivative was then converted to 2-bromopropenamide by dehydrobromination with triethylamine and analyzed by GC–MS, employing $^{13}$C$_3$acrylamide as internal standard. In-house validation data for commercial and experimental cereal products showed good precision of the method, with repeatability and intermediate reproducibility RSDs below 10%. The LOD and LOQ were 2 and 5 $\mu$g kg$^{-1}$, respectively, and recoveries of acrylamide from samples spiked at levels of 5–500 $\mu$g kg$^{-1}$ ranged between 93 and 104% after correction for analyte loss using an internal standard. Finally, a comparative test organized with two independent laboratories provided additional validation of the good performance of this method, particularly at very low concentration levels.

Lee et al. (2007a) developed a method to determine acrylamide in aqueous matrices by using DI-SPME coupled to gas chromatography–positive chemical ionization tandem mass spectrometry (GC–PCI-MS/MS) in the selected reaction monitoring (SRM) mode. The optimized SPME experimental procedures to extract acrylamide from aqueous solutions were the use of a Carbowax–divinylbenzene (CW–DVB)-coated fiber at pH 7, extraction time of 20 min and analyte desorption at 210 $^\circ$C for 3 min. A detection limit of 0.1 $\mu$g L$^{-1}$ was obtained.

Wenzl et al. (2006) validated two analytical procedures for the determination of acrylamide in bakery products (crispbreads, biscuits) and potato products (chips) in the concentration range 20–9000 $\mu$g kg$^{-1}$. The methods were based on GC–MS of the derivatized analyte and on HPLC–MS/MS of native acrylamide. Isotope dilution with isotopically labeled acrylamide was an integral part of both methods. The study was evaluated according to internationally accepted guidelines. The performance of the HPLC–MS/MS method was found to be superior to that of the GC–MS method and to be suited to the purpose of determining acrylamide in bakery and potato products.

Dunovská et al. (2006) developed and validated a simple and rapid GC method employing a high-resolution TOF mass analyzer that permits the direct analysis (no derivatization) of acrylamide in various heat-processed foods. Co-isolation of acrylamide precursors (e.g., sugars and asparagine) that constitute a risk of overestimation of the results due to additional formation of analyte in the hot GC injector was avoided by extraction with $n$-propanol followed by solvent exchange to acetonitrile (MeCN). Based on the addition of PSA sorbent to the defatted extract in MeCN, introduction of dispersive SPE as a novel purification strategy provided a significant reduction of some abundant matrix co-extracts (mainly free fatty acids). The isotope dilution technique (with acrylamide-$d_3$ as an internal standard) was employed to compensate potential target analyte losses and/or matrix-induced chromatographic response enhancement. The LOQs ranged between 15 and 40 $\mu$g kg$^{-1}$ and recoveries were between 97 and 108% depending on the food matrix examined. The repeatability of the measurements (expressed as RSD) was as low as 1.9% for potato crisps containing acrylamide at a level of 1 mg kg$^{-1}$. Slightly higher values (RSD < 4.0%)
were achieved for breakfast cereals and crisp bread with an ~10 times lower content of this processing contaminant.

Xu et al. (2013) developed a quantitative method for the simultaneous determination of 3-monochloropropane-1,2-diol (3-MCPD) and acrylamide in foods by GC–MS/MS. The analytes were purified and extracted using the matrix solid-phase dispersion extraction (MSPDE) technique with Extrelut NT. A coupled column system (3 m Innowax combined with 30 m DB-5 ms) was developed to separate the two compounds efficiently without derivatization. Triple-quadrupole MS in the multiple reaction monitoring (MRM) mode was applied to suppress matrix interferences and obtain good sensitivity in the determination of both analytes. The LOD in the sample matrix was 5 μg kg\(^{-1}\) for both 3-MCPD and acrylamide. The average recoveries of 3-MCPD and acrylamide in different food matrices were 90.5–107 and 81.9–95.7%, respectively, with RSDs of 5.6–13.5 and 5.3–13.4%, respectively. The inter-day RSDs were 6.1–12.6% for 3-MCPD and 5.0–12.8% for acrylamide. Both contaminants were found in samples of bread, fried chips, fried instant noodles, soy sauce, and instant noodle flavoring. Neither 3-MCPD nor acrylamide was detected in samples of dairy products (solid or liquid samples) and non-fried instant noodles.

### 1.3.2 Heterocyclic amines

Heterocyclic amines (HAs), a typical class of food-derived contaminants, are formed during heating of various proteinaceous foodstuffs, such as pork, poultry, beef, and fish (Wakabayashi et al., 1995; Murkovic, 2004). These amines have also been found in various environments (Kataoka, 1997). The mechanisms of the formation of HAs in foods have been known to occur via two pathways. The formation of some HAs is based on the reaction of creatinine with fragmented hexoses and pyrazine or pyridine derivatives that are formed during the cooking of foods (Gross et al., 1993). Other HAs, the pyrolysis-type HAs, are formed directly through thermal decomposition of proteins or amino acids. It was also demonstrated that the types and amounts of HAs formed can be attributed to parameters such as cooking time, temperature, and procedures. These compounds have been validated to be mutagenic, and some of them have also been shown to be carcinogenic in animal experiments (in vivo) (Felton and Knize, 1990). For these reasons, it is important to determine the amount of these mutagens present in cooked foods to estimate the intakes and the risks to human health. Since HAs are present at low concentration levels in complex matrices, it is necessary to develop sensitive and selective methodologies to determine these compounds in food samples.

The determination of HAs in proteinaceous foods has involved both LC and GC coupled to MS or other detection modes. LC is a suitable method because it provides both qualitative and quantitative information without the need for analyte derivatization. However, some HAs are nonpolar compounds and therefore show low ionization efficiency in ESI-MS, leading to high detection limits. In this case,
1.3 ANALYSIS OF CONTAMINANTS FORMED DURING FOOD PROCESSING

GC appears to be the technique of choice for the analysis of HAs due to its favorable combination of high selectivity and resolution, good accuracy and precision, wide dynamic concentration range, and high sensitivity. The detection systems commonly used are nitrogen–phosphorus-selective detection (NPD) and MS (Kataoka and Kijima, 1997). However, both methods have their limitations: GC-NPD cannot offer abundant structure information and GC–MS sometimes cannot provide the sufficient sensitivity in the determination of trace amount of HAs in complex matrix.

Zhang et al. (2008) developed a GC–MS/MS method for the simultaneous quantitation and confirmation of 13 HAs in Chinese cooked foods. The use of a triple-quadrupole analyzer (QqQ) in the MRM mode led to excellent selectivity and sensitivity. The procedure, which included an SPE step with a LiChrolut EN cartridge and a derivatization step with \(N,N\)-dimethylformamide di-\textit{tert}-butylacetal as the reagent, was validated by obtaining satisfactory accuracy and precision for most of the analytes. The applicability of the method was studied using a meat sample without detectable quantities of HAs. Good repeatability and sufficiently low LODs of the instrumental method ranging from 1.1 to 7.5 pg were determined. The LOQs of the complete method were established by low-level spiking of reference standards into a meat sample without detectable quantities of HAs. The LOQs varied from 0.12 to 0.48 ng g\(^{-1}\), with 2-amino-3,4,8-trimethylimidazo[4,5-\textit{f}]quinoxaline and 2-amino-1-methyl-6-phenylimidazo[4,5-\textit{b}]pyridine present at the higher values. The analysis of 10 Chinese cooked food samples validated the ubiquitous presence of HAs in these foods. The results demonstrated the feasibility of the GC–MS/MS method for the determination of trace levels of food-derived hazardous compounds in complex food matrices such as meat samples.

Skog et al. (1998) introduced a GC–MS technique for the analysis of nonpolar heterocyclic amines in common cooked meats, pan residues, and meat extracts after SPE. The mutagenic heterocyclic amines 3-amino-1,4-dimethyl-\(5\text{H}\)-pyrido[4,3-\textit{b}]indole (Trp-P-1), 3-amino-1-methyl-\(5\text{H}\)-pyrido[4,3-\textit{b}]indole (Trp-P-2), 2-amino-\(9\text{H}\)-pyrido[2,3-\textit{b}]indole (AaC), and 2-amino-3-methyl-\(9\text{H}\)-pyrido[2,3-\textit{b}]indole (MeAaC) were identified in several samples at concentrations up to 8 ng g\(^{-1}\). The co-mutagenic substances 1-methyl-\(9\text{H}\)-pyrido[3,4-\textit{b}]indole (harman) and \(9\text{H}\)-pyrido[3,4-\textit{b}]indole (norharman) were also detected in the samples at concentrations up to almost 200 ng g\(^{-1}\). The GC–MS method can be applied without derivatization of the sample. The technique offers high chromatographic efficiency, yielding LODs for pure references in the range 0.1–2 ng per injection.

Murray et al. (1993) developed a GC–MS assay for the measurement of 2-amino-3,8-dimethylimidazo[4,5-\textit{f}]quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo[4,5-\textit{f}]quinoxaline (DiMeIQx), and 2-amino-1-methyl-6-phenylimidazo[4,5-\textit{b}]pyridine (PhIP) in foods. Stable isotope-labeled analogs of MeIQx and PhIP were used as internal standards and the synthesis of deuterated PhIP was described. The mass spectrometer was operated in the electron-capture negative ion CU mode and the amines were chromatographed as their di-\textit{3},\textit{5}-bistrifluoromethylbenzyl derivatives. All three compounds could be measured in a single chromatographic run and detection limits of 0.05, 0.1, and 0.2 ng g\(^{-1}\) for MeIQx, DiMeIQx, and
PhIP, respectively, in foods were obtained. Various home-cooked and commercially prepared foods were analyzed with this assay and several were found to contain measurable amounts of one or more of the three amines.

### 1.3.3 Furan

Furan is a colorless, volatile, and lipophilic organic compound. Owing to its toxicity and carcinogenicity, furan has been included by the International Agency for Research on Cancer in Group 2B as possibly carcinogenic to humans (IARC, 1995) and has been classified by the US Department of Health and Human Services as a human pathogen (National Toxicology Program, 2005). The formation mechanisms of furan in foods are known to occur via three pathways: thermal degradation and rearrangement of carbohydrates, thermal oxidation of polyunsaturated fatty acids, and/or thermal decomposition of ascorbic acid or its derivatives (Perez Locas and Yaylayan, 2004).

Therefore, developing rapid, selective, and sensitive analytical methods to determine actual levels of furan in foods has become one of the major dominant themes in recent research on furan. The analysis of furan in food samples is complicated because of its extremely high volatility (Altaki et al., 2007), low molecular weight, and low concentration levels (ng g\(^{-1}\) to pg g\(^{-1}\)) present in foods. For the accurate analysis of furan in food samples, the US Food and Drugs Administration (FDA) selected an automated headspace sampling GC–MS analysis with the standard additions method. However, the method was time consuming and labor intensive since seven sample preparation steps were required for each sample (Goldmann et al., 2005).

Two common methodologies are currently used for the determination of furan: headspace (HS) GC–MS and SPME-GC–MS. These methodologies are continuously being modified and updated to improve their sensitivity and robustness and also to reduce or eliminate the influence of interfering ions/molecules originating from compounds derived from the matrices (Nyman et al., 2006; Nie et al., 2013).

Kim et al. (2010) studied and optimized the sample preparation for furan analysis using SPME to replace the FDA method. SPME is a solvent-free sampling technique and involves simple and minimized sample preparation procedures. It can also bring high sensitivity and selectivity and minimum interferences from matrix compounds. The authors also performed a monitoring and risk assessment of furan in various food products. The optimized fiber exposure temperature, time, and amount of sample of liquid, semi-solid, and paste foods were 50°C, 20 min, and 5 g (mL), respectively. The level of furan in canned meat (32.16 ng g\(^{-1}\)) was the highest among the samples studied. The furan levels in canned fish, canned vegetable, nutritional/diet drinks, canned soups, and jarred sauces were 29.40, 22.86, 7.28, 18.54, and 21.52 ng g\(^{-1}\), respectively. Furan concentrations in baby food products were between 3.43 and 97.21 ng g\(^{-1}\). The exposure estimate (14.59 ng kg\(^{-1}\) body weight per day) for baby foods was the highest among all the food samples tested. However, this exposure estimate for baby foods was lower than that prescribed by the FDA.
Bianchi et al. (2006) developed and validated a simpler method for the selective and sensitive analysis of furan in baby foods based on SPME-GC–MS. HS-SPME is a simple and a solvent-free alternative method to the traditional HS method for food analysis. Since furan occurs at trace levels in foods, HS-SPME can provide sufficient selectivity and sensitivity for the analysis of furan with low LODs and minimum interferences from compounds derived from the matrix. Altaki et al. (2007) carried out a systematic study of the HS-SPME parameters that affect the extraction and desorption process. In addition, the applicability of the isotope dilution method for the quantification of furan in solid and liquid food samples was investigated and the proposed method was applied to the analysis of furan in several food samples. Carboxen–polydimethylsiloxane fiber was found to be the most effective coating for the extraction of furan, with sensitivity higher than that for other commercially available fibers. Maximum responses were obtained using extraction conditions of 25°C for 30 min with 20% w/w NaCl and a headspace/aqueous phase volume ratio of 25 mL/15 mL. The isotope dilution method provided similar results to the standard additions method recommended the FDA for the quantification of furan in foods. The combination of HS-SPME and GC–MS showed good linearity, and the run-to-run and day-to-day precisions for foods were RSD <6% and <10%, respectively. The LODs were at pg g⁻¹ levels. The HS-SPME-GC–MS method was successfully applied to the analysis of furan in selected food products and can be proposed as a simple and accurate alternative to the FDA method for the analysis of furan in foods.

Nie et al. (2013) developed and validated an optimized automated HS-GC–MS method to assess the furan content in thermally processed commercial foods available in China. Furan-d₄ was used as an internal standard with the addition of salt (NaCl) to the headspace with an oven temperature of 70°C and an equilibration time of 30 min, coupled with an HP-PLOT/Q GC column. The proposed HS-GC–MS method was applied to the quantification of furan in 11 categories of foods (total 133 food samples). The average levels of furan detected in the various food products were infant formula 15.0, bread 4.0, coffee 60.6, fruit juice 5.3, dairy product 1.5, nutritional drink 16.2, canned jam 30.4, spice 9.3, vinegar, 38.3, beer 4.9, and soy sauce 128.8 ng g⁻¹. The results indicated that soybean is a type of raw materials or precursor that can easily form furan during heat processing of foods because soybean contains high fat and protein levels.

Bononi and Tateo (2009) used an HS-SPME-based analytical method and matrix-matched calibration curves to screen 21 commercial samples of “aceto balsamico di Modena” (Modena balsamic vinegar) purchased in an Italian market. This work enabled a global risk assessment linked to home consumption of this product to be obtained. The isotope dilution methods used in these evaluations are known for their general robustness, and quantification by internal standardization using isotopically labeled furan has been adopted by the majority of workers using a simple HS method or HS-SPME analysis.

Sarafraz-Yazdi et al. (2012) optimized the HS-SPME method with two different coatings synthesized by sol–gel technology [such as poly(ethylene glycol) (PEG) and PEG reinforced with multi-walled carbon nanotubes (CNTs)] and determined
furan at trace levels in food samples. Under optimized conditions, the linear ranges for furan with PEG and PEG–CNT fibers were 0.005–10 and 0.0005–10 ng mL\(^{-1}\), the LODs (S/N = 3) were 0.001 and 0.00025 ng mL\(^{-1}\), and the LOQs were 0.005 and 0.0005 ng mL\(^{-1}\), respectively. For PEG and PEG–CNT fibers, the repeatabilities (n = 5) were 6.2 and 4.9% and the reproducibilities (n = 3) were 6.6 and 5.3%, respectively. Relative recoveries for samples spiked with 0.1 ng mL\(^{-1}\) of furan ranged from 92 to 103%. The proposed HS-SPME-GC–FID (flame ionization detection) method was successfully applied to the extraction of furan from two environmental food samples (baby food and fruit juice).

Altaki et al. (2009) proposed a simple, fast, and fully automated method based on HS-SPME coupled online with GC–IT-MS for the determination of furan in foods. The performance of the proposed method was compared with that of the automated HS-GC–MS method proposed by the FDA in terms of repeatability, LOD and LOQ. Both methods provided similar results for the determination of furan in selected food samples, although slightly worse precision (RSD 9–12%) and higher LODs (5–20 times higher) were obtained with the HS method. In addition, a higher sample throughput in routine analyses for furan was obtained using the proposed HS-SPME-GC–IT-MS method with isotope dilution than using the FDA method, which recommends standard addition for quantification. The proposed method provides good precision (RSD <10%) and low LODs, ranging from 0.02 to 0.12 ng g\(^{-1}\) depending on the sample. The developed HS-SPME-GC–MS method was used to determine furan in several Spanish food commodities and concentrations ranging from 0.1 ng g\(^{-1}\) to 1.1 g g\(^{-1}\) were found.

5-Hydroxymethylfurfural or 5-hydroxymethyl-2-furaldehyde (HMF) is a heat-induced product of the well-known Maillard reaction that occurs in many carbohydrate-rich foods such as biscuits, bread, marmalade, breakfast cereals, and honey (Tomlinson et al., 1993; Berg and Boekel, 1994). HMF has been found to exhibit mutagenic and DNA strand-breaking activity. Moreover, the presence of HMF in foods has raised toxicological concerns because this compound and its derivatives, 5-chloromethylfurfural and 5-sulfooxymethylfurfural, have been found to be cytotoxic, genotoxic, mutagenic, and carcinogenic, inducing colo-rectal, hepatic, and skin cancers (Teixidó et al., 2006).

Teixidó et al. (2006) proposed a new, simple, and selective method for the determination of HMF in foods by GC–MS. Several derivatization procedures based on the formation of an HMF silylated derivative using different reagents were studied. Among the derivatization reagents examined, N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA) provided the best derivatization yield. Sample clean-up was also optimized, using either LLE with dichloromethane or SPE with several commercially available cartridges, and the best results were obtained using ENV+ cartridges. Quality parameters such as day-to-day and run-to-run precision (RSD <10%), linearity (between 25 and 700 ng g\(^{-1}\)) and LOD (6 ng g\(^{-1}\)) were established. This method was successfully applied to the determine the HMF content in several Spanish food samples from a local market, such as jam, honey, orange juice, and bakery products.
1.3 Analysis of Contaminants Formed During Food Processing

1.3.4 Polycyclic Aromatic Hydrocarbons (PAHs)

PAHs, also known as polyaromatic hydrocarbons or polynuclear aromatic hydrocarbons, are groups of different chemicals formed during the incomplete burning of coal, oil, gas, wood, and other organic materials such as tobacco and charbroiled meat. The possible sources of PAHs in foods are environmental contamination and thermal treatment of varying severity used in the preparation and manufacture of foods (Guillén, 1994). Studies have shown that high levels of PAHs were present in meat cooked at high temperatures, such as in grilling or barbecuing, and in smoked fish (Larsson et al., 1983). Owing to their potential carcinogenic properties, the US Environmental Protection Agency (EPA) has included 16 PAHs in its list of persistent organic pollutants (POPs).

Yurchenko and Mölder (2005) determined the levels of six PAHs (benzo[a]pyrene, benz[a]anthracene, benzo[k]fluoranthene, benzo[b]fluoranthene, benzo[ghi]perylene, and indeno[1,2,3-cd]pyrene) in 97 samples of smoked fish, 11 samples of fresh fish, and 18 olive oil and 14 rape oil samples. For cleaning of the sample, gel chromatography was used. PAHs were separated by GC and detected by positive-ion CI using ammonia as reagent gas. An HP 6890 Plus GC–HP 5973MS system with positive-ion CI option was used in the SIM mode. The LOD for PAHs using this method was ∼0.3 ppb with a recovery of ∼75%. The results were confirmed by HPLC.

Aguinaga et al. (2007) developed a method for the determination of 16 PAHs in milk and related products based on DI-SPME followed by GC–MS. The influence of various parameters on the extraction efficiency of PAHs was carefully monitored. Good performance (e.g., recovery, precision, and quantitation limits) was attained when a PDMS–DVB fiber was immersed in the sample for 60 min at 55 °C. Detection limits ranged from 0.003 to 1.5 μg L$^{-1}$ (S/N = 3), depending on the compound and the sample. The proposed method was successfully applied to infant formulas and milk and related products and the presence of both fluoranthene and pyrene in two samples was confirmed.

El-Beqqali et al. (2006) developed a new sensitive, selective, fast, and accurate technique for online sample preparation. Microextraction in a packed syringe (MEPS) is a new miniaturized SPE technique that can be connected online to GC or LC without any modifications. In MEPS, ∼1 mg of the solid packing material is inserted into a syringe (100–250 mL) as a plug. Sample preparation takes place on the packed bed. The bed can be coated to provide selective and suitable sampling conditions. The new method is very promising: it is very easy to use, fully automated, inexpensive, and rapid in comparison with previously applied methods. The determination of PAHs in water was performed using MEPS as the sample preparation method online with GC–MS. The results with MEPS used for sample preparation were compared with those from other techniques such as SBSE and SPME. The method was validated and the calibration curves were evaluated by means of quadratic regression and weighted by the inverse of the concentration: 1/x for the calibration range 5–1000 ng L$^{-1}$. The MEPS applied
polymer (silica-C$_8$) could be used more than 400 times before the syringe was discarded. The extraction recovery was about 70%. The results showed close correlation coefficients ($R > 0.998$) for all analytes in the calibration range studied. The accuracy of MEPS-GC–MS was between 90 and 113% and the inter-day precision ($n = 3$ days), expressed as RSD, was 8–16%. MEPS reduced the handling time 30- and 100-fold compared with SPME and SBSE, respectively.

Veyrand et al. (2007) developed an efficient and selective analytical method for the determination and quantification of 19 PAHs in food and oil samples. This method included the monitoring of 15 PAHs stated as priority in the EU 2005/108 recommendation. The samples were extracted according to a selective extraction step using pressurized liquid extraction followed by purification using polystyrene–divinylbenzene SPE. Identification and quantification were performed using GC–MS/MS, with an isotope dilution approach using $^{13}$C-labeled PAHs. The novel combination of selective extraction followed by purification provided highly purified analytes combined with a fast and automated method. The advantages of GC–MS/MS over other detection methods were tremendous in terms of sensitivity, selectivity, and interpretation facility. LODs varied between 0.008 and 0.15 µg kg$^{-1}$ and LOQs between 0.025 and 0.915 µg kg$^{-1}$ for PAHs in foods. The calibration curves showed good linearity for all the PAHs ($R^2 > 0.99$) and the precision and recovery were fit for purpose. The trueness (accuracy) of the method was established using the US National Institute of Standards and Technology (NIST) SRM 2977 reference material.

Ghasemzadeh-Mohammadi et al. (2012) developed a simple and efficient method using MAE and dispersive liquid–liquid microextraction (DLLME) coupled with GC–MS for the extraction and quantification of 16 PAHs in smoked fish. Benzo[a]pyrene, chrysene, and pyrene were employed as model compounds and spiked into smoked fish to assess the extraction procedure. Several parameters, including the nature and volume of hydrolysis, extraction and dispersion solvents, microwave time, and pH were optimized. Under the optimum conditions for MAE, 1 g of fish sample was extracted with 12 mL of KOH solution (2 M) and ethanol in a ratio of 50:50 in a closed-vessel system. For DLLME, 500 µL of acetone (dispersion solvent) containing 100 µL of ethylene tetrachloride (extraction solvent) were rapidly injected by syringe into 12 mL of the sample extract solution (previously adjusted to pH 6.5), thereby forming a cloudy solution. Phase separation was performed by centrifugation and a volume of 1.5 µL of the sedimented phase was analyzed using GC–MS in the SIM mode. Satisfactory results were achieved when this method was applied to determine PAHs in smoked fish samples. The MAE–DLLME method coupled with GC–MS provided excellent enrichment factors (in the range 244–373 for 16 PAHs) and good repeatability (with an RSD between 2.8 and 9%) for spiked smoked fish. The calibration graphs were linear in the range 1–200 ng g$^{-1}$, with $R^2 > 0.981$, and detection limits were between 0.11 and 0.43 ng g$^{-1}$. The recoveries of PAHs in smoked fish were 82.1–105.5%. A comparison with previous methods demonstrated that the proposed method is an accurate, rapid, and reliable sample pretreatment method that provides good enrichment factors and detection limits for extracting and determining PAHs in smoked fish.
Quinto et al. (2012) calculated the partition equilibria and extraction rates of different PAHs through multivariate nonlinear regression from data obtained after microextraction with a packed sorbent (MEPS) of 16 PAHs from water samples. The MEPS-GC–MS method was optimized to investigate the partitioning parameters for a priori prediction of solute sorption equilibrium, recoveries, and preconcentration effects in aqueous and solvent systems. Finally, real samples from the sea, agricultural irrigation wells, streams, and domestic supplies were analyzed. LODs (S/N ≥ 3) and LOQs (S/N ≥ 10) were strictly dependent on the volume of water and methanol used during the extraction process. Under the experimental conditions, the LODs and LOQs ranged from 0.5 to 2 ng L$^{-1}$ and from 1.6 to 6.2 ng L$^{-1}$, respectively.

### 1.3.5 Tetramine

Tetramethylenedisulfotetramine, also known as tetramine or TETS, is odorless, tasteless, and water-soluble and is stable in a wide variety of matrices. In most cases, the route of exposure is ingestion of intentionally contaminated foods. Therefore, it is important to have a method capable of determining tetramine at low concentrations in foods (De Jager et al., 2008).

De Jager et al. (2008) developed an automated SPME-GC–MS method for the determination of tetramine. A comparison of DI and HS extraction techniques using a 70 μm Carbowax–divinylbenzene (CW–DVB) fiber was presented. The optimized DI-SPME method provided an aqueous extraction LOD of 9.0 ng g$^{-1}$ and the HS-SPME LOD was 2.7 ng g$^{-1}$. In both SPME modes, the recovery was highly matrix dependent and quantification required standard addition calibrations. Analysis of foods using DI-SPME encountered many obstacles, including fiber fouling, low recovery, and poor reproducibility. HS-SPME was successfully applied to food analysis with minimal interferences. Standard addition calibration curves for foods resulted in high linearity ($R^2 > 0.98$), reproducibility (RSD < 12%), and sensitivity with LODs ranging from 0.9 to 4.3 ng g$^{-1}$.

De Jager et al. (2009) also presented an SBSE-GC–MS method for the determination of tetramine. The LOD of the optimized method was 0.2 ng g$^{-1}$ for extractions from water and 0.3–2.1 ng g$^{-1}$ for extractions from foods. The recovery was highly matrix dependent (36–130%) and quantification required standard addition calibrations. The standard addition calibration lines had high linearity ($R^2 > 0.97$) and replicate extractions had good reproducibility (RSD 4.4–9.8%). A comparison of the SBSE method and a previously developed HS-SPME method was performed. Generally, SBSE provided higher sensitivity with shorter analysis times.

### 1.3.6 Chloropropanols

3-Chloropropane-1,2-diol (3-MCPD) and 1,3-dichloro-2-propanol (1,3-DCP), known as chloropropanols, are commonly produced by acid-hydrolyzed vegetable protein and in various heat-processed foods. 3-MCPD and 1,3-DCP are two of the
most toxic chloropropanols. 3-MCPD is harmful to both male fertility and kidney functions when given regularly in high doses (FAO/WHO, 2006). The maximum level of 0.02 mg kg\(^{-1}\) for 3-MCPD in soy sauce and acid-HVP was established by the European Community [Commission Regulation (EC) No. 466/2001; European Commission, 2001].

Racamonde et al. (2011) determined 3-MCPD and 1,3-DCP for the first time in bakery foods using pressurized liquid extraction (PLE) combined with \textit{in situ} derivatization and GC–MS analysis. To reduce the time of analysis and improve the sensitivity, derivatization of the compounds was performed in the cell extraction. A 1 g amount of sample dispersed with 0.1 g of anhydrous sodium sulfate and 2.5 g of diatomaceous earth was extracted with ethyl acetate. A 1 g amount of Florisil as clean-up adsorbent and 70 \(\mu\)L of BSTFA were used for 3 min at 70 °C. Under the optimum conditions, the calibration curves showed good linearity (\(R^2 >0.9994\)) and precision (RSD \(\leq2.4\%\)) within the tested ranges. The LOQs for 1,3-DCP and 3-MCDP were 1.6 and 1.7 \(\mu\)g kg\(^{-1}\), respectively, which were far below the established limits in European and US legislation. The method was applied to the analysis of several toasted bread, snacks, cookies, and cereal samples.

van Bergen et al. (1992) developed a method for the identification and determination of a wide range of chloropropanols in protein hydrolyzates and composite savory food products. Based on capillary GC of heptafluorobutyrate derivatives with ECD and MS, the methods showed that the main chloropropanols found in traditionally produced hydrolyzates were 3-chloro-1,2-propanediol, 2-chloro-1,3-propanediol, 1,3-dichloro-2-propanol, and 2,3-dichloro-1-propanol. The LODs were 5–100 \(\mu\)g kg\(^{-1}\) for the chloropropanediols and 10 \(\mu\)g kg\(^{-1}\) for the dichloropropanols.

Xu et al. (2013) developed a quantitative method for the simultaneous determination of 3-monochloropropane-1,2-diol (3-MCPD) and acrylamide in foods by GC–MS/MS. The analytes were purified and extracted using the MSPDE technique with Extrelut NT. A coupled column system (3 m Innowax combined with 30 m DB-5 ms) was developed to separate the two compounds efficiently without derivatization. Triple-quadrupole MS in the MRM mode was applied to suppress matrix interferences and obtain good sensitivity in the determination of both analytes. The LOD in the sample matrix was 5 \(\mu\)g kg\(^{-1}\) for both 3-MCPD and acrylamide. The average recoveries for 3-MCPD and acrylamide in different food matrices were 90.5–107 and 81.9–95.7\%, respectively, with RSDs of 5.6–13.5 and 5.3–13.4\%, respectively. The inter-day RSDs were 6.1–12.6\% for 3-MCPD and 5.0–12.8\% for acrylamide.

Chung et al. (2002) developed a highly selective and sensitive method for the determination of ppb levels of 1,3-dichloro-2-propanol (1,3-DCP) and 3-chloropropane-1,2-diol (3-MCPD) in soy sauce using capillary GC with MS detection. Samples were homogenized, mixed with sodium chloride solution, and then adsorbed on silica gel. The loaded silica gel was packed into a chromatographic column, from which chloropropanols were extracted by elution with ethyl acetate. Heptafluorobutyric anhydride was added to the concentrated eluate to derivatize the
chloropropanols and the derivatized analytes were separated by GC and identified and quantified by MS. A linear relationship between the concentration of the two chloropropanols and the detector response was obtained over the concentration range 10–1000 mg kg\(^{-1}\). The precision of the method was satisfactory at about 5\%, and recoveries of 1,3-DCP and 3-MCPD from soy sauce samples spiked at 25 mg kg\(^{-1}\) were 77 and 98\%, respectively. The LOQ of the method was \(\sim\)5 mg kg\(^{-1}\) for both 1,3-DCP and 3-MCPD, meeting the requirements of tolerance limits adopted by different international institutions and governments around the world.

Meierhans et al. (1998) improved a routine method for the determination of 3-chloropropene-1,2-diol (3-MCPD) and 2-chloropropene-1,3-diol (2-MCPD) in different savory foods using capillary GC with MS detection. The monochloropropanediols were extracted from Extrelut columns with diethyl ether and derivatized with acetone to the corresponding dioxolanes. Detection was performed on two different mass spectrometers using SIM to reach an ultimate sensitivity in the low \(\mu\)g kg\(^{-1}\) range. An LOD of 1 \(\mu\)g kg\(^{-1}\) was achieved using Extrelut 20 for extraction. The method was validated to be fast and reliable and showed good recoveries and accuracy, confirmed by successful participation in two ring test studies. Results of analyses of finished goods (e.g., seasonings and soy sauces) containing MCPDs in sub-ppb level were presented.

Abu-El-Haj et al. (2007) developed a method for the determination of 3-monochloropropanediol (3-MCPD) and 1,3-dichloropropanol (1,3-DCP) in small specimens of food products. 3-MCPD and 1,3-DCP were isolated from soy sauce and other food products using alumina column extraction. Sample portions (1–2 g) spiked with deuterated 3-MCPD-\(d_5\) and 1,3-DCP-\(d_5\) as internal standards were mixed with 2 g of aluminum oxide and packed into a small disposable column. The compounds were eluted with 25 mL of dichloromethane, concentrated, and derivatized with heptafluorobutyric anhydride. GC–MS analysis was conducted in the SIM mode. Four ion fragments were used for the identification of 3-MCPD and 3-MCPD-\(d_5\), and three ions were used for the identification of 1,3-DCP. The LOD and LOQ were 1 ng g\(^{-1}\) and 3 ng g\(^{-1}\), respectively, and the recovery was \(\sim\)80\%. Observed matrix effects were negligible for 3-MCPD and were around a 20\% signal decrease for 3-DCP.

Lee et al. (2007c) developed a method for identifying 1,3-dichloro-2-propanol and 3-chloro-1,2-propanediol in aqueous matrices by using headspace on-fiber derivatization following SPME combined with GC–MS. The optimized SPME experimental procedures for extracting 1,3-dichloro-2-propanol and 3-chloro-1,2-propanediol in aqueous solutions involved an 85 \(\mu\)m polyacrylate-coated fiber at pH 6, a sodium chloride concentration of 0.36 g mL\(^{-1}\), extraction at 50 °C for 15 min, and desorption of analytes at 260 °C for 3 min. Headspace derivatization was conducted in a laboratory-made design with \(N\)-methyl-\(N\)-(trimethylsilyl)trifluoroacetamide vapor following SPME by using 3 \(\mu\)L of \(N\)-methyl-\(N\)-(trimethylsilyl)trifluoroacetamide at an oil-bath temperature of 230 °C for 40 s. This method had good repeatability (RSD \(\leq\)19\%, \(n = 8\)) and good linearity (\(R^2 \geq 0.9972\)) for ultrapure water and soy sauce samples that were spiked with two analytes. LODs at the ng mL\(^{-1}\) level were
obtained. The results demonstrated that headspace on-fiber derivatization following SPME is a simple, fast, and accurate technique for identifying trace levels of 1,3-dichloro-2-propanol and 3-chloro-1,2-propanediol in soy sauce.

1.4 Analysis of environmental contaminants

1.4.1 Organometallic compounds

Food is usually the main source of human exposure to heavy metals. Amongst the heavy metals present in foods, methylmercury (MeHg) and organotins are of particular concern in terms of food safety and public health.

Mercury exists naturally in abundance in the environment. It enters the environment by both natural and human means. Mercury in the environment can be oxidized to inorganic bivalent mercury with the presence of organic matter in waters. Inorganic mercury can also be converted to the methylated form by microorganisms, especially in the aquatic systems (Eisler, 2000).

Mercury can exist in inorganic form in foods and the more toxic organic forms such as MeHg exist in fish and shellfish. Mercury present in other foods is mainly in inorganic form. Dietary inorganic mercury is of little toxicological concern (Morita et al., 1998). Fish and other seafood products are the major source of MeHg, especially large predatory species such as tuna and swordfish. A number of analytical methods have been developed to monitor organomercury compounds, especially MeHg, in the marine environment. However, there is little literature regarding the determination of MeHg and EtHg simultaneously in foods. Amongst the reported studies, different analytical techniques have been developed for the speciation of mercury. These methods usually combine a separation and a detection technique, such as GC or LC coupled with an element-selective detection method [e.g., atomic emission, atomic absorption, atomic fluorescence, or inductively coupled plasma mass spectrometry (ICP-MS)]. Among these methods, the coupling of GC with ICP–MS combines high sensitivity with the possibility of speciated isotope dilution measurements (Chung et al., 2011).

Chung et al. (2011) developed a reliable and sensitive method for simultaneous determination of MeHg and EtHg in various types of foods by GC–ICP-MS. Samples were digested with pancreatin and then hydrochloric acid. MeHg and EtHg in the extract were derivatized in an aqueous buffer with sodium tetraphenylborate. After phase separation, the extract was directly transferred to analysis. The analyses were conducted by GC–ICP-MS with monopropylmercury chloride (PrHgCl) as surrogate standard.

Organotin compounds (OTCs) are applied commercially as heat and light stabilizers for poly(vinyl chloride) (PVC) plastics, wood preservatives, biocides in marine antifouling paint, fungicides, insecticides, and acaricides (Rosenberg, 2005). Organotins enter the marine environment through various routes. Tributyltin has been
extensively used in ship antifouling paint formulations. Tributyltin released from the paint has been found to have adverse effects on many nontarget organisms, particularly mollusks. As a result, many countries have banned or restricted the use of tin-based antifouling paints (Forsyth and Clearoux, 1991). These compounds have been forbidden by the International Maritime Organization (Gajda and Jancso, 2010). Most OTCs show a wide range of toxic effects on humans; the exact level of toxicity depends on the compound used. Tetraorganotins are very stable molecules with low toxicity and low biological activity, but they can be metabolized to toxic triorganotin compounds. GC has usually been used to determine the OTCs.

Campillo et al. (2012) developed an environmentally friendly method for the determination of seven OTCs in honey and wine samples, using HS-SPME and GC. The analytes were derivatized in situ with sodium tetraethylborate and the derivatization and preconcentration steps were optimized. A 100 mm polydimethylsiloxane fiber was most suitable for preconcentrating the derivatized analytes from the headspace of an aqueous solution containing the sample. When microwave-induced plasma atomic emission detection (MIP-AED) and MS detection were compared, higher sensitivity was attained for all compounds by using MS, although MIP-AED showed more specific chromatograms. Using MS, LODs ranged roughly from 0.3 to 4.3 pg (Sn) g$^{-1}$ for honey samples, and from 0.1 to 2 pg (Sn) mL$^{-1}$ for wine samples. The optimized method was successfully applied to different samples, and the compounds studied were detected in some wines at 0.05–5 ng (Sn) mL$^{-1}$ levels.

1.4.2 Polychlorinated biphenyls

Polychlorinated biphenyls (PCBs) are soluble in fatty and lipid-rich tissue and are associated with specific types of cancer in humans, such as cancer of the liver and biliary tract (Borlakoglu and Haegele, 1991). The WHO has recommended an acceptable daily intake for dioxins, furans, and PCBs including non-ortho-substituted (77, 81, 126, and 169) and mono-ortho substituted (105, 114, 118, 123, 156, 157, 167, and 189) CBs. This recommendation has also been adopted by the European Scientific Committee for Food (SCF, Brussels, Belgium). Therefore, it is likely that these non- and mono-ortho-substituted CBs will be included in food monitoring programs. Consequently, there is a significant need for unambiguous measurements of these CBs (Korytár et al., 2002). It is therefore of critical importance to monitor the presence of these pollutants in food samples. In order to solve these problems, rapid and reliable analytical procedures for the determination of these pollutants in a variety of complex matrices at very low concentration levels are needed (Fuoco et al., 2005).

Beyer and Biziuk (2010), used various SPE columns to clean up a model mixture (containing a-linolenic acid as interfering substance) for PCBs and organochlorine pesticides. Octadecylsilyl (C$_{18}$), graphitized nonporous carbon (Envi-Carb), aminopropyl (NH$_{2}$), Florisil, and alumina SPE sorbents were evaluated. The
Florisil, alumina, and NH$_2$ columns were found to provide the most effective clean-up, remove the greatest amount of interfering substance, and simultaneously ensure analyte recoveries higher than 70% for most compounds. The Envi-Carb and C$_{18}$ columns did little to eliminate matrix interferences. The developed clean-up procedure was applied to real low-fat food samples, such as herring (Clupea harengus). Recoveries of $>78\%$ with standard deviations (SDs) $<15\%$ were obtained for all compounds under the selected conditions. Method quantification limits (MQLs) were in the range 5–10 μg kg$^{-1}$.

Müller et al. (2001) developed a fast and simple pressurized liquid extraction method for the determination of PCBs in feeding stuffs and food matrices. The method was based on a simultaneous extraction/clean-up step requiring a minimum of sample handling. The final analysis was performed with GC–MS. Seven PCBs (28, 52, 101, 118, 138, 153, and 180) were analyzed. All of these PCBs are indicator congeners and should be included in an analytical monitoring program, according to European legislation. The extracted matrices were spiked feed for poultry and two certified reference materials naturally contaminated with PCBs (cod-liver oil and milk powder), showing excellent conformity with the certified data.

Matsui et al. (2011) developed a gas chromatography–multi-photon ionization–time-of-flight mass spectrometry (GC–MPI–TOF–MS) method, using four types of laser sources to determine PCBs in a sample mixture. When a fourth harmonic emission (266 nm) of a picosecond Nd:YAG laser (1064 nm) was utilized, highly chlorinated PCBs larger than hepta-CBs were not observed. A fifth harmonic emission (213 nm) of the picosecond Nd:YAG laser allowed the measurement of PCBs from di- to octa-CBs, and the LOD was several picograms for each component of PCBs. The LOD for the total amount of PCBs, which was calculated using the protocol provided by the Ministry of the Environment, Japan, was 1000 pg. The signal intensity of the congeners with chlorine atoms at the ortho positions (noncoplanar PCBs) was enhanced by using the fifth harmonic emission. When the fourth harmonic emission remaining after fifth harmonic generation was simultaneously used, the LOD for total PCBs was improved to 667 pg. The PCB sample was also analyzed using a third harmonic emission (267 nm) of a femtosecond Ti:sapphire laser (800 nm), providing an LOD of 677 pg. Thus, the two-color beam (266/213 nm) of a picosecond Nd:YAG laser had a comparable or even slightly superior performance to the more expensive femtosecond Ti:sapphire laser.

Focant et al. (2004) studied the separation of the 209 PCB congeners by using GC × GC–TOF-MS. Four column combinations based on thermally stable phases, DB-1–HT-8, DB-XLB–HT-8, DB-XLB–BPX-50, and HT–8–BPX-50, were investigated. The HT–8–BPX-50 set produced the best separation. The distribution of the 100–150 ms wide peaks was highly structured in the chromatographic space and based on the degree of ortho substitution within each separated homologous series. A total of 192 congeners were resolved in 146 min (1.3 analytes per minute) using this column set. Eight coelutions involved 17 congeners. Among them, seven congeners were present in Aroclors at levels >1.0 wt.% (CBs 33, 47, 48, 95,
97, 163, and 187). Except for CBs 47 and 48, none of the major constituents of commercial mixtures coeluted. CB 138 was well separated from CBs 163 and 164 in the second dimension. For all column sets, CBs 20, 33, and 109 always coeluted with other PCBs. The 12 toxic dioxin-like congeners (CBs 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, and 189) and the seven European Union marker PCBs (CBs 28, 52, 101, 118, 138, 153, and 180) were separated from any interfering congeners.

Popp et al. (2005) developed an optimized method using SBSE for the determination of 25 PCBs from water samples among three of the most toxic coplanar PCBs (PCBs 77, 126, and 169). Since the investigated PCBs comprise all steps of chlorination (from PCB 1 as monochlorobiphenyl to PCB 209 as decachlorobiphenyl), the results should be representative of the total class of the 209 PCB congeners. For 8 mL spiked water samples with a 2 mL addition of methanol and a 2 h exposure time of the stir bars, recoveries between 28% (PCB 209) and 93% (PCB 1, 52, and 77) were found. LODs between 0.05 and 0.15 ng L\(^{-1}\) were calculated for the combination of SBSE and thermodesorption-GC–MS. The procedure was applied to the investigation of groundwater and river water samples from the industrial region of Bitterfeld, near Leipzig, Germany.

Focant et al. (2005) tested a comprehensive GC×GC–TOF-MS experimental setup for the measurement of seven 2,3,7,8-substituted polychlorinated dibenzo-p-dioxins (PCDDs), ten 2,3,7,8-substituted polychlorinated dibenzofurans (PCDFs), four non-ortho-substituted PCBs, eight mono-ortho-substituted PCBs, and six indicator PCBs (Aroclor 1260) in food samples. A 40 m × 0.18 mm i.d. RTX-500 column with 0.10 μm film thickness was used as the first dimension (1D) and a 1.5 m × 0.10 mm i.d. BPX-50 column with 0.10 μm film thickness as the second dimension (2D). The GC×GC separation was completed in 45 min. Quantification was performed using \(^{13}\)C-labeled isotope dilution (ID). Isotope ratios of the selected quantification ions were checked against theoretical values prior to peak assignment and quantification. The dynamic working range spanned three orders of magnitude. The lowest detectable amount of 2,3,7,8-TCDD was 0.2 pg. Fish, pork, and milk samples were tested. On a congener basis, the GC×GC–ID-TOF–MS method was compared with the reference GC–ID high-resolution mass spectrometry (HRMS) method and with the alternative GC-ID tandem-in-time quadrupole ion storage mass spectrometry (QIST-MS/MS). PCB levels ranged from low picograms to low nanograms per gram of sample and the data compared very well between the different methods. For all matrices, PCDD/Fs were at low picogram levels (0.05–3 pg) on a fresh weight basis. Although congener profiles were accurately described, the RSDs with GC×GC–ID-TOF-MS and GC–QIST-MS/MS were much higher than those with GC–ID-HRMS, especially for low-level pork and milk. On a toxic equivalent (TEQ) basis, all methods, including the dioxin-responsive chemically activated luciferase gene expression (DR-CALUX) assay, produced similar responses.
1.4.3 Monocyclic aromatic hydrocarbons

Monocyclic aromatic hydrocarbons (MAHs), including benzene, toluene, ethylbenzene, and xylenes, are important organic air contaminants. Unlike PAHs, MAHs in foods come chiefly from environmental contamination. MAHs have attracted increasing attention owing to their toxicity towards human health. After the first reports on the presence of benzene in foods, several studies investigated the presence of this molecule in different food systems, mostly beverages (Aprea et al., 2008).

Lee et al. (2007b) developed an analytical method for detecting trace levels of benzene, toluene, ethylbenzene, and xylenes (BTEX) in water by using headspace solid-phase microextraction (HS-SPME) coupled with cryo-trap GC–MS. The shape of the chromatographic peaks for BTEX was improved by using cryo-trap equipment. The HS-SPME experimental procedures for extracting BTEX from water were optimized with a 75 μm Carboxen–polydimethylsiloxane (CAR–PDMS)-coated fiber at a sodium chloride concentration of 267 g L⁻¹, extraction for 15 min at 25 °C and desorption at 290 °C for 2 min. Good linearity was verified in the range 0.0001–50 μg L⁻¹ for each analyte (R² = 0.996–0.999). The LODs of BTEX in water reached sub-ng L⁻¹ levels. The LODs of benzene, toluene, ethylbenzene, m/p-xylene, and o-xylene were 0.04, 0.02, 0.05, 0.0, 1 and 0.02 ng L⁻¹, respectively. The proposed method was successfully used for the quantification of trace levels of BTEX in ground water. The results indicated that HS-SPME coupled with cryo-trap GC–MS is an effective tool for the analysis of BTEX in water samples at the sub-ng L⁻¹ level.

In another study, Bianchin et al. (2012) proposed a new optimization approach for the simultaneous determination of PAHs, benzene, toluene, ethylbenzene, and xylene isomers (BTEX) in water samples using the SPME technique followed by GC–MS separation and detection. The objective was to achieve compromise extraction conditions suitable for all semi-volatile and volatile compounds, under which the amount extracted was maximized for all of the analytes. This was achieved by careful optimization of the fiber coating, salting-out effect, extraction time and temperature and extraction mode (headspace or direct immersion). With the optimized fiber coating (i.e., 65 μm PDMS–DVB), other selected factors were optimized using a response surface methodology through central composite designs. As expected, the optimized results for each class of analytes varied significantly, probably due to the differences in their volatility and the equilibrium constants for the analyte–fiber coating. In order to overcome this issue, a new optimization approach was proposed based on a combination of extraction modes and extraction temperatures in a single extraction procedure. The final optimized procedure was 48 min of extraction in direct immersion mode with the sample maintained at 80 °C, followed by a further 32 min of headspace extraction with the sample temperature kept at 10 °C. The proposed procedure was compared with conventional methods based on the use of a single extraction mode and temperature (80 min of headspace extraction at 60 °C or 80 min of direct immersion extraction at 50 °C). The newly proposed method was shown to be more attractive as it extracted larger amounts of both
semi-volatile and volatile compounds in a single extraction procedure compared with the conventional approaches. The optimized method was validated and excellent results were obtained.

Fabietti et al. (2001) used the purge-and-trap technique and GC–MS to evaluate the presence and content of the principal aromatic hydrocarbons responsible for environmental pollution and 60 samples of different types of soft drinks with an aqueous base were analyzed. In another study, Górna-Binkul et al. (1996) used a solvent extraction technique to isolate MAHs from fruits. GC–MS (SIM mode) was applied to determine the isolated pollutants. It was observed that the uptake of MAHs depended on the species and took place in different morphological parts of the biological material. The highest concentrations of MAHs were found in parsley leaves (m- and p-xylene) and orange peel (toluene). Estimation of the daily human exposure to MAHs through eating contaminated fruit and vegetables was performed.

1.5 Analysis of contaminants from packaging materials

Packaging has become an important part in the food manufacturing process. In order to improve the performance and extrinsic features of packaging materials, some types of additives, such as plasticizers, ink, adhesives, and anti-statics, have been developed and added to the packaging materials. These compounds applied on packaging material may be non-intentionally added to foods and have attracted increasing concern because they may migrate into foods and subsequently influence food safety. Although packaging materials are not typically associated with pathogenic outbreaks or biological contamination, several commonly used plasticizers have been demonstrated in toxicity studies to have carcinogenic effects in rodents and potential estrogenic effects in humans (NTP Technical Report Series 212, 1980 and NTP Technical Report Series 217, 1982; National Toxicology Program, 1980, 1982).

Inks employed on the printed surface of the packaging are cured thermally and eliminated with a drying process. The residues of the ink could contaminate the food surrounded by the packaging material. As a result, a class of molecules used in ultraviolet-cured inks may migrate into foods.

Negreira et al. (2010) developed a novel, single-step method for the determination of seven ink photoinitiators in carton-packed milk samples. SPME and GC, combined with MS, were used as sample preparation and determination techniques, respectively. Parameters affecting the performance of the microextraction process were thoroughly evaluated using uni- and multivariate optimization strategies based on the use of experimental factorial designs. The coating of the SPME fiber, together with the sampling mode and the temperature, were the factors that had a major influence on the efficiency of the extraction. Under the final conditions, 1.5 mL of milk and 8.5 mL of ultrapure water were poured into a glass vessel, which was closed and immersed in a boiling water bath. A polydimethylsiloxane–divinylbenzene (PDMS–DVB)-coated fiber was exposed directly to the diluted sample for 40 min. Subsequently, the fiber was desorbed in the injector of the GC–MS system for
The optimized method provided LOQs between 0.2 and 1 μg L\(^{-1}\) and good linearity in the range 1–250 μg L\(^{-1}\). The inter-day precision remained below 15% for all compounds in spiked whole milk. The efficiency of the extraction varied for whole, semi-skimmed, and skimmed milk. However, no differences were observed among the relative recoveries achieved for milk samples of different brands and with the same fat content.

Sagratini et al. (2008) developed a new analytical method using GC–MS and LC–MS for the determination of five ink photoinitiator residues in packaged food beverages, namely 2-isopropylthioxanthone (ITX), benzophenone, 2-ethylhexyl 4-dimethylaminobenzoate (EHDAB), 1-hydroxycyclohexyl phenyl ketone (Irgacure 184), and ethyl 4-dimethylaminobenzoate (EDAB). Samples extracted from selected beverages (milk, fruit juices, and wine) and related packagings using \(n\)-hexane and dichloromethane were purified on SPE silica gel cartridges and then analyzed by GC–MS and LC–MS. The recoveries obtained by spiking the beverage samples at concentrations of 4 and 10 μg L\(^{-1}\) with a standard mixture of photoinitiators were in the ranges 42–108% for milk, 50–84% for wine, and 48–109% for fruit juices. The reproducibility of the method was assessed in all cases from the correlation coefficient, and was lower than 19%. The LODs and LOQs obtained using GC–MS were in the ranges 0.2–1 and 1–5 μg L\(^{-1}\), respectively. The method was applied to analyze 40 packaged food beverages (milk, fruit juices, and wine samples). The most significant contamination was that of benzophenone found in all the samples in the concentration range 5–217 μg L\(^{-1}\). Its presence was confirmed by LC–atmospheric-pressure photoionization (APPI)-MS/MS analysis. The photoinitiator (EHDAB) was found in 11 out of the 40 beverages in the concentration range 0.13–0.8 μg L\(^{-1}\). Less important was the ITX contamination found in three out of the 40 samples in the range 0.2–0.24 μg L\(^{-1}\). This work led to the proposal of a new method for the analysis of ink photoinitiator residues in polycoupled carton packaging and in contained food beverages.

Benzophenone (BP) and 4-methylbenzophenone (4MBP) are photoinitiators that are generally used to cure ink on carton boards. Van Hoeck et al. (2010) developed a fast and reliable method for the determination of BP and 4MBP in breakfast cereals. The sample was extracted ultrasonically using a mixture of dichloromethane and acetonitrile (1:1), followed by clean-up of the extract using SPE with a silica cartridge. Finally, the extract was analyzed by GC–MS. Benzenophenone-\(d_{10}\) was used as an internal standard. The presented method was validated in terms of linearity, recovery, repeatability and intra-laboratory reproducibility, specificity, LOD, and LOQ, and was able to detect both BP and 4MBP at very low concentrations (LOD 2 μg kg\(^{-1}\)) in breakfast cereals.

Acrylic adhesives are used to manufacture multilayer laminates that are used in food packaging to form the geometric shape of the package and also to stick labels on the packages. Once applied on the packaging, adhesives can provide potential migrant compounds that could contaminate the packaged food.
Adhesives are complex matrices where intentionally and non-intentionally added substances are present, and the identification of the migrant compounds is required by law. Canellas et al. (2012) developed a method of atmospheric pressure GC coupled with a quadrupole (Q) hyphenated to a TOF mass spectrometer (APGC–MS/Q-TOF) for identification of unknowns coming from three different acrylic adhesives. The results were compared with those obtained by conventional GC–MS-Q. Sixteen compounds were identified by GC–MS/Q and five of them were confirmed by APGC–MS/Q-TOF as their molecular ions were found. Moreover, additional new compounds, 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one, were identified and their structures were elucidated using the spectra obtained by APGC–MS/Q-TOF. This finding of the new identified compounds was very relevant as these compounds were biocides suspected to be allergenic and cytotoxic in humans. Migration studies were carried out using Tenax as solid food simulant and the results showed that the three acrylic adhesives tested in this work were safe for use in food packaging materials since the migration of compounds previously identified was below the limit established in current legislation.

Mineral oil contamination in foods has been well known for a long time. Most recently, attention has been focused on migration from cardboard packaging. Mineral oils contaminating foodstuffs consist of mineral oil saturated hydrocarbons (MOSHs) comprising a complex mixture of linear, branched, and cyclic compounds, and variable amount of mineral oil aromatic hydrocarbons (MOAHs), mainly alkylated. Both MOSHs and MOAHs form “humps” of unresolved peaks with the same range of volatility. Since these two fractions have different toxicological relevances, it is important to quantify them separately both in cardboard and in foods.

Moret et al. (2012) developed a rapid off-line SPE-LVI-GC–FID method based on the use of silver silica gel and low solvent consumption for the determination of MOSHs and MOAHs in cardboard and dried foods packaged in cardboard. The SPE method was validated using LVI with a conventional on-column injector and the retention gap technique (which allowed the injecting of up to 50 μL of sample). The detector response was linear over all the concentration range tested (0.5–250 μg mL⁻¹) and recoveries were practically quantitative. Further, the reproducibility was good (RSDs lower than 7%) and the LOQ was adequate to quantify the envisioned limit of 0.15 mg kg⁻¹ proposed in Germany for MOAH analysis in food samples packaged in recycled cardboard. Rapid heating of the GC oven allowed the sample throughput to be increased (3–4 samples per hour) and enhanced the sensitivity. The proposed method was used for the determination of MOSHs and MOAHs in selected food samples usually sold commercially in cardboard packaging. The most contaminated item was a tea sample (102.2 and 7.9 mg kg⁻¹ of MOSHs and MOAHs below n-C₂₅, respectively), followed by a rice and a sugar powder sample. All were packaged in recycled cardboard.
Alkylphenols (APs) are degradation products of alkylphenol polyethoxylates (APEOs), which are added as nonionic surfactants to cleaning agents, including food detergents. The use of detergents containing nonyl- or octylphenol ethoxylates to wash vegetables prior to their packaging can lead to contamination by their degradation products.

Cacho et al. (2012) developed a method for the determination of three APs [4-\textit{tert}-octylphenol (tOP), 4-\textit{n}-octylphenol (OP), and 4-nonylphenol (NP)], and six phthalate esters (PEs) [dimethyl phthalate (DMP), diethyl phthalate (DEP), di-\textit{n}-butyl phthalate (DBP), \textit{n}-butylbenzyl phthalate (BBP), di-2-ethylhexyl phthalate (DEHP), and di-\textit{n}-octyl phthalate (DOP)] in vegetables using SBSE in combination with thermal desorption gas chromatography–mass spectrometry (TD-GC–MS). Ultrasonic radiation was used to extract the analytes from the solid food matrix and the extract obtained was preconcentrated by SBSE. The different parameters affecting both stages were carefully optimized. The method was applied to analyze commercial vegetables in the form of plastic-packed salads and canned greens and also the corresponding filling liquids of the canned foods. Quantification of the samples was carried out against aqueous standards using an internal standard (anthracene). The analysis of a 2 g vegetable sample provided LODs between 12.7 and 105.8 pg g\textsuperscript{−1} for OP and DEHP, respectively. Studies of migration from the plastic packaging of the vegetable samples were carried out. DEP, DBP, and DEHP were found to have migrated from the bags to the simulant and the same compounds were quantified in lettuce, corn salad, arugula, parsley, and chard at concentration levels in the range 8–51 ng g\textsuperscript{−1}. However, OP and NP were found in only two vegetable samples and one filling liquid, and neither was detected in any package. The proposed method provided recoveries of 83–118%.

Plasticizers are mainly organic esters with high boiling points, which are often employed in significant amounts in food contact materials to increase flexibility and durability (Tester, 1982). When the packaging material directly contacts foods, the plasticizers could migrate into and contaminate the foods. Owing to the carcinogenic effects of plasticizers at high doses in mice and rats of both genders, several analytical methods have been developed for their analysis.

Lau and Wong (1996) developed a method for the determination of plasticizers in foods using GC–MS with ion-trap mass detection. The plasticizers were quantified by an internal standard addition method using diisobutyl phthalate as the internal standard. Four selected food samples were spiked with nine different plasticizers at about 0.3 μg g\textsuperscript{−1}. The recoveries of the plasticizers were in the range 90–106%. The proposed method showed an improvement in precision and exhibited good linearity over a wide range of concentrations. The new method was applied to analyze real samples and the results were found to be in agreement with those obtained using the well-established isotope dilution technique.

Lu et al. (2012) developed a GC–MS-based isotope dilution technique for the determination of environmental estrogens in vegetables and fruits. The isotopically labeled standards of related environmental estrogens were used as the
isotope dilution standards (IDS) to form the following analyte–surrogate pairings: octylphenol–[13C₆]-4-n-nonylphenol, 4-n-nonylphenol–[13C₆]-4-n-nonylphenol, 4-nonylphenol–[13C₆]-4-n-nonylphenol, bisphenol A–[13C₁₂]bisphenol A, estrone–[13C₆]estrone, 17α-estradiol–[13C₆]-17β-estradiol, 17β-estradiol–[13C₆]-17β-estradiol, 17α-ethynylestradiol–[13C₂]–17β-ethynylestradiol, and estriol–[2H₄]estriol. Plant samples were homogenized and extracted ultrasonically with acetone. Acid pretreatment greatly increased the peak intensities for the analytes. Acid hydrolysis pretreatment was important for liberating conjugates of estrogenic contaminants in plant materials. Recoveries of the spiked analytes were >90%. The LOD ranged from 0.01 to 0.20 μg kg⁻¹ and the LOQ ranged from 0.04 to 0.60 μg kg⁻¹. Bisphenol, nonylphenol, and natural estrogens were detected in vegetable and fruit samples obtained from local markets, illustrating the feasibility of this method to determine trace levels of estrogenic contaminants in vegetables and fruits. The method had significant environmental implications in terms of the simultaneous analysis of estrogenic contaminants in vegetables and fruit samples.

1.6 Nitrite

Nitrite is widespread in Nature and is also used as a food additive for preservation, as in color fixers, and as a corrosion inhibitor. There has been concern about the potential health impact of nitrite additives in foods because the reactions of nitrite with various amines, amino acids, and endogenous enzymes form carcinogenic nitrosamines.

The maximum permissible limit of nitrite in drinking water specified by the EU is 100 μg L⁻¹ and by the US Public Health Service 60 μg L⁻¹ (18 μg L⁻¹ NO-N) (Jain et al., 1997). It is important that sensitive and accurate methods are available for the determination of nitrite and its derivatives. Such methods should also be simple and rapid and be capable of determining nitrite and its derivatives in various types of real samples (Tanaka et al., 1980).

Akyüz et al. (2009) developed GC–MS and LC with fluorescence detection (LC–FL) methods for the determination of low levels of nitrite and nitrate in biological, food, and environmental samples. The methods included derivatization of aqueous nitrite with 2,3-diaminonaphthalene (DAN), enzymatic reduction of nitrate to nitrite, extraction with toluene, and chromatographic analysis of the highly fluorescent 2,3-naphthotriazole (NAT) derivative of nitrite using GC–MS in the SIM mode and LC–FL. Nitrite and nitrate ions in solid samples were extracted with 0.5 M aqueous NaOH by sonication. The recoveries of nitrite and nitrate ions based upon GC–MS and LC–FL results were 98.40 and 98.10%, respectively, and the precision of these methods as indicated by the RSDs were 1.00% for nitrite and 1.20% for nitrate. The LODs of the GC–MS (SIM mode) and LC–FL methods based on S/N = 3 were 0.02 and 0.29 pg mL⁻¹ for nitrite and 0.03 and 0.30 pg mL⁻¹ for nitrate, respectively.
Summary

In this chapter, the basics of GC and MS techniques were introduced, and state-of-the-art GC–MS methods used in determining food chemical hazards were described. There are a great number of compounds in food samples and the concentration of chemical hazards is relatively low in the food matrices. In order to deal with the analysis of a large number of compounds in foods, effective sample preparation is essential for achieving precise and reliable analytical results. Some sample preparation methods, such as SPE, LLE, SBSE, and MEPS, were described. A variety of food contaminants, such as pesticides, veterinary drugs, food additives, organometallics, process toxins, and contaminants from packaging materials, that have been determined by GC–MS methods were highlighted.

Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ASE</td>
<td>accelerated solvent extraction</td>
</tr>
<tr>
<td>CI</td>
<td>chemical ionization</td>
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<tr>
<td>DI-SPME</td>
<td>direct immersion solid-phase microextraction</td>
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<tr>
<td>DLLME</td>
<td>dispersive liquid–liquid microextraction</td>
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<tr>
<td>DMI</td>
<td>difficult matrix introduction</td>
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<tr>
<td>DTD</td>
<td>direct thermal desorption</td>
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<td>ECD</td>
<td>electron-capture detection</td>
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<td>EI</td>
<td>electron impact ionization</td>
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<td>EPA</td>
<td>Environmental Protection Agency</td>
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<tr>
<td>ESI</td>
<td>electrospray ionization</td>
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<tr>
<td>FDA</td>
<td>Food and Drugs Administration</td>
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<tr>
<td>FID</td>
<td>flame ionization detection</td>
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<tr>
<td>FT-ICR</td>
<td>Fourier transform ion cyclotron resonance</td>
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<td>GC</td>
<td>gas chromatography</td>
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<td>GPC</td>
<td>gel permeation chromatography</td>
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<td>HA</td>
<td>heterocyclic amine</td>
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<td>HAc</td>
<td>acetic acid</td>
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<tr>
<td>HPLC</td>
<td>high-performance liquid chromatography</td>
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<td>HRMS</td>
<td>high-resolution mass spectrometry</td>
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<tr>
<td>HS</td>
<td>headspace</td>
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<tr>
<td>ICP</td>
<td>inductively coupled plasma</td>
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<td>IT</td>
<td>ion trap</td>
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<tr>
<td>LLE</td>
<td>liquid–liquid extraction</td>
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<td>LOD</td>
<td>limit of detection</td>
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<tr>
<td>LOQ</td>
<td>limit of quantification</td>
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<td>LP-GC</td>
<td>low-pressure gas chromatography</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>LVI</td>
<td>large-volume injection</td>
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<td>MAE</td>
<td>microwave-assisted extraction</td>
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<td>MAH</td>
<td>monocyclic aromatic hydrocarbon</td>
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<tr>
<td>MEPS</td>
<td>microextraction in a packed syringe</td>
</tr>
<tr>
<td>MIP-AED</td>
<td>microwave-induced plasma atomic emission detection</td>
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<tr>
<td>MPI</td>
<td>multi-photon ionization</td>
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<td>MRL</td>
<td>maximum residue limit</td>
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<td>MRM</td>
<td>multiple reaction monitoring</td>
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<td>MS</td>
<td>mass spectrometry</td>
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<tr>
<td>MSPD</td>
<td>matrix solid-phase dispersion</td>
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<tr>
<td>MSPDE</td>
<td>matrix solid-phase dispersion extraction</td>
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<tr>
<td>NFA</td>
<td>National Food Administration</td>
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<tr>
<td>NPD</td>
<td>nitrogen–phosphorus selective detection</td>
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<tr>
<td>PAH</td>
<td>polycyclic aromatic hydrocarbon</td>
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<tr>
<td>PCI</td>
<td>positive chemical ionization</td>
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<tr>
<td>POP</td>
<td>persistent organic pollutant</td>
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<tr>
<td>PTV</td>
<td>programmed-temperature vaporization</td>
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<tr>
<td>QIST-MS–MS</td>
<td>in-time quadrupole ion storage mass spectrometry</td>
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<tr>
<td>QuEChERS</td>
<td>quick, easy, cheap, effective, rugged, and safe</td>
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<tr>
<td>RSD</td>
<td>relative standard deviation</td>
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<td>SBSE</td>
<td>stir-bar sorptive extraction</td>
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<tr>
<td>SIM</td>
<td>selected ion monitoring</td>
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<tr>
<td>SLE-LTP</td>
<td>solid–liquid extraction with low-temperature purification</td>
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<tr>
<td>S/N</td>
<td>signal-to-noise ratio</td>
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<tr>
<td>SPE</td>
<td>solid-phase extraction</td>
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<tr>
<td>SRM</td>
<td>selected reaction monitoring</td>
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<td>TOF</td>
<td>time-of-flight</td>
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