Chapter 12

Gas Chromatography–Time-of-Flight Mass Spectrometry in Food and Environmental Analysis

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1 INTRODUCTION

Hyphenation of gas chromatography (GC) with mass spectrometry (MS) allows identification and quantification of a wide range of even trace amounts of GC-amenable compounds in complex matrices. Until now, low-resolution (unit mass) mass spectrometric detectors employing either single quadrupole or ion trap mass analyzers have been used in most of the food and environmental analysis applications.

While quadrupole is mainly operated in selected ion monitoring (SIM) mode for enhanced sensitivity in ultra-trace analysis, the ion trap (except for full scan mode) is used in MS–MS (tandem-in-time) mode to increase selectivity. In addition, triple quadrupole (tandem-in-space) or high-resolution magnetic double-focusing sector instruments can be employed for specific analyses [1].
Recent progress in instrumentation design (optics mainly) as well as the use of fast recording electronics (which were not available or were too expensive until a few years ago) together with improvements in signal-processing techniques have led to the renaissance of time-of-flight (TOF) mass analyzers for the determination of a wide range of both target and nontarget organic components occurring in various biotic and abiotic matrices [2].

This chapter provides a general overview of gas chromatography–time-of-flight mass spectrometry (GC–TOF-MS) basic features, highlighting its advantages and limitations compared to GC using conventional mass analyzers. Examples of results obtained for food and environmental contaminants, aroma and flavor components, and food authenticity assessment are described to illustrate the potential of this technique.

2 GAS CHROMATOGRAPHY–TIME-OF-FLIGHT MASS SPECTROMETRY

Historically, TOF-MS represents one of the oldest MS systems. The idea of TOF-MS was first proposed by Stephens et al. in 1946 [3] followed by construction of the first TOF mass spectrometer by Cameron and Eggers 2 years later [4]. The most outstanding contribution to resolution improvements arose from the fundamental TOF-MS publication by Wiley and McLaren in 1955 [5]. Although the first commercial TOF device (Bendix Corporation) was produced as early as 1957 [6], the interest in TOF-MS waned in the 1960s due to the popularity of magnetic sector and quadrupole instruments. The instrumental innovation of the “reflectron” by Mamyrin in the early 1970s [7] and the development of orthogonal acceleration in the late 1980s by Dawson and Guilhaus [8], and Dodonov et al. [9] were other milestones in TOF-MS development. In 1990, the renaissance of GC–TOF-MS started followed by the introduction of first commercial GC–TOF-MS instruments in 1995–2005 (LECO, Micromass, Jeol, Thermo).

Currently, three types of GC–TOF-MS instruments differing in their basic characteristics are available:

i. High-resolution/accurate mass analyzers (5000–12,500 full width at half maximum, FWHM) providing only moderate acquisition speed (20–50 spectra/s),

ii. Unit-resolution instruments that feature high acquisition speeds (500–1000 spectra/s),

iii. High-speed high-resolution/accurate mass analyzers permitting high acquisition speeds (up to 200 spectra/s) as well as high mass resolving power (50,000 FWHM).

In addition to these TOF-MS instruments for single (MS$^1$) analysis, a hybrid instrument combining quadrupole and TOF-MS has recently been introduced allowing either analysis under the conditions of high-resolution time-of-flight mass spectrometry (HR-TOF-MS) (MS$^1$) or Q/HR-TOF-MS (MS/MS) with
selection of precursor ions and monitoring of product ions through the entire mass range with high mass accuracy.

The application potential of these approaches is obviously complementary. The technical features of current available GC–TOF-MS systems are summarized in Table 1.

In the following paragraphs, advantages and limitations of TOF-MS instruments allowing comparison with conventional scanning MS detectors are summarized [1,2,10–13].

1. **Acquisition speed.** Time needed to obtain one mass spectrum is in the range of tens to hundreds of microseconds. In total, 1000–40,000 primary spectra that are hereby obtained in 1 s are summed and, as the final result, 1–1000 spectra/s are then stored in a computer depending on the type of TOF-MS instrument. **Maximal spectral acquisition speed** is a critical parameter in detection of very narrow peaks generated during fast chromatographic separation. The moderate acquisition rates of the HR-TOF instruments predetermine their use as the detector for conventional and fast GC; the high-speed low-resolution TOF and high-speed high-resolution TOF instruments are suitable for detection of very narrow chromatographic peaks generated by very fast and ultra-fast GC or comprehensive two-dimensional GC (GC × GC). The “optimal” acquisition speed depends on various parameters such as (i) required number of data points per chromatographic peak; (ii) ability to resolve closely coeluted analytes by means of spectral deconvolution; and (iii) signal-to-noise (S/N) parameters. Figure 1 shows the influence of acquisition rate on peak shapes. A low spectral acquisition rate unavoidably results in rather poor peak shapes that obviously do not represent classic Gaussian curve. Although Baumann et al. [14] showed that seven to eight points per peak are required for obtaining the 99.99% peak recovery and having available only three to four points resulted only in a small degradation (1.4%) of peak recovery. Thus, more data points and high acquisition speed are typically needed for efficient deconvolution of eluting compounds.

2. **Mass resolving power.** Good mass resolving power is achieved by orthogonal sampling of generated ions, which is important for their spatial focusing (ions are ejected to a mass analyzer at practically the same instant). Further improvement of mass resolution is obtained using reflectron for energy focusing. This “ion mirror” consists of a series of ring electrodes with linearly increasing voltage creating retarding fields. After reaching the reflectron area, ions with higher energy penetrate more deeply inside, which extends the time until they are reflected. As a consequence of this phenomenon, the ions of the same m/z value with different initial energies reach the detector at almost the same time. In addition, the mass resolving power is substantially improved by making the ions pass twice along a TOF flight tube before reaching the detector. High-speed low-resolution TOF-MS instruments provide only a unit mass resolution, whereas
<table>
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<tr>
<th>Instrument (Company)</th>
<th>Upper Mass Limit (Da)</th>
<th>Mass Resolving Power</th>
<th>Maximal Acquisition Rate (spectra/s)</th>
<th>Mass Accuracy (ppm)</th>
<th>Linearity (Orders of Magnitude)</th>
<th>Acquisition System</th>
<th>Ionization Mode</th>
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<td>Tempus (Thermo)</td>
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ADC, analogue-to-digital converter; EI, electron ionization; FWHM, full width at half maximum; NCI, negative chemical ionization; PCI, positive chemical ionization; ppm, parts per million; Q, quadruple; TDC, time-to-digital converter; TOF, time-of-flight.
HR-TOF analyzers offer mass resolving power of about 5000–50,000 FWHM. The mass resolving power of HR-TOF instruments is not constant throughout the entire mass range (Figure 2); typically for ions <m/z 100, these values are lower as compared to values used for instrument’s specification. On the other hand, the mass resolving power of one novel instrument reaches even higher values (25,000–50,000 FWHM corresponding to 12,500–25,000 (10% valley definition)) than sector instruments operating typically at a mass resolving power of >10,000 (10% valley definition). The advantage of high resolution is the possibility to partially or completely resolve matrix components yielding ions with the same
nominal mass as that of the target analyte, hence, significantly reducing background interferences and, consequently, improving the analyte identification.

3. **Mass accuracy.** In the case of HR-TOF-MS system, mass accuracy <5 ppm is attainable by using a lock mass approach, that is, introducing a reference compound into the ion source during analyses or at the end of the each analysis. On the basis of previously performed mass calibration over a given mass range and defined value (ion) of a lock mass, or several masses, the software automatically corrects the values of all masses in the acquired spectra. Under these conditions, the determination of elemental composition is possible; also the specificity for the identification of unknowns is enhanced. However, mass accuracy is not constant throughout the wide concentration range of analytes. Typically, some deviations are observed at low- and high-signal intensity (see Figure 3 as an example). While at low concentrations, too weak analytical signal can lead to worsened mass accuracy, at high analyte concentration saturation of the detector (multichannel plate) and/or saturation of a recording device (time-to-digital converter (TDC) or analogue-to-digital converter (ADC)) used in TOF-MS instruments can lead to the same phenomenon.

4. **Acquisition of complete spectra.** Contrary to scanning instruments that provide enhanced selectivity and sensitivity only when operated in a SIM mode (quadrupole) or when a measurement of product fragmentation

![FIGURE 3](image)

**FIGURE 3** Mass accuracy in mDa (x-axis) of fragmentation ion of dimethoate (theoretical mass 124.9826 Da) in dependence on concentration of analyte in matrix-matched standards (y-axis). Reprinted with permission from Ref. [10].
ions in MS–MS mode is employed (ion trap), TOF-MS instruments allow acquisition of full mass spectra even at these very low concentration levels thanks to higher mass analyzer efficiency. This efficiency is, for a quadrupole mass analyzer scanning over a 500 amu mass range, only about 0.1%, while 25% efficiency is obtained for oa-TOF instrument. This enables the use of the full capabilities of library reference spectra to search for identification/confirmation of trace analytes identity.

5. Absence of spectral skew. There are no changes in the ratios of analyte ions across the peak during the acquisition of the mass spectrum, and consequently, no spectral skew (observed commonly by scanning instruments) is encountered. This allows automated deconvolution of partially overlapped peaks on the basis of increasing/decreasing ion intensities in collected spectra and background subtraction followed by identification using a library search. Figure 4 shows an example of spectral deconvolution of nonan-2-ol and (1S)-1,7,7-trimethylbornan-2-one (camphor) isolated by headspace solid-phase microextraction (HS-SPME) from beer [15]. In this particular case, the peak apex separation of these compounds was 1.7 s, which required relatively high acquisition rate (10 spectra/s) for automated peak finding.

The deconvolution function (employing software correction for spectral skewing) is currently available also for scanning instruments in AMDIS (Automated Mass Spectral Deconvolution and Identification System) software [16]. However, the low signal intensity during full spectra acquisition and the relatively low acquisition rate of common scanning instruments are parameters that make this feature of high importance in fast GC analysis (i.e., under conditions of lower chromatographic resolution of eluted components).

6. Extended mass range. Although there is theoretically no upper mass limit for the TOF-MS analyzers, this parameter is not critical in combination with GC because volatility/thermolability of target compounds effectively dictates the scope of this technique. Compared with common mass analyzers with an upper mass limit of \( m/z \) 600–1050, the TOF analyzers coupled to GC operate up to \( m/z \) 1000–2000.

7. Detector. On the contrary to many scanning instruments, in which an electron multiplier is integrated as a detection device, the TOF-MS employs the multichannel plate (MCP) detector, which allows simultaneous analysis of all masses across the whole mass range within a few microseconds. However, one must be aware of its limited lifetime, that is, 1–3 years (the replacement of a conventional electron multiplier is required in a 5- to 7-year period). Depending on the frequency of the instrument use, the potential to detect compounds drops by time; the key factor in this context is the value of voltage set to the MCP. In general, sensitivity improvement requires a higher MCP voltage setting, which unavoidably leads to a reduced lifetime of the MCP.
FIGURE 4 Spectral deconvolution of two closely eluted beer markers of the GC–HS-TOF-MS fingerprint. (1) Nonan-2-ol, $m/z$ 45 displayed; (2) (1S)-1,7,7-trimethylnorbornan-2-one (camphor), $m/z$ 95 displayed. Reprinted with permission from Ref. [15].
8. **Linear dynamic range.** The linear dynamic range of common scanning instruments varies between five and six orders of magnitude. The current TOF-MS instruments generally suffer from the limited linear dynamic range compared with conventional MS instrumentation. The ADC offers linear dynamic range of four to five orders of magnitude but, at low analyte signal intensities, noise becomes a limiting factor for its use. The TDC, on the contrary, is very suitable for detection of weak signals, which is the case of analytes at ultra-trace levels. Although the linear dynamic range of this device typically does not exceed two orders of magnitude, it can be expanded to approximately three orders of magnitude by application of the dead time correction function. Moreover, because of the continuing improvements in both hardware and software features, the dynamic range of some recent instruments employing the TDC is as high as four orders of magnitude. Application of a high voltage to a specific focusing lens reducing the intensity of ions passing into the TOF analyzer represents a technical solution that allows replacement of saturated data in a mass spectrum with unsaturated ones acquired when the ion beam has been defocused by the lens.

9. **Cost of the instrument.** An important factor when considering a TOF-MS system purchase is undoubtedly its cost. Unfortunately, the cost of TOF-MS is still substantially higher (approximately two to three times) compared with low-resolution quadrupole or ion trap instruments. However, in line with dropping cost of sophisticated electronics integrated in these instruments, prices may fall.

3 **APPLICATIONS OF GC–TOF-MS IN FOOD AND ENVIRONMENTAL ANALYSIS**

In recent years, application of GC–TOF-MS (both high-resolution and high-speed instruments) has been demonstrated as a powerful and highly effective analytical tool in analysis of food and environmental contaminants (e.g., pesticide residues, polychlorinated biphenyls, brominated flame retardants (BFRs), dioxins, polycyclic aromatic hydrocarbons, toxaphene, and acrylamide), flavor compounds, drug screening, petrochemical analysis, and metabolomic studies, demonstrating great potential of this technique not only for quantification of target analytes but also for identification of nontarget compounds in diverse (often complex) matrices [1,2]. In the following sections, examples of TOF-MS performance in these applications will be outlined.

3.1 **Food and Environmental Contaminants**

3.1.1 **Pesticide Residues**

Currently, more than 800 pesticide active ingredients in a wide range of commercial products are registered for use in agriculture to meet food supply
demands. Under certain circumstances, however, residues of active ingredients occur in treated crops at the time of harvest. Because of potential health risk for consumers, resulting from acute and/or chronic dietary exposure, maximum residue limits (MRLs) for many pesticides have been established around the world. The rapid and cost-effective multiple residue analysis at very low levels within a single run represents, therefore, a challenging task for both regulatory agencies and food producers [17].

During the recent decade, both HS-TOF-MS and HR-TOF-MS instrumental platforms were reported to be a useful tool in trace analysis of pesticide residues in foods. Typically, HS-TOF-MS is used in either 1D-GC or GC × GC setup; in the latter case, further enhancement of separation power is achieved together with improvement of sensitivity [18]. These instruments employed in most cases spectral deconvolution of the acquired GC(×GC)–MS records, while the use of HR-TOF-MS allowed the unbiased identification and reliable quantification of pesticide residues through the application of a narrow mass window (0.02 Da) for extracting analyte ions [10].

The first comprehensive studies focusing on advantages and limitations of GC–HR-TOF-MS in trace analysis were published by Dalluge et al. [11] and Cajka and Hajslova [10]. In these studies, various aspects such as signal intensity versus acquisition rate, mass accuracy, selectivity of detection, limits of quantification/detection, working range, and repeatability of responses were evaluated. In general, unbiased identification and reliable quantification of target analytes are possible due to (i) application of narrow mass window (0.02–0.05 Da) for extracting analyte ions and (ii) availability of full spectral information even at very low levels of target analytes.

In a follow-up study, Cajka et al. [17] developed a rapid method using programmed temperature vaporizer injection–low-pressure gas chromatography–high-resolution time-of-flight mass spectrometry (PTV–LP-GC–HR-TOF-MS) for the analysis of multiple pesticide residues in fruit-based baby food. Using fast GC, analysis of 100 pesticide residues within a 7-min runtime was achieved. The benefit of using HR-TOF-MS to eliminate background interferences (chemical noise originating mainly from matrix coextractives) through the use of narrow mass window setting for extracting target ions, thereby increasing selectivity, is illustrated in Figure 5 with the example of phosalone. A 1 Da mass window gave peak-to-peak S/N ratio of 6, but setting the mass window to 0.1 Da or even as low as 0.02 Da led to an S/N of 25 and 74, respectively, in a baby food extract. With only a few exceptions, the lowest calibration levels (LCLs) for the pesticides tested were ≤0.01 mg/kg, which meets the EU MRL set for pesticide residues in cereal-based foods and baby foods (2003/13/EC).

Leandro et al. [19] developed a GC–HR-TOF-MS method for the quantification of approximately 100 pesticides in baby food, pear, and lettuce samples. The previously observed limitation of relatively narrow linear range [10] for HR-TOF-MS instruments with 1 and 3.6 GHz TDC was overcome by
using the new DRE (dynamic range enhancement) function. Acquiring with DRE ON, the instrument is capable of managing the large number of ions by switching to low-sensitivity mode and then applying the DRE magnification factors to correct the response. Acquiring with DRE OFF, the analyte at high concentration leads to saturation of the detector on the TDC, in which consequence the response falls outside the extracted mass chromatogram window (0.05 Da), as illustrated in Figure 6A. The spectrum is also indicative of saturation, as illustrated in Figure 6B. Targeted quantification, exact mass peak detection, and deconvolution and library searching packages were used successfully to detect and identify incurred residues present in the samples at concentrations above 0.01 mg/kg.

FIGURE 5 Influence of mass window setting for detection of 0.01 mg/kg phosalone ($t_R = 4.11$) in apple baby food extract using HR-TOF-MS. Target ion $m/z$ 182.001 extracted using a mass window of (A) 1 Da, (B) 0.1 Da, and (C) 0.02 Da. Reprinted with permission from Ref. [17].
FIGURE 6  (A) Extracted ion chromatograms of chlorpyrifos (m/z 313.9574) with DRE ON and OFF (HR-TOF-MS instrument with the TDC) and (B) its spectra, in a lettuce matrix-matched standard at 1.0 μg/mL. The saturated ions are marked by question-marks. Reprinted with permission from Ref. [19].
In the case of HS-TOF-MS, several papers describing coupling to either 1D-GC or GC × GC were published. While for 1D-GC–HS-TOF-MS analyses, acquisition speeds between 10 and 20 spectra/s were used, for GC × GC setup acquisition, speeds up to 250 spectra/s were needed to obtain sufficient number of points per chromatographic peak.

Koesukwiwat et al. [20] used LP-GC–HS-TOF-MS for the identification and quantification of 150 pesticides in tomato, strawberry, potato, orange, and lettuce samples. The results from this work demonstrated the potential for routine use of LP-GC–HS-TOF-MS to achieve faster individual sample turnaround time and higher throughput than with common GC–MS methods. Furthermore, LP-GC–HS-TOF-MS attained greater ruggedness than alternate fast GC–MS approaches. The major limitation of the method so far was the time it took to process the results using the software. Although the signal of target analytes was automatically checked, assigned, integrated, and compared to the reference file based on their mass spectra, the manual checking to better assign and identify peaks and correct integration errors was a very time-consuming and onerous process. The spiking of 150 pesticides to so many samples in this study contributed to this drawback.

Mastovska et al. [21] employed 1D-GC–HS-TOF-MS for the analysis of 150 pesticides in various cereal grain matrices (corn, oat, rice, and wheat). Both sample preparation and injection were optimized to be compatible with GC–MS. To obtain deconvoluted reference spectra even for closely eluting peaks, analytes (in total 185 compounds monitored by GC–HS-TOF-MS, including important pesticide degradation products) were divided into two groups for two separate injections into the GC system. The authors pointed out that the HS-TOF-MS instrument does not require presetting of analyte-specific conditions for each individual pesticide as opposed to, for example, single ion monitoring with quadrupole or tandem MS with a triple quadrupole or an ion trap mass analyzer. Therefore, the analysis (data acquisition) is nontargeted. However, for routine pesticide residue analysis, it is difficult to process the data in a completely nontargeted fashion, relying only on spectral deconvolution, peak finding, and spectral matching algorithms provided by the data processing software. Instead, they preferred to create templates (in the calibration portion of the software) that enabled fast data review for pesticides on their target list by extracting traces of their quantitation ions in expected retention time windows and comparing their deconvoluted and raw MS spectra with library and reference spectra.

The capability of spectra deconvolution of complex chromatograms was also investigated by Patel et al. [22], de Koning et al. [23], who used an automated difficult matrix introduction technique for the injection of pesticide residues in nonpurified food extracts. Since the nonvolatiles do not enter the GC column using this sample introduction technique, even the injection of nonpurified sample extracts is feasible. However, one should be aware that more (semi) volatiles enter the GC column, thus increasing the risk of coelutions.
In recent years, comprehensive two-dimensional GC (GC × GC) has attained increasing attention for its outstanding separation potential and capability to solve demanding analytical tasks. Trace-level analysis of pesticide residues in complex food matrices represents such a demanding task [24].

Zrostlikova et al. [18] explored the potential of GC × GC–HS-TOF-MS with 20 modern pesticides with a broad range of physicochemical properties in apple and peach samples. It has been demonstrated that the application of GC × GC brings distinct advantages such as enhanced separation of target pesticides from matrix coextracts as well as their improved detectability. The limits of detection (LODs) of the pesticides comprised in the study (determined at S/N = 5) ranged from 0.2 to 30 pg injected with the exception of the last eluted deltamethrin, for which 100 pg could be detected. When compared to 1D-GC–TOF MS analysis under essentially the same conditions, the detectability enhancement was 1.5- to 50-fold. Full mass spectral information by HS-TOF-MS and the deconvolution capability of the dedicated software allowed for reliable identification of most pesticides at levels below 0.01 mg/kg (<10 pg injected) in fruit. Figure 7 shows an example of dichlorvos in an apple extract at 10 ng/mL under the conditions of 1D-GC (a) and GC × GC (b). In general, performance characteristics of the GC × GC–HS-TOF-MS method, such as linearity of calibration curves, repeatability of (summed) peak areas, as well as repeatability of first and second dimension retention times, were shown to fully satisfy the requirements for trace-level analysis of the pesticide residues in food.

The superiority of GC × GC over 1D-GC in pesticide residue analysis was also documented by other authors (Banerjee et al. [25], Dasgupta et al. [26], and Schurek et al. [27]).

3.1.2 Polychlorinated Dibenzo-p-Dioxins and Dibenzofurans

Quantitative determination of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDDs/PCDFs) occurring in biological matrices at ultra-trace levels is typically performed using GC coupled to a high-resolution sector analyzer. High-resolution systems (mass resolving power of >10,000, 10% valley) provide higher selectivity compared to unit mass resolution instruments especially when the levels of potentially interfering compounds are too great. However, this instrumentation is very expensive, bulky, and requires operation by a highly trained specialist. Therefore, alternate analytical instruments (less expensive) have been investigated for dioxin analysis in several laboratories. Among others, GC × GC–HS-TOF-MS has been reported as a valuable technique for improved selectivity in dioxin analysis. In the case of GC × GC–HS-TOF-MS, the improvement of selectivity is achieved employing the secondary column with different polarities that can better separate the target compounds from coeluting matrix components [1].

Hoh et al. [28] evaluated and optimized GC × GC–HS-TOF-MS parameters to yield complete separation of the 17 most important PCDD/PCDF
congeners from polychlorinated biphenyl (PCB) interferences and to attain the lowest detection limits. After optimization, all 17 PCDDs/PCDFs were separated in <60 min and, in particular, the critical pair of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and pentachlorobiphenyl congener CB126 did not coelute chromatographically. Accurate identification and determination of all analytes could be made using their deconvoluted full mass spectra. The method could identify 0.25 pg of TCDD with standard injection from its full mass spectrum.

In a follow-up study, Hoh et al. [29] applied direct sample introduction (DSI)–GC × GC–HS-TOF-MS as a screening method for 17 PCDDs/PCDFs and 4 non-ortho PCBs in fish oil. Comparison of instrumental performance
between DSI–GC\texttimes{}GC–HS-TOF-MS and the traditional 1D-GC–HRMS method showed good agreement of results for standard solutions analyzed in blind fashion. The limit of quantification (LOQ) of TCDD was 0.94 pg/g (Figure 8). This value was suitable for analytical screening of a large number of fish oil (or fish) samples using 2,3,7,8-tetrachlorodibenzofuran and CB126 as markers.

3.1.3 Polybrominated Diphenyl Ethers

Polybrominated diphenyl ethers (PBDEs) belong to a group of BFRs, which are chemicals widely used in various products, for example, plastics, textiles, and furnishing foams to prevent a fire hazard. Currently, there is a growing interest in PBDE analysis in environmental and food samples because of the continual increase in the levels of these compounds in the general environment and human tissues during the past decade [1]. Considering PBDE accumulation potential and with regard to the growing toxicological concerns, unbiased control of PBDE occurrence in the environment is recommended by the recently introduced EU Regulation (2003/11/EC) [30].

Cajka et al. [31] explored the potential of GC–HR-TOF-MS in the analysis of PBDEs in fish and sediment. Two ionization techniques, viz., electron ionization (EI) and negative chemical ionization (NCI), the latter with methane as a reagent gas, were used in this study. While the instrumental LCLs obtained in EI were in the range of 1–5 pg, their values ranged from 10 to 250 fg in NCI mode. This enhancement in detectability of target analytes enabled identification/quantification of even minor PBDE congeners (Figure 9) and, consequently, improved characterization of particular sample
contamination patterns. In general, the quality of the generated data was still comparable to that obtained by a quadrupole analyzer when the amount of sample taken for analysis was higher by one order of magnitude (instruments operated in NCI mode compared). However, due to a limited linear range of the HR-TOF-MS instrument (saturation of the TDC) and taking into account a typically large concentration range of persistent organohalogen pollutants in environmental matrices, it was often possible to obtain accurate quantification of major congeners only by reanalysis of diluted samples.

### 3.1.4 Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are formed by the incomplete combustion of organic matter. They are widely distributed in the environment and human exposure to them is unavoidable. A number of them are carcinogenic and mutagenic. Their presence in the environment is reflected in their presence at detectable levels in many types of uncooked food. In addition, cooking processes can generate PAHs in food. PAHs can also be formed during the curing and processing of raw food prior to cooking [32]. Measurements of PAHs in food and environmental matrices represent a challenging task because of the complexity of food and environmental samples.

Purcaro et al. [33] developed a simple and fast SPME method coupled with GC × GC–HS-TOF-MS for analysis of PAHs in edible oil, performed directly in a hexane solution of the oil. Sampling conditions were optimized by using a sample of oil fortified with a standard solution of PAHs. Figure 10 shows a GC × GC chromatogram of oil fortified with PAHs demonstrating the complexity of the sample.

Drabova et al. [34] developed and validated a simple, fast, and cost-effective sample preparation procedure for the determination of 15 + 1 European Union Polycyclic Aromatic Hydrocarbons (15 + 1 EU PAHs) in dry tea leaf samples. For the final identification/quantification of target PAHs, GC × GC–HS-TOF-MS was used. High peak capacity, provided by GC × GC, enabled separation of otherwise well-known critical groups of PAHs.
represented by (i) benz[a]anthracene, cyclopenta[cd]pyrene, and chrysene; (ii) benzo[b]fluoranthene, benzo[c]fluoranthene (BcF), benzo[j]fluoranthene (BjF), benzo[k]fluorene (BkF), benzo[a]pyrene (BaP), benzo[ghi]perylene (BghiP), chrysene (Ch), cyclopenta[cd]pyrene (CPP), dibenz[ah]anthracene (DBahA), dibenzo[ae]pyrene (DBaeP), dibenzo[ah]pyrene (DBahP), dibenzo[ai]pyrene (DBaiP), dibenzo[al]pyrene (DBalP), indeno [1,2,3-cd]pyrene (IP), and 5-methylchrysene (5MeCh).) Reprinted with permission from Ref. [33].

3.1.5 Comprehensive Contaminants Profiling

Most analytical methods for contaminants focus on individual groups of targeted analytes. Therefore, analysis of multiple classes of contaminants typically entails several sample preparations, fractionations, and injections, whereas other chemicals of possible interest are neglected or lost. A comprehensive contaminant profiling is a novel instrumental approach employing 1D-GC or GC × GC–HS-TOF-MS. Thanks to the recent revival of TOF-MS instruments, several hundreds of analytes, belonging to different classes of organic pollutants such as PCBs, PAHs, BFRs, and pesticides, can be theoretically measured in one run. During recent years, some effort has been spent to develop such a profiling approach, resulting in the introduction of 1D-GC and GC × GC–HS-TOF-MS methods. Typically, these methods are in combination
with large volume injection in order to achieve low LODs of target compounds, allowing simultaneous analysis of various groups of contaminants in food and environmental matrices [35].

Focant et al. [36] presented a GC×GC–HS-TOF-MS method for the simultaneous measurement of selected PCBs, organochlorine pesticides (OCPs), and BFRs in human serum and milk. Using GC×GC ensured the chromatographic separation of most compounds and TOF-MS allowed mass spectral deconvolution of coeluting compounds as well as the use of 13C-labeled internal standards for quantification.

Hernandez et al. [37] explored the potential of GC–HR-TOF-MS for screening of organic pollutants in water. For the extraction, SPME was employed. Investigation of 60 target organic pollutants, including pesticides, octyl/nonyl phenols, pentachlorobenzene, and PAHs, was carried out by evaluating the presence of up to five representative \( m/z \) ions per analyte, measured at high mass accuracy and the attainment of their \( Q/q \) (\( Q \), quantitative ion; \( q \), confirmative ion) intensity ratio. This strategy led to the detection of 4-tert-octylphenol, simazine, terbuthylazine, chlorpyrifos, terbumeton, and terbutryn in several water samples at low part-per-billion levels. Full-spectrum acquisition data generated by the HR-TOF-MS analyzer also allowed subsequent investigation of the presence of PBDEs and several fungicides in samples after MS data acquisition, without the need to reanalyze the water samples. In addition, nontarget analysis was also tested by application of a deconvolution software. Several organic pollutants that did not form a part of the list of contaminants investigated were identified in the water samples, thanks to the excellent sensitivity of HR-TOF-MS in full-spectrum acquisition mode and the valuable accurate mass information provided by the instrument. Bisphenol A, the antioxidant 3,5-di-tert-butyl-4-hydroxy-toluene (BHT), its metabolite 3,5-di-tert-butyl-4-hydroxybenzaldehyde (BHT-CHO), the polycyclic musk galaxolide, and the UV filter benzophenone were some of the compounds present in the water samples analyzed.

Hoh et al. [38] optimized an analytical method using gel permeation chromatography (GPC) followed by DSI–GC×GC–HS-TOF-MS to quantify multiple groups of targeted persistent organic pollutants and halogenated natural products (HNPs) simultaneously in fish oil samples. This new method has a wider analytical scope than the traditional approach that uses multiple methods to cover each class of compounds. The analysis revealed that the relatively more volatile and lighter organic compounds, such as PCBs, OCPs, and other smaller organohalogen compounds, were still present in two brands of “PCB-free” cod liver oils, albeit at much lower levels than in an untreated commercial sample. Moreover, the less volatile organic compounds, such as PBDEs and brominated HNPs, were detected at similar levels in all three cod liver oils. This suggests that the commercial molecular distillation treatment used for removal of organic/inorganic toxic contaminants is only effective for the lighter organic contaminants.
Kalachova et al. [39] developed and validated a rapid and flexible method for the simultaneous determination of 18 key representatives of PCBs, 7 PBDEs, and 32 PAHs in fish and shrimp by GC–HS-TOF-MS. Using a streamlined sample preparation procedure, six samples could be processed in less than 1 h; moreover, the volume of the extraction solvent and consumption of other chemicals can be significantly reduced compared to, for example, traditional Soxhlet extraction followed by GPC. Under optimized GC–HS-TOF-MS conditions, the LOQs were as follows: PCBs 0.1–0.5 \( \mu \text{g/kg} \), PBDEs 0.5 \( \mu \text{g/kg} \), and PAHs 0.05–0.25 \( \mu \text{g/kg} \). In Figure 11, an example of a chromatogram of fish muscle tissue spiked with PCBs, PBDEs, and PAHs is shown.

In a follow-up study, Kalachova et al. [40] used GC × GC–HS-TOF-MS as a tool for the simultaneous determination of various groups of contaminants including 18 PCBs, 7 PBDEs, and 16 PAHs. Since different groups of analytes (traditionally analyzed separately) were included into one instrumental method, significant time savings were achieved. Using large volume PTV, the following LOQs were achieved—PCBs, 0.01–0.25 \( \mu \text{g/kg} \); PBDEs, 0.025–5 \( \mu \text{g/kg} \); PAHs, 0.025–0.5 \( \mu \text{g/kg} \). An acquisition speed of 100 spectra/s was required for sufficient identification/quantification of target analytes. Besides the focus on target analytes, the acquired chromatographic records were submitted after deconvolution to a mass spectral library to identify other contaminants (nontarget screening). Using this approach, other PCB congeners as well as OCPs were identified.

### 3.1.6 Acrylamide

Acrylamide represents a processing contaminant, the presence of which was reported at increased amounts in starch-enriched food such as potato chips, French fries, roast potatoes, breakfast cereals, and crisp bread. Direct analysis of acrylamide in complex food matrices is not an easy task since the \( m/z \) 71 and 55 ions yielded by electron ionization (EI) fragmentation are of low value and nonspecific. Intensive chemical background noise at low \( m/z \) range does not allow obtaining low detection limit and adequate precision when using commonly available unit mass resolution instruments (in this context, bromination provides improved detectability of the analyte) [41].

Dunovska et al. [42] developed a method for direct detection of acrylamide in food employing GC–HR-TOF-MS. Extraction by \( n \)-propanol followed by solvent exchange to MeCN avoided coisolation of acrylamide precursors (sugars and asparagine) that could yield additional analytes in the hot splitless GC injector. Extensive reduction of matrix components in sample extracts, hence improvement of method robustness, was obtained by dispersive solid-phase extraction employing a primary–secondary amine sorbent. Isotopically labeled \( d_3 \)-acrylamide was employed for compensation of potential target analyte losses and/or matrix-inducted chromatographic response enhancement. Using a HR-TOF-MS instrument and using a narrow mass window setting...
FIGURE 11 An example of a GC–HS-TOF-MS chromatogram of fish muscle tissue spiked with PCBs, PBDEs, and PAHs at 5 μg/kg (major PCBs 138, 153, and 180 and PBDE 47 at 25 μg/kg). (Note: acenaphthene (AC), acenaphthylene (ACL), anthracene (AN), benz[a]anthracene (BaA), benzo[a]pyrene (BaP), benzo[b]fluoranthene (BbFA), benzo[c]fluorene (BcFL), benzo/[fluoranthene (BjFA), benzo[g,h,i]perylene (BghiP), chrysene (CHR), cyclopenta[cd]pyrene (CPP), dibenz[a,h]anthracene (DBahA), dibenzo[a,l]pyrene (DBaeP), dibenzo[a,h]pyrene (DBahP), dibenzo[a,l]pyrene (DBalP), dibenzothiophene (DBT), fluoranthene (FA), fluorene (FL), indeno[1,2,3-cd]pyrene (IP), naphthalene (NA), phenanthrene (PHE), pyrene (PY), 1-methylchrysene (1MC), 3-methylchrysene (3MC), 5-methylchrysene (5MC), 1-methylnaphthalene (1MN), 2-methylnaphthalene (2MN), 1-methylphenanthrene (1MPH), and 1-methylpyrene (1MP)). Reprinted with permission from Ref. [39].
(0.02 Da in this study), both acrylamide and $d_3$-acrylamide (internal standard) were unequivocally identified by monitoring the ions at $m/z$ 71.036 (55.018) and 74.056 (58.039), respectively (Figure 12). LOQ values obtained using this method were only slightly higher (15–40 $\mu$g/kg) compared to those attainable by GC–MS, which uses a laborious and time-consuming bromination step (2–25 $\mu$g/kg).

### 3.2 Aroma and Flavor Compounds

A wide range of volatile compounds occur in food headspace, including components responsible for typical flavor, off-flavor, and other quality/safety parameters. SPME in combination with the GC–MS technique is one of the methods of choice for these types of compounds. GC–TOF-MS has also been demonstrated as a tool for food authenticity assessment (e.g., coffee, honey, cacao bean, beer, ice wine, apples) based on the analysis of volatiles [43].

#### 3.2.1 Characterization of Volatiles in Food

Song et al. [44] used SPME–GC–HS-TOF-MS to examine suitability and compatibility for rapid sampling, separation, and detection of apple flavor volatiles. The rapid spectral acquisition rate of 40 spectra/s of the HS-TOF-MS permitted 40–80 spectra to be collected over the typical 1- to 2-s peak
widths. Coeluting compounds such as butyl hexanoate and hexyl butanoate were successfully deconvoluted using $m/z$ 117 and 89, respectively, even though elution times differed by only 0.2 s (Figure 13). A similar approach was applied by these authors [45] also for the analysis of tomato volatiles.

Ryan et al. [46] focused on the analysis of roasted coffee bean volatiles using SPME–GC × GC–HS-TOF-MS. The complexity of the headspace volatile composition of roasted coffee beans is such that it yielded thousands of chromatographic peaks, which were clearly apparent by the peak density in the corresponding two-dimensional contour plots generated for each sample. Semiquantitative analysis was restricted to the 44 selected components (mainly pyrazines). The authors concluded that although HS-TOF-MS suffers from large data files, which leads to slow data processing, it is the most appropriate technology for accurate peak identification and quantitation of the fast chromatographic peaks generated in the GC × GC method.

Kanavouras et al. [47] used SPME–GC–HS-TOF-MS for the analysis of olive oil volatiles. HS-TOF-MS permits identification of compounds in about 7–8 min compared to the approximately 1 h required by the conventional purge and trap-GC analysis. The analyses performed on the GC–HS-TOF-MS-system demonstrated high sensitivity and also high selectivity due to the high quality of mass spectra obtained.

Zhu et al. [48] used GC × GC–HS-TOF-MS to characterize the volatile compounds in Chinese liquors. According to the automated data processing by TOF-MS software, combined with the ordered chromatogram and the

![FIGURE 13](image.png) Demonstration of high-speed spectral generation (40 spectra/s) enabling the detection and quantification of coeluting compounds by using GC–HS-TOF-MS. The solid line represents the reconstructed total ion current (RTIC). Retention times differ by approximately 0.2 s. Reprinted with permission from Ref. [44].
retention index database, a total of 528 components were identified in a Moutai liquor sample, including organic acids, alcohols, esters, ketones, aldehydes, acetics, lactones, nitrogen- and sulfur-containing compounds, etc. In addition, the contribution of some important aroma compounds to the flavor of Moutai liquor was also studied.

Rochat et al. [49] utilized GC × GC–HS-TOF-MS to study sulfur compounds in roast beef headspace. More than 70 sulfur compounds were found by this approach and the identification of 50 of them was confirmed.

Robinson et al. [50] used SPME–GC × GC–HS-TOF-MS for characterization of wine volatiles. This study demonstrates an important advancement in wine volatile analysis as the method allows for the simultaneous analysis of a significantly larger number of compounds found in the wine headspace compared to other current single-dimensional GC–MS methodologies. The methodology allowed for the simultaneous analysis of over 350 different tentatively identified volatile and semivolatile compounds found in the wine headspace. These included potent aroma compound classes such as monoterpenes, norisoprenoids, sesquiterpenes, and alkyl-methoxypyrazines, which have been documented to contribute to wine aroma.

Setkova et al. [51] developed a rapid method for characterization of wine volatiles using SPME–GC–HS-TOF-MS. A high acquisition rate (50 spectra/s) allowed for a very effective spectral deconvolution while utilizing only a relatively short (10 m) narrow-bore column. Figure 14 illustrates the deconvolution procedure and peak find algorithm used to distinguish between peaks with poor chromatographic resolution. Under the 2.5-s segment of the analytical ion chromatogram (the total ion current after the deconvolution and baseline correction), seven different compounds were assigned with peak apexes very close to each other (the distance between the peak apexes was only 200–400 ms). Using this approach, 201 volatiles were tentatively assigned based on library search and retention index [52].

The GC × GC–HS-TOF-MS approach has also been used to characterize and identify volatiles in butter [53], olive oil [54], honey [55], wine [56], potato chips [57], basil [58], and pepper [59].

The identification of substance(s) responsible for unpleasant sensory properties represents a somewhat demanding task because a wide range of volatile compounds are released from food. Cajka et al. [60] used SPME for the extraction of volatiles from the contaminated and reference soft drink samples to compare the GC–HS-TOF-MS profiles of volatile compounds. A careful examination of the contaminated sample chromatogram showed a small, narrow peak (2.8 s at the baseline) of 2-chloro-5-methyl-phenol (MW = 142.6), completely overlapped by a broad, fronting peak of sorbic acid (Figure 15). Under the conditions of “manual” examination of recorded data, this compound was “invisible”; however, using the automated deconvolution function of the data processing software, this taint compound was identified even in the presence of sorbic acid, which was at approximately 55-fold higher intensity.
3.2.2 Food Authenticity and Origin

Risticic et al. [61] used 29 volatiles determined by SPME–GC–HS-TOF-MS in differentiation of Arabica coffee samples of different origins. The utilization of the HS-TOF-MS instrument ensured the completion of one GC–MS run of a complex coffee sample in 7.9 min and the complete list of benefits provided by ChromaTOF software, including fully automated background subtraction, baseline correction, peak find, and mass spectral deconvolution algorithms were exploited during the data evaluation procedure. Using principal component analysis (PCA), the corresponding geographical origin discriminations of coffees originating from South and Central America, Africa, and Asia were successfully established. In addition to successful geographical discrimination of (1) authentic sample collections from Brazil and Colombia and (2) non-authentic sample collections from South America, Central America, Africa, and Asia, this classification study was also successful in detecting potential...

FIGURE 14 Automated peak find algorithm in the ChromaTOF software (HS-TOF-MS instrument); (A) analytical ion chromatogram (total ion current after the baseline correction and spectral deconvolution), (B) seven different compounds assigned under the analytical ion chromatogram. Reprinted with permission from Ref. [51].
compositional changes that coffee undergoes due to the limited shelf-life stability over extensive storage conditions.

Cajka et al. [62] employed a SPME–GC × GC–HS-TOF-MS procedure for fast characterization of honey volatiles. Thanks to high separation efficiency of GC × GC and automated deconvolution function of the data processing software, only 19 min were needed for separation of the sample components. In total, 374 samples were collected over two production seasons in Corsica (n = 219) and other European countries (n = 155) with the emphasis to confirm the authenticity of the honeys labeled as “Corsica” (protected denomination of origin region). Using artificial neural networks with multilayer perceptrons (ANN-MLP) for chemometric analysis, a high prediction ability of 95% was obtained, indicating that this approach was successful, fitting to the authenticity purpose.

Humston et al. [63] used SPME–GC × GC–HS-TOF-MS for the analysis of cacao bean volatiles from six geographical origins (Costa Rica, Ghana, Ivory Coast, Venezuela, Ecuador, and Panama). Twenty-nine analytes that change in concentration levels via the time-dependent moisture damage process were measured using chemometric software. Biomarker analytes that were independent of geographical origin were found. Furthermore, prediction algorithms were used to demonstrate that moisture damage could be verified before there were visible signs of mold by analyzing subsets of the 29 analytes.

Cajka et al. [15] applied SPME–GC–HS-TOF-MS for obtaining fingerprints (GC profiles) of beer volatiles. In total, 265 speciality beer samples were collected over a 1-year period with the aim to distinguish, based on analytical (profiling) data, (i) the beers labeled as Rochefort 8; (ii) a group consisting of Rochefort 6, 8, and 10 beers; and (iii) Trappist beers. Although the profile of volatiles of beer samples was rather complex, only 21 min were
needed for the separation of compounds isolated by SPME. This relatively short GC run, which was approximately two to four times shorter than in previously published studies employing GC, was possible by the use of the deconvolution function of the HS-TOF-MS instrument. In this way, the lower chromatographic resolution of partially coeluted compounds was resolved spectrometrically; thus, pure mass spectra of volatile compounds were obtained, allowing reliable identification based on a library search. An additional benefit of the use of TOF-MS was simultaneous acquisition of full mass spectra even at very low concentration of particular compounds (due to the high mass analyzer efficiency), as compared to quadrupole MS operated in full scan mode used in previous studies. Thus, similar intensity of volatiles could be achieved but with shorter SPME extraction time (5 vs. 30–60 min). The best prediction ability was obtained for the model that distinguished a group of Rochefort 6, 8, and 10 beers and the rest of the beers. In this case, all chemometric tools employed, that is, partial least squares discriminant analysis (PLS-DA), linear discriminant analysis, and ANN-MLP, provided 100% correct classification. As an example, Figure 16 shows first and second PLS scores of different PLS-DA models, demonstrating separation between classes.

Giraudel et al. [64] utilized a SPME–GC–HS-TOF-MS method previously developed by Setkova et al. [51,52] for the analysis of volatile and semivolatile components of ice wine that originated from Canada and the Czech Republic. Using Kohonen self-organizing maps, a clear discrimination of the 137 samples,

FIGURE 16  First and second PLS scores for Rochefort 6, 8, and 10 (red) versus the rest (green) of beer samples (SPME–GC–HS-TOF-MS analysis of volatiles). Graph constructed using calibration data set (n = 166). Reprinted with permission from Ref. [15].
according to their Canadian and Czech origins, was obtained from a 300-cell trained map, without any outlying sample or analysis constituent.

Aprea et al. [65] used SPME–GC–HR-TOF-MS method for the acquisition of metabolite profiles of apple volatiles. Untargeted TOF-MS analysis revealed markers specific for each apple variety and was proven a useful tool for further studies on the apple metabolome. Advanced chemometric/statistical techniques (PCA and PLS-DA) were used to explore data and extract useful information. Processing of the data by MarkerLynx provided 2320 features from which 1019 were reported to be important using the variable importance values (VIP >1) of the developed PLS-DA model. Using a more strict criterion (VIP >1.7), only 30 variables were retained. However, the authors pointed out that variables sharing the same retention time window were scrutinized as if originating from the same metabolite since MS data were acquired in EI mode that causes strong ion fragmentation. This implied that the 30 variables corresponded to much fewer compounds. Therefore, these ions were further examined by the inspection of the GC mass chromatograms. The corresponding peaks (10) were finally annotated by retention time indices and spectral matching using NIST library. Figure 17 shows first, second, and third PLS scores, demonstrating separation among classes of examined samples.

FIGURE 17  PLS-DA plot showing grouping of samples according to apple variety (SPME–GC–HR-TOF-MS analysis of volatiles). Loadings with variable importance values (VIP >1 are also shown (in gray). Significant characteristic loadings (VIP >1.7) for each variety are shown as stars. Reprinted with permission from Ref. [65].
4 CONCLUSIONS

Over the past few years, there has been substantial progress in technologies employing GC coupled to orthogonal acceleration TOF-MS for improved performance. High-resolution and high-speed TOF analyzers represent complementary approaches for target as well as nontarget analysis of a wide range of (semi)volatile organic compounds present in food and environmental matrices.

The availability of sophisticated data systems and data processing algorithms has enabled automated and faster data handling, which is an important requirement for implementation of this mass spectrometric technique into routine use.

REFERENCES


